The effect of various priming techniques on drought stress tolerance and recovery kinetics of *Vigna unguiculata* (L.) Walp.

Thesis submitted to
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the requirements for the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

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Certificate

This is to certify that the thesis entitled **"The effect of various priming techniques on drought stress tolerance and recovery kinetics of *Vigna unguiculata* (L.) Walp."** submitted by **Aswathi Raj K. P.** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Botany** of the **University of Calicut,** is a *bonafide* record of the research work undertaken by her in this department under my supervision and guidance and no part thereof has been submitted for the award of any other degree.

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Declaration

I hereby declare that the work presented in the thesis entitled "**The effect of various priming techniques on drought stress tolerance and recovery kinetics of *Vigna unguiculata* (L.) Walp.**" is based on the original work done by me under the guidance of **Dr. Jos T. Puthur, Professor, Plant Physiology and Biochemistry Division, Department of Botany, University of Calicut** and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

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Dedicated to

My family and to the power of resilience…

May it inspire you to face challenges with unwavering inner strength and hope…

ABSTRACT

This research work was conducted to investigate the impact of different seed priming treatments on the drought stress tolerance potential and recovery kinetics of cowpea. The seeds of four cowpea varieties were obtained from the Regional Agricultural Research Station (RARS) in Pattambi, Kerala, India, and the Kerala Agricultural University (KAU) in Thrissur. Healthy and randomly selected cowpea seeds were surface sterilized and germinated in the culture bottles that contained distilled water (control) and varying concentrations of PEG 6000 (0%, 5%, 10%, 15%, 20%, and 25%), and maintained in a plant growth chamber under controlled conditions. Following the screening procedure, out of the four cowpea varieties, Anaswara was chosen as the sensitive variety and PGCP 6 as the tolerant variety. Priming treatments were conducted in these two varieties with contrasting stress tolerance potential. In order to optimize seedling performance, the concentrations/dosages and duration of priming treatments, such as BABA priming, hydropriming, PEG priming, and UV-B priming, were standardized for the two varieties. According to the preliminary findings, BABA, PEG, and UV-B priming were found to be more efficient against PEG stress in cowpea. Therefore, detailed analysis was performed in the BABA, PEG, and UV-B primed cowpea to evaluate the role of priming on drought stress tolerance. Control plants were kept under well-watered conditions, whereas drought stress was imposed by ceasing irrigation until the leaf relative water content (RWC) decreased to 50%. Subsequent to the stress, the plants were rehydrated for four days to recuperate from drought stress. Osmolytes and osmolality increased during drought stress and reduced during recovery from stress. The content of total soluble sugars, total free amino acids, proline, and total soluble proteins in plants that emerged from primed seeds was significantly higher than in the plants that developed from non-primed seeds. The increased accumulation of osmoprotectants safeguards biomembranes, organelles, and cytosolic enzymes. The drought induced stress effects were assessed by evaluating the accumulation of reactive oxygen species including hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), and the membrane stability index (MSI), electrolyte leakage, and the degree of lipid peroxidation. During drought stress, cowpea exhibited elevated levels of reactive oxygen species (ROS), leading to membrane damage and lipid peroxidation. The accumulation of ROS was most pronounced in the non-primed sensitive cowpea, causing significant organelle damage. Consequently, there was a reduction in chlorophyll content, reduced absorption,

trapping, and electron transport flux per cross section, as well as a lowered performance index, decreased PSI and PSII activity, and a reduced net photosynthetic rate. Seed priming markedly improved the stress tolerance mechanism in cowpea by elevating antioxidant activities. A notable augmentation in enzymatic antioxidants, including SOD, CAT, APX, and GPOX, was reported in drought exposed primed cowpea and it served a vital function in cellular protection and the mitigation of oxidative damage. Upon rewatering, the activities of SOD, CAT, GPOX, and APX were downregulated in cowpea, as their function became negligible due to the rapid recovery of primed cowpea plants from stress. Together with these, non-enzymatic antioxidants were also increased, which offered additional protection. Root length exhibited a slight increase during drought stress, facilitating the extraction of additional water from deeper soil layers; concurrently, lateral root growth, as well as the number and size of root nodules reduced during drought stress. In addition, seed priming with UV-B and BABA enhanced both the number and size of nodules, maybe attributable to the influence of UV-B and BABA on the expression of nod genes or genes involved in flavonoid production. Drought stress significantly reduced the nitrogen (N), molybdenum (Mo) and iron (Fe) content in the root nodules of cowpea varieties studied and the reduction was less in primed plants. RT-qPCR analysis was performed to evaluate the expression of dehydrin genes (*DHN*) in cowpea under well-watered, drought-stressed, and recovery conditions. The gene expression study revealed that not all dehydrin genes in cowpea are drought-inducible. In the tolerant variety (PGCP 6), only three DHN genes (*Vu400*, *Vu500*, and *Vu600*) were activated during drought stress. The study demonstrates that seed priming with BABA, PEG, and UV-B enhances drought stress tolerance in tolerant cowpea variety and impart drought tolerance in sensitive variety and the extent of tolerance generated by priming was more pronounced in the tolerant variety. Among the priming treatments, BABA priming was proved to be more efficient in mitigating drought stress in the cowpea varieties Anaswara and PGCP 6. BABA priming involves trans-priming, in which the priming stimuli and the prior stress differ in characteristics, perhaps promoting cross-tolerance to stress responses in cowpea. Rewatering alleviated the intensity of drought stress in primed cowpea plants more effectively, promoting a rapid recovery during the recuperation phase. As a result, the BABA primed Anaswara and PGCP 6 exhibited fastest recovery compared to plants subjected to other priming treatments.

Keywords: Antioxidation, Drought, Photosynthesis, Recovery, Seed priming

സംഗ്രഹം

വൻപയറുകൾ നേരിടുന്ന വരൾച്ചാസമ്മർദ്ദത്തെ ലഘൂകരിക്കാനും അതുവഴി ജലം ലഭ്യമാകുമ്പോൾ ജീവൽ പ്രവർത്തനങ്ങളെ ത്വരിത ഗതിയിൽ വീണ്ടെടുക്കാനും സീഡ് പ്രൈമിംഗ് ട്രീറ്റ്മെന്റുകൾ ചെലുത്തുന്ന സ്വാധീനം കണ്ടെത്തുന്നതിനാണ് ഈ ഗവേഷണ പ്രവർത്തനം. കേരള കാർഷിക സർവകലാശാല, തൃശ്ശൂർ, പ്രാദേശിക കാർഷിക ഗവേഷണ കേന്ദ്രം, പട്ടാമ്പി എന്നിവിടങ്ങളിൽ നിന്ന് 4 ഇനം വൻപയർ വിത്തുകൾ ശേഖരിച്ചു. വിത്തുകൾ വ്യത്യസ്ത ഗാഢതയിലുള്ള പോളി എഥിലിൻ ഗ്ളൈകോൾ (PEG) ലായനികളിൽ വളർത്തിയ ശേഷം പ്രതിരോധ നില നിർണ്ണയിച്ചു. അനശ്വരയെ പ്രതിരോധം കുറഞ്ഞതും പി ജി സി പി 6 നെ സഹിഷ്ണുതശേഷി കൂടിയതുമായ ഇനമായി തെരഞ്ഞെടുത്തു. തുടർന്ന് അന്തർലീനമായ സമ്മർദ്ദപ്രതിരോധശേഷി വർദ്ധിപ്പിക്കുന്നതിനായി ഈ രണ്ട് വൻപയർ ഇനങ്ങളെ പ്രൈമിംഗ് ന് വിധേയമാക്കി. ബീറ്റാ അമിനോ ബ്യൂട്ടിറിക് അസിഡ് (ബാബ), പോളി എഥിലിൻ ഗ്ളൈക്കോൾ, ഹൈഡ്രോപ്രൈമിംഗ്, അൾട്രാ വയലറ്റ്-ബി (യു വി-ബി) പ്രൈമിംഗ് എന്നിവയുടെ ഗാഢതയും ദൈർഘ്യവും കണ്ടെത്തി.

പഠനത്തിനായി സസ്യങ്ങളെ കാലിക്കറ്റ് സർവകലാശാല ബോട്ടണി പഠനവകുപ്പിലെ പോളിഹൗസിൽ വളർത്തി. തുടർന്ന് ഇലയുടെ ആപേക്ഷിക ജലാശം പകുതിയാകുന്നത് വരെ ജലസേചനം നിർത്തി വരൾച്ചാസമ്മർദ്ദം നൽകി. തുടർന്ന് 4 ദിവസത്തെ തുടർച്ചയായ ജലസേചനത്തിലൂടെ വീണ്ടെടുക്കാൻ നടത്തി. ഓസ്മോളാലിറ്റിയും ഓസ്മോലൈറ്റുകളും വരൾച്ച ഘട്ടത്തിൽ വർധിച്ചു. പ്രൈം ചെയ്ത സസ്യങ്ങളിൽ ഇവയുടെ അളവ് ഗണ്യമായി കൂടുതലായിരുന്നു. റിയാക്റ്റീവ് ഓക്സിജൻ സ്പീഷിസുകളുടെ അളവ് ക്രമാതീതമായി വർധിക്കുന്നത് സസ്യകോശങ്ങളുടെയും അവയവങ്ങളുടെയും തകരാറുകൾക്ക് കാരണമാകുന്നു. തൽഫലമായി പ്രകാശസംശ്ലെഷണ നിരക്ക് കുറഞ്ഞു. എന്നാൽ, ആന്റിഓക്സിഡന്റുകളുടെ ശ്രദ്ധേയമായ വർദ്ധനവ് റിയാക്റ്റീവ് ഓക്സിജൻ സ്പീഷിസുകളെ ക്രമീകരിച്ചുകൊണ്ട് കോശസംരക്ഷണത്തിന് സഹായകരമാകുന്നു. വരൾച്ച ഘട്ടത്തിൽ സസ്യത്തിന്റെ വേരിന്റെ നീളത്തിൽ നേരിയ വർദ്ധനവ് കാണപ്പെട്ടു. ആഴത്തിലുള്ള മൺപാളികളിൽ നിന്നും ജലംശം വലിച്ചെടുക്കാൻ ഇത് സഹായകമാകുന്നു. കൂടാതെ, വേരിലെ നൊഡ്യൂളുകളുടെ എണ്ണത്തിലും വലുപ്പത്തിലും പ്രകടമായ കുറവ് കാണപ്പെട്ടു. എന്നാൽ യു വി-ബിയും ബാബയും ഇവയെ മെച്ചപ്പെടുത്തി. ഇത് ഫ്ലാവോനോയ്ഡ് ഉത്പാദനത്തിന്റെയോ നോഡ് ജീനുകളുടെ പ്രകടനത്തിൽ ഇവയ്ക്കുള്ള പങ്കിനെ രേഖപെടുത്തുന്നു. വൻപയറിലെ ഡീഹൈഡ്രിൻ ജീനുകളുടെ പ്രവർത്തനത്തെ മനസിലാക്കാൻ ആർ ടി-കൃ പി സി ആർ നടത്തി. എല്ലാ ഡീഹൈഡ്രിനുകളും വരൾച്ചാസമയത്ത് പ്രേരിപ്പിക്കുന്നില്ല എന്നത് പഠനം വെളിപ്പെടുത്തി. പ്രൈമിംഗ് വഴി ഉണ്ടാകുന്ന സഹിഷ്ണുതയുടെ വ്യാപ്തി പ്രതിരോധശേഷി കൂടിയ പയറിനമായ പി ജി സി പി 6 ൽ ആണ് കൂടുതൽ പ്രകടമായത്. കൂടാതെ, മറ്റ പ്രൈമിംഗ് കളെ അപേക്ഷിച്ച് ബാബ പ്രൈമിംഗ് കൂടുതൽ കാര്യക്ഷമമാണെന്ന് തെളിയിക്കപ്പെട്ടു.

ഇതിൽ പ്രൈമിംഗ് ഉത്തേജകവും പിൻകാല സമ്മർദ്ദവും വ്യത്യസ്ത സ്വഭാവത്തിൽ പെട്ടതാണെങ്കിലും ഇവ പരസ്പരം ബന്ധിതമാണ്. പ്രൈം ചെയ്ത സസ്യങ്ങളിൽ സമ്മർദ്ദതീവ്രത ലഘൂകരിക്കുന്നത് വഴി ജലം ലഭ്യമാകുന്ന ഘട്ടത്തിൽ ജീവൽപ്രവർത്തങ്ങൾ പെട്ടെന്നുതന്നെ പൂർവ്വസ്ഥിതിയിലെത്തുന്നു. അതിനാൽ വരൾച്ചയെ അതിജീവിക്കാനും ജലം ലഭ്യമാകുമ്പോൾ അവയിൽ നിന്നും ത്വരിത ഗതിയിൽ പുനർജീവിക്കാനുമുള്ള ശേഷി പ്രൈമിംഗ് വഴി വൻപയറുകൾക്ക് സാധ്യമാക്കുന്നു എന്ന് തെളിയിക്കപ്പെട്ടു.

സൂചകപദങ്ങൾ: വരൾച്ച, പ്രകാശസംശ്ലേഷണം, സീഡ് പ്രൈമിംഗ്, പുനരുജ്ജീവനം

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ABBREVIATIONS

ABA	-	Abscisic acid
ABS	-	Absorption flux
APX	-	Ascorbate peroxidase
AsA	-	Ascorbate
BABA	-	β-amino butyric acid
CAT	-	Catalase
Chl	-	Chlorophyll
CSm	-	Cross section related to Fm
DCMU	-	3(3,4 dichlorophenyl)-1,1-dimethyl urea
DCPIP	-	2,6-dicholrophenolindophenol
DHN	-	Dehydrin
DIo	-	Dissipated energy flux
DTNB	-	5-dithio-bis-2-nitrobenzoic acid
EL%	-	Electrolyte leakage %
ETo	-	Electron transport flux
ETR	-	Electron transport rate
Fm	-	Maximum chl *a* fluorescence
F_O	-	Initial chl *a* fluorescence
FTIR	-	Fourier Transform Infrared
Fv	-	Variable chl *a* fluorescence
FW	-	Fresh weight
GABA	-	γ-amino butyric acid
gs	-	Stomatal conductance
GSH	-	Glutathione
H_2O_2	-	Hydrogen peroxide
HEPES	-	N-(2-Hydroxyethyl)piperazine-N-(2-Ethanesulphonic acid)
KBr	-	Potassium bromide
LHC	-	Light harvesting complex
MDA	-	Malondialdehyde
MSI	-	Membrane stability index
MV	-	Methyl viologen

NaN_3	-	Sodium azide
NBT	-	Nitroblue tetrazolium
$O_2^{\bullet-}$	-	Superoxide
pBQ	-	para-Benzoquinone
PEG	-	Polyethylene glycol
PI	-	Performance index
Pn	-	Net photosynthetic rate
POD	-	Guaiacol peroxidase
PSI	-	Photosystem I
PSII	-	Photosystem II
r	-	Pearson's correlation coefficient
RC	-	Reaction center
ROS	-	Reactive oxygen species
RWC	-	Relative water content
SEM	-	Scanning Electron Microscope
SOD	-	Superoxide dismutase
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TRo	-	Trapping energy flux
UV	-	Ultraviolet

1. INTRODUCTION

One of the biggest challenges of the 21st century is climate change, which has adverse consequences on ecosystems and human population (Eckardt et al., 2023). There are severe concerns about climatic extremes on a global scale, and as cities grow and the world's population rises, more organisms are at risk from these extremes (Zhou et al., 2023). The fact that abiotic stressors brought on by global climate change have already caused a 60% drop in crop yields worldwide raises even greater concerns. Plants that are subjected to these severe and frequent abiotic stress conditions undergoes irreversible morpho-physiological and molecular changes, and future climate conditions are predicted to exacerbate this damage (Fan et al., 2024). Elevation in atmospheric carbon dioxide concentration, high temperature, drought and salinity as a consequence of climate change is the major concern of the era (Chaudhry and Sidhu, 2022). A plethora of abiotic stresses have caused damage to plants at different phases of their growth and development, and as a result, plants have been trying to evolve adaptation mechanisms to lessen the consequences of stress. The complex mechanism involves the stress signal perception, signal transduction, activation of physiological and biochemical pathways and expression of stress responsive genes (Ma et al., 2024).

Among the various abiotic stresses, drought is the predominant one that severely hampers plant growth and performance. Globally, dry areas are expected to surpass half of the total land area by the end of the 21st century. The Food and Agriculture Organisation of the United Nations estimates that the world's highly arid, arid, semi-arid, and semi-humid arid areas cover approximately 6.1 billion hectares of land, or 41% of the planet's land area (FAO, 2019). Global climate change exacerbates the occurrence of

drought, and the United Nations World Water Development report (WWAP, 2018) states that 55 million people are globally affected by drought and 700 million people could be at risk of displacement by 2030. Plants are more susceptible to drought stress due to their sessile nature. Hence, plants have robust stress responses that involve a wide range of interactions at several regulatory levels, such as metabolic control, morphological adaptation, physiological response and gene expression (Farooq et al., 2012; Mansoor and Chung, 2024).

Drought stress considerably impacts crop yield and must be mitigated to ensure food security for 9 billion individuals by 2050, as food demand is projected to be doubled by that year (Tilman et al., 2011; He et al., 2021). The Sustainable Development Goals (SDGs), sets a collection of goals within a worldwide consensus to eradicate poverty, save everything that makes the earth habitable, and guarantee that everyone lives in peace and prosperity. These goals were officially ratified in 2015 by all member states of the United Nations for the duration of 2016–2030 (Biermann et al., 2022). Therefore, it is relevant to study about the effect of drought stress on crop plants and the possible ways to reduce its impacts. Diverse approaches, including traditional breeding and contemporary techniques like genetic engineering, mutation breeding, and polyploidy breeding, are being tested to generate plants capable of withstanding environmental stresses. Dating back centuries, traditional breeding has played a crucial role in producing different crops adaptable to diverse environmental circumstances, including drought.

The first and most crucial step of plant growth is seed germination, which is negatively impacted by drought stress. It has an impact on imbibition, germination, and seedling establishment, which results in inadequate seedling establishment and decreased germination rate (Toscano

et al., 2017). Reduction in plant height, number of leaves, fresh and dry biomass were also observed under water stress (Khan et al., 2016). Another commonly observed morphological symptom is leaf rolling, which is the loss of pressure potential resulting from water loss from the upper epidermis of the leaf when plants are under water deficit condition. Studies have found that, during the progression of drought stress, leaves showed curling and wilting syndrome (Pandey et al., 2023).

There are notable changes in the internal structure of plants subjected to drought stress. The leaf epidermis's outer wall develops a cuticle protection layer. Under the circumstances of water scarcity, the content of cuticular lipids in leaves considerably gets increased. Also wax alkanes increases under drought stress, which is primarily responsible for the rise in epidermal wax per unit area. Additionally, the water shortage raises the amount and alters the proportion of cutin monomers, and increases the cuticular thickness (Kosma et al., 2009). Furthermore, plant leaves exhibit a tendency to increase the mesophyll palisade tissue, lessen the spongy tissue, enhance the number of cell layers, and also reduces the volume and the intercellular space in order to adapt towards drought conditions (Bai et al., 2019).

Likewise, drought stress have a negative impact on various physiological processes including net photosynthesis (*Pn*), stomatal conductance (*gs*), internal CO_2 concentration (*Ci*), transpiration rate (*E*), carboxylation efficiency, and water-use efficiency (WUE). The impact of drought stress on leaf gaseous exchange rate and chlorophyll fluorescence can be significant. It relies on the plant's growth stage and the period of drought stress (Mathobo et al., 2017). *Pn*, *gs* and *Ci* decreased under drought stress along with reduction in maximum quantum yield of PSII (Fv/Fm), effective quantum yield of PSII photochemistry (ΦPSII), and photochemical

quenching coefficient (qp) (Hassan, 2006). Moreover, during drought conditions, the light-harvesting complexes (LHCs) and certain core proteins of PSI and PSII were found to be disassembled, but these complexes were regrouped during the process of recovery. Profound drought stress resulted in a decrease in both LHCI and LHCII, as well as a few PSI and PSII reaction centre proteins, suggesting a notable disruption of the photosynthetic apparatus (Pandey et al., 2023).

Enhancement in reactive oxygen species (ROS) generation in several cellular compartments, including chloroplasts, peroxisomes, and mitochondria, is an unavoidable outcome of drought stress. Drought hampers the regular transportation of solutes, leads to electron leakage, and stimulates the generation of reactive oxygen species (ROS), which induce oxidative damage. These reactive oxygen species (ROS) interact with several cellular components including the nucleus, proteins, and membranes, therefore compromising their structural integrity. Nevertheless, when present in low quantities, ROS function as crucial signaling molecules by activating several stress-responsive pathways and promote cross talk among various pathways. ROS are associated with the detection and stimulation of ABA, other hormones, Ca^{2+} fluxes and respiratory burst oxidase homologs (RBOH). They are expected to play a role in both the upstream and downstream routes of the ABA-dependent/independent signaling pathways during drought stress. By adjusting the levels of ROS, it is possible to improve the tolerances of crop plants to drought and other abiotic stress conditions (Verma, et al., 2019). Increased generation of ROS is tightly regulated by a flexible and collaborative antioxidant system that controls the concentration of ROS inside the cell and determines its redox state (Cruz de Carvalho, 2008; Noctor et al., 2014). Antioxidative mechanisms, including non-enzymatic compounds such as ascorbate, glutathione, carotenoids and α-tocopherol, as well as enzymatic antioxidants like superoxide dismutase,

catalase, peroxidases and enzymes of the ascorbate-glutathione (ASC-GSH) cycle, that scavenge surplus reactive oxygen species (ROS) generated in plant cells either by partial inhibition of ROS generation and/or by neutralisation of ROS that have been previously generated (García-Caparrós et al., 2021).

Plants exhibit acclimation processes in response to drought stress, which leverage secondary metabolites (SMs) and phytohormones to modify physiochemical, and molecular responses. Phytochemicals, including flavonoids, polyphenols, volatile isoprene, and non-volatile isoprenoid, are the most often researched SMs in drought adverse situations. As an adaptive reaction, the plant accumulates SMs which serve as a potent antioxidant, synthesise and transport metabolites and enzymes, stabilise cellular components, as well as signal and regulate genes. Typically, SMs interact with PHs to overcome limitations of drought stress. PHs including abscisic acid (ABA), auxins, cytokinins, gibberellic acid, ethylene, salicylic acid, jasmonic acid, brassinosteroids, and strigolactones are essential in the signaling pathways of plants under drought stress (Ahmad et al., 2023).

In order to counteract the harmful impacts of drought stress, drought-tolerant plants recruit a diverse range of metabolites and low molecular weight proteins. Dehydrins (DHNs) are a specific category of proteins that build up in plants in response to drought and other related stress. These proteins exhibit a strong affinity for water and has diverse functions in safeguarding plant cells during conditions of drought stress. For nearly three decades, dehydrins have been recognised as chaperones that facilitate ion-binding activities, serve as cryo-protectants, and assist in the scavenging of free radicals. Additional activities of dehydrin have been investigated in recent times, and the findings indicate that dehydrin may have functions beyond its chaperone features. The new discovery of dehydrin's role in

regulating stress-responsive genes has facilitated its involvement in the cell's transcription regulatory machinery during the stress response. Furthermore, dehydrins have been documented to perform an indirect function in the modification of histones. Within this epigenetic mechanism, the change of H3K4me3 is directly linked to the upregulation of dehydrin and other genes that respond to drought (Tiwari and Chakrabarty, 2021). Extensive evidence indicates that DHN proteins contribute to drought stress tolerance by improving water retention capacity, increasing chlorophyll content, preserving photosynthetic machinery, initiating ROS detoxification, and facilitating the accumulation of compatible solutes, among other mechanisms (Riyazuddin et al., 2022).

Conventional breeding has contributed to enhance crop yield; but with the global population approaching 7 billion and continuing to rise, it is not feasible to meet the increasing demands for food and shelter using traditional methods. Conventional breeding techniques often require extensive timeframes to produce new varieties with desirable traits. The transfer of traits between genotypes occurs via sexual reproduction, a process characterised by its slow pace (Moose and Mumm, 2008). These methods may lack precision because they are based on the possibility of gene recombination and hence this complicates the selection of specific traits and may result in the incorporation of undesirable traits (Lyzenga et al., 2021). Introducing new traits into a crop or enhancing existing traits absent from the gene pool can also be challenging (Sharma et al., 2013). A primary disadvantage of traditional breeding is its dependence on germplasm crossover or random mutagenesis, which is a laborious and frequently ineffective method, particularly for intricate traits such as drought tolerance. These traits are typically regulated by multiple genes, making it challenging to select all relevant genes at once (Tanksley and McCouch, 1997; Acquaah, 2024). Overcoming these difficulties poses a significant challenge, as

backcrossing demands substantial time and resources. The selection of superior genotypes based on phenotypic features has been constrained by poor heritability, genetic interactions including epistasis, and environmental-genotype interactions.

In light of these constraints, there has been a transition towards the incorporation of genomics and genome editing tools with conventional breeding practices (Şimşek et al., 2024). In this context, genome editing and genetic engineering serve as effective methods for enhancing crops by focusing on specific traits such as drought. These methods may prove beneficial in instances where the genes for desired traits are absent from the gene pool (Ahmed et al., 2024). The extensive applicability of biotechnology suggests that biotechnology could serve as a crucial tool for achieving the UN's Sustainable Development Goals. Innovative biotechnology methods have improved our comprehension of the molecular and entire plant mechanisms involved in drought responses. Numerous genes triggered by drought stress have been found, and several of these have been cloned. Plant genetic engineering and molecular marker techniques facilitate the formation of drought-resistant germplasm. Transgenic plants possessing genes for abiotic stress tolerance are being engineered for abiotic stress management. Transgenics possessing several genes associated with drought tolerance have been generated in various plants via *Agrobacterium* and particle gun technologies. Typically, drought stress-tolerant transgenics are subjected to pot trials or contained field evaluations (Gosal et al., 2009)

Biotechnological methods to enhance drought stress tolerance in plants may include the overexpression of genes associated with cellular homeostasis, such as osmotic adjustment, chaperones, or antioxidants. Furthermore, advancements in the identification of plant microRNAs, especially those whose expression is modified by drought stress, present

intriguing new targets for the modulation of drought response pathways. Collaboration between academia and industry could speed up the development of new drought resistant traits (Umezawa et al., 2006; Zhang et al., 2011; Deikman et al., 2012). Research emphasising the significant advancements in genetic engineering techniques, particularly CRISPR-Cas9 genome editing, has revolutionised the development of drought tolerant crops. There are studies which examines the crucial function of microbial biotechnology, including plant growth-promoting rhizobacteria (PGPR) and mycorrhizae, in improving plant resilience to drought. The integration of advanced biotechnology techniques with conventional breeding practices is proposed as a comprehensive strategy for enhancing crop resilience to drought stress (Şimşek et al., 2024).

Marker-assisted breeding and the application of "omics" technology have been accepted as effective methods for enhancing drought tolerance, allowing for the identification of drought-related quantitative trait loci (QTLs) and improving the efficiency of drought screening approaches (Jaafar et al., 2021). The advancement of genetically modified organisms (GMOs) for agricultural improvement has used many genetic engineering methodologies, such as genome editing, transgenesis, cisgenesis and intragenesis (Holme et al., 2013). Among these, the alteration of regulatory genes, which encode proteins crucial in plant stress responses has emerged as a particularly effective technique for developing crops adaptable to drought conditions. This method utilises the inherent ability of plants to adjust with environmental stressors by altering key regulatory processes. To achieve this objective, scientists have explored extremophiles, like halophytes and thermophiles, organisms thrive under extreme conditions, to get heterologous genes that provide stress resistance. The integration of these genes into agricultural crop genomes seeks to confer improved

survival mechanisms under unfavourable environments (Şimşek et al., 2024).

Notably, a variety of crop species, such as maize, rice, wheat and soybean has been the target of genetic changes to generate cultivars with increased drought tolerance. This novel genetic engineering exemplifies the integration of bacterial cold shock proteins (csp) into *Arabidopsis*, maize and rice to enhance their resilience to drought stress (Şimşek et al., 2024). The studies by Campo et al. (2012) on the *ZmGF14-6* gene and Yu et al. (2013) on *OsSNAC1* and Yang et al. (2019) on *OsbZIP62* in transgenic rice highlight the significance of these stress related genes in enhancing drought tolerance. These findings demonstrate that genetic alterations can control essential processes such as ROS homeostasis, stomatal closure, and transpiration rate, hence improving the plant's capacity to endure water scarcity. These studies emphasize the potential of genetic engineering to enhance drought resistance. Diverse green biotechnology methods have been employed on crops to enhance their drought resistance and augment the yield. One of these methods resulted in the development of Golden Rice by Syngenta™. It was created by incorporating a gene from daffodil into rice, yielding rice that exhibited enhanced drought tolerance and contained 23 times the beta-carotene of conventional rice (Nasser et al., 2021). Drought resistance was attained by engineering rice to overexpress the trait locus *DEEPER ROOTING* 1 (DRo1), resulting in increased root depth in the soil, hence improving rice yield under drought conditions (Uga et al., 2013).

The transfer of genes between species may also transfer traits that are problematic. Hence, robust antitrust legislation and enforcement agencies are essential, especially in small developing nations. Efficient legislation is necessary to uphold intellectual property rights in accordance with the agreements established by the World Trade Organisation (WTO) and the

Convention on Biological Diversity. Nonetheless, worry for the unknown and insufficient study on the long-term impact of green biotechnology on animal and human and health has led to concerns about its potential hazards to humans, animals, and ecosystem health. The presence of such dangers implies that, in the absence of appropriate measures, certain aspects of green biotechnology may fail to meet the criteria of the UN Sustainable Development Goal pertaining to health (Nasser et al., 2021). In addition to this significant limitation, these technologies are costly, cumbersome, and subject to biosafety rules and constraints that impede the utilization of transgenics in the field. Given the aforementioned limits of existing methodologies, it is essential to consider alternate solutions to enhance plant tolerance to various stimuli. The alternative option would be more acceptable if it is simple, cost-efficient, and easily adoptable by farmers, while also effectively demonstrating tolerance (Jisha et al., 2013).

Priming, in contrast to other complex methods such as breeding or genetic engineering, is simple, eco-friendly, cost effective and farmer friendly so that it can readily implemented to increase stress tolerance of plants. Besides this, priming approach is metabolically cost-effective as it is component of induced resistance in plants (Vijayakumari et al., 2016). Because of the overall benefits, priming is a widely acceptable and ever growing area in the field of stress physiology. Priming is the selective application of several natural and synthetic factors to seeds and/or seedlings in order to elicit a moderate level of stress (Paparella et al., 2015), through which plants achieve a distinguishing physiological state known as the 'primed' state following the pretreatment with a priming agent, which enhances certain cellular reactions (Wojtyla et al., 2016). Consequently, plants are well-prepared to promptly react and endure to later, more severe stress (Farooq et al., 2020). The primed defense response constitutes a component of induced resistance in plants, hence augmenting their intrinsic

potential, enabling primed plants to react more swiftly and effectively when subjected to stress conditions (Vijayakumari et al., 2016).

In the early 19th century, investigations helped to define seed priming. This empirical understanding led to a well-defined idea of seed priming as a replicable method for seed workers in the 1960s, when May et al. (1962) demonstrated that seeds subjected to treatment and then dried under regulated conditions sprouted rapidly under stress environments. Successful results encouraged the authors to note the fascinating physiological effects underlying this technique (Pagano et al., 2023). Since that time, the application of seed priming has steadily progressing, with hydro, osmo, chemical, hormonal, radiation and nano-priming are extensively used (Sherin et al., 2022). Research proved that the seedlings emerging from primed seeds exhibit early and uniform germination, resulting in an overall improvement in several growth characteristics throughout their lifespan (Jisha et al., 2013; Guo et al., 2022).

Seed priming facilitates controlled water uptake, pre-germinative metabolism and speed up the germination; yet, it is essential to prevent the radical protrusion (Soeda et al., 2005). Pre-germinative metabolism is triggered when water uptake begins and continues throughout imbibition phase. This phase triggers key physiological processes, such as the de novo synthesis of proteins and nucleic acids, ATP production, activation of DNA repair mechanisms, and antioxidant systems (Paparella et al., 2015). In recent years, chemical priming has appeared as a feasible approach for addressing diverse abiotic challenges in crop plants (Sako et al., 2020; Aswathi et al., 2023; Gohari et al., 2024). Enhancing tolerance to abiotic stress can be accomplished through the administration of diverse natural or synthetic substances. Among many compounds evaluated for their efficacy in mitigating abiotic challenges, certain compounds have demonstrated a

priming impact in activating defence responses in plants. This encompasses β-amino butyric acid (BABA), γ-amino butyric acid (GABA), reactive oxygen–nitrogen–sulfur species, melatonin, spermine, spermidine, choline, to mitigate stressors like drought and other osmotic stresses (Savvides et al., 2016). Research findings have demonstrated the significance of chemical priming agents in enhancing plant growth, photosynthesis and alleviating the inhibitory impact of drought stress on plant development (Khan et al., 2019). Priming GABA and BABA is gathering increased attention, as these non-protein amino acids enhance plants resilience to abiotic stressors without incurring significant energy expenditures on defensive systems. The precise mechanism of action of priming with GABA/BABA in plants remains unclear, while their significance as signaling molecules during stress is indisputable.

Studies indicate that hydropriming is an efficient, economical, and secure technique for enhancing the tolerance of seeds to osmotic stress, consequently facilitating seedling growth and agricultural productivity in adverse conditions (Kaur et al., 2002). In this priming procedure, the seeds are immersed in sterilized distilled water kept at the appropriate temperature. Following hydration, the seeds were re-dried to their original weight (Aswathi et al., 2023). It is crucial to thoroughly dry the seeds after soaking, as the storage of inadequately dried seeds would provide more detrimental effects than beneficial effects (Thomas et al., 2000).

Osmopriming is another practical seed priming technique involving the immersion of seeds in osmotic solutions of low water potential to promote controlled hydration (Marthandan et al., 2020). Research has demonstrated the beneficial impact of osmopriming on germination patterns, seedling development, photosynthetic performance and stress tolerance. The osmopriming agent serves as a moderate stressor, enhancing

stress memory in plants and subsequently activating tolerance mechanisms upon exposure to further stress. Polyethylene glycol (PEG) serves as an efficient osmopriming agent, capable of inducing mild osmotic stress in plants, hence enhancing their tolerance to subsequent stress exposure.

While UV light is known to induce stress in plants, modest doses of it elicit a priming effect. Numerous reports have addressed the beneficial impact of low levels of UV irradiation on plant growth (Hideg et al., 2013; Bornman et al., 2015). Irradiating low doses of UV-B light can positively influence the morpho-physiological and molecular traits of plants (Kacharava et al., 2009). Suboptimal UV-B radiation levels augment the production of secondary metabolites, accumulation of compatible solutes, antioxidant activity, improvement of pigment composition, and activation of stress-responsive gene expression. These impacts augment the drought tolerance potential of plants, hence facilitating stress acclimatization (Thomas and Puthur, 2020; Luo et al., 2022; Sen et al., 2022). UV-B radiation has multiple advantages as it can promote plant hardiness, augment resistance to various stresses and can play pivotal role in enhancing the agricultural productivity and thus impacting food security (Sen and Puthur, 2021).

Priming relies on the principle that, prior low-level exposure can equip a plant for future stress or elicit an adaptive state that may persist until a further exposure to stress occurs. Plants are recognized for their ability to retain and retrieve information, which can be characterized as memory (Crisp et al., 2016). Evidence indicates that early exposure to mild stress might establish epigenetic memory in plants, preparing them to face similar or different stressors in the future (Hossain et al., 2018; Marcos et al., 2018). Exposure to abiotic stress modifies the expression levels of numerous transcription factors associated with stress metabolism. Prolonged

alterations in gene expression are also induced by epigenetic modifications, allowing the plant to retain a form of memory of past experiences, even after the stress has been alleviated (Bruce et al., 2007). Alterations in chromatin architecture regulate gene expression by controlling gene accessibility for transcriptional machinery (Banerjee and Roychoudhury, 2017). These alterations encompass DNA methylation, histone modification, or chromatin remodelling (Bruce et al., 2007).

In nature drought is succeeded by a beneficial phase characterized by water availability through precipitation or irrigation, prompting plants to attempt recovery. Plants require recuperation from the harm inflicted by drought during stress recovery. The intensity of the drought and the degree of damage imposed by the stress determines the recovery kinetics of plants (Rivas et al., 2016). A reduced extent of drought-induced damage correlates with an accelerated rate and kinetics of recovery (Chen et al., 2016). Consequently, drought recovery plays a crucial role in the adaptation of plants to drought stress. Research has demonstrated that diverse priming approaches, including hydropriming, osmopriming, UV-B priming, and chemical priming, are typically employed to boost drought stress tolerance in plants (Wojtyla et al., 2016). The identical priming approaches may also be beneficial in facilitating the plant's rapid and efficient recovery from drought stress.

To speed up the process of recovery it is essential to maintain optimal tissue water status inside the plant cell. This was primarily achieved by the increased accumulation of osmolytes, including sugars, amino acids, proline, and glycine betaine. The maintenance of elevated water content by the priming-induced enhancement of osmolytes may facilitate faster recovery from drought stress. Moreover, pre-germinative activities occurring during seed priming activate the antioxidant mechanism to eliminate reactive

oxygen species and mitigate oxidative stress (Paparella et al., 2015). Hence, the lesser the damage to the cellular structure, greater is the recovery rate (Rivas et al., 2016). Studies revealed that the increase of antioxidants resulting from a plethora of seed priming leads to mitigation of oxidative damage induced by drought stress (Zheng et al., 2016; Khan et al., 2020). This effective operation of antioxidant mechanisms, facilitated by various priming strategies, will undoubtedly assist plants in recuperating and reinstating their usual functions promptly upon alleviation of stress (Aswathi et al., 2022).

Photosynthesis, among the several biochemical processes, exhibits significant sensitivity to drought and recovery (Hayano-Kanashiro et al., 2009; Zhang et al., 2018). The immediate outcome of rewatering is photosynthetic recovery. Priming alleviate the drought induced damage to the photosynthetic machinery and facilitates rapid recovery. Diverse seed priming methods enhanced the photosynthetic efficacy of various plants. Among this tolerant genotypes exhibited swift recovery for net photosynthetic rate, stomatal conductance, and plant water status (Hayano-Kanashiro et al., 2009). This was corroborated by the observation that increased modulation in the expression of differentially expressed genes encoding various transcription factors occurs in the tolerant genotype during drought and subsequent recovery (Hayano-Kanashiro et al., 2009; Zheng et al., 2010; Zhang et al., 2018). It was also discovered that seed priming improves the tolerance capability of tolerant types more significantly than sensitive kinds. It was also discovered that seed priming improves the tolerance capability of tolerant plants more significantly than sensitive plants (Sen and Puthur, 2020).

Analogous to the DNA repair mechanisms observed during initial germination, similarly stress recovery depends on the repair mechanisms for

DNA and organelle damage. In recovery, plants reorganize majority of metabolic pathways to repair drought-induced damage (Chen et al., 2016). The retention of priming memory in seedlings derived from primed seeds enhances their drought tolerance and facilitates quick recovery upon rewatering. Consequently, these findings indicate that halo and UV-B priming of seedlings effectively mitigates drought stress effects and improves recovery rates throughout the seedling stage (Sen and Puthur, 2021). Likewise, Plant Growth-Promoting Rhizobacteria (PGPR) enhance the drought tolerance of both sensitive and tolerant barley cultivars, facilitating the recovery of plants to a physiological state comparable to that of unstressed plants (Ferioun et al., 2024).

There are limited studies on the beneficial effects of seed priming for complementing the recovery of plants after the withdrawal of drought stress. Therefore, it is crucial to concentrate on the drought tolerance potential generated by priming in plants and the recovery kinetics of drought-stressed plants as affected by seed priming. Additionally, investigations must examine the potential processes by which priming facilitates accelerated and effective recovery from drought. A significant information void exists concerning the impact of seed priming on the recovery kinetics of plants exposed to drought stress. These findings enhance the intrinsic stress tolerance capacity of plants, consequently facilitating improved crop yield. The environmental and ethical safety provided by several seed priming procedures renders it a promising and sustainable approach for securing food supply (Aswathi et al., 2022).

The demand for food crops, particularly legumes rich in protein, is rising due to their ability to address hidden hunger, primarily manifested as protein shortage (Jha et al., 2022). *Vigna unguiculata* (cowpea), is a diploid annual herbaceous legume recognized for its substantial protein content

(18%–25%), and is considered as an exceptional source of plant-based protein. It is an ancient agricultural species that originated in Africa and subsequently disseminated across Latin America and Southeast Asia. Africans have been cultivating and domesticating cowpeas for decades to provide protein for both human use and cattle feed. It is presently cultivated globally, with a specific focus on tropical regions. It is a warm-season crop, which thrives in flat terrain, with temperatures between 25 and 35° C and yearly precipitation of 750 to 1100 mm. It has greater resilience to sandy soils and drought compared to soybeans. For almost 6000 years, cowpea has served as a predominant and economical source of protein in Africa, and it has progressively integrated into global diets. It can be consumed whole, canned, or frozen, and can also be ground into flour for baking applications (Abebe and Alemayehu, 2022).

Besides protein contents cowpea also contains carbohydrate, possesses low-fat content, and have an amino acid profile that complements cereal grains; it additionally provides a substantial amount of minerals and vitamins. Since the crop exhibits resilience to heat and drought conditions, it is integrating into many global cropping systems (Narayana and Angamuthu, 2021). Its protein and lysine content are 2–4 times higher than cereal and root crops (Gonçalves et al., 2016). Cowpea whole grain protein has more amino acids than cereals but less methionine and cysteine than livestock-origin proteins. Animal-derived proteins, vitamins, and a vital mineral are in demand and often more expensive. Legumes like cowpea could improve feed accessibility and protein absorption for humans and animals. In developing countries, cowpea seeds and leaves are cheaper than cattle, dairy, shellfish, fish, meat, or chicken and provide protein, vitamins, and minerals, helping low-income farmers to fight protein deficiency (Dakora and Belane, 2019; Abebe and Alemayehu, 2022).

The vegetative portion of the cowpea plant contains elevated contents of vitamins compared to whole or germinated grains. Moreover, vitamin C contents in leaves exceed those in grains, whereas vitamin C in grains increase by 4 to 38 times during sprouting. Whole grains of cowpea is rich in vitamin A, thiamin, riboflavin, niacin, pantothenic acid, vitamin B6, and Vitamin C, containing a minor quantity of folate. They are necessary for blood coagulation, muscular contraction, neurone activity, digestive functions, and acid-base balance in animal and human physiology. Cowpea leaves, aerial parts and grains offer substantial amounts of total dietary fiber. In humans, dietary fiber significantly reduces the risk of chronic diseases, including cancer and diabetes (Hardisson et al., 2001; Gonçalves et al., 2016).

Cowpea has recently gathered increased interest from consumers and academics globally due to its possible health advantages, which encompass anti-diabetic, anti-cancerous, and anti-hypertensive properties (Jayathilake et al., 2018). Cowpea leaves and green pods are used as remedy for several human diseases, such as adenitis, smallpox, measles, burns, and ulcers. Cowpea seeds are significant for the treatment of several diseases, including antipyretic, astringent and diuretic conditions. Decoction or soup is used for intestinal cramps, liver and spleen disorders, leucorrhoea, menustrual irregularities, and urinary expulsions (Narayana and Angamuthu, 2021). Additional health benefits of omega-3 fatty acids present in cowpea encompass premature neonatal health, asthma, mental disorders, dysmenorrhea, and diabetes (Das et al., 2012; Abebe and Alemayehu, 2022).

Cowpea is a crucial component of traditional agricultural systems; in addition to its agronomic benefits as human food, it serves as important fodder and significantly contributes to atmospheric nitrogen fixation, enhancing soil fertility and supporting ecosystem dynamics through nitrogen recycling (Kyei Boahen et al., 2017). Decomposition of its leaves

litter, roots, and nodes provides residual nitrogen, making it a crucial companion crop for cereal-pulse farming. Nevertheless, minimal research has been conducted on it, and it is the least utilized pulse crop relative to others and has attracted less international research (Chivenge et al., 2015). Cowpea seeds and leaves have not been extensively studied or blended for human and animal nutrition. European cowpea production is low and no advanced nation produces and exports cowpeas like the United States. Asia has historically lagged behind Africa in cowpea output (Abebe and Alemayehu, 2022).

The drought resistance of cowpea influences nearly all growth phases, and hydraulic responses possess a genetic component (Muchero et al., 2008), rendering the species a captivating model for exploring the fundamentals of drought adaptation (Goufo et al., 2017). Survival in water-scarce conditions may primarily rely on the safeguarding of vital cellular components as well as the ability to recuperate following the restoration of moisture. The recovery following drought stress has infrequently been examined, especially at the biochemical and molecular dimensions. The metabolic mechanisms following rehydration remain little understood and primarily pertain to desiccation-tolerant flora, such as mosses, resurrection ferns, and angiosperms (Cooper and Farrant, 2002).

The growth, cellular redox equilibrium, photosynthetic activity, and nitrogen fixation of plants are negatively affected by frequent and severe episodes of drought stress, which in turn hinders cowpea production (Goufo et al., 2017; Nunes et al., 2022). Drought during various growth phases can inhibit cell division and expansion, resulting in stunted growth and significant production loss (Fatokun et al., 2012). Drought during the reproductive phase might significantly reduce cowpea productivity (Hamidou et al., 2007). A comparable decline in grain production and fodder

by 65% and 40%, respectively, was noted under post-flowering drought stress in field-grown cowpea (Belko et al., 2014). Moreover, dehydration experienced during vegetative and flowering stages has been shown to reduce leaf area and impact grain quality (Tankari et al., 2021).

FAOSTAT (2017) reported that cowpea was cultivated on around 11 million hectares in Africa in 2017, predominantly in West Africa (10.6 million hectares). Global cowpea production exceeds 7.4 million tons, with Africa contributing approximately 5.2 million tons. FAOSTAT (2017) indicates that more than 87% of cowpeas are cultivated in Africa. In South America, Brazil has increased cowpea farming and is currently ranked third in global production (Nkomo et al., 2021). Growing cowpea is quite sensitive to shifts in water supply, despite the fact that this is a warm-weather, semi-arid crop. Furthermore, osmotic stress constrains the growth and productivity of cowpea. As a result, we need to figure out how to make this crop more resistant to stress by finding the appropriate priming strategy for enhancing the desired traits in the target plant (Aswathi et al., 2022). In order to feed the world's population and improve soil fertility, it is more important to develop drought-stress resilient crops like cowpea. This will decrease the ecological and economic implications of the current climate scenario on the production of cowpea. Limited research has been conducted till date to evaluate the comparative performance of cowpea seedlings derived from differently primed seeds against drought stress and to investigate the mechanisms behind priming-induced drought stress tolerance and recovery kinetics.

No prior findings exist regarding the comparative study of priming on cowpea to improve drought stress tolerance and recovery. The current study centres on four cowpea varieties (Anaswara, Bhagyalakshmi, Kanakamony, PGCP 6), which are prevalent in Kerala. This study analyses

the comparative efficacy of BABA, hydro, PEG, and UV-B priming in modulating various physiochemical responses of cowpea varieties, which exhibit differing stress tolerance, when subjected to stress-inducing concentrations of polyethylene glycol (PEG). The persistence of stress memory was also investigated by inducing drought stress by withholding water and later the plant performance was further assessed during the time of stress recovery through rewatering. It emphasises the impact of drought stress and recovery on morphological, physiological, biochemical, and molecular processes, as well as the mechanisms of tolerance that may help to differentiate between drought-tolerant and drought-sensitive cowpea varieties. Consequently, the current study was developed to achieve the following aims.

1. Screening of drought sensitive and tolerant cowpea varieties by imparting PEG-induced drought stress in cowpea grown under in vitro conditions.

2. To analyze the optimal duration and concentration/dosage of different priming treatments (BABA priming, hydropriming, PEG priming and UV-B priming), which can specifically prime *Vigna unguiculata* seeds for enhancing tolerance of the seedlings towards PEG-induced drought stress.

3. To assess the impact of seed priming treatments of cowpea grown in soil and subjected to drought and recovery.

    a) by monitoring growth features and tissue water status.

    b) by evaluating osmolytes and osmolality.

    c) by evaluating photosynthetic efficiency.

    d) by evaluating cellular redox homeostasis.

4. To study the modulations in root and root nodules of primed cowpea subjected to drought stress and recovery.

5. Gene expression analysis of dehydrins in the leaves of primed and non-primed cowpea during well-watered, drought-stressed and recovery conditions.

2. REVIEW OF LITERATURE

Plants are often subjected to numerous adverse environmental conditions, including high light intensity, heat, UV radiation, drought, cold, salt, nutritional deficiency and environmental pollutants (Banerjee and Roychoudhury, 2017; Martinez et al., 2018). Frequent and severe instances of stress exposure negatively impact plant performance and productivity, thus adversely affecting food and agriculture system that may not be possible to meet the requirements of the expanding global population (Zandalinas et al., 2017; Zhou et al., 2017). The plants thriving in these challenging environments adjust and tune their metabolic processes to sustain optimal functions (Hussain et al., 2019; Ansari et al., 2021). The modifications in the morphological, physiochemical and molecular attributes enable them to endure adverse conditions. The drastic increase in the atmospheric temperature and the ensuing effects of global warming significantly damage global agricultural productivity by restricting water availability and intensifying the frequency of drought. Drought is the most significant abiotic stress, directly impacting plant metabolism, growth, and productivity (Reddy et al., 2004; Abdel-Ghany et al., 2020). Extended and repeated drought occurrences may ultimately result in the desertification of arable land and exacerbate the severity of famine. Current research is concentrated on identifying ideal strategies to mitigate the impacts of drought stress on crop production and to enhance overall plant performance and yield in the era of frequent and intense climate change scenario.

2.1 Effects of drought stress on plant growth and development

Drought stress affects various morphological and physiological processes such as seed germination, seedling establishment, shoot and root growth, photosynthesis, crop yield and quality. Vegetative growth begins

with the germination of seeds. In order to reactivate metabolic processes and promote the formation of the embryonic axis, seeds must attain a suitable hydration during the water absorption phase for successful germination. During drought, the water absorption process is delayed and subsequently the seedling formation is hindered (Oguz et al., 2022). Studies reported the adverse effects of drought stress on various germination and growth parameters of several plant species.

During drought stress, reduction in germination rate, plumule and radicle dry weight were observed in soybean (*Glycine max*) (Karami et al., 2016). Similarly, drought stress caused reduction in leaf area index and root yield in *Beta vulgaris* (Khodadadi et al., 2020). Significant reduction in biomass and leaf area index were also noticed under drought stress in *Sesamum radiatum and Sesamum indicum* (Jeyaraj et al., 2024). It is found that the shoot dry weight and root dry weight were reduced under drought stress in different plant species viz. *Glycine max* (Du et al., 2020), *Avena sativa* L. (oats) (Ghimire et al., 2024), and *Triticum aestivum* (Wang et al., 2024). Ghimire et al. (2024) revealed that the root length, area, volume and root-shoot biomass ratio were lowered in oats under drought stress. Likewise, Batool and co-workers (2020) reported that the relative growth rate of plant, number of leaves, leaf area, biomass production, number of tuber, tuber weight and yield were reduced in *Solanum tuberosum* L. (potato) subjected to drought stress. Water stress reduced plant height, shoot and root dry biomass and fruit yield, while increased the root length in *Solanum lycopersicum* L. (tomato) (Escobar-Hernández et al., 2024). Water deficit condition leads to the rolling of leaves and in line with this, flag leaves of wheat rolled extremely under drought stress (Willick et al., 2018) and sesame leaves were rolled during severe water stress with the highest rolling of leaves observed in the cultivars than the wild plants (Jeyaraj et al., 2024).

Drought stress can impair stomatal conductance, photosynthesis, ion homeostasis, and induce oxidative damage through the excessive accumulation of reactive oxygen species (ROS), hence hindering normal plant development (Farooq et al., 2009; Matos et al., 2010). Studies revealed that apple varieties with thickened cuticle layer, longer palisade cells, and a thicker layer of spongy parenchyma exhibited better ability to withstand drought (Bai et al., 2019). Comparative study in two olive (*Olea europaea* L.) cultivars revealed that tolerant cultivar had a thicker palisade parenchyma, higher trichome and stomatal density, and a greater ability to withstand water stress. Additionally, the leaves showed a smaller specific leaf area, a higher foliar tissue density, and a slower decline in the net CO_2 assimilation rate (Guerfel et al., 2009). In wheat, it was discovered that drought led to a reduction in stomatal conductance, an increase in stomatal resistance, and a drop in both photosynthetic rate and transpiration rate (Ahmed and Stockle, 2017). Similar results were observed by Rahbarian et al. (2011), wherein drought stress reduced the PSII photochemical efficiency (Fv/Fm), CO_2 assimilation rate and transpiration rate. Tolerant variety exhibited higher PSII photochemical efficiency than the sensitive one at seedling and podding stages. Moreover, the tolerant genotypes exhibited better water use efficiency (WUE) and CO_2 absorption rate compared to sensitive genotypes at all stages of drought. Likewise, cowpea plants experiencing water stress exhibited decreased CO_2 assimilation rate, stomatal conductance and transpiration rates (Souza et al., 2004). Reduction in stomatal conductance, net photosynthesis, mesophyll conductance, the maximum rate of electron transport and the maximum rate of carboxylation by Rubisco were observed in wheat under drought stress (Fang et al., 2023). An evident and meaningful positive linear correlation was found between net photosynthesis and stomatal conductance, suggesting that an increase in stomatal conductance enhances net photosynthesis (Sikder et al., 2016).

Likewise, when subjected to increasing drought stress a substantial decrease in gas exchange characteristics were observed in *Pisum sativum* leaves. The results indicated a decrease in the photosynthetic rate (Pn), transpiration rate, and stomatal conductance, intercellular CO_2 concentration and water use efficiency under drought stress conditions (Pandey et al., 2023).

Upon exposure to drought stress, the PSII efficiency and associated parameters of photochemical quenching diminished, but non-photochemical parameters exhibited an increase in lettuce (*Lactuca sativa*) seedlings. In the analysis of ten chlorophyll fluorescence parameters in lettuce, the coefficient of photochemical quenching (qP), the effective quantum yield of photochemical energy conversion in PSII (Y(PSII)), and the coefficient of photochemical quenching of variable fluorescence exhibited a significant drop in drought-stressed seedlings from day 6 of treatment relative to the control. Conversely, fluorescence ratio (Rfd), maximal quantum yield (Fv/Fm), and quantum yield of non-regulated energy dissipation in PSII (Y(NO)) were significantly affected solely at the extreme stress, confirming that the PSII reaction center became deactivated due to photoinhibition, exclusively when drought stress reached an extreme level (Shin et al., 2021).

Reactive oxygen species (ROS) has a significant role as signal transduction molecules at a level below the threshold level, but are toxic when present at high level. Oxidative stress induced by ROS compromises cellular integrity by disrupting biomembranes, and resulting in the destruction of proteins and nucleic acids (Gill and Tuteja, 2010). A tight balance exists between the production of ROS and their scavenging (Choudhury et al., 2017), because an effective ROS detoxification is crucial for the proper functioning of a cell. To mitigate the oxidative stress induced by accumulating ROS, plants have evolved robust antioxidative mechanisms, including non-enzymatic compounds such as ascorbate,

glutathione, phenolics, α-tocopherol and carotenoids, along with enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate-glutathione (ASC-GSH) cycle enzymes, which mitigate excess ROS in plant cells through either the partial inhibition of ROS production or the scavenging of pre-existing ROS (Sharma et al., 2012). However, under extreme stress circumstances, this redox equilibrium is disturbed. Drought exposure leads to the accumulation of ROS in numerous plants including rice, wheat (Vijayaraghavareddy et al., 2022), soybean (Ji et al., 2023), *Pisum sativum* L. (pea) (Dutta et al., 2024) etc.

Studies have found that, total antioxidant activity and flavonoid content were increased in the leaves and roots of citrus exposed to drought stress. As compared to the drought susceptible species *Citrus limon* (lemon), the tolerant citrus species *Citrus aurantium* (sour orange) exhibited high antioxidant activities. Flavonoid contents such as silibinin, trilobatin, benzylideneacetophenone, isorhamnetin, liquiritigenin etc. were significantly accumulated in sour orange subjected to drought stress. In line with this, upregulation of genes involved in the pathway of flavonoid biosynthesis was observed during drought stress in sour orange and it contribute towards the drought tolerance potential (Rao et al., 2024).

According to the study of Cheng and co-workers (2024), drought reduced the activity of SOD and CAT, resulting in the buildup of superoxide and hydrogen peroxide. Disruption in the antioxidant balance led to membrane damage and subsequently an elevated lipid peroxidation was noted. The study concluded that drought hinders ovule production by disrupting the pistil's antioxidant metabolic balance, hence damaging the cytological structure of the developing ovules. Another finding revealed that the activities of enzymatic antioxidants such as SOD, CAT, POD, APX and GPX were increased in chickpea subjected to drought stress (Abdoli et al.,

2024). In another study, ascorbate concentration, total phenol content (TPC), and total flavonoid content (TFC), as well as antioxidant activity were reduced in drought-stressed lettuce seedlings (Shin et al., 2021).

Plants have evolved many survival methods during drought conditions. Stress signals cause the buildup of compatible solutes, which serve as osmoprotectants to maintain osmotic equilibrium and stabilise structural integrity of cell (Bohnert et al., 1995; Choluj et al., 2008). Enhanced plant water status attained through the buildup of osmolytes, facilitating the alleviation of oxidative damage (Wang et al., 2019). These osmoprotectants are primarily categorised into three groups such as sugars and polyols, betaines, amino acids and their derivatives. The osmolyte, proline functions as a buffer to mitigate water deficiency, and its accumulation is a crucial indication of several stress response mechanisms, especially in plants subjected to drought stress (Jaleel et al., 2007). Drought tolerant cultivars had the highest rate of proline accumulation under stress conditions. This may be due to the function of proline as an osmolyte, preserving osmotic potential following stress alleviation. Proline aids in maintaining subcellular structures (such as membranes and proteins), scavenges free radicals, and regulates cellular redox potential during stress (Hayat et al., 2012). Findings have proven a considerable increase in glycine betaine content also under stress conditions. The tolerant cultivars exhibited a greater enhancement in glycine betaine than the sensitive cultivars during stress exposure, which was further corroborated by the positive association between glycine betaine contents and drought tolerance. Glycine betaine aids in preserving cell membrane integrity and facilitating osmotic adjustment during drought stress in cotton (*Gossypium hirsutum*) (Singh et al., 2021). Similarly, proline content significantly increased in *Amaranthus tricolor* under low, moderate and severe drought (Sarker and Oba, 2018). In rice, the content of total free

amino acids, proline, soluble sugar and soluble protein were found to be increased during drought stress (Sen and Puthur, 2021).

Plant responses to drought stress entail intricate signaling mechanisms (Takahashi et al., 2018). In a drought stressed condition, plants regulate their gene expression so as to adapt to the adverse conditions, and this results in physiochemical alterations within the plant cell (Abdel-Ghany et al., 2020). As the water supply diminishes, plants rapidly exhibit drought tolerance responses (Pandey and Shukla, 2015). Understanding the precise mechanism of drought resistance is crucial for alleviating the detrimental impacts of drought stress on plant performance. Genes regulated by drought exhibit intricate regulatory mechanisms and subsequent responses during water scarcity, conferring stress tolerance to plants (Chiappetta et al., 2015; Begcy et al., 2019). Gene expression regulation occur at several levels, encompassing transcriptional, post-transcriptional, translational, and post-translational stages (Wang et al., 2010; Zhang et al., 2014). Transcriptional signal transduction occurs via ABA-dependent and ABA-independent mechanisms. It encompasses several transcription factors, including apetala2/ethylene responsive factor (*AP2/ERF*), myeloblastosis (*MYB*), myelocytomatosis (*MYC*), *WRKY*, basic-leucine zipper (*bZIP*), *NAC*, and dehydration-responsive element binding protein (*DREB*), which subsequently activate genes that promote drought tolerance. Mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinases (CDPK) are significant in drought signaling (Singh and Laxmi, 2015). A comprehensive understanding of these molecular pathways will facilitate the enhancement of the genetic capacity of plants for drought resistance.

MdbHLH160, an ABA-responsive bHLH transcription factor, that enhances drought tolerance in *Arabidopsis thaliana* and *Malus domestica*. During drought stress, MdbHLH160 directly binds to the promoter of

MdSOD1 and stimulates transcription, which initiates ROS scavenging and improves drought resistance. Furthermore, MdbHLH160 enhanced the expression of the gene *MdDREB2A-like*, a gene belonging to the DREB (dehydration-responsive element binding factor) family that enhances the ability of apples to withstand drought stress. Protein degradation and ubiquitination experiments demonstrated that drought and ABA effectively stabilised MdbHLH160. Further it was discovered that the BTB protein MdBT2 interact with MdbHLH160, facilitating the ubiquitination and degradation of MdbHLH160. But, the treatment of ABA significantly suppressed the ubiquitination and degradation activity. Hence, it was proven that drought-induced ABA signaling operate at both the transcriptional and post-translational stages to augment MdbHLH160 mediated drought resistance (Mao et al., 2024).

Protein kinase encoded by General Control Nonderepressible 2 (*GCN2*) is responsive to a range of biotic and abiotic stressors. Wang et al. (2023a) shown that *NtGCN2* may modulate drought tolerance in tobacco by controlling proline accumulation, reactive oxygen species (ROS) scavenging, and stomatal closure. These findings suggest that *NtGCN2* has the potential to be used in genetically modifying cultivar drought resistance. They examined the drought tolerance role of *NtGCN2* by studying *NtGCN2*-overexpressed transgenic tobacco plants. Compared to wild-type (WT) plants, transgenic plants overexpressing *NtGCN2* has shown greater tolerance to drought stress. Under conditions of drought stress, the transgenic tobacco plants displayed increased activity of enzymatic antioxidants, higher levels of abscisic acid and proline content and upregulation of genes encoding proline synthase and important antioxidant enzymes. However, they showed reduced levels of malondialdehyde and reactive oxygen species. Experiments using next-generation sequencing (NA-seq) revealed that the increased expression of *NtGCN2* in guard cells

regulated the expression of genes associated with proline biosynthesis and breakdown, ABA synthesis and breakdown, ion channels and antioxidant enzymes in response to drought stress (Wang et al., 2023a). During periods of drought, plants exhibit alterations in the expression levels of genes, particularly those associated with phenol production. Moderate drought stress maintained cell homeostasis with no effect on plant growth, improved protective enzyme activity and osmolytes, and boosted accumulation of isoflavones (Ai et al., 2024).

Molecular and genomic surveys in *Arabidopsis*, rice, and other plants have found several drought-inducible genes with diverse activities. These genes include several transcription factors that control the expression of stress-inducible genes. The products derived from stress-inducible genes exert dual roles in the initial stress response and in the establishment of plant stress tolerance (Shinozaki and Yamaguchi-Shinozaki, 2007). Dehydrins, or Group II late embryogenesis abundant (LEA) proteins, are inherently disordered proteins characterised by their strong hydrophilicity. Many adverse environmental circumstances, such as low temperature, drought, and excessive salinity, induce the expression of dehydrins (Sun et al., 2021). Dehydrins are a versatile and heterogeneous class of protein. They are characterized by conserved sequence motifs, containing a lysine rich K-segment, an N-terminal Y-segment, and S motifs. Selected dehydrins and a few poorly characterized dehydrin-like proteins are involved in stress acclimation and have been shown interactions with organelles. By stabilizing biological membranes and binding ROS, dehydrins and dehydrin-like proteins play a role in protecting delicate organellar structures under adverse conditions (Szlachtowska and Rurek, 2023).

Legumes are integral to traditional cuisines across several cultures, possess low greenhouse gas and water footprints, are cost-effective, and

represent a sustainable protein supply. Legumes offer additional environmental benefits by improving the quality of soil via nitrogen fixation, diminishing the necessity for fertilizers, enhancing crop yield and disease resistance through crop rotation or intercropping, and remaining cost-effective. Cowpea is a warm-season crop predominantly cultivated in the semiarid areas of Africa, Asia, and Latin America. Cowpea exhibits tolerance to drought and heat, and possesses the ability to thrive in low-fertility soils and marginal regions (Semba et al., 2021).

The recurrent incidence of drought stress negatively impacts the growth, cellular redox balance, photosynthetic efficiency, and nitrogen fixation of cowpea, hence impeding its yield (Goufo et al., 2017). Consequently, there is an urgent necessity to concentrate research on enhancing the drought resilience of legumes to guarantee global food security in the face of climate change issues. Drought impairs the initiation of symbiosis, as water scarcity hinders the exchange of signalling molecules essential for communication between host legumes and rhizobia, leading to inadequate nodulation and nitrogen fixation (Miransari et al., 2013). The suppression of nodule nitrogen fixation during drought stress is associated with carbon deficiency for rhizobia, reduced nitrogenase activity due to oxygen shortage, and feedback inhibition of nitrogen fixation (Valentine et al., 2011; Dollete et al., 2024).

Contemporary breeding, together with genetic and molecular approaches, has revealed numerous genes associated with abiotic stress tolerance (Atkinson and Urwin, 2012; Vijayakumari et al., 2016). Hence, considerable progress has been made for the enhancement of abiotic stress tolerance in specific crops by breeding and genetic engineering. Nonetheless, comprehending the functions and intricate connections of abiotic stress-related genes remains a difficulty (Wang et al. 2003; Arbona et al. 2013),

rendering the development of crop varieties with abiotic stress tolerance by gene manipulation a difficult task. Furthermore, transgenic plants are expected to maintain productivity and other essential agronomic traits, while their stress tolerance is enhanced by genetic engineering. Gao et al. (2008) found that, unlike most monogenic features, the engineering of stress-related genes for abiotic stresses can influence many economically significant traits in crops when they are constitutively overexpressed. Despite significant advancements in enhancing plant tolerance to abiotic stresses through breeding and genetic transformation, the aforementioned limitations necessitate the continuous pursuit of improved methodologies that confer abiotic stress tolerance to plants while minimally impacting productivity (Vijayakumari et al., 2016).

2.2 Priming

In recent years priming has appeared as an emerging and viable technique for addressing diverse abiotic challenges faced by plants. Priming is a process via which plants achieve a distinctive physiological condition known as the 'primed' state, following a pretreatment with a priming agent. It augments the defensive strategies by recalling previous stresses, enabling a more effective response to analogous future stressors. Mild exposure to stress might prime a plant for future stressors or elicit a state of adaptation that persists until further exposure occurs. Plants are recognised for their ability to store and retrieve information, referred to as memory (Crisp et al., 2016). The notion of plant memory, despite being bolstered by increasing scientific data, continues to be regarded with scepticism by numerous professionals, including certain plant biologists (Galviz et al., 2020). Multiple studies illustrate the presence of plant stress memory, which is essential for comprehending plant stress responses in the current climatic context.

The capacity to remember past events is a fundamental benefit for living being in unpredictable and adverse circumstances. Priming, a specific strategy in stress physiology, has significant promise for cultivating stress-resilient plants (Paparella et al., 2015). Priming entails the application of various natural or synthetic substances to seeds or seedlings to elicit specific immunological responses in plants. This approach resembles vaccination, as mild stress exposure enhances plant immunity by activating stress-responsive genes and proteins, hence increasing stress tolerance (Jisha et al., 2013). Research persists in investigating the stress alleviating abilities of various priming agents in response to different stressors across multiple plant species.

If the nature of initial and subsequent stresses are same, it is referred to as cis-priming. In trans (cross)-priming, the priming stimuli differ from the subsequent stress to which the plants are subjected and it involves interaction among several stressors. The efficacy of cross-stress priming results from the activation and interplay of synergistic stress signaling pathways common to several stressors, enabling primed plants to develop enhanced stress tolerance through cross-stress memory. Evidences substantiates the efficacy of cis-priming techniques, including drought priming in wheat (*Triticum aestivum* L.) (Wang et al., 2021), UV priming to mitigate UV stress in rice (*Oryza sativa* L.) (Thomas and Puthur, 2020), NaCl-priming in chickpea (*Cicer arietinum* L.) and lentils (*Lens culinaris* Medik.) (Paul et al., 2023), and thermopriming in mustard (*Brassica juncea*) (Samantaray et al., 2023). Likewise, there are reports revealing the trans priming effects in plants including NaCl and UV-B priming against drought stress (Sen and Puthur, 2021); heat priming against drought (Hossain et al., 2013) and cold shock against drought stress (Yamamoto et al., 2014).

2.2.1 Priming and memory imprint

Epigenetic and chromatin-based processes involved in priming/stress memory include DNA methylation, histone modifications, and nucleosome positioning. According to Ge and Brickner (2024), these epigenetic changes have the potential to impact priming-induced transgenerational stress memory. It has been postulated that plants may have inherited some sort of "stress memory" based on how they react to repeated stresses. Epigenetic modifications provide a dynamic and long-lasting way for plants to react to environmental cues, altering or amplifying responses to future challenges even after the stimulus has been eliminated (Bruce et al., 2007). Priming memory transmission from parents to offspring was also proven (Wojtyla et al., 2016). Researchers are trying to figure out if this transgenerational memory will remain strong from one generation to the next or if it will gradually diminish as time goes on. This memory helps future generations deal with stress. After plants are exposed to the stress of an unfavourable environment, the priming effects may be imprinted in them, allowing them to enhance their abiotic stress tolerance response when faced with the same or different types of stress in the future (Martinez-Medina et al., 2016).

Epigenetic alterations, changes in gene expression patterns, and transcript accumulation are all outcomes of prolonged drought. The steady increase in H3K4 trimethylation and RNA polymerase II phosphorylation is likely responsible for the accumulation of gene expression and transcripts in *Arabidopsis*, as noted by Ding et al. (2012). These alterations show that drought stress is imprinted in memory because they remain after stress recovery. Similarly, histone changes associated with defensive mechanisms and potentially facilitating gene expression due to priming include acetylation of lysine residues on histones H3 and H4 (H3ac, H4ac) in the promoters of *WRKY* genes and methylation of histone H3K4me (Jaskiewicz

et al., 2011). Hence, one under-recognized strategy for increasing crop stress tolerance could be to activate priming responses or to make targeted alterations to the epigenome.

2.2.2 Seed priming

Seed priming is the treatment of seeds with a variety of synthetic and natural agents, so that the plants develops a potential to withstand stress (Jisha et al., 2013; Thomas and Puthur, 2020). As a result, plants experience a modest stress response, which in turn triggers an alarm system and partially activates proteins and genes that are involved in stress tolerance (Chen et al., 2013). Priming has been shown to improve the performance of newly emerging seedlings as well as to promote early and uniform germination of seeds (Farooq et al., 2013; Jisha et al., 2013). Research on rice has shown that some seed priming methods can make sensitive types more resistant to stress and increase the tolerance potential of a tolerant variety (Sen and Puthur, 2021). It is evident that priming imprints are transmitted to distinct phases of a plant's life cycle because in both instances, priming done in the seed stage permits the priming effects to be transmitted to the seedlings (Sen and Puthur 2021). Priming is beneficial due to its low risk and cost-effectiveness while being relevant in fostering stress tolerance.

The stress stimuli employed for priming are short and mild yet sufficient to induce stress tolerance, creating metabolic markers (elicitor factors) or chromatin alterations that influence signal transduction and gene expression when plants face a subsequent stress stimulus (van Buer et al., 2019). Consequently, several defence systems are activated rapidly and robustly in primed plants compared to non-primed plants subjected to stress conditions. The primary advantages of priming are augmented antioxidant mechanisms, improved photosynthetic efficiency, increased stress tolerance, and cross-tolerance. The comprehensive improvement in growth and

performance of primed plants ultimately enhances yield, and productivity. Understanding the morpho-physiological and molecular changes involved with priming is essential for developing plants that are tolerant to multitudes of biotic and abiotic stresses to ensure that productivity is maintained (Sherin et al., 2022). Multiple priming approaches, including hydropriming, osmopriming, chemical priming, amino acid priming, radiation priming, magnetopriming, drought priming, heat priming, and nanopriming, have been shown to improve the stress tolerance of a wide range of plant species such as rice, maize, cowpea, chickpea (Paparella et al., 2015; Bera et al., 2022; Nile et al., 2022).

2.2.2.1 Hydropriming

Hydropriming is recognized as a simple, safe and cost-effective method for improving seed germination, seedling growth as well as enhancing the ability of seeds to achieve osmotic adjustment, hence improving seedling establishment and crop production in adverse conditions (Kaur et al., 2002). This priming technique involves immersing seeds in sterilized distilled water maintained at an optimal temperature, with the period of hydropriming regulated by monitoring seed imbibition during germination (Kaya et al., 2006), subsequently dehydrating them to their initial moisture content prior to sowing. Hydropriming facilitates the stimulation of physiological and biochemical processes in seeds. It improves moisture levels through a constant supply of oxygen and stimulates the production of hydrolytic enzymes (amylase, cellulase, and xylanase) to transform stored nutrients such as carbohydrates, proteins, and lipids into simpler compounds (ATP) for pre-germinative metabolic activities.

Hydropriming significantly enhanced germination rate, seedling vigor, promote uniform germination, crop growth and development in various plants including faba bean (Damalas et al., 2019), and *Vigna radiata*

(Jisha and Puthur, 2018) under drought stress. Nevertheless, its benefits, the primary drawback of this approach is the unregulated absorption of water by the seeds, which is depending upon the seed tissue's affinity for water. It is advisable to specify precise volume of water, temperature, and time of soaking to achieve an optimal degree of hydration and prevent the radicle protrusion (Jisha et al., 2013). Hydropriming of maize (*Zea mays* L.) significantly enhanced germination and seedling development under drought stress conditions (Janmohammadi et al., 2008). Similarly, hydroprimed green gram seeds exhibited greater germination, enhanced growth, and increased activity of antioxidants and nitrate reductase enzyme under drought stress (Jisha and Puthur, 2018). Hydropriming treatments mitigated the adverse impacts of drought stress in sunflower, by enhancing soluble sugars and chlorophyll (Bourioug et al., 2020). The study demonstrated that the priming treatments enhanced germination traits specifically and augmented growth and yield parameters.

2.2.2.2 Osmopriming

Osmopriming is a method of treating the seeds with solutions of low water potential (Marthandan et al., 2020). Osmopriming was found to enhance the stress tolerance potential of plants by improving germination, growth, antioxidation and photosynthesis. Priming with polyethylene glycol (PEG) established tolerance to drought stress, especially during the reproductive stages of wheat, which was evident in the higher net photosynthetic rate. The improved photo-protective mechanism and the antioxidative mechanism by priming decrease the transfer of excess excitation energy to the PSII reaction centers and thus protect the photosystems. Primed plants have higher carotenoid contents than non-primed plants under stress conditions. Carotenoids help the plants to survive the detrimental impacts of drought stress by protecting

photosynthetic machinery that are required for better photosynthesis reflecting in plant growth and yield (Abid et al., 2018; Sherin et al., 2022).

Seed osmopriming enabled barley (*Hordeum vulgare*) plants to sustain a higher relative growth rate and grain yield during drought conditions (Kaczmarek et al., 2017). Osmopriming improved the percentage and mean emergence time (MET) of sorghum seeds at sub- optimal temperature (Moradi and Younesi, 2009). Osmopriming the seeds of lentil mitigate oxidative stress effect by enhancing seed germination, seedling development, biomass, chlorophyll, sugar accumulation, and mitigated oxidative stress in lentils under water stress conditions (Farooq et al., 2020). Another study revealed that under drought stress, primed plants exhibited reduced production of ROS and lipid peroxidation, together with elevated activities of CAT, APX, and GR compared to non-primed- plants. Likewise, the osmopriming treatment of seeds enabled wheat plants to sustain a higher relative growth rate during drought conditions, leading to increased dry matter and grain yield compared to non-primed treatments. It was determined that seed osmopriming induced lasting stress memory, which enhanced plant growth in wheat under drought stress (Abid et al., 2018). The beneficial effects of $CaCl_2$ pretreatment on drought stress adaptation in barley are evidenced by an increase in divalent cations such as Mg^{2+}, Zn^{2+} that are crucial for calcium-related drought adaptation, enhanced seedling vigor, and the preservation of higher leaf water potential, which correlates with improved drought recovery and overall plant performance during early season drought (Kaczmarek et al., 2017).

2.2.2.3 BABA priming

β-amino butyric acid (BABA) is the beta isomer of amino butyric acid, a non-protein amino acid. It has been the subject of extensive research due to its well-known defense stimulatory potential. According to studies, BABA

activates a wide range of metabolic pathways. Thus, it can aid plants in managing both biotic and abiotic stresses by acting as an external bioactive priming agent (Decsi et al., 2024). BABA promotes drought tolerance and serves as an effective strategy to alleviate drought induced damages in a variety of plants such as *Brassica napus* (Mohamadi et al., 2017), *Vigna radiata* (Jisha and Puthur, 2016), *Vigna unguiculata* (Aswathi et al., 2023) and *Zea mays* (Shaw et al., 2016). Application of BABA enhanced relative water content, photosynthetic rate (*Pn*), stomatal conductance (*gs*) and transpiration rate (*E*), thus reducing water usage efficiency. Additionally, the application of BABA reduced the generation of hydrogen peroxide (H_2O_2), malondialdehyde content, and electrolyte leakage, resulting in reduced cell membrane damage from oxidative stress. The administration of BABA increased the accumulation of proline and soluble carbohydrates, hence enhancing the osmotic adjustment ability of plants during drought stress. Notably, enhanced activities of antioxidant enzymes such as SOD, CAT, POD and APX along with their transcript levels, may mitigate the detrimental effects of oxidative stress and decrease the accumulation of harmful substances in BABA-treated plants. Furthermore, exogenous BABA markedly stimulated the accumulation of drought tolerance-associated genes such as *VfMYB*, *VfDHN*, *VfLEA*, *VfERF*, *VfNCED*, *VfWRKY*, *VfHSP*, and *VfNAC* in both leaves and roots of *Vicia faba*, indicating that BABA may function as a signaling molecule to modulate the expression of drought tolerance-related genes (Abid et al., 2020).

2.2.2.4 UV-B priming

Approximately 5% of solar ultraviolet-B radiation (280-315 nm) penetrates the Earth's surface, and the bulk is absorbed by the atmospheric ozone layer. Nonetheless, various anthropogenic and environmental factors have contributed to ozone depletion, which results in more penetration of

UV-B radiations (Lawrence et al., 2020). The incidence of solar UV radiation on the Earth's surface has escalated in recent years due to the progressive depletion of the ozone layer, which has been shown to be harmful to both flora and fauna (Dowlath et al., 2021; Barnes et al., 2022). UV-B exposure results in damage to proteins, lipids, and DNA, inducing covalent linkages between adjacent pyrimidine bases, which leads to the formation of photoproducts, predominantly cyclobutane pyrimidine dimers (CPDs) and pyrimidine-6,4-pyrimidinone photoproducts (6,4PPs), ultimately causing genomic instability (Takahashi et al., 2011). However, UV-B radiation can beneficially influence the morpho-physiological and molecular characteristics of plants, even at low levels (Kacharava et al., 2009). Suboptimal UV-B radiation levels enhance antioxidant activity, secondary metabolite synthesis, compatible solute accumulation, pigment composition, and the expression of stress-responsive genes, thereby increasing the drought tolerance potential of plants and promoting stress acclimatization (Thomas and Puthur, 2020; Luo et al., 2022).

The dosage of UV radiation used for seed priming treatment is crucial, as it had significant role in plant development and performance. Elevated radiation levels are fatal to plants, although sensitivity to ionising radiation varies significantly across various plant species. The application of several non-ionizing physical agents, including UV radiation, microwave radiation, magnetic fields, and ultrasonic waves, is currently regarded as a promising method for seed improvement (Araújo et al. 2016). These physical agents are typically recognised as harmful or genotoxic, and induce stress in plants. But, low to moderate doses can elicit a priming effect. This biphasic response is generally defined by a low dose stimulating or advantageous effect, contrasted with an inhibitory or toxic effect at elevated dosages of the same substance. The response of cells or organisms to modest doses of harmful foreign substances is regarded as an adaptive mechanism

subsequent to an initial disruption of homeostasis. Hormesis is the term used to describe the phenomena of any system being stimulated by low concentrations of any physical agent (Mattson, 2008; Bera et al., 2022).

UV-B priming enhanced the harvest index and reduced panicle sterility in rice and preserved priming imprints throughout the plant life cycle, ultimately resulting in enhanced yield-related parameters in rice (Sen and Puthur, 2021). It was found that, in basil plants (*Ocimum basilicum*) subjected to UV-B light (222.6 μWm^{-2} for 3 hours each morning for two weeks) caused significant increase in leaf area (Chang et al., 2009). In kidney bean and rice, UV treatment enhanced anthocyanin levels, which mitigate ROS and provide protection against stress-induced damage (Kacharava et al., 2009; Thomas and Puthur, 2020). Rice seedlings emerged from UV-B primed seeds exhibited larger stomatal pore apertures on the adaxial surface and the open stomata facilitated an enhancement in light-saturated net CO_2 assimilation rate (*Pn*), stomatal conductance (*gs*), and intercellular CO_2 (*Ci*) in UV-B primed rice subjected to drought (Thomas et al., 2020).

2.3 Recovery

In nature, plants are experiencing continuous cycles of drought stress and recovery (Aswathi et al., 2022). Upon alleviation of water scarcity, plants must resume growth quickly. The recovery from stress is a complex process that entails the reorganization of numerous metabolic pathways to rectify drought-induced damage and restore plant development. It needs far more than only reverting to the condition prior to the initiation of stress (Vanková et al., 2012). Drought recovery is described as the ability of a plant to recuperate from drought stress. Drought adaptability is described as the holistic ability that encompasses both drought resistance and recovery for coping with drought stress and subsequent rewatering. Although drought resistance, particularly drought avoidance and tolerance, has been a

significant focus in prior research on plant drought adaptation, the importance of drought recovery in this context has gathered far less attention (Chen et al., 2016; Aswathi et al., 2022). Recently, there has been a growing emphasis on drought recovery in crops suggesting that drought recovery is integral to drought resistance in crops.

The time required for an ecosystem to revert to its pre drought functioning condition is a significant indicator of drought induced damage. If droughts become more frequent, the interval between them may shorten to a point shorter than the recovery period, resulting in irrevocably harmed ecosystems and extensive degradation of the terrestrial carbon sink. The duration required for the recovery of plant function is crucial for ecosystem stability, as the onset of a subsequent drought prior to complete recovery may result in an ecosystem transitioning to a different state. In *Ficus carica* L., water stress resulted in elevated leaf temperature and significant leaf abscission, whereas rehydration prompted the development of new leaves and reinstated the functionality of the photosynthetic apparatus in previously stressed plants. The recovery by rehydration enhanced the vegetative growth and extended the duration of the vegetation cycle (Ammar et al., 2020). The loss of structural integrity and the disruption of chloroplast organisation can quickly recover upon rewatering after drought. A prolonged drought may cause a reduction in OEC capacity due to the disruption of electron transport at the PSII donor side. However, some plants have demonstrated the capability to recover and restore to the pre drought condition effectively. The quinoa PSII effectively withstands extreme stress conditions, including drought, and demonstrates significant potential to repair and recoup the degraded PSII protein complexes post-stress (Manaa et al., 2021).

Visentin and coworkers (2020) evaluated ABA accumulation and sensitivity, along with the performance of wild-type and miR156-overexpressing (miR156-oe) tomato plants under drought conditions. They assessed miR156 levels in wild-type, strigolactone treated and strigolactone depleted plants subjected to drought. The "after-effect" of drought, characterised by incomplete stomatal re-opening after recovery, was exacerbated by both miR156 and strigolactones. The transcript patterns of many miR156 targets were modified in plants lacking strigolactone. This finding indicate that strigolactones serve as a molecular connection between miR156 and drought in tomato, identifying miR156 as the mediator of the ABA-dependent influence of strigolactones on the residual effects of drought stress on stomata. Consequently, it offers insights into the roles of strigolactone and miR156 in stomatal function (Visentin et al., 2020)

To elucidate the mechanisms underlying post drought recovery in soybean, protein alterations were examined in the roots, including the hypocotyl. The proteome analysis revealed that proteins across many functional categories exhibit altered abundance in soybean seedlings during the recovery phase following stress removal. Several proteins associated with cell wall integrity, hormone metabolism and secondary metabolism demonstrated alterations in abundance throughout the four day of recovery phase. The abundance and activity levels of aldehyde dehydrogenase and peroxidase were significantly modified after the alleviation of drought. The activity of peroxidase was elevated in soybean roots at the time of recovery phase and it is associated with the detoxification of harmful ROS, notably peroxides, which are augmented in response to oxidative stress. The activity of aldehyde dehydrogenase augmented during drought, redirecting cellular metabolism towards the production of less toxic derivatives that can generate acetyl CoA and facilitate fatty acid biosynthesis, thus alleviating toxicity. Aldehyde dehydrogenase activity was normalised in the soybean

root during the recuperation phase. The current findings indicate that peroxidase and aldehyde dehydrogenase are crucial for post-drought recovery in soybean by diminishing the cellular problem of detrimental chemicals that accumulate under stress conditions (Khan and Komatsu, 2016).

Alterations in chromatin state are associated with the control of genes involved in biological processes, including development and stress responses in plants. The work by Kim et al. (2012), examined the alteration of chromatin status associated with gene repression during the recovery from drought stress of drought-inducible genes (*RD20*, *RD29A*, and *AtGOLS2*) and a rehydration-inducible gene (*ProDH*). During drought stress, RNA polymerase II recruited to drought-responsive genes and as a result the increased mRNA levels of *RD20*, *RD29A* and *AtGOLS2* was observed. While, the mRNA levels of these genes decreased following rehydration, and were partially sustained for 5 h post-rehydration, indicating that the transcriptional activities of these genes were rapidly inactivated during recovery. Histone H3K9ac was augmented by dehydration and swiftly eliminated from these locations upon rehydration. Conversely, histone H3K4me3 exhibited a progressive decline during rehydration but remained at diminished levels post-rehydration, indicating that H3K4me3 serves as an epigenetic marker of stress memory. The results indicate that transcriptional activity and chromatin status swiftly undergo transition from an active to an inactive state throughout the restorative process. These findings indicate that histone changes are associated with the silencing of drought-inducible genes throughout the rehydration recovery process (Kim et al., 2012).

Proline, total free amino acids, total sugars, reducing sugars, and polyphenol concentrations increased in drought-stressed plants and tended to decline throughout the recovery phase. The drought-induced elevations

in total free amino acids, proline, sugars, and polyphenols were markedly greater in the moderately tolerant genotype (GM 090304) compared to the sensitive genotype (Ca/H 631). These findings indicate that proline, sugars, and polyphenols serve as primary compatible solutes facilitating osmotic equilibrium, safeguarding cellular macromolecules, detoxifying cells, and scavenging free radicals during water stress conditions (Parida et al., 2007).

It is plausible to speculate that during drought recovery, the activation of aquaporins is entirely adaptive. The upregulation of aquaporin genes in response to water stress (Yamada et al., 1997) suggests an increase in cellular conductivity, primarily facilitating water loss to the environment under such conditions. However, the upregulation during recovery occurs when soil moisture is more abundant, creating a beneficial water potential gradient from the soil to the plant so as to mitigate the risk of heavy water loss. Secchi et al. (2007) examined the impact of drought and subsequent recovery on the transcript levels of *OePIP2.1* (a PIP2 aquaporin gene) in twigs of 2-year-old olive trees exposed to a drought/rewatering regimen. It has been discovered that during drought conditions, there is a reduction in water potential and an elevation in hydraulic resistance, coinciding with a reduction in aquaporin gene expression and contrasting trends were observed upon rewatering.

Zhang et al. (2018) examined the differentially expressed genes (DEGs) associated with photosynthesis and found that the expression levels of two photosystem I (PSI) genes, specifically a PSAL-encoding gene (*GRMZM2G026015*) and a PSAN-encoding gene (*GRMZM2G019807*), were markedly downregulated following a 3-day drought treatment. Furthermore, the expression levels of ten genes, including those encoding subunits of photosystem I and II such as *PSAL*, *PSAO*, *PSAG*, *PSAD*, *PSAN*, and *PSAE*, were downregulated following the six-day drought treatment.

Notably, in rewatered plants, the expression levels of the majority of these genes reverted to control levels, even the expression of certain genes was upregulated. The expression levels of *GRMZM2G012397* (*PSAK*) and *GRMZM2G451224* (*PSAH-2*) significantly diminished throughout the 6-day drought treatment, but were elevated during the water recovery phase. A similar expression pattern was noted for PSII genes. The expression levels of several genes, including *PSB28* (*GRMZM2G005433*), *PSBQ* (*GRMZM2G008892*), and *PSBP* (*GRMZM2G172723*), were significantly downregulated due to drought conditions, especially following a 6-day treatment. The expression of the majority of these genes returned to normal levels in rewatered plants. The expression levels of four genes (*GRMZM2G429955*, *GRMZM2G057281*, *GRMZM2G092427*, and *GRMZM2G117412*) associated with LHC protein were diminished during three days of drought stress. Expression levels of over 10 LHC genes were reduced following the 6-day drought treatment. Subsequent to the water recovery phase, the expression levels of the majority of these LHC genes reverted to control levels or exhibited upregulation (Zhang et al., 2018).

Drought stress adversely affected barley seedling growth by disrupting both primary and secondary metabolism, evidenced by elevated levels of H_2O_2, MDA, and electrolyte leakage, alongside reduced levels of relative water content, membrane stability index, and photosynthetic pigments. After the removal of drought stress, rehydration did not restore the peak growth and performance levels in barley seedlings. Nevertheless, the recovery under drought conditions using Si NPs and silicate proved to be more efficacious, associated with alterations in chlorophyll content, osmolyte and metabolite profiles, cellular damage and membrane stability, as well as the activity of antioxidant enzymes (SOD, CAT, POD, and APX). The efficacy of Si treatments in recovery was contingent upon the intensity of drought stress; nonetheless, the findings demonstrate that barley exposed

to severe drought can recover with low doses of Si NPs. This recovery can be ascribed to enhanced availability of Si NPs to seedlings, more uniform distribution of Si within plant cells, diminished oxidative stress, safeguarding of cell membranes, and the biosynthesis of specific defense-related metabolites (Ghorbanpour et al., 2020).

2.4 Seed priming complement recovery from drought stress

Studies revealed that various priming techniques such as hydropriming, osmopriming, UV-B priming and chemical priming generally result in the enhancement of drought stress tolerance in plants (Wojtyla et al., 2016). The same priming techniques could be equally good in aiding the plant for a quick and effective recovery from drought stress. It will be interesting to see what all features and effects of priming would aid the plant in recovery from drought stress. Yi et al. (2016) suggested that the improved activity of antioxidant machinery helps to protect the cellular structures from drought stress and aid in rapid stress recovery. Drought-resistant variety showed greater modulation of antioxidants and thereby showed faster recovery (Wang et al., 2019). Such a response was reflected in the case of seed priming also, wherein priming offered more stress tolerance to the tolerant variety (Sen and Puthur, 2020). The efficient functioning of antioxidant machinery attributed to various priming techniques will certainly help plants to recover and restore their normal activity as earlier as possible, on being relieved from stress. Another important parameter is photosynthesis; photosynthetic recovery is the immediate result of rewatering. The extent of stress recovery relies on the pre-drought intensity, duration and plant species (Rivas et al., 2016). Various seed priming technologies were found to enhance the photosynthetic performance of plants under drought stress and hence alleviate drought induced damage, which compliment faster recovery from stress (Aswathi et al., 2022).

Hydropriming of *Medicago truncatula* seeds resulted in the upregulation of genes involved in DNA damage repairs and antioxidation machinery. Hydropriming treatment for 4 h enhanced the activity of formamidopyrimidine DNA glycosylase (FPG) involved in base excision repair (Forti et al., 2020). Accumulation of tubulin subunits upon hydropriming and osmopriming in *Arabidopsis* seeds also indicated the role of seed priming in the reactivation of the cell cycle (Gallardo et al., 2001). Recovery was aided by the reactivation of cell cycle events (Wojtyla et al. 2016). Similar to the DNA repair processes during early germination, stress recovery also relies on the DNA and organelles' damage repair mechanism. During recovery, plants rearrange most of the metabolic pathways to repair drought-induced damages (Chen et al., 2016).

The augmented stress tolerance may help plants to recover quickly at the time of rewatering. Drought caused substantial disruptions in PSII photochemistry in both Chinese and white cabbage. The diminished assembly of PSII units and the decoupling of the OEC, along with the inactivation of reaction centres and the reduced electron transfer rate between Q_A and Q_B, impaired the efficient utilisation of absorbed and stored light energy. Nonetheless, the elevated dissipation of surplus light diminished the ability for photochemical Q_A lowering by augmenting the PQ pool in both Chinese and white cabbage. In white cabbage, reduced stress on PSI resulted in a diminished flow of electrons to the PSI acceptor side, potentially serving as a compensatory mechanism to safeguard PSI from over reduction and, so, to manage drought stress. The complete restoration of PSII photochemistry in Chinese cabbage indicated the reversible downregulation of PSII processes. Nevertheless, the rewatering of white cabbage did not facilitate a complete restoration of most parameters, indicating more extensive damage to the photosynthetic units and confirming its intensified vulnerability to drought (Antunović Dunić et al.,

2023). Findings of Jahan et al. (2023) indicate that gas exchange indices, such as mesophyll conductance, diminished with the ageing of wheat leaves; however, the rate of loss was mitigated under conditions of moderate water stress. However, the recovery rate following rewatering of water-stressed plants differed among leaves of various ages (Jahan et al., 2023).

Retention of the stress tolerance mechanism even after recovery is helpful to keep the plant ready for repeated drought events (Nawaz and Wang, 2020). The priming memory can be accomplished through epigenetics and metabolic imprinting (Schwachtje et al., 2019). Priming mediated stress memory helps to improve the tolerance potential of plants through the accumulation of stress-responsive proteins and activation of genes, which will facilitate quick recovery from stress. The exposure of mild stress during the vegetative stage of a plant exhibits an enhanced tolerance potential during the second exposure to severe stress. Drought stress recovery in tomato showed upregulated expression of histone variants, which lead to the reactivation of DNA replication and restoration of cell cycle activity (Iovieno et al., 2016). Likewise, seed priming also promote the activation of specific enzymes, early DNA replication, and synthesis of DNA and RNA (Bray et al., 1989). These changes contribute to early and uniform germination, improved growth, and performance of seedlings emerged from primed seeds (Parveen et al., 2019; Farooq et al., 2020).

The enhancement of stress tolerance capacity via diverse seed priming methods is a promising approach to alleviate the adverse effects of drought stress on plant growth and development. Priming not only enhances drought tolerance potential but also facilitates speedy and effective recovery from stress effects. Maintaining optimal plant water status, enhancing antioxidative mechanisms, improving photochemical efficiency, repairing DNA and organelle damage, and facilitating de novo synthesis of

nucleic acids and proteins through seed priming mitigates the degree of drought-induced damage to plant performance. The physiochemical alterations occurring during seed priming may enhance the mechanisms critical for a plant's rapid recovery from drought stress. Consequently, in the presence of a favourable environment aided by precipitation or irrigation, plants emerged from primed seeds recover more rapidly and effectively. A significant information void exists concerning the impact of seed priming on the recovery kinetics of plants exposed to drought stress.

3. MATERIALS AND METHODS

3. 1 Plant material

The legume crop *Vigna unguiculata* (L.) Walp., known as cowpea, was used for the present research work. Seeds from four different cowpea varieties viz. Anaswara, Bhagyalakshmi, Kanakamony, and PGCP 6 were procured from Kerala Agricultural University (KAU) in Thrissur and the Regional Agricultural Research Station (RARS) in Pattambi, Kerala, India. The study was conducted mainly in two phases. In the preliminary phase, evaluation of different priming treatments, including BABA priming, hydropriming, PEG priming and UV-B priming on enhancing the innate tolerance potential of cowpea varieties subjected to polyethylene glycol (PEG 6000) stress, by analyzing various morphological and physio-chemical parameters were carried out. In the second phase of the study, two cowpea varieties, drought tolerant PGCP 6 and sensitive Anaswara, were chosen based on the initial analysis and subjected to drought stress and recovery to assess the effects of BABA, PEG, and UV-B priming on the drought stress tolerance mechanisms and recovery kinetics.

3.2 Experimental design

3.3 Screening of varieties for stress tolerance and determination of stress imparting concentrations of PEG 6000

Healthy, randomly selected cowpea seeds were surface sterilized using 0.1% $HgCl_2$ solution for 5 min, followed by thorough washing using distilled water. The seeds were allowed to germinate in culture bottles (22 × 12 cm) lined with absorbent cotton containing distilled water (control) and various concentrations of PEG 6000 (0, 5, 10, 15, 20, and 25%). The culture bottles were maintained in a plant growth chamber under controlled

conditions of temperature (25±2°C) and relative humidity (55±5%), subjected to a 14/10 h light–dark cycle with a light intensity of 300 µmol m^{-2} s^{-1}. Morphological parameters and total chlorophyll contents were assessed in 8 d old seedlings.

3.4 Standardisation of seed priming duration and concentration/dosage of BABA, Hydro, PEG and UV-B priming

For BABA priming, the seeds of sensitive and tolerant varieties were subjected to different concentrations of BABA (0, 0.5, 1.0, 1.5, 2, 2.5 mM) for durations of 3, 6, and 9 h, respectively. Hydropriming was conducted by soaking the seeds in distilled water for 3, 6, and 9 h. For osmopriming, sterilized seeds were immersed in polyethylene glycol solution (PEG 6000) of different concentrations (5, 10, 15, 20, and 25%) for durations of 3, 6, and 9 h. Subsequent to the priming treatments, seeds were rinsed thrice with distilled water and then dried to their initial moisture content. For UV-B priming, surface sterilized seeds were exposed to low doses of UV-B radiation using Philips TL 20W/01 RS narrowband UV-B tubes. The biologically effective UV-B (UV-BBE) was obtained through normalization at 300 nm. The dosage was determined by regulating the exposure time and intensity as inputs, using the method established by Caldwell (1971). Seeds were treated with 0, 0.9, 1.8, 2.7, and 3.6 kJm^{-2} s^{-1} of UV-B radiation for priming purposes. The PMA2106 UV-B sensor (Solar Light Co., USA) in combination with the PMA 2200 radiometer (Solar Light Co., USA) was employed to measure the UV-B intensity. To prevent any incident UV-C, UV-B tubes (20 W, Philips, Germany) were encased with cellulose diacetate filters (Johnston Industrial Plastics, Toronto, Canada). After priming of seeds, both the primed and non-primed seeds were transferred to distinct culture bottles (11 x 22 cm) packed with absorbent cotton soaked with double distilled water (control), as well as polyethylene glycol-6000 (stress).

These were incubated in a plant growth chamber under regulated conditions of light intensity (300 µmol m^{-2} s^{-1}), temperature (25±2°C), and relative humidity (55±5%), maintaining a photoperiod of 14/10 h. The growth and biochemical characteristics of all seedlings were documented on 8 d of germination.

3.5 Drought stress and recovery kinetics study under polyhouse conditions

3.5.1 Plant growth conditions and experiment design

Primed and non-primed seeds were grown in the polyhouse under controlled conditions at the Department of Botany, University of Calicut, at 28 ± 2°C, 60±5% relative humidity and 12 h photoperiod ranging from 28-900 µmol m^{-2} s^{-1} (6 am-6 pm) (Figure 1). The trials were conducted using plastic pots of size 16 cm × 14 cm (diameter × height) with drainage holes. Each pot was filled with 2 Kg of soil with known properties (Table 1). The experiments were performed in a completely randomized factorial design. Three weeks old plants with fully developed trifoliate leaves were subjected to two different watering regimes. Controlled plants were maintained under well-watered conditions, while drought stress was induced by withholding irrigation until the leaf relative water content (RWC) approached 50%. Non-primed sensitive variety was subjected to progressive drought stress by withholding watering until day 7 (7 d), and the primed sensitive variety was subjected to drought stress up to day 9 (9 d). While, non-primed tolerant variety was progressively stressed up to day 11 (11 d) and primed tolerant variety up to day 14 (14 d). Following the stress, the seedlings were rehydrated for four days for recovery from drought stress and then analysed for the kinetics of recovery. All physio-chemical analyses were conducted on the first completely developed trifoliate leaves.

Table 1. Physiochemical characteristics of soil.

Sl. No.	Soil parameters	Value	Units
Physical properties			
1.	Soil texture	Sandy loam	
2.	Porosity	38.22±1.45	%
3.	Water holding capacity	43.78±2.19	%
4.	Field capacity	26.91±1.35	%
Chemical properties			
1.	pH	6.9	
2.	Organic matter	1.725±0.086	%
3.	Availiable nitrogen	335.20±16.75	kg/ha
4.	Available phosphorus	17.04±0.85	kg/ha
5.	Available potassium	283.12±14.15	kg/ha

3.5.2 Relative water content

The relative water content of the plant samples was assessed as per the methodology of Weatherly (1950). Fresh leaves were weighed and thereafter submerged in double-distilled water for 6 h. The leaf samples were wiped using filter paper, and the turgid weight was noted. The sample was subsequently placed in an oven at 100°C for 1 h, then maintained at 60°C, and the dry weight was measured.

$$\text{RWC} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

3.5.3 Soil moisture content

Gravimetric method was used to determine the moisture content of the soil. To begin with, soil was taken out from the pots and properly homogenised and the wet soil sample was weighed. The soil was then dried in an oven at 100°C for 24 hours and the dry weight was noted. Dry weight of the soil was monitored until the moisture content was stabilised.

Figure 1. Cowpea grown under polyhouse conditions of Department of Botany, University of Calicut.

3.5.4 Phenotypic traits

3.5.4.1 Plant height and leaf area

The height of the plant was measured using a graduated scale. The leaf area of both non-primed and primed plants under well-watered conditions, drought stress, and post-drought stress recovery was assessed using the graph paper method by tracing the leaf edges on graph paper. The leaves were rinsed with distilled water and dried with filter paper prior to measurement.

3.5.5 Osmotic adjustments

3.5.5.1 Osmolality

Osmolality was measured using a vapour pressure osmometer (Wescor 5520, California, USA). The freeze-thaw method was employed to estimate leaf osmolality. Leaf samples were thawed at room temperature, and sap exuding from the discs of the thawed leaves was collected using a pipette and promptly transferred to the disc chamber of the osmometer. Osmolality measurements were conducted according to Hura et al. (2007).

3.5.5.2 Osmolytes

3.5.5.2.1 Total soluble sugars content

The total soluble sugars content was determined based on the procedure of Dubois et al. (1956).

Extraction: Plant samples, previously weighed, were homogenised in 5 mL of 80% ethanol in a pre-chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was collected. The pellet was re-extracted with 80% ethanol, and the supernatant was collected to assess the total soluble sugars content.

Estimation: A known aliquot volume was taken from the supernatant and made up to 2 mL with distilled water. Subsequently, 1 mL of 5% (w/v) phenol was added and thoroughly mixed. Further, 5 mL of concentrated sulphuric acid was added into the tube and allowed to cool. The optical density of the mixture was measured at 490 nm with a microplate reader. D-glucose served as the standard. The total soluble sugar concentration was quantified in milligrams per gram fresh weight.

3.5.5.2.2 Total free amino acids content

The methodology of Moore and Stein (1948) was used to estimate free amino acids, and L-leucine was used as the standard.

Extraction: Pre-weighed leaf samples were homogenised in 5 mL of 80% ethanol using a pre-chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was obtained. The pellet was re-extracted with 80% ethanol, and the supernatant was collected to quantify the total free amino acids content.

Estimation: A specified volume of aliquot was take out from the supernatant and diluted to 1 mL with distilled water. Subsequently, 1 mL of a freshly prepared reagent solution was added and kept in a boiling water bath for 15 min. Subsequent to cooling, 5 mL of diluent (n-propanol and distilled water in a 1:1 ratio) was added, thoroughly mixed, and incubated at room temperature for 15 min. Absorbance was noted at 570 nm with a microplate reader. L-leucine served as the standard. The concentration of free amino acids in the plant samples was quantified in milligrams per gram fresh weight.

3.5.5.2.3 Proline

The proline content was determined following the procedure of Bates et al. (1973) using L-proline as the standard.

Extraction: Fresh leaf samples, pre-weighed, were homogenised in 5 mL of 3% (w/v) aqueous sulfosalicylic acid with a pre-chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was obtained and used for proline estimation. **Estimation:** An equal volume of glacial acetic acid and ninhydrin reagent was added to a known volume of supernatant and thoroughly mixed. The mixture was subjected to heating in a boiling water bath for 1 h, after which the reaction was stopped by transferring the tubes to an ice bath. Subsequent to cooling, 4 mL of toluene was added into the reaction mixture and thoroughly agitated for 30 s using a vortex mixer. The upper chromophoric toluene layer was isolated, and the optical density was quantified at 520 nm using microplate reader. The proline concentration in the plant samples was quantified in milligrams per gram fresh weight.

3.5.5.2.4 Total soluble proteins

The total soluble protein of the leaf sample was assessed by the Bradford (1976) method.

Extraction: 0.5 g of leaf sample was homogenised in 5 mL of phosphate buffer (pH 7.0) with a pre-chilled mortar and pestle. The protein precipitate from the sample was obtained using centrifugation at 5,000 rpm for 10 min at 4°C.

Estimation: Following centrifugation, 0.1 mL of aliquot was pipetted and made up to a final volume of 1 mL with distilled water. 5 mL of Bradford reagent were added to each aliquot, and the mixture was completely

homogenised using a vortex mixer. The optical density of the solution was measured using microplate reader at 575 nm. The standard was bovine serum albumin (BSA) fraction V powder. The total soluble protein content was quantified as milligrams of protein per gram fresh weight of plant tissue.

3.5.6 Photosynthetic performance

3.5.6.1 Total chlorophyll

Total chlorophyll contents in leaves were estimated using the method of Arnon (1949). A 500 mg fresh leaf sample was weighed on an electronic balance and subsequently ground in 5 mL of 80% acetone using a pre-chilled mortar and pestle. The supernatant was obtained by centrifugation at 5000 rpm for 10 min at 4°C. Re-extraction using 80% acetone and centrifugation was conducted repeatedly until the pellet attained a colourless state. The absorbance at 663, 646, 750, and 470 nm was measured using a microplate reader. The total chlorophyll (Chl *a*+*b*) and carotenoids content in the extract was quantified in micrograms per gram fresh weight using the formula;

$$Chlorophyll\ \ a+b\ = \frac{20.12\ (A_{646}\text{-}A_{750})+8.02\ (A_{663}\text{-}A_{750})}{Fresh\ weight\ of\ the\ sample} \times volume$$

3.5.6.2 Chlorophyll (Chl) *a* fluorescence analysis

Chlorophyll *a* fluorescence parameters were assessed on the fully expanded trifoliate leaf using the Plant Efficiency Analyzer-Handy PEA (Hansatech Ltd., Norfolk, UK), a portable, high-resolution fluorometer (Strasser et al., 2004). All measurements were conducted on the upper surfaces of the leaves following a 20 min dark adaptation, using leaf exclusion clips. A continuous red light (3000 µmol photons m^{-2} s^{-1}, 650 nm) was projected from an array of three light-emitting diodes onto a 5 mm diameter area of the leaf surface, with fluorescence measurements recorded

for a duration of 1 s at a data acquisition rate of 10 µs for the initial 2 ms and 1 ms thereafter. The acquired data were analyzed using Biolyzer HP3 software from the Bioenergetics Laboratory at the University of Geneva, Switzerland. Various Chl *a* fluorescence parameters are listed in the table below (Table 2).

Table 2. List of chlorophyll *a* fluorescence parameters.

Parameters	Description
Phases in induction curve	
O=F_o	Minimal fluorescence/ first step of chl *a* fluorescence transient
I=F_I	Intermediate step in the chl *a* fluorescence transient at 2 ms
J=F_J	Intermediate step in the chl *a* fluorescence transient at 30ms
P=F_p=F_m	Maximal fluorescence level/ final step of chl *a* fluorescence transient
K	Intermediate step in the chl *a* fluorescence transient at 0.3 ms
OJ-phase	It represents the reduction of the acceptor side of PSII
JI-phase	It represents the reduction of the PQ (Plastoquinone) pool
IP-phase	It represents the reduction of the acceptor side PS I
Area	Area above tha fluorescence induction curve
t_{Fm}	Time taken to reach F_m
Other JIP parameters	
F_v	Maximal variable fluorescence (F_m-F_o)
F_v/F_m	It represent maximum quantum yield of PSII
F_v/F_o	It represents the maximum efficiency of water splitting complex
S_M	It represents multiple turnover of Q_A reductions
S_M/ t_{Fm}	It representing the average redox state of Q_A in the time span from 0 to t_{Fm}
N	Turn over number of Q_A indicates the number of times Q_A was reduced from 0 to t_{Fm}
SFI $_{abs}$	An indicator of PSII structure and functioning
V_J	Relative variable fluorescence at phase J of the fluorescence induction curve

V_I	Relative variable fluorescence at phase I of the fluorescence induction curve
PI_{abs}	Performance index of PSII on absorption basis
PI_{total}	Performance index of electron flux to the final PSI electron acceptors
10RC/abs	Absorption per RC
RC/CS_M	density of active PS II reaction centers per cross section
DF_{ABS}	PSII-relative driving force index on an absorption basis
Kn	Non-photochemical de-excitation rate constant
Kp	Photochemical de-excitation rate constant
Yield parameters	
φPo	Maximum quantum yield of primary PSII photochemistry (at t = 0)
φ (Do)	Quantum yield of energy dissipation
φ(Eo)	Quantum yield (at t = 0) for electron transport from QA^- to plastoquinone
Ψo	Probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond QA
Specific energy flux	
ABS/RC	Absorption flux per RC corresponding directly to its apparent antenna size
TRo/RC	Trapping flux leading to QA reduction per RC at t = 0
ETo/RC	Electron transport flux from QA^- to plastoquinone per RC at t = 0
DIo/RC	Dissipated energy flux per RC at the initial moment of the measurement, i.e., at t = 0
Phenomenological energy flux	
ABS/CSm	Absorption of energy per excited cross-section (CS) approximated by F_m
TRo/CSm	Excitation energy flux trapped by PSII of a photosynthesizing sample cross-section (CS) approximated by F_m
ETo/CSm	Electron flux transported by PSII of a photosynthesizing sample cross-section (CS) approximated by F_m
DIo/CSm	Heat dissipation of excitation energy by PSII of a photosynthesizing sample cross-section (CS) approximated by F_m

3.5.6.3 Photosystem (PS) I and II activities

PSI and PSII activities were analyzed polarographically by using a Clark-type oxygen electrode (DW1/AD, Hansatech, Norfolk, UK) connected to a digital control box (OXYG1, Hansatech) at 4°C. The Hansatech Instruments oxygen electrode disc is a specialised electrochemical cell classified as a Clark-type polarographic sensor. It consists of a resin-bonded central platinum cathode and a concentric silver anode connected by an electrolytic bridge, continually polarised by the oxygen electrode control unit. A homogeneous electrolyte layer is maintained over the electrode's dome surface by a paper spacer and a P.T.F.E. (polytetrafluoroethylene) membrane, which is secured by an O-ring around the dome. The well containing the anode functions as a reservoir, with the electrolyte being dragged to the cathode by the paper spacer, which acts as a wick at the base. An external O-ring groove encircles the entire device. The external O-ring secures the electrodes within the base of the electrode chamber. The oxygen electrode is a specific type of electrochemical cell comprising two electrodes submerged in an electrolyte solution. A 50% saturated KCl solution is commonly utilised in oxygen electrode system. The application of a polarising voltage of 700 mV ionises the electrolyte and initiates current flow through a sequence of electrochemical processes. Upon proper preparation and polarisation at 700 mV, the electrochemical reactions occurring at the disc induce a current flow in the presence of oxygen. This current is directly proportional to the concentration of dissolved oxygen in the sample contained within the reaction vessel.

Isolation of thylakoids and assay of photosynthetic electron transport activities of the isolated thylakoids were carried out according to the method of Puthur (2000). The light-dependent O_2 uptake/evolution was quantified by irradiating the sample with saturating white light intensity (1800 µmol

photons $m^{-2}s^{-1}$) from a 100W halogen lamp (LS2, Hansatech). The activities of PSI and PSII were quantified as µmol of O_2 consumed (PSI) or evolved (PSII) per min per milligram of chlorophyll.

Isolation of thylakoid membranes: 0.5 g of fresh tissue was homogenised in 5 mL of ice-cold isolation buffer (pH 7.8) comprising of 400 mM sucrose, 10 mM NaCl, and 20 mM tricine, using a pre-chilled mortar and pestle. The homogenate was filtered through 6 layers of cheese cloth to avoid debris and subsequently centrifuged at 4°C for 6 min at 5000 rpm. Following centrifugation, the supernatant was discarded, and the pellet was resuspended in 500 µl of suspension buffer (pH 7.5) comprising 100 mM sucrose, 10 mM NaCl, 20 mM HEPES [N(2-hydroxyethyl) piperazine-N-(2-ethanesulphonic acid)], and 2 mM $MgCl_2$, and transferred to a sterile chilled tube and maintained on ice for several hours with negligible activity loss.

Calculation of the total chlorophyll content in the thylakoid solution

To compare data obtained from various chloroplast preparations, the total chlorophyll content of the thylakoid samples was assessed using the method of Arnon (1949). 20 µl of the thylakoid suspension was added into the test tube containing 3 ml of 80% acetone. The tube was sealed with parafilm, and the contents were vigorously mixed using a vortex mixer to dissolve the chlorophyll. The homogenate was then centrifuged at 5000 rpm for 5 min to pellet any particulate matter, and the supernatant was collected. The absorbance of the supernatant was measured at 645, 663, and 750 nm relative to the solvent blank (80% acetone). The overall chlorophyll concentration was determined using the following equation:

$$Total\ Chl = 20.12\ (A_{646}\text{-}A_{750}) + 8.02\ (A_{663}\text{-}A_{750}) \times Dilution\ factor$$

Analysis of thylakoid electron transport activities: PSI activity was quantified by assessing oxygen consumption following the inhibition of PSII

activity through the addition of 500 mM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to the medium. The reaction mixture comprised reaction buffer, 10 mM reduced 2,6-dichlorophenolindophenol (DCPIP), 500 µM ascorbate, 5 mM methyl viologen (MV), 1 M sodium azide (NaN_3), 5 µM DCMU and 40 µL thylakoid extract. Electron transport to PSI was sustained by artificial electron donors, ascorbate and DCPIP, present in the medium. Ascorbate functioned as a reductant by donating electrons to DCPIP, which subsequently transferred the electrons to plastocyanin, and then to PSI. Electrons from PSI are redirected to an artificial electron acceptor, MV, in the reaction mixture rather than being received by the FeS centre. Ultimately, MV interacts with oxygen molecules in the medium, resulting in the production of H_2O_2. The dissociation of H_2O_2 into oxygen and H_2O catalysed by catalase in plant tissue is inhibited by the addition of NaN_3 to the reaction mixture. Hence, the oxygen consumption attributable to the activity of PSI alone is quantified using an oxygen electrode device.

To assess PSII activity, the artificial electron acceptor para-benzoquinone (pBQ) was introduced to the medium, with the competence to receive electrons from plastoquinone. The electron transfer from plastoquinone to cytochrome was halted due to the increased reducing potential of pBQ, resulting in the measurement of PSII activity alone. The dissociation of water for electron transfer to PSII leads to the release of oxygen molecules in the medium, quantified using an oxygen electrode system. The reaction mixture (2 mL) in the electrode chamber comprised the reaction buffer, 20 µL pBQ (500 µM), and 40 µL thylakoid extract, prepared in suspension buffer (pH 7.5).

3.5.6.4 Leaf gas exchange parameters

Leaf gas exchange parameters were analyzed using a LI-6400 portable photosynthesis system (infra-red gas analyzer, LI-COR, Lincoln, Nebraska,

USA). Leaf surfaces were cleansed and dried using tissue paper prior to being placed in the leaf chamber for gas exchange measurements. Measurements were conducted at room temperature and ambient CO_2 concentrations. All measurements were conducted on fully opened trifoliate leaves, with readings taken between 9:00 and 10:00 AM. The light intensity of the internal light source in LI-6400 was adjusted to 1500 µmol (photon) m^{-2} s^{-1} to guarantee a consistent and even light throughout all measurements. The various photosynthetic parameters, such as net photosynthetic rate (*Pn*), stomatal conductance (*gs*), intercellular CO_2 concentration (*Ci*), and transpiration rate (*E*), were recorded.

3.5.7 Leaf micromorphology

3.5.7.1 Scanning electron micrograph of stomata

The stomatal characteristics were examined by scanning electron microscopy (SEM). The sample preparation was performed by fixing the leaves in 2.5% (w/v) glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) and prior to imaging, the samples were dehydrated with a series of graded acetone. The dehydrated samples were mounted on aluminium stub and sputtering was made by gold-palladium coater and the SEM photomicrographs of stomata were taken (Carl-Zeiss, Gemini 300, Jena, Germany).

3.5.7.2 Epicuticular wax

3.5.7.2.1 Quantification: Cuticular wax was extracted from cowpea leaves as per the method outlined by Walton (1990) with few changes. Fresh leaf samples were soaked twice for 30 s each in a test tube containing 25 mL of chloroform for extraction. The solvent was agitated for 30 s during each extraction using a Pasteur pipette. The test tubes were weighed to determine the wax content following the evaporation of chloroform.

3.5.7.2.2 Fourier transform infrared spectroscopic analysis of epicuticular wax

Spectrum of the epicuticular wax was measured using a Fourier Transform Infrared Spectrometer (FT-IR-Agilent Technologies, Cary 660, Malaysia). For IR analysis, KBr discs were prepared by mixing the dried KBr with the sample in the ration 1:150 mg (sample:KBr) and a hydraulic pressure of 10 ton was applied. Wax extracts were analysed in the infrared range of 500 to 4000 cm^{-1} with a resolution of 2 cm^{-1}.

3.5.7.2.3 Scanning electron microscopic investigation of epicuticular wax

The modulations in the cuticular wax deposition was studied by examining the foliar micromorphology through scanning electron microscopy (SEM). The leaf segments were fixed in 2.5% (w/v) glutaraldehyde, prepared in 0.1 M phosphate buffer solution (pH 7.4). Fixed specimens were dehydrated with an ascending series of graded acetone and the samples were mounted using carbon tape aluminium stub. The specimens were sputter coated with gold-palladium coater. Photomicrographs were taken using a field emission scanning electron microscope with photographic attachment (FESEM, Carl-Zeiss, Gemini 300, Jena, Germany).

3.5.8 Free radical production

3.5.8.1 Superoxide ($O_2^{\bullet -}$)

The superoxide content in the leaves was assessed according to the methodology of Doke (1983).

Extraction: Pre-weighed leaf samples were cut into 1×1 mm pieces and submerged in 5 mL of 0.01 M potassium phosphate buffer (pH 7.8) containing 0.05% NBT and 10 mM NaN_3. The mixture was first maintained at room temperature for 1 h, then transferred to a water bath at 85°C for a 15

min incubation. Subsequent to incubation, the mixture was promptly transferred to an ice bath to lower the temperature.

Estimation: Following cooling, the absorbance of the mixture was assessed at 580 nm using a microplate reader (Multiskan sky, Thermo Fisher Scientific Oy, Ratastie 2, FI-01620 Vantaa, Finland). The quantification of $^{\bullet}O_2^-$ concentration in the plant samples was done using an extinction coefficient of 12.3 $mM^{-1}cm^{-1}$. The $O_2^{\bullet -}$ concentration in the plant samples was quantified in millimoles per gram fresh weight.

3.5.8.2 Hydrogen peroxide (H_2O_2)

The hydrogen peroxide content was measured as per the protocol of Junglee et al. (2014).

Extraction: Pre-weighed leaf samples were homogenized in 5 mL of 0.1% ice-cold trichloroacetic acid (TCA) using a mortar and pestle. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was collected and used for the assessment of H_2O_2 concentration.

Estimation: One mL of the supernatant was combined with an equivalent volume of potassium phosphate buffer (pH 7), subsequently 1 mL of 1 M potassium iodide (KI) was added. The absorbance of the mixture was quantified at 390 nm with a microplate reader, using hydrogen peroxide as the reference standard. The H_2O_2 concentration in the plant samples was quantified in micrograms per gram fresh weight.

3.5.8.3 *In situ* localization of $O_2^{\bullet -}$

To assess the *in situ* localization of $O_2^{\bullet -}$, cowpea leaves were submerged in 6 mM NBT (nitroblue tetrazolium) at 25°C for 4 h under light conditions until the formation of dark blue spots, indication of insoluble formazan generated

by the reaction of NBT with $O_2^{\bullet-}$ Leaves were decolorized by immersing in boiling ethanol for 15-20 min. Subsequent to cooling, the leaves were photographed with a microscope (Leica DM 2000 LED, Wetzlar, Germany).

3.5.9 Stress intensity assessment

3.5.9.1 Membrane stability index (MSI)

The membrane stability index (MSI) was calculated following the methodology proposed by Sairam et al. (1997). Fresh tissue samples were pre-weighed, cut into 10 mm² pieces, and immersed in 20 mL of distilled water contained in two distinct sets of tubes. The first set was maintained at a temperature of 40°C for 30 min (C_1), while the second set (C_2) was subjected to boiling in a water bath at a pre-determined temperature of 100°C for 15 min. The Multi-parameter PCSTestr (Eutech Instruments, Vernon Hills, USA) was employed to measure the electrical conductivities (EC_1 and EC_2). The MSI was subsequently calculated using the formula,

$$MSI = [1- (EC_1/EC_2)] \times 100$$

Where EC_1 and EC_2 were the respective electrical conductivities at 40°C and 100°C temperatures.

3.5.9.2 Electrolyte leakage (EL%)

Electrolyte leakage (EL%) was assessed as per the protocol outlined by Lutts et al. (1996), with minor modifications. Fresh leaf samples, pre-weighed, were divided into segments measuring 10 mm² and subsequently placed in tubes containing 20 mL of distilled water. The samples were stored at 4°C for 24 h, subsequently allowed to reach room temperature, and the solution was then transferred to a different tube (C_1). The tissue was subsequently immersed in 20 mL of distilled water and autoclaved for 15 min at 120°C (C_2). Upon reaching room temperature, the electrical

conductivities of the two solutions (EC_1 and EC_2) were measured using a Multi-parameter PCSTestr. The EL% was determined as

$$EL\% = (EC_1/EC_2) \times 100$$

3.5.9.3 Lipid peroxidation

The extent of lipid peroxidation was evaluated using the quantification of malondialdehyde (MDA) levels. The MDA content was determined following the standard methodology established by Heath and Packer (1968).

Extraction: 0.5 g of tissue was homogenized in 5 mL of 5% trichloroacetic acid (TCA), and the homogenate was centrifuged at 12,000 rpm for 15 min. The supernatant was used for the measurement of MDA.

Estimation: A volume of 2 mL of the supernatant was mixed with an equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). The mixture was subjected to heating for 20 min at 95°C, subsequently cooled, and then centrifuged for 2 min at 3000 rpm. The absorbance of the supernatant was recorded at 532 and 600 nm using a microplate reader, and the MDA concentration was determined using its molar extinction value of 155 mM^{-1} cm^{-1}. The MDA concentration in the leaf samples was quantified in micromoles per gram fresh weight.

3.5.10 Free radical scavenging system

3.5.10.1 Enzymatic antioxidant system

3.5.10.1.1 Extraction for enzyme assay

The extraction of all antioxidant enzymes was performed using the methodology of Polle et al. (1994). Fresh leaf samples, pre-weighed, were homogenised in 5 mL of 0.1 M potassium phosphate buffer (pH 7.0) using a

pre-chilled mortar and pestle. The homogenate was filtered through four layers of muslin cloth, and the filtrate was centrifuged at 14,000 rpm for 15 min at 4°C; the supernatant obtained was used for the enzyme assay.

3.5.10.1.2 Superoxide dismutase (SOD, EC 1.15.1.1)

SOD activity in fresh samples was assessed using a modified method of Giannopolitis and Ries (1977).

The superoxide dismutase (SOD) activity was measured by assessing its capacity to prevent the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture comprised 0.1 mL of 1.5 M sodium carbonate, 0.3 mL of 0.13 M methionine, 0.3 mL of 10 µM EDTA, 0.3 mL of 13 µM riboflavin, 0.3 mL of 0.63 mM NBT, and 0.1 mL of enzyme extract. A phosphate buffer (0.1 M, pH 7.0) was employed to dilute the reaction mixture to a final volume of 3 mL. Various assay systems, including dark-control, light-control, and test samples, were kept. Test tubes containing all assay mixtures, including enzyme extract, illuminated under a fluorescent lamp for 30 min constituted the test sample, while the set of tubes kept in darkness served as the dark control. The assay mixture devoid of enzyme extract was exposed to a fluorescent lamp for 30 min, serving as the light control. The combination devoid of NBT and enzyme extract functioned as the blank. The buildup of blue formazan in different tubes was assessed using a microplate reader by measuring the absorbance at 560 nm relative to the blank. One unit of SOD activity is defined as the quantity of enzyme necessary to reduce the photochemical reduction of NBT to blue formazan by 50%.

3.5.10.1.3 Catalase (CAT, EC 1.11.1.6)

The enzyme activity of CAT in fresh leaf samples was assessed according to the protocol of Kar and Mishra (1976).

Enzyme assay: Catalase activity was assessed by quantifying the decrease in absorbance at 240 nm over a duration of 1 min following the breakdown of H_2O_2. One enzyme unit (EU) was defined as the moles of H_2O_2 degraded per min per milligram of protein. The assay system consisted of 2.4 mL of 50 mM phosphate buffer (pH 7.0), 0.3 mL of enzyme extract, and 0.3 mL of 30 mM H_2O_2. Phosphate buffer and enzyme extract were transferred into a test tube and thoroughly mixed. A very minute quantity of H_2O_2 was added to the mixture in the test tube to begin enzyme activity. Enzyme activity was assessed at 240 nm for 90 s at 15 s intervals following the addition of H_2O_2. The CAT activity was quantified as µmol H_2O_2 oxidised per minute per gram fresh weight.

3.5.10.1.4 Ascorbate peroxidase (APX, EC 1.11.1.11)

The APX activity in fresh samples was quantified according to Nakano and Asada (1981).

Enzyme assay: The reaction mixture (3 mL) comprised 2.589 mL of 100 mM potassium phosphate buffer (pH 7.0), 15 µL of 0.5 mM ascorbate, 296 µL of 30% (w/v) hydrogen peroxide, and 100 µL of enzyme extract. The activity of APX was measured by monitoring the reduction in absorbance at 290 nm over 3 min at 30 s intervals using a microplate reader. One unit of APX activity is defined as the quantity of enzyme necessary to oxidise 1 µmol of ascorbate per min. The specific activity of each enzyme was estimated following the determination of soluble protein concentration in the enzyme extract, as per Bradford (1976).

Specific activity = (Enzyme activity in Units) / (mg protein/mL enzyme extract)

3.5.10.1.5 Guaiacol peroxidase (GPOX, EC 1.11.1.7)

GPOX activity in fresh samples was assessed following the modified methodology of Gaspar et al. (1975).

Enzyme assay: The reaction mixture (3 mL) comprised 2.858 mL of 100 mM potassium phosphate buffer (pH 7.0), 30 µL of 1% guaiacol, 12 µL of 30% (w/v) hydrogen peroxide, and 100 µL of enzyme extract. The GPOX activity was measured by monitoring the increase in absorbance at 420 nm over 3 min at 30 s intervals using a microplate reader. A single unit of GPOX activity is defined as the quantity of enzyme necessary to oxidise 1 µmol of guaiacol per minute.

3.5.10.2 Non-enzymatic antioxidants

3.5.10.2.1 Ascorbate (AsA) content

The quantification of AsA content was conducted using the methodology of Chen and Wang (2002) with minor changes.

Extraction: Fresh plant samples, pre-weighed, were homogenised using a mortar and pestle in 5 mL of 5% (w/v) trichloroacetic acid (TCA). The homogenate centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was collected and used for the assessment of AsA content.

Estimation: An aliquot of 0.5 mL of the supernatant was mixed with 0.25 mL of 0.2 M sodium phosphate buffer (pH 7.4). To this, 0.5 mL of 10% (w/v) trichloroacetic acid, 0.5 mL of 42% (v/v) phosphoric acid, 0.5 mL of 4% (w/v) bipyridyl (dissolved in 70% alcohol), and 0.25 mL of 3% (w/v) ferric chloride were added. The mixture was incubated at 40°C for 30 min. Absorbance was quantified at 525 nm utilising a microplate reader. The pure form of AsA served as the standard. The ascorbic acid concentration in the plant samples was quantified in milligrams per gram fresh weight.

3.5.10.2.2 Glutathione (GSH) content

The assessment of GSH content was conducted using the methodology of Chen and Wang (2002) with some modifications.

Extraction: Pre weighed fresh leaf samples were homogenised in 5 mL of 5% (w/v) trichloroacetic acid (TCA) with a mortar and pestle. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was obtained and used for the estimation of GSH.

Estimation: A 0.5 mL of the supernatant was mixed with 2.3 mL of 0.2 M sodium phosphate buffer at pH 6.8. To this, 0.2 mL of 6 mM 5,5'-dithiobis-2-nitrobenzoic acid (DNTB/Ellman's reagent) diluted in 0.2 M sodium phosphate buffer at pH 6.8 was added. The mixture was incubated at 30°C for 5 min. Absorbance was quantified at 412 nm using a microplate reader. Oxidised glutathione served as the standard. The GSH levels in the plant samples were quantified in milligrams per gram fresh weight.

3.5.11 Secondary metabolites

3.5.11.1 Anthocyanin content

The anthocyanin content was assessed using the method of Mancinelli et al. (1975), with minor modifications.

Extraction: Fresh leaf samples were homogenised and extracted in 5 mL of acidified methanol (methanol: HCl, 99:1). The extract was maintained at low temperatures for 24 h, and the volume was adjusted to 5 mL.

Estimation: The concentration of anthocyanin was quantified via a microplate reader. The absorbance at 530 nm was recorded, and the anthocyanin content was expressed in micromol per gram fresh weight, using an extinction coefficient of 33 mM^{-1} cm^{-1} to calculate the anthocyanin concentration.

3.5.11.2 Flavonoids content

The extraction and quantification of flavonoid content was conducted following the method of Mirecki and Teramura (1984) with minor modifications.

Extraction: Fresh leaf samples, pre-weighed and cut into 1×1 mm pieces, were immersed in 5 mL of a solvent composed of methanol, hydrochloric acid, and water in a 79:1:20 ratio. The mixture was incubated at room temperature for 24 h. Following centrifugation, the resultant supernatant was used for the quantification of flavonoid content.

Estimation: The optical density of the supernatant was measured at 315 nm with a microplate reader. The flavonoid content was determined using its molar extinction value of 33 mM^{-1} cm^{-1} and expressed in millimoles per gram fresh weight.

3.5.11.3 Total phenolics content

The estimation of total phenolics content was conducted using Folin-phenol Ciocalteu's reagent, following the method proposed by Folin and Denis (1915).

Extraction: Freshly weighed leaf samples were homogenised in a mortar and pestle with 5 mL of 80% ethanol. The homogenate was centrifuged for 10 min at 10,000 rpm at 4°C. The supernatant was obtained, and the pellet was subjected to re-extraction with 80% ethanol. The pooled supernatant was analysed to determine the total phenolics content.

Estimation: A specified volume of an aliquot from the supernatant was extracted and diluted with distilled water to a total volume of 3 mL. The mixture was subsequently combined with 0.5 mL of 1 N Folin-Ciocalteu reagent. After 3 min of incubation, 2 mL of 20% sodium carbonate was

added. The solution was thoroughly mixed for 1 min prior to being allowed to develop its colour. A microplate reader was used to measure the optical density of the solution at 650 nm. The standard used was catechol. The total phenolics content was quantified in milligrams per gram fresh weight.

3.5.12 Root and root nodule study

3.5.12.1 Root length, number and size of root nodules

For the purpose of analysing the properties of the roots and root nodules, the plants were uprooted. The soil that was associated with the roots was removed by gently washing with water. The length of the roots was determined with the help of a graduated scale, and the growth of the lateral roots was observed. The amount of root nodulation was measured by isolating the nodules and counting the number of the same. The estimation of the size of the nodule was made with a microscope (Leica DM 2000 LED, Wetzlar, Germany).

3.5.12.2 Elemental analysis (ICPMS) of root nodules

Key elements in the root nodule were analysed using inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent 7800, Santa Clara, United States). Nodules were collected, kept at 100°C for 1 h, and subsequently transferred to 60°C until a stable dry weight was attained. The Kjeldahl flask contained pre-weighed dried sample, to which 5 ml of concentrated HNO_3 was added. The solution was heated in a heating mantle until it became colourless. Subsequent to digestion, the solution was cooled, filtered, and diluted with distilled water to a final volume of 50 mL. The instrument was calibrated using internal multielement standards (Merck, Germany). The subsequent operational criteria were implemented during the measurement. RF Power was 1500 watts; gas flow rate was 13.0 litres per

minute for the plasma gas. Agilent ICP-MS MassHunter software was used for the elemental analysis.

3.5.12.3 Determination of nitrogen content of root nodules

The CHNS-O Analyser (Thermo Scientific FLASH 2000 HT) was employed to determine the nitrogen content percentages in the sample. The "Dumas method" was employed, which entails the "flash combustion" of the material. A chromatographic column segregates the products after combustion. A thermal conductivity detector (TCD) and Eager Xperience software, which generates an output signal proportional to the concentration of each component in the mixture, were employed for detection of nitrogen present in the root nodule of cowpea.

3.5.13 Gene expression studies of dehydrins

RNA was isolated according to the protocol of Valenzuela-Avendaño et al. (2005). Leaves were ground in liquid nitrogen using an extraction buffer composed of 38% buffer-saturated phenol, 0.8 M guanidine thiocyanate, 0.4 M ammonium thiocyanate, 0.1 M sodium acetate, and 5% glycerol. The extracts were incubated at ambient temperature for 10 min and subsequently centrifuged at 9200 rpm for 10 min. To the supernatant, 300 µL of chloroform-isoamyl alcohol was added, followed by vortexing and centrifugation at 9200 rpm for 10 min at 4°C. The top aqueous phase was transferred to a new tube, and 375 µL of isopropanol and 0.8 M sodium citrate/1 M sodium chloride were added. The tubes were incubated at ambient temperature for 10 min and subsequently centrifuged at 10,100 rpm for 10 min at 4°C. The supernatant was removed, and the pellet was rinsed with chilled 70% ethanol, followed by centrifugation at 10,100 rpm for 10 min at 4°C. The pellets obtained were dried and dissolved in RNase-free water. The isolated total RNA was transferred to a deep freezer (-80°C) until

further use. The RNA quantification was performed with a Genova nano spectrophotometer (Jenway, Cole-Parmer Ltd, UK) and 1 µg of RNA was used to synthesize cDNA using an iScript cDNA synthesis kit (Bio-Rad Laboratories Inc., USA). RT-qPCR was performed to determine the expression levels of *DHN* genes in 96 well plates using TB GREEN Premix Ex Taq Ii (2X) SYBR qPCR Master Mix using CFX96 Real- Time PCR detection system (Bio-Rad Laboratories Inc., USA). The PCR was performed in a final volume of 20 µl. The reaction mixture contained 5 µl of diluted cDNAs, 10 µl of qPCR mastermix (2×) and 0.3–0.4 µM of each primer with the following PCR conditions. 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s followed by 95°C for 15 s. The melting curves were analyzed at 60°C – 95°C after 40 cycles in sequential steps by increasing the temperature in 0.5°C steps for 20 min. The average Ct values for actin gene was used as the internal control and for normalization. The relative expression was calculated using the $2^{-\Delta\Delta CT}$ method. All the experiments were performed with three biological replicates and three technical replicates.

The five *DHN* genes sequences from *Vigna unguiculata* retrieved from the Phytozome v13 database with the names *Vigun11g006400*, *Vigun03g008500*, *Vigun09g268600*, *Vigun04g020700*, and *Vigun09g267800* were used for analyzing the gene expression patterns. The gene-specific primers were designed for dehydrins and actin (internal control) using the Primer-3 software. The abbreviated names of the *DHN* genes are provided in the table below (Table 3). The primer details are listed in Table 4.

Table 3. Abbreviated names of the dehydrin (*DHN*) genes used in the study.

Name of the gene given in the phytozome	Abbreviated name
Vigun11g006400	*Vu400*
Vigun03g008500	*Vu500*
Vigun09g268600	*Vu600*
Vigun04g020700	*Vu700*
Vigun09g268600	*Vu800*

Table 4. List of primers used for RT-qPCR analysis.

Gene abbreviation	Primer sequences	Amplicon size	Tm	GC%	Length (bp)
Vu400	F-GGAACTGAGAGATGAACATGGA	92	59.14	45.45	22
	R-GCACTGGGTTACCTTTCTCAT		58.19	47.62	21
Vu500	F-GGCGGTGGAAGTACACAAGT	83	60.04	55.00	20
	R-CCACTGTTGTTGCTGTTGCT		59.95	50.00	20
Vu600	F-GAAGCCTCAGGAAGAGGTGAT	95	59.83	52.38	21
	R-GGCTGTGCTTCTTCTCTCCTT		60.15	52.38	21
Vu700	F-GTGGAGCTGGGTATGGTATGA	100	59.83	52.38	21
	R-TTGTCATGATCTCCACGAGAC		58.66	47.62	21
Vu800	F-GAGGAGGAAGAAGGGAGTGAA	90	59.80	52.38	21
	R-GTGGTTGTTGTTGGTGAATGC		61.25	47.62	21
Actin	F-TGATAACGATCGGTGCTGAA	80	60.22	45.00	20
	R-GCCAGAAGATTCCATCCCTA		59.08	50.00	20

3.6 Statistical analysis

Duncan's multiple range tests were employed to statistically assess the data at a 5% significance level. One-way ANOVA was conducted using SPSS version 21.0. Pearson's correlation analysis was used to assess the

relationship between various metrics (SPSS version 21.0) and cluster heat map were performed with R software version 4.4.2.

3.7 Chemicals

Analytical reagent grade chemicals from Himedia, SRL, GMBH, Merck, Qualigens, BDH, and Spectrochem. Chemicals such as β-amino butyric acid (BABA) bovine serum albumin, sodium azide, riboflavin, 3-(3,4-dichlorophenyl)-1, L-ascorbate, methyl viologen, 1-dimethyl urea and glutaraldehyde from Sigma Aldrich Co., USA were used.

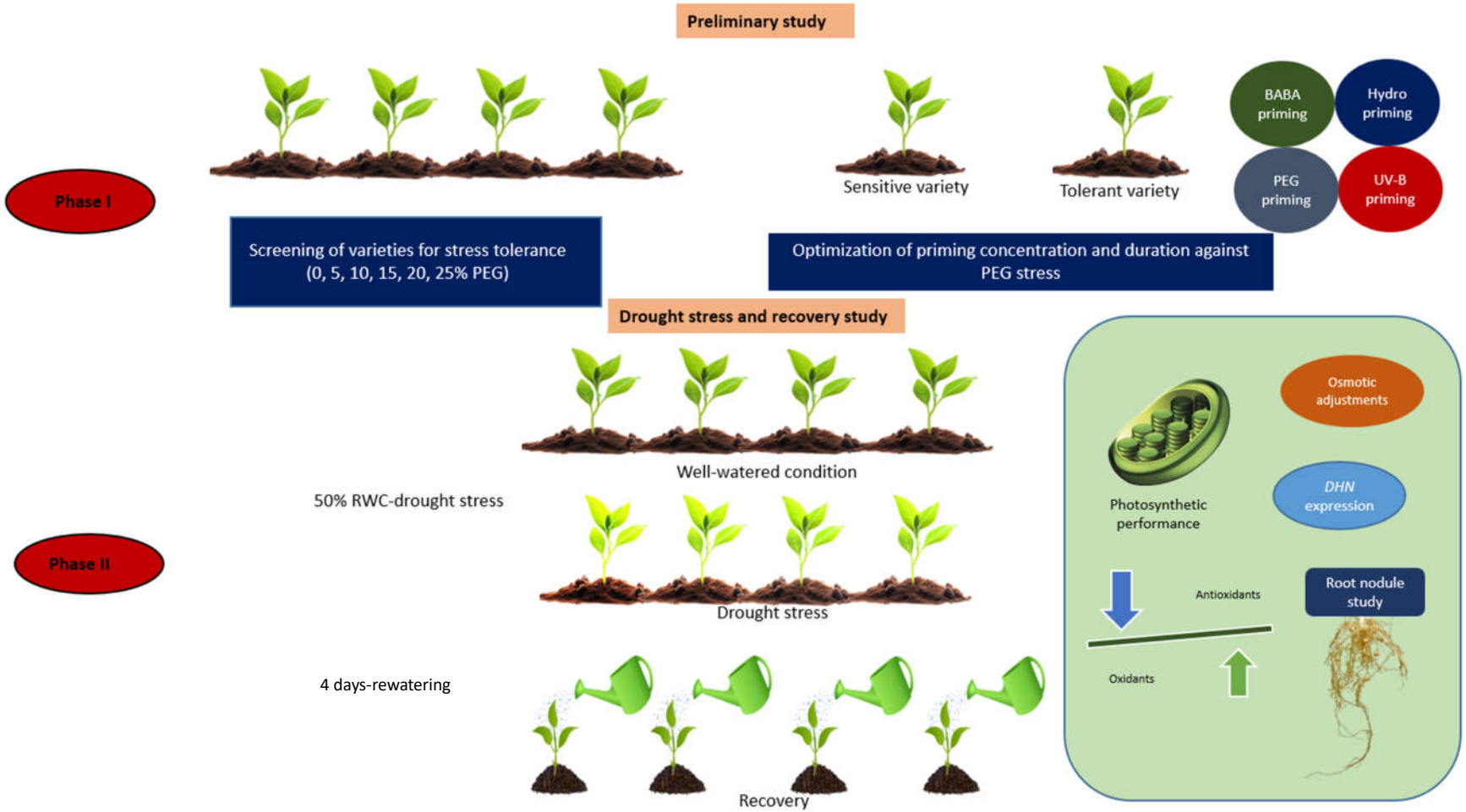

Figure 2. Scheme of the study to analyze the priming induced drought tolerance mechanism and the beneficial role of priming for complementing recovery.

4. RESULTS

4.1. Screening of varieties for stress tolerance and determination of stress-imparting concentrations of PEG 6000

Four different varieties of cowpea viz. Anaswara, Bhagyalakshmi, Kanakamony and PGCP 6 were selected for the present study. The concentration of PEG which imparted ~50% reduction in various morphological and physiological attributes such as shoot length, fresh weight, dry weight, total chlorophyll contents was selected as the stress imparting concentration of PEG. The maximum rate of increase in shoot length occurred up to 8 d of seedlings growth in all cowpea varieties evaluated. Similar trends were observed in the case of fresh weight and dry weight also.

Shoot length progressively decreased with increasing concentration of PEG in all the four cowpea varieties studied. In the varieties Anaswara and Bhagyalakshmi, a reduction of ~50% in shoot length of 8 d old seedlings was observed at 15% PEG stress. Whereas, in the case of varieties Kanakamony and PGCP 6, ~50% reduction was observed at 20% PEG stress. Reduction in fresh weight and dry weight were also noticed when the seedlings were subjected to PEG stress. The highest reduction was observed in Anaswara followed by Bhagyalakshmi, Kanakamony and PGCP 6. Retardation of ~50% in fresh weight and dry weight was observed in both Anaswara and Bhagyalakshmi on exposure to 15% PEG. Whereas in Kanakamony and PGCP 6 it was at 20% PEG (Table 5).

A prominent decrease in total chlorophyll contents was observed in all the varieties of cowpea on exposure to PEG stress. Total chlorophyll contents exhibited a reduction of 50% in Anaswara and Bhagyalakshmi

imparted with 15% PEG stress. Whereas 20% PEG resulted in a reduction of ~50% total chlorophyll content in Kanakamony and PGCP. As compared to other varieties, lesser reduction was noted in the variety PGCP 6 subjected to varying concentrations of PEG (Figure 3).

The degree of lipid peroxidation in the control and PEG stressed seedlings was assessed by measuring the MDA content. The MDA content increased with increasing concentration of PEG stress in all the cowpea varieties studied. Maximum increase in MDA content occurred in variety Anaswara followed by Bhagyalakshmi. There was an increase of 153% and 144% in MDA content at 15% PEG in Anaswara and Bhagyalaksmi respectively. Whereas an increase of only 71 and 69% was observed respectively in Kanakamony and PGCP 6 grown in 15% PEG solution (Figure). The increase in MDA content was 109% and 93% respectively in cowpea varieties Kanakamony and PGCP 6 grown under 20% of PEG stress (Figure 4).

The findings from the first phase of the study, which focused on shoot length, fresh weight, dry weight, total chlorophyll contents and MDA content in the plantlets, indicated that the cowpea variety Anaswara could tolerate up to 15% PEG, while PGCP 6 could endure up to 20% PEG; however, stress effects were severe in seedlings grown at concentrations exceeding these thresholds. Consequently, for the further phase of the study, cowpea varieties Anaswara and PGCP 6 were subjected to 15% and 20% PEG respectively, and all analyses were conducted on 8 d old seedlings, as the plant exhibited maximum growth and performance on this day.

Table 5 A. Shoot length, fresh weight and dry weight of Anaswara subjected to different concentrations of PEG 6000 stress. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Anaswara					
Growth parameter	**Concentration of PEG**	**4 d**	**6 d**	**8 d**	**10 d**
Shoot length (cm)	0%	6.133±0.120 ^{a}	10.500±0.289 ^{a}	12.033±0.120 ^{a}	12.433±0.120 ^{a}
	5%	5.600±0.115 ^{b}	8.567±0.145 ^{b}	11.500±0.057 ^{b}	11.100±0.153 ^{b}
	10%	3.067±0.145 ^{c}	7.367±0.145 ^{c}	9.533±0.145 ^{c}	8.567±0.120 ^{c}
	15%	2.467±0.273 ^{d}	4.400±0.058 ^{d}	6.333±0.089 ^{d}	5.833±0.067 ^{d}
	20%	1.567±0.120 ^{e}	2.600±0.058 ^{e}	3.367±0.089 ^{e}	3.167±0.177 ^{e}
	25%	1.067±0.088 ^{f}	1.500±0.153 ^{f}	1.933±0.089 ^{f}	2.167±0.203 ^{f}
Fresh weight (g)	0%	0.308±0.006 ^{a}	0.464±0.003 ^{a}	0.750±0.004 ^{a}	0.749±0.012 ^{a}
	5%	0.212±0.004 ^{b}	0.302±0.005 ^{b}	0.676±0.012 ^{b}	0.648±0.004 ^{b}
	10%	0.152±0.005 ^{c}	0.203±0.006 ^{c}	0.571±0.005 ^{c}	0.569±0.004 ^{c}
	15%	0.134±0.003 ^{d}	0.159±0.003 ^{d}	0.409±0.003 ^{d}	0.388±0.003 ^{d}
	20%	0.066±0.003 ^{e}	0.083±0.004 ^{e}	0.260±0.005 ^{e}	0.248±0.004 ^{e}
	25%	0.013±0.002 ^{f}	0.016±0.003 ^{f}	0.036±0.003 ^{f}	0.033±0.002 ^{f}
Dry weight (g)	0%	0.023±0.0002 ^{a}	0.040±0.0020 ^{a}	0.073±0.0020 ^{a}	0.070±0.0020 ^{a}
	5%	0.015±0.0005 ^{b}	0.033±0.0020 ^{b}	0.062±0.0010 ^{b}	0.053±0.0020 ^{b}
	10%	0.009±0.0004 ^{c}	0.023±0.0020 ^{c}	0.057±0.0020 ^{c}	0.046±0.0030 ^{c}
	15%	0.008±0.0003 ^{cd}	0.014±0.0008 ^{d}	0.037±0.0020 ^{d}	0.033±0.0020 ^{d}
	20%	0.007±0.0006 ^{d}	0.012±0.0005 ^{d}	0.014±0.0010 ^{e}	0.014±0.0010 ^{e}
	25%	0.005±0.0007 ^{e}	0.0053±0.0004 ^{e}	0.006±0.0002 ^{f}	0.006±0.0002 ^{f}

Table 5 B. Shoot length, fresh weight and dry weight of Bhagyalakshmi subjected to different concentrations of PEG 6000 stress. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Bhagyalakshmi					
Growth parameter	**Concentration of PEG**	**4 d**	**6 d**	**8 d**	**10 d**
Shoot length (cm)	0%	5.400±0.115 a	7.533±0.145 a	10.400±0.115 a	10.867±0.176 a
	5%	4.967±0.145 b	6.233±0.088 b	10.000±0.115 a	9.800±0.115 b
	10%	2.733±0.088 c	5.633±0.088 c	8.233±0.1089 b	8.200±0.115 c
	15%	2.200±0.115 d	3.333±0.088 d	6.100±0.208 c	6.100±0.173 d
	20%	1.633±0.203 e	2.200±0.058 e	3.600±0.208 d	3.533±0.145 e
	25%	1.400±0.058 f	1.067±0.145 f	2.233±0.120 e	2.000±0.153 f
Fresh weight (g)	0%	0.326±0.003 a	0.518±0.007 a	0.656±0.003 a	0.652±0.003 a
	5%	0.264±0.003 b	0.454±0.003 b	0.608±0.008 b	0.589±0.004 b
	10%	0.178±0.003 c	0.383±0.010 c	0.500±0.006 c	0.498±0.005 c
	15%	0.149±0.005 d	0.283±0.008 d	0.373±0.002 d	0.368±0.020 d
	20%	0.101±0.003 e	0.161±0.006 e	0.257±0.002 e	0.264±0.007 e
	25%	0.018±0.003 f	0.022±0.003 f	0.046±0.003 f	0.037±0.004 f
Dry weight (g)	0%	0.021±0.0005 a	0.042±0.003 a	0.077±0.002 a	0.076±0.003 a
	5%	0.014±0.0005 b	0.036±0.003 b	0.067±0.002 b	0.060±0.002 b
	10%	0.009±0.0003 c	0.025±0.001 c	0.060±0.002 c	0.053±0.002 c
	15%	0.007±0.0004 d	0.017±0.002 d	0.041±0.003 d	0.039±0.003 d
	20%	0.006±0.0004 e	0.013±0.0008 e	0.017±0.001 e	0.016±0.001 e
	25%	0.003±0.0002 f	0.006±0.0004 f	0.008±0.0004 f	0.007±0.0004 f

Table 5 C. Shoot length, fresh weight and dry weight of Kanakamony subjected to different concentrations of PEG 6000 stress. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Kanakamony					
Growth parameter	**Concentration of PEG**	**4 d**	**6 d**	**8 d**	**10 d**
Shoot length (cm)	0%	7.800±0.173 ^{a}	11.267±0.145 ^{a}	14.000±0.115 ^{a}	14.800±0.153 ^{a}
	5%	7.567±0.233 ^{a}	10.467±0.145 ^{b}	13.467±0.145 ^{b}	13.767±0.145 ^{b}
	10%	6.400±0.153 ^{b}	8.000±0.115 ^{c}	12.233±0.176 ^{c}	12.200±0.173 ^{c}
	15%	5.100±0.115 ^{c}	6.867±0.088 ^{d}	9.633±0.203 ^{d}	9.533±0.145 ^{d}
	20%	3.667±0.088 ^{d}	5.300±0.115 ^{e}	6.333±0.088 ^{e}	6.500±0.289 ^{e}
	25%	1.533±0.176 ^{e}	2.433±0.088 ^{f}	3.833±0.088 ^{f}	4.200±0.153 ^{f}
Fresh weight (g)	0%	0.462±0.003 ^{a}	0.534±0.003 ^{a}	0.777±0.008 ^{a}	0.774±0.004 ^{a}
	5%	0.446±0.008 ^{a}	0.472±0.003 ^{b}	0.740±0.004 ^{b}	0.676±0.012 ^{b}
	10%	0.293±0.006 ^{b}	0.436±0.004 ^{c}	0.611±0.020 ^{c}	0.640±0.030 ^{b}
	15%	0.201±0.010 ^{c}	0.298±0.003 ^{d}	0.526±0.004 ^{d}	0.532±0.010 ^{c}
	20%	0.103±0.005 ^{d}	0.167±0.005 ^{e}	0.428±0.003 ^{e}	0.447±0.003 ^{d}
	25%	0.054±0.001 ^{e}	0.066±0.003 ^{f}	0.156±0.004 ^{f}	0.156±0.004 ^{e}
Dry weight (g)	0%	0.035±0.0020 ^{a}	0.055±0.0006 ^{a}	0.079±0.0030 ^{a}	0.077±0.0020 ^{a}
	5%	0.031±0.0003 ^{b}	0.051±0.0008 ^{b}	0.070±0.0020 ^{b}	0.067±0.0020 ^{b}
	10%	0.025±0.0010 ^{c}	0.038±0.0007 ^{c}	0.066±0.0020 ^{b}	0.059±0.0010 ^{c}
	15%	0.017±0.0002 ^{d}	0.037±0.0020 ^{c}	0.050±0.002 ^{c}	0.047±0.0020 ^{d}
	20%	0.009±0.0004 ^{e}	0.026±0.0005 ^{d}	0.033±0.0020 ^{d}	0.031±0.0030 ^{e}
	25%	0.005±0.0003 ^{f}	0.017±0.0020 ^{e}	0.019±0.0008 ^{e}	0.018±0.0008 ^{f}

Table 5 D. Shoot length, fresh weight and dry weight of PGCP 6 subjected to different concentrations of PEG 6000 stress. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple

PGCP 6					
Growth parameter	**Concentration of PEG**	**4 d**	**6 d**	**8 d**	**10 d**
Shoot length (cm)	0%	8.233±0.145 a	11.033±0.145 a	14.667±0.440 a	15.000±0.115 a
	5%	8.167±0.120 a	10.733±0.120 a	14.333±0.219 a	14.667±0.088 a
	10%	7.633±0.088 b	7.700±0.153 b	13.067±0.176 b	12.933±0.120 b
	15%	6.267±0.145 c	6.967±0.145 c	10.133±0.203 c	9.937±0.088 c
	20%	4.333±0.240 d	5.667±0.240 d	7.300±0.115 d	7.233±0.120 d
	25%	1.833±0.145 e	2.600±0.145 e	4.133±0.088 e	4.933±0.233 e
Fresh weight (g)	0%	0.444±0.003 a	0.560±0.005 a	0.808±0.004 a	0.798±0.003 a
	5%	0.413±0.002 b	0.510±0.004 b	0.802±0.004 a	0.760±0.006 b
	10%	0.297±0.002 c	0.460±0.002 c	0.656±0.006 b	0.750±0.007 b
	15%	0.207±0.006 d	0.309±0.003 d	0.561±0.003 c	0.644±0.005 c
	20%	0.120±0.004 e	0.178±0.003 e	0.450±0.010 d	0.448±0.003 d
	25%	0.057±0.004 f	0.086±0.003 f	0.194±0.005 e	0.200±0.004 e
Dry weight (g)	0%	0.038±0.0030 a	0.062±0.0010 a	0.080±0.0020 a	0.079±0.0030 a
	5%	0.036±0.0010 a	0.058±0.0010 a	0.077±0.0040 a	0.070±0.0020 b
	10%	0.027±0.0020 b	0.052±0.0010 b	0.070±0.0020 b	0.063±0.0020 c
	15%	0.018±0.0010 c	0.042±0.0010 c	0.053±0.0010 c	0.050±0.0020 d
	20%	0.010±0.0002 d	0.030±0.0020 d	0.041±0.0005 d	0.037±0.0020 e
	25%	0.005±0.0002 e	0.019±0.0004 e	0.026±0.0007 e	0.026±0.0007 f

range tests at $p \leq 0.05$.

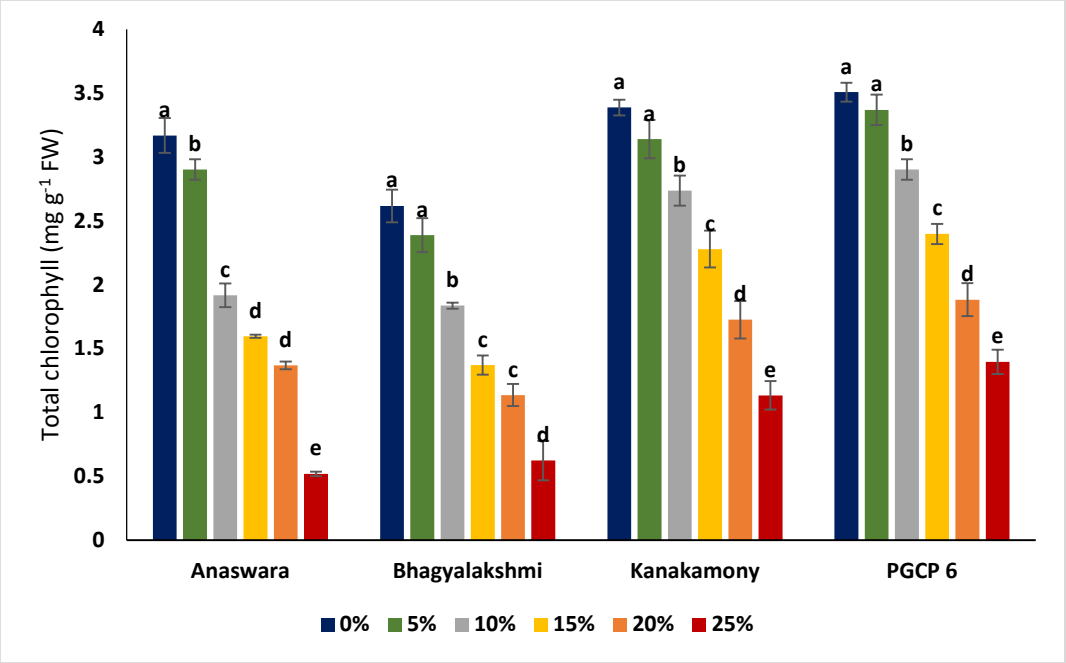

Figure 3. Photosynthetic pigment of four cowpea varieties (Anaswara, Bhagyalakshmi, Kanakamony and PGCP 6) subjected to different concentrations of PEG 6000. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

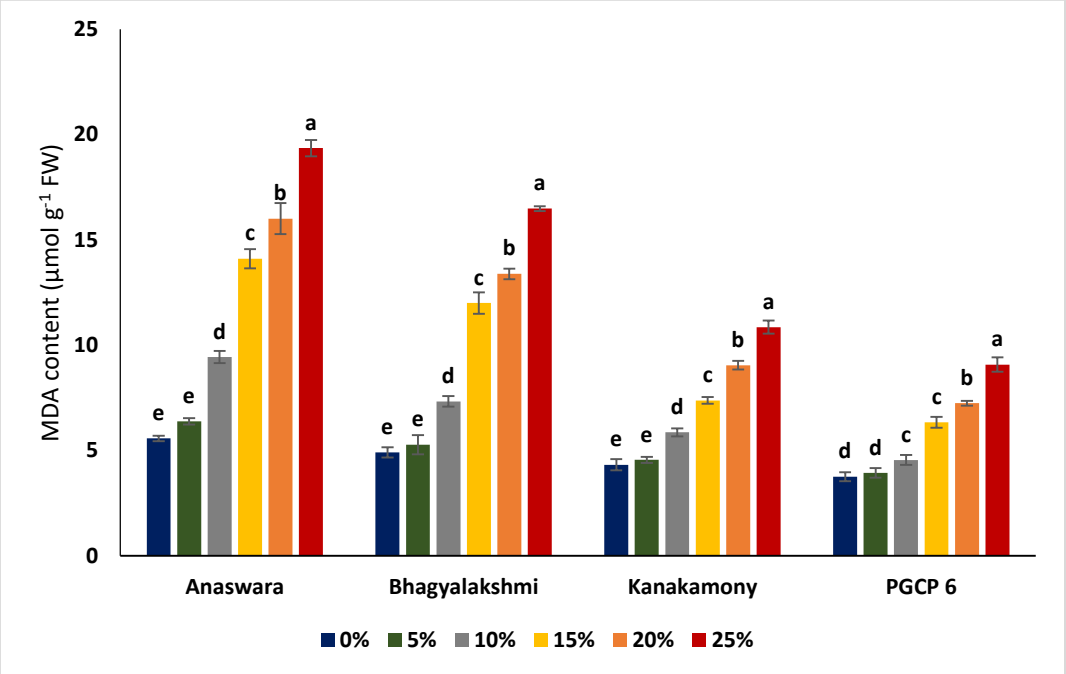

Figure 4. MDA content of four cowpea varieties (Anaswara, Bhagyalakshmi, Kanakamony and PGCP 6) subjected to different concentration of PEG 6000. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

4.2. Standardisation of seed priming duration and concentration/ dosage of BABA, Hydro, PEG and UV-B priming

Following the initial phase of the study, the sensitive variety Anaswara and the tolerant variety PGCP 6 were chosen for the subsequent stage, during which the seeds were subjected to different concentrations/ dosages of priming treatments, viz. BABA, Hydro, PEG and UV-B priming, to determine the optimal priming dosage. Following seed priming, tolerant variety was grown in higher PEG concentration (20%), whereas sensitive variety was grown in lower PEG concentration (15%). The duration of seed priming, which resulted in the greatest enhancement of growth parameters such as shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea seedlings were noted. Among the different priming durations (3, 6, and 9 h), 6 h priming period exhibited the greatest enhancement in growth characteristics of the selected sensitive variety Anaswara and tolerant cowpea variety PGCP 6 under unstressed and stressed conditions.

For BABA priming, cowpea seeds were treated with different concentrations of BABA (0, 0.5, 1.0, 1.5, 2.0, and 2.5 mM) for 3, 6 and 9 h, after these treatments, the seeds were allowed to germinate. The growth parameters like shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea seedlings raised from primed and non-primed seeds were recorded. Stress sensitive variety Anaswara and the stress tolerant variety PGCP 6 showed maximum seedling growth attributes and total chlorophyll when the seeds were primed with 1.5 mM BABA (Table 6). The ideal concentration of PEG for effective priming was determined from several concentrations (0, 5, 10, 15, 20, and 25%). According to the growth parameters and total chlorophyll contents, the selected priming concentration for Anaswara was 10% PEG for 6 h, whereas for PGCP 6, it was 15% for 6 h (Table 7). UV-B priming were carried out by irradiating the

seeds with low dosage of UV-B. A gradual increase in total chlorophyll content was observed in Anaswara with increasing UV-B doses (0.9, 1.8), but doses exceeding 1.8 kJm^{-2} s^{-1} (3.6, 4.5 and 5.4 kJm^{-2} s^{-1}) resulted in a rapid decline in total chlorophyll content. Therefore, 1.8 kJm^{-2} s^{-1} was selected as the effective priming dose for Anaswara. Exposure to a dosage exceeding 3.6 kJm^{-2} s^{-1} led to a reduction in shoot length, fresh weight, dry weight, and total chlorophyll content in PGCP 6 seedlings emerged from primed seeds and grown under both stressed and unstressed conditions. In contrast to higher dosage, a mild dosage of UV-B radiation, specifically 3.6 kJm^{-2} s^{-1}, positively influenced the growth and total chlorophyll of PGCP 6 (Table 8). In the case of hydropriming, seeds of both the varieties soaked in water for 6 h recorded highest increment in various growth parameters and in the content of total chlorophyll than the seeds soaked for 3 h and 9 h (Table 9).

4.3 Drought stress and recovery kinetics study under polyhouse conditions

4.3.1 Relative water content (RWC)

To study the impact of various seed priming treatments viz. BABA priming, PEG priming and UV-B priming, cowpea plants (3 weeks old) were exposed to drought stress. Drought stress was imposed by withholding watering until the leaf relative water content (RWC) reached nearly 50%. The time taken to reach the desired RWC in each treatments was monitored regularly. Drought stress adversely affects the plant water status. There was a progressive reduction in the relative water content in sensitive and tolerant cowpea varieties subjected to drought. Compared to the non-primed plants subjected to drought, primed ones under drought stress exhibited a lesser reduction in RWC%. Non-primed sensitive variety (Anaswara) reached 50% RWC on day 7 of water stress (7 d), and the primed plants took 9 days (9 d). Whereas the tolerant cowpea variety (PGCP 6) attained 50% reduction in

Table 6 A. Shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea (var. Anaswara) seedlings emerged from seeds primed with different concentrations of BABA and subjected to PEG 6000 stress (S). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Concentration of BABA	Anaswara											
	Shoot length (cm)			Fresh weight (g)			Dry weight (g)			Total chlorophyll (mg g^{-1} FW)		
	3 h	6 h	9 h	3 h	6 h	9 h	3 h	6 h	9 h	3 h	6 h	9 h
0 mM	12.000± 0.208 b	11.917± 0.130 c	12.000± 0.288 c	0.732± 0.011 a	0.736± 0.007 b	0.737± 0.008 a	0.0706± 0.0038 a	0.0708± 0.0040 a	0.0706± 0.0038 a	3.348± 0.044 b	3.197± 0.031 c	3.270± 0.085 c
0.5 mM	12.867± 0.176 a	13.000± 0.115 ab	13.000± 0.153 a	0.733± 0.008 a	0.750± 0.008 a	0.745± 0.008 a	0.0710± 0.0005 a	0.0730± 0.0019 a	0.0723± 0.0018 a	3.537± 0.093 ab	3.450± 0.076 ab	3.438± 0.067 b
1.0 mM	13.066± 0.120 a	13.033± 0.088 ab	12.733± 0.145 ab	0.74± 0.006 a	0.751± 0.015 a	0.747± 0.018 a	0.0720± 0.0011 a	0.0736± 0.0039 a	0.0730± 0.0042 a	3.857± 0.043 a	3.731± 0.06 ab	3.625± 0.010 a
1.5 mM	13.1± 0.057 a	13.233± 0.145 a	12.667± 0.176 ab	0.743± 0.012 a	0.752± 0.011 a	0.751± 0.012 a	0.0723± 0.0014 a	0.0740± 0.0015 a	0.0726± 0.0009 a	3.924± 0.0333 a	3.947± 0.039 a	3.631± 0.0105 a
2.0 mM	13.000± 0.115 a	12.933± 0.145 ab	12.567± 0.233 abc	0.747± 0.009 a	0.740± 0.010 ab	0.747± 0.009 a	0.0726± 0.0039 a	0.0726± 0.0039 a	0.0713± 0.0037 a	3.875± 0.024 a	3.781± 0.014 ab	3.501± 0.098 ab
2.5 mM	12.733± 0.240 a	12.567± 0.233 b	12.233± 0.145 bc	0.739± 0.015 a	0.733± 0.009 b	0.740± 0.015 a	0.0711± 0.0039 a	0.0701± 0.0045 a	0.0710± 0.0038 a	3.842± 0.0153 a	3.701± 0.048 ab	3.267± 0.060 c
S+0 mM	6.033± 0.120 d	5.967± 0.088 d	5.933± 0.067 e	0.396± 0.032 b	0.393± 0.032 d	0.393± 0.030 b	0.0360± 0.0049 b	0.0363± 0.0046 c	0.0363± 0.0046 c	1.613± 0.016 c	1.624± 0.008 e	1.628± 0.022 d
S+ 0.5 mM	6.800± 0.173 c	6.933± 0.23 a	6.733± 0.145 d	0.397± 0.021 b	0.398± 0.026 cd	0.400± 0.0306 a	0.0387± 0.0023 b	0.0399± 0.0034 c	0.0397± 0.0032 bc	1.645± 0.028 c	1.76± 0.018 de	1.650± 0.028 d
S+1.0 mM	7.167± 0.12 c	7.300± 0.173 c	7.033± 0.267 d	0.400± 0.032 b	0.407± 0.043 c	0.410± 0.032 b	0.0399± 0.0035 b	0.0409± 0.0038 bc	0.0406± 0.0034 bc	1.705± 0.024 c	1.779± 0.025 de	1.687± 0.029 a
S+ 1.5 mM	7.233± 0.176 c	7.317± 0.208 c	6.933± 0.296 d	0.403± 0.027 b	0.412± 0.036 c	0.407± 0.035 b	0.0440± 0.0015 b	0.0520± 0.0030 b	0.0500± 0.003 b	1.722± 0.029 c	1.791± 0.027 d	1.717± 0.037 d
S +2.0 mM	7.200± 0.115 c	6.833± 0.088 cd	6.767± 0.145 d	0.407± 0.022 b	0.403± 0.023 cd	0.400± 0.025 b	0.0457± 0.0040 b	0.0467± 0.0046 bc	0.0450± 0.005 bc	1.626± 0.024 c	1.750± 0.028 de	1.650± 0.013 d
S +2.5 mM	6.800± 0.115 c	6.067± 0.120 d	6.000± 0.115 e	0.400± 0.050 b	0.400± 0.050 cd	0.400± 0.032 b	0.0407± 0.0023 b	0.0400± 0.0029 c	0.0397± 0.0031 bc	1.621± 0.029 c	1.690± 0.050 e	1.630± 0.049 d

Table 6 B. Shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea (var. PGCP 6) seedlings emerged from seeds primed with different concentrations of BABA and subjected to PEG 6000 stress (S). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Concentration of BABA	PGCP 6											
	Shoot length (cm)			Fresh weight (g)			Dry weight (g)			Total chlorophyll (mg g^{-1} FW)		
	3 h	6 h	9 h	3 h	6 h	9 h	3 h	6 h	9 h	3 h	6 h	9 h
0 mM	14.733± 0.392 c	14.667± 0.441 c	14.633± 0.409 c	0.804± 0.007 b	0.807± 0.005 c	0.807± 0.008 b	0.0800± 0.0029 a	0.0797± 0.0026 a	0.0800± 0.0023 a	3.503± 0.057 d	3.475± 0.029 e	3.510± 0.061 c
0.5 mM	15.533± 0.290 b	15.600± 0.300 b	15.567± 0.296 b	0.820± 0.006 b	0.825± 0.003 b	0.820± 0.006 ab	0.0817± 0.0020 a	0.082± 0.0023 a	0.0813± 0.0017 a	3.973± 0.076 b	3.971± 0.077 c	3.957± 0.068 a
1.0 mM	15.833± 0.203 ab	15.900± 0.208 ab	15.733± 0.267 ab	0.837± 0.003 a	0.840± 0.003 ab	0.835± 0.003 a	0.0823± 0.0014 a	0.0827± 0.0015 a	0.082± 0.0012 a	4.231± 0.0176 a	4.219± 0.015 b	3.744± 0.053 b
1.5 mM	16.400± 0.231 a	16.500± 0.173 a	16.400± 0.231 a	0.840± 0.006 a	0.843± 0.004 a	0.838± 0.009 a	0.0830± 0.0015 a	0.0833± 0.0012 a	0.0833± 0.0012 a	4.277± 0.057 a	4.353± 0.024 a	3.617± 0.026 bc
2.0 mM	16.233± 0.145 a	16.200± 0.115 ab	16.100± 0.173 ab	0.841± 0.005 a	0.842± 0.004 ab	0.836± 0.003 a	0.0823± 0.0018 a	0.0820± 0.0015 a	0.0813± 0.0019 a	4.053± 0.038 b	4.049± 0.0364 c	3.610± 0.049 bc
2.5 mM	16.00± 0.231 d	15.933± 0.176 ab	15.600± 0.231 b	0.835± 0.003 a	0.837± 0.004 ab	0.835± 0.003 a	0.0813± 0.0009 a	0.0810± 0.0006 a	0.0807± 0.0009 a	3.631± 0.0139 c	3.580± 0.055 d	3.585± 0.057 c
S+0 mM	7.300± 0.153 e	7.333± 0.120 f	7.300± 0.1528 e	0.451± 0.011 d	0.450± 0.010 e	0.450± 0.011 c	0.0407± 0.0005 d	0.0407± 0.0005 d	0.0407± 0.0005 d	1.750± 0.021 h	1.747± 0.019 j	1.757± 0.015 f
S+ 0.5 mM	8.233± 0.120 d	8.300± 0.152 e	8.033± 0.203 d	0.458± 0.007 cd	0.460± 0.008 e	0.453± 0.009 c	0.0433± 0.0012 d	0.0437± 0.0009 d	0.043± 0.0015 d	1.919± 0.011 fg	1.922± 0.0128 gh	1.919± 0.011 de
S+1.0 mM	8.400± 0.208 d	8.500± 0.115 de	8.233± 0.145 d	0.463± 0.004 cd	0.466± 0.005 de	0.460± 0.006 c	0.0517± 0.0012 c	0.0520± 0.0011 c	0.0500± 0.0011 c	1.970± 0.043 f	1.997± 0.025 g	1.969± 0.043 d
S+ 1.5 mM	8.800± 0.173 d	9.100± 0.208 d	8.267± 0.176 d	0.475± 0.007 c	0.478± 0.004 d	0.470± 0.006 c	0.0567± 0.0017 b	0.0583± 0.0009 b	0.0557± 0.0018 b	2.10± 0.019 e	2.113± 0.030 f	1.995± 0.027 d
S +2.0 mM	8.766± 0.145 d	8.800± 0.173 de	8.233± 0.145 d	0.468± 0.007 cd	0.465± 0.006 de	0.463± 0.007 c	0.0573± 0.0017 b	0.0573± 0.0017 b	0.0543± 0.0020 b	1.88± 0.037 fg	1.867± 0.026 hi	1.883± 0.037 def
S +2.5 mM	8.567± 0.120 d	8.600± 0.153 de	8.167± 0.120 d	0.463± 0.009 cd	0.460± 0.006 e	0.457± 0.009 c	0.0430± 0.0012 d	0.0433± 0.0009 d	0.0430± 0.0011 d	1.811± 0.034 gh	1.783± 0.033 ij	1.810± 0.034 ef

Table 7 A. Shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea (var. Anaswara) seedlings emerged from seeds primed with different concentrations of PEG and subjected to PEG 6000 stress (S). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Concentration of PEG	Anaswara											
	Shoot length (cm)			Fresh weight (g)			Dry weight (g)			Total chlorophyll (mg g^{-1} FW)		
	3 h	6 h	9 h	3 h	6 h	9 h	3 h	6 h	9 h	3 h	6 h	9 h
0%	12.133± 0.088 ^{b}	12.067± 0.120 ^{c}	12.100± 0.153 ^{c}	0.733± 0.012 ^{a}	0.737± 0.012 ^{a}	0.733± 0.009 ^{a}	0.0707± 0.0029 ^{a}	0.0700± 0.0057 ^{a}	0.0703± 0.0057 ^{a}	3.178± 0.141 ^{c}	3.170± 0.137 ^{c}	3.177± 0.143 ^{bc}
5%	12.500± 0.289 ^{ab}	12.533± 0.260 ^{bc}	12.433± 0.296 ^{abc}	0.737± 0.009 ^{a}	0.750± 0.006 ^{a}	0.747± 0.0089 ^{a}	0.0723± 0.0009 ^{a}	0.0730± 0.0015 ^{a}	0.0717± 0.0009 ^{a}	3.345± 0.106 ^{c}	3.380± 0.083 ^{b}	3.249± 0.143 ^{b}
10%	13.000± 0.115 ^{a}	13.300± 0.153 ^{a}	13.033± 0.121 ^{a}	0.748± 0.011 ^{a}	0.752± 0.010 ^{a}	0.75± 0.011547 ^{a}	0.0733± 0.0032 ^{a}	0.0740± 0.0032 ^{a}	0.0730± 0.0032 ^{a}	3.7715± 0.0692 ^{a}	3.790± 0.051 ^{a}	3.789± 0.0513 ^{a}
15%	12.967± 0.120 ^{ab}	13.067± 0.067 ^{ab}	12.900± 0.208 ^{ab}	0.750± 0.0115 ^{a}	0.750± 0.0115 ^{a}	0.747± 0.0145 ^{a}	0.0720± 0.0006 ^{a}	0.0730± 0.0011 ^{a}	0.0720± 0.0015 ^{a}	3.762± 0.077 ^{a}	3.785± 0.079 ^{a}	3.781± 0.025 ^{a}
20%	12.600± 0.208 ^{ab}	12.700± 0.153 ^{abc}	12.567± 0.233 ^{abc}	0.738± 0.009 ^{a}	0.739± 0.010 ^{a}	0.739± 0.010 ^{a}	0.0713± 0.0040 ^{a}	0.0730± 0.0036 ^{a}	0.0710± 0.0038 ^{a}	3.606± 0.003 ^{ab}	3.606± 0.003 ^{ab}	3.006± 0.014 ^{cd}
25%	12.433± 0.328 ^{ab}	12.500± 0.305 ^{bc}	12.300± 0.208 ^{bc}	0.737± 0.014 ^{a}	0.733± 0.012 ^{a}	0.731± 0.016 ^{a}	0.0690± 0.0040 ^{a}	0.0697± 0.0046 ^{a}	0.0707± 0.0020 ^{a}	3.558± 0.020 ^{b}	3.559± 0.0215 ^{b}	2.975± 0.055 ^{d}
S+0%	6.000± 0.115 ^{f}	6.0333± 0.145 ^{f}	6.067± 0.176 ^{e}	0.380± 0.041 ^{b}	0.383± 0.044 ^{b}	0.380± 0.042 ^{b}	0.0360± 0.0032 ^{c}	0.0360± 0.0032 ^{c}	0.0356± 0.0035 ^{c}	1.594± 0.0185 ^{d}	1.647± 0.031 ^{d}	1.611± 0.028 ^{e}
S+ 5%	6.600± 0.264 ^{de}	6.667± 0.318 ^{ef}	6.600± 0.265 ^{e}	0.390± 0.015 ^{b}	0.397± 0.022 ^{b}	0.400± 0.025 ^{b}	0.0390± 0.0036 ^{bc}	0.0395± 0.0037 ^{bc}	0.0443± 0.0023 ^{bc}	1.670± 0.016 ^{d}	1.692± 0.030 ^{d}	1.625± 0.022 ^{e}
S+10%	7.100± 0.058 ^{cd}	7.367± 0.185 ^{d}	7.233± 0.145 ^{d}	0.397± 0.035 ^{b}	0.403± 0.033 ^{b}	0.397± 0.0318 ^{b}	0.0463± 0.0032 ^{b}	0.0475± 0.0025 ^{b}	0.0467± 0.0017 ^{b}	1.6712± 0.0140 ^{d}	1.732± 0.050 ^{d}	1.659± 0.022 ^{e}
S+ 15%	7.200± 0.115 ^{c}	6.833± 0.145 ^{de}	6.533± 0.240 ^{e}	0.400± 0.021 ^{b}	0.400± 0.0208 ^{b}	0.393± 0.017 ^{b}	0.0456± 0.0018 ^{b}	0.0473± 0.0017 ^{b}	0.0447± 0.0029 ^{bc}	1.637± 0.013 ^{d}	1.697± 0.01 ^{d}	1.635± 0.013 ^{e}
S +20%	6.800± 0.231 ^{cde}	6.733± 0.348 ^{de}	6.433± 0.285 ^{e}	0.387± 0.009 ^{b}	0.393± 0.009 ^{b}	0.390± 0.0058 ^{b}	0.0440± 0.0017 ^{bc}	0.0460± 0.0026 ^{bc}	0.0437± 0.0032 ^{bc}	1.631± 0.132 ^{d}	1.634± 0.014 ^{d}	1.608± 0.0125 ^{e}
S +25%	6.333± 0.088 ^{ef}	6.40± 0.100 ^{ef}	6.100± 0.173 ^{e}	0.383± 0.044 ^{b}	0.392± 0.042 ^{b}	0.387± 0.045 ^{b}	0.0400± 0.0023 ^{bc}	0.0410± 0.0026 ^{bc}	0.0417± 0.0022 ^{bc}	1.621± 0.026 ^{d}	1.624± 0.023 ^{d}	1.606± 0.021 ^{e}

Table 7 B. Shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea (var. PGCP 6) seedlings emerged from seeds primed with different concentrations of PEG and subjected to PEG 6000 stress (S). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Concentration of PEG	PGCP 6											
	Shoot length (cm)			Fresh weight (g)			Dry weight (g)			Total chlorophyll (mg g^{-1} FW)		
	3 h	6 h	9 h	3 h	6 h	9 h	3 h	6 h	9 h	3 h	6 h	9 h
0%	14.733± 0.393 b	14.700± 0.416 c	14.667± 0.441 c	0.817± 0.009 a	0.810± 0.006 b	0.803± 0.009 b	0.0797± 0.0020 a	0.0800± 0.0017 a	0.0807± 0.0012 a	3.573± 0.066 d	3.565± 0.068 c	3.481± 0.113 c
5%	15.433± 0.233 a	15.500± 0.289 b	15.400± 0.305 b	0.822± 0.006 a	0.825± 0.003 ab	0.817± 0.009 ab	0.0810± 0.0026 a	0.0820± 0.0021 a	0.0817± 0.0022 a	3.974± 0.076 c	3.983± 0.085 b	3.93± 0.044 a
10%	15.800± 0.416 a	16.000± 0.289 ab	15.833± 0.203 ab	0.830± 0.006 a	0.835± 0.003 a	0.832± 0.004 a	0.0830± 0.0015 a	0.0840± 0.0006 a	0.0827± 0.0009 a	4.152± 0.031 ab	4.164± 0.042 ab	3.766± 0.018 b
15%	16.067± 0.176 a	16.300± 0.208 a	16.200± 0.153 a	0.837± 0.009 a	0.840± 0.003 a	0.834± 0.007 a	0.084± 0.0011 a	0.0843± 0.0009 a	0.0837± 0.0014 a	4.275± 0.058 a	4.302± 0.078 a	3.589± 0.013 c
20%	15.700± 0.252 a	15.633± 0.186 b	15.600± 0.153 ab	0.830± 0.006 a	0.832± 0.004 a	0.830± 0.006 a	0.083± 0.0011 a	0.0827± 0.0009 a	0.0823± 0.0012 a	4.052± 0.037 bc	4.060± 0.045 b	3.580± 0.017 c
25%	15.533± 0.203 a	15.500± 0.173 b	15.433± 0.233 b	0.821± 0.006 a	0.825± 0.003 ab	0.824± 0.003 ab	0.0803± 0.0007 a	0.0807± 0.0003 a	0.0810± 0.0015 a	3.678± 0.060 d	3.629± 0.037 c	3.557± 0.0267 c
S+0%	7.400± 0.1155 d	7.367± 0.088 e	7.333± 0.120 e	0.452± 0.007 b	0.450± 0.009 d	0.447± 0.009 c	0.0407± 0.0005 c	0.0407± 0.0005 d	0.0407± 0.0006 d	1.807± 0.062 f	1.802± 0.039 e	1.803± 0.039 d
S+ 5%	8.267± 0.186 c	8.400± 0.208 d	8.033± 0.145 b	0.453± 0.006 b	0.457± 0.006 cd	0.452± 0.004 c	0.0430± 0.0015 c	0.0433± 0.0012 d	0.0430± 0.0015 d	1.975± 0.064 ef	1.981± 0.0707 de	1.855± 0.025 d
S+10%	8.333± 0.203 c	8.500± 0.115 d	8.267± 0.066 d	0.463± 0.006 b	0.465± 0.008 cd	0.458± 0.0093 c	0.051± 0.0015 b	0.0517± 0.0009 c	0.0510± 0.0015 c	1.9908± 0.0436 ef	1.991± 0.0437 de	1.858± 0.0796 d
S+ 15%	8.767± 0.145 c	9.100± 0.2081 d	8.500± 0.289 d	0.467± 0.012 b	0.470± 0.0076 c	0.467± 0.0088 c	0.0560± 0.0015 b	0.0580± 0.0011 b	0.0573± 0.0012 b	2.11± 0.085 e	2.137± 0.081 d	1.892± 0.041 d
S +20%	8.667± 0.167 c	8.733± 0.145 d	8.233± 0.145 d	0.462± 0.004 b	0.463± 0.004 cd	0.463± 0.004 c	0.0553± 0.0027 b	0.0563± 0.0018 b	0.0560± 0.0021 b	1.884± 0.036 f	1.876± 0.030 e	1.805± 0.041 d
S +25%	8.500± 0.115 c	8.533± 0.088 d	8.100± 0.115 d	0.455± 0.008 b	0.458± 0.0044 cd	0.450± 0.0058 c	0.0437± 0.0032 c	0.0430± 0.0025 d	0.0427± 0.00218 d	1.845± 0.050 f	1.805± 0.059 e	1.767± 0.015 d

Table 8 A. Shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea (var. Anaswara) seedlings emerged from seeds primed with different dosages of UV-B and subjected to PEG 6000 stress (S). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Dosage of UV-B	Anaswara			
	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Total chlorophyll (mg g^{-1} FW)
0 kJm^{-2} s^{-1}	11.983±0.101 ^{c}	0.737±0.006 ^{ab}	0.0703±0.0040 ^{a}	3.176±0.015 ^{c}
0.9 kJm^{-2} s^{-1}	12.967±0.203 ^{ab}	0.749±0.005 ^{a}	0.0728±0.0020 ^{a}	3.547±0.016 ^{b}
1.8 kJm^{-2} s^{-1}	13.266±0.120 ^{a}	0.752±0.002 ^{a}	0.0737±0.0020 ^{a}	3.890±0.019 ^{a}
2.7 kJm^{-2} s^{-1}	13.000±0.115 ^{ab}	0.748±0.013 ^{a}	0.0733±0.0020 ^{a}	3.555±0.034 ^{b}
3.6 kJm^{-2} s^{-1}	12.867±0.203 ^{ab}	0.739±0.009 ^{ab}	0.0723±0.0040 ^{a}	3.177±0.015 ^{c}
4.5 kJm^{-2} s^{-1}	12.500±0.289 ^{bc}	0.730±0.011 ^{b}	0.0697±0.0040 ^{a}	3.172±0.011 ^{c}
5.4 kJm^{-2} s^{-1}	12.467±0.240 ^{bc}	0.704±0.012 ^{c}	0.0693±0.0040 ^{a}	3.140±0.022 ^{c}
S+0 kJm^{-2} s^{-1}	6.000±0.115 ^{f}	0.390±0.029 ^{c}	0.0363±0.0009 ^{d}	1.628±0.011 ^{e}
S+0.9 kJm^{-2} s^{-1}	6.900±0.208 ^{de}	0.392±0.024 ^{c}	0.0407±0.0030 ^{cd}	1.745±0.042 ^{d}
S+1.8 kJm^{-2} s^{-1}	7.333±0.176 ^{d}	0.407±0.089 ^{c}	0.0517±0.0030 ^{b}	1.787±0.023 ^{d}
S+2.7 kJm^{-2} s^{-1}	6.900±0.208 ^{de}	0.397±0.022 ^{c}	0.0470±0.0050 ^{bc}	1.750±0.015 ^{d}
S+3.6 kJm^{-2} s^{-1}	6.767±0.145 ^{e}	0.390±0.006 ^{c}	0.0423±0.0010 ^{cd}	1.674±0.020 ^{e}
S+4.5 kJm^{-2} s^{-1}	6.033±0.088 ^{f}	0.388±0.006 ^{c}	0.0420±0.0020 ^{cd}	1.626±0.013 ^{e}
S+5.4 kJm^{-2} s^{-1}	6.000±0.115 ^{f}	0.382±0.004 ^{c}	0.0395±0.0003 ^{cd}	1.619±0.013 ^{e}

Table 8 B. Shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea (var. PGCP 6) seedlings emerged from seeds primed with different dosages of UV-B and subjected to PEG 6000 stress (S). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Dosage of UV-B	PGCP 6			
	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Total chlorophyll (mg g^{-1} FW)
0 kJm^{-2} s^{-1}	14.700±0.416 ^{d}	0.808±0.004 ^{c}	0.0797±0.0020 ^{b}	3.469±0.031 ^{c}
0.9 kJm^{-2} s^{-1}	15.600±0.346 ^{bc}	0.825±0.003 ^{bc}	0.0820±0.0010 ^{ab}	3.869±0.064 ^{b}
1.8 kJm^{-2} s^{-1}	16.000±0.289 ^{b}	0.835±0.003 ^{ab}	0.0827±0.0010 ^{ab}	3.973±0.067 ^{b}
2.7 kJm^{-2} s^{-1}	16.300±0.153 ^{ab}	0.839±0.002 ^{ab}	0.0830±0.0006 ^{ab}	3.975±0.064 ^{b}
3.6 kJm^{-2} s^{-1}	16.900±0.208 ^{a}	0.850±0.003 ^{a}	0.0840±0.0010 ^{a}	4.317±0.005 ^{a}
4.5 kJm^{-2} s^{-1}	15.933±0.240 ^{b}	0.847±0.004 ^{a}	0.0810±0.0006 ^{ab}	4.205±0.013 ^{a}
5.4 kJm^{-2} s^{-1}	15.200±0.231 ^{cd}	0.808±0.004 ^{c}	0.0805±0.0030 ^{ab}	3.946±0.050 ^{b}
S+0 kJm^{-2} s^{-1}	7.300±0.115 ^{g}	0.450±0.010 ^{f}	0.0407±0.0005 ^{f}	1.743±0.020 ^{g}
S+0.9 kJm^{-2} s^{-1}	8.200±0.115 ^{f}	0.458±0.007 ^{ef}	0.0440±0.0006 ^{f}	1.864±0.019 ^{g}
S+1.8 kJm^{-2} s^{-1}	8.400±0.115 ^{f}	0.462±0.007 ^{ef}	0.0477±0.0020 ^{e}	1.992±0.032 ^{f}
S+2.7 kJm^{-2} s^{-1}	8.800±0.115 ^{ef}	0.470±0.008 ^{de}	0.0530±0.0006 ^{d}	2.122±0.040 ^{de}
S+3.6 kJm^{-2} s^{-1}	9.233±0.145 ^{e}	0.480±0.003 ^{d}	0.0600±0.0006 ^{c}	2.180±0.050 ^{d}
S+4.5 kJm^{-2} s^{-1}	8.700±0.152 ^{ef}	0.461±0.010 ^{ef}	0.0543±0.0020 ^{d}	2.033±0.012 ^{ef}
S+5.4 kJm^{-2} s^{-1}	7.500±0.287 ^{g}	0.455±0.005 ^{ef}	0.0430±0.0010 ^{f}	1.782±0.020 ^{g}

Table 9 A. Shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea (var. Anaswara) seedlings emerged from hydroprimed seeds and subjected to PEG 6000 stress (S). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Hydropriming (hour of soaking)	Anaswara			
	Shoot length (cm)	Fresh weight (g)	Dry weight (g)	Chlorophyll (mg g^{-1} FW)
0 h	12.000±0.115 ^{b}	0.738±0.004 ^{a}	0.0700±0.0058 ^{a}	3.170±0.010 ^{b}
3 h	12.867±0.176 ^{a}	0.743±0.014 ^{a}	0.0730±0.0015 ^{a}	3.262±0.018 ^{ab}
6 h	13.000±0.231 ^{a}	0.753±0.018 ^{a}	0.0740±0.0015 ^{a}	3.387±0.076 ^{a}
9 h	12.967±0.203 ^{a}	0.750±0.023 ^{a}	0.0730±0.0025 ^{a}	3.205±0.099 ^{ab}
S+0 h	6.000±0.115 ^{d}	0.390±0.029 ^{b}	0.0357±0.0035 ^{b}	1.615±0.022 ^{c}
S+3 h	6.400±0.416 ^{cd}	0.397±0.032 ^{b}	0.0403±0.0034 ^{b}	1.632±0.015 ^{c}
S+6 h	7.000±0.208 ^{c}	0.407±0.037 ^{b}	0.0443±0.0023 ^{b}	1.665±0.013 ^{c}
S+9 h	7.033±0.145 ^{c}	0.403±0.038 ^{b}	0.045±0.0011 ^{b}	1.616±0.021 ^{c}

Table 9 B. Shoot length, fresh weight, dry weight and total chlorophyll contents of cowpea (var. PGCP 6) seedlings emerged from hydroprimed seeds and subjected to PEG 6000 stress (S). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Hydropriming (hour of soaking)	PGCP 6			
	Shoot length	Fresh weight (g)	Dry weight (g)	Chlorophyll (mg g^{-1} FW)
0 h	14.667±0.441 ^{b}	0.808±0.009 ^{b}	0.080±0.0023 ^{a}	3.579±0.071 ^{b}
3 h	15.300±0.153 ^{ab}	0.820±0.023 ^{b}	0.0823±0.0014 ^{a}	3.628±0.017 ^{b}
6 h	15.400±0.115 ^{a}	0.843±0.014 ^{ab}	0.0833±0.0017 ^{a}	3.896±0.126 ^{a}
9 h	15.267±0.145 ^{ab}	0.863±0.009 ^{a}	0.0827±0.0029 ^{a}	3.694±0.057 ^{b}
S+0 h	7.267±0.145 ^{c}	0.452±0.008 ^{c}	0.0408±0.0006 ^{c}	1.807±0.062 ^{c}
S+3 h	7.333±0.120 ^{c}	0.467±0.009 ^{c}	0.049±0.0015 ^{b}	1.900±0.017 ^{c}
S+6 h	7.467±0.203 ^{c}	0.473±0.014 ^{c}	0.048±0.0017 ^{b}	1.948±0.027 ^{c}
S+9 h	7.300±0.173 ^{c}	0.470±0.006 ^{c}	0.0457±0.0014 ^{bc}	1.823±0.010 ^{c}

RWC on day 11 (11 d) and the primed PGCP 6 plants reached 50% RWC on day 14 (14 d). Rewatering led to the restoration of RWC, with the non-primed tolerant variety (PGCP 6) achieved initial RWC levels on 3 d after rewatering (R3). The leaf RWC of primed PGCP 6 recovered within 2 d of rewatering (R2), while the sensitive variety Anaswara returned to initial RWC levels by d 4 (R4), although primed plants recovered by d 3 (R3) (Table 10).

4.3.2 Soil moisture content (SMC)

The soil moisture content was 31-32% at well-watered condition and there was a gradual decline in the percentage of soil moisture content during the progress of drought stress (DS). On 7 d of imposing drought (7 d), the soil moisture content was 22-23%, followed by 19-20% on 9 d after drought stress exposure (9 d). The soil moisture content reached 18% on 11 d of stress (11 d), 14-15% on D13 and 10-11% on 14 d of drought treatment (14 d). Upon recovery, SMC returned to a level same as that of the control soil. On the first day of recovery itself, the SMC was between 30% and 32% (Table 11).

4.3.3 Phenotypic traits

4.3.3.1 Shoot length and leaf area

There was a significant reduction in shoot length and leaf area of both the varieties during drought stress condition. As compared to the control plants (non-primed plants under well-watered conditions), the reduction was more prominent in the sensitive variety. A significant reduction of 40% was observed in the variety Anaswara followed by 13% reduction in shoot length of PGCP 6. The reduction in shoot length was less in the primed plants subjected to drought stress than the non-primed plants under drought stress. Similarly, the reduction in leaf area was less in tolerant

variety (20%) than the sensitive variety (31%) during drought stress. Also, there was lesser reduction in the leaf area of primed plants as compared to the non-primed plants subjected to drought stress. There was a reduction of only 12-13% in primed PGCP 6, and 24-25% reduction in primed Anaswara under drought stress, compared to the control.

There were no significant changes in the shoot length and leaf area of primed and non-primed cowpea after the recovery from drought stress, but with a slight increase in the shoot length of tolerant variety PGCP 6 (Figure 5 and 6).

4.3.4 Osmolality

There was no much variation in the leaf osmolality of the primed plants of both the cowpea varieties studied under the non-stressed condition. However increased level of osmolality was noted when the plants were exposed to drought stress, with the highest osmolality in primed tolerant variety. There was an increase of 61% in non-primed PGCP 6 followed by 46% increase in non-primed Anaswara. The highest increase was noted in BABA and PEG primed PGCP 6 (89%), followed by UV-B primed PGCP 6 (82%). There was an increase of 64-68% in leaf osmolality of primed sensitive variety (Anaswara).

During recovery, the leaf osmolality was reduced than the drought-stressed plants in both the cowpea varieties studied. The highest reduction was observed in tolerant variety during the recuperation from drought stress. There was a reduction of 42%-47% in primed tolerant plants and a reduction of 37%-41% in primed sensitive plants after recovery (Figure 7).

Table 10 A. Relative water content of trifoliate leaves of cowpea variety Anaswara as influenced by seed priming, drought stress and recovery. NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants), 0 d-11 d-different days of drought stress, R1-R4-days of recovery from drought stress. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Anaswara Relative water content (%)											
Treatment	**0 d**	**1 d**	**3 d**	**5 d**	**7 d**	**9 d**	**11 d**	**R1**	**R2**	**R3**	**R4**
NP	90.161± 1.277^{a}	88.358± 2.267^{a}	75.320± 4.292^{b}	59.847± 1.309^{c}	48.209± 2.030^{d}	40.112± 1.642^{e}	35.672± 1.345^{e}	58.935± 2.832^{c}	77.483± 4.374^{b}	87.191± 4.018^{a}	90.006± 2.144^{a}
BP	92.016± 3.445^{a}	90.002± 2.609^{ab}	81.447± 2.034^{b}	71.010± 2.656^{c}	60.587± 1.232^{d}	46.353± 2.031^{e}	39.745± 1.343^{e}	65.019± 2.307^{cd}	82.822± 5.109^{ab}	92.124± 5.978^{a}	92.193± 2.684^{a}
PP	92.899± 3.045^{a}	90.464± 2.491^{abc}	81.815± 1.919^{bcd}	71.661± 1.325^{de}	59.843± 1.196^{f}	45.438± 1.344^{g}	38.454± 1.373^{g}	64.879± 6.339^{ef}	80.738± 4.547^{cd}	92.381± 4.616^{ab}	92.517± 4.369^{ab}
UP	92.632± 2.955^{a}	90.353± 3.213^{ab}	81.406± 0.915^{b}	72.727± 1.638^{c}	60.738± 2.919^{d}	45.387± 2.752^{e}	39.298± 1.967^{e}	64.879± 5.698^{cd}	82.719± 2.189^{b}	92.484± 1.841^{a}	92.530± 3.234^{a}

Table 10 B. Relative water content of trifoliate leaves of cowpea variety PGCP 6 as influenced by seed priming, drought stress and recovery. NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants), 0 d-14 d-different days of drought stress, R1-R4-days of recovery from drought stress. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

PGCP 6 Relative water content (%)														
Treatment	**0 d**	**1 d**	**3 d**	**5 d**	**7 d**	**9 d**	**11 d**	**13 d**	**14 d**	**15 d**	**R1**	**R2**	**R3**	**R4**
NP	92.311± 1.975 a	91.166± 1.658 ab	83.744± 3.077 b	75.224± 1.049 c	63.065± 2.029 d	52.097± 2.773 e	45.572±1 .393 ef	40.108± 1.951 fg	38.022± 1.136 fg	35.669± 1.600 g	67.201± 4.482 d	87.074± 4.129 b	92.389± 4.095 a	92.092± 2.101 a
BP	95.216± 0.782 a	94.627± 1.250 a	88.497± 2.391 ab	80.012± 3.873 cd	72.683± 2.299 d	64.060± 1.997 e	54.0598± 3.040 f	49.245± 1.052 f	47.004± 1.226 fg	40.085± 2.271 g	81.763± 5.728 bc	95.013± 3.348 a	95.130± 3.242 a	95.741± 1.778 a
PP	95.814± 1.081 a	94.798± 3.678 a	87.174± 2.423 ab	81.025± 3.533 bc	73.296± 5.117 c	64.143± 4.267 d	52.205±2 .029 e	49.048± 1.773 e	46.085± 2.699 ef	39.784± 1.663 f	83.447± 4.043 b	95.131± 2.471 a	96.216± 1.663 a	95.419± 1.829 a
UP	95.473± 2.150 a	94.173± 2.531 a	87.015± 3.693 ab	80.684± 4.914 bc	72.937± 4.467 c	63.503± 2.197 d	53.904±1 .779 e	49.815± 3.229 ef	46.830± 1.269 ef	41.211± 2.351 f	80.220± 4.143 bc	95.584± 1.841 a	95.391± 1.719 a	95.760± 2.128 a

Table 11. Effect of drought stress and recovery on soil moisture content (%) of cowpea variety Anaswara (A) and PGCP 6 (B) subjected to seed priming. NP (non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants), NP+DS (non-primed plants under drought stress), BP+DS (BABA primed plants under drought stress), PP+DS (PEG primed plants under drought stress), UP+DS (UV-B primed plants under drought stress), 0 d-14 d-different days of drought stress, R1-R4-days of drought stress recovery. Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Table 11 A.

Soil moisture content (%)										
Treatment	0 d	7 d	9 d	11 d	13 d	14 d	R1	R2	R3	R4
NP	31.663± 1.80 ^{a}	31.160± 1.63 ^{a}	32.0587± 2.04 ^{a}	32..104± 2.04 ^{a}	31.927± 2.05 ^{a}	32.423± 2.61 ^{a}	30.952± 1.81 ^{a}	31.406± 1.96 ^{a}	31.219± 1.72 ^{a}	31.706± 2.18 ^{a}
BP	31.825± 2.00 ^{a}	31.623± 2.44 ^{a}	32.5861± 1.41 ^{a}	32.541± 2.27 ^{a}	32.496± 1.50 ^{a}	32.761± 2.06 ^{a}	30.763± 2.39 ^{a}	31.654± 2.21 ^{a}	31.888± 2.26 ^{a}	31.666± 2.40 ^{a}
PP	31.514± 1.79 ^{a}	31.564± 1.44 ^{a}	32.014± 2.08 ^{a}	32.014± 2.07 ^{a}	32.015± 2.08 ^{a}	32.825± 2.34 ^{a}	31.249± 1.61 ^{a}	32.065± 1.24 ^{a}	32.297± 1.21 ^{a}	32.065± 1.23 ^{a}
UP	31.914± 1.97 ^{a}	31.652± 1.34 ^{a}	32.897± 1.98 ^{a}	32.848± 1.95 ^{a}	32.860± 1.21 ^{a}	32.893± 2.03 ^{a}	31.969± 1.44 ^{a}	32.098± 1.92 ^{a}	31.825± 2.03 ^{a}	32.053± 1.96 ^{a}
NP+DS	30.768± 2.51 ^{a}	22.620± 2.08 ^{b}	19.016± 2.18 ^{bc}	18.027± 2.47 ^{bc}	14.022± 1.52 ^{cd}	10.030± 1.82 ^{d}	30.483± 1.77 ^{a}	31.764± 2.46 ^{a}	31.850± 2.37 ^{a}	32.079± 2.32 ^{a}
BP+DS	31.181± 1.04 ^{a}	23.027± 1.49 ^{b}	19.978± 1.83 ^{bc}	18.128± 1.80 ^{cd}	15.193± 1.09 ^{d}	10.969± 1.75 ^{e}	30.842± 2.40 ^{a}	31.234± 1.24 ^{a}	31.045± 1.07 ^{a}	31.044± 1.07 ^{a}
PP+DS	31.654± 1.38 ^{a}	22.383± 1.46 ^{b}	19.945± 2.45 ^{bc}	18.039± 2.13 ^{cd}	14.438± 2.47 ^{d}	10.730± 1.58 ^{e}	31.239± 1.38 ^{a}	31.840± 1.41 ^{a}	32.026± 1.41 ^{a}	32.164± 1.37 ^{a}
UP+DS	31.502± 2.01 ^{a}	22.813± 1.96 ^{b}	19.536± 1.41 ^{bc}	18.376± 2.18 ^{cd}	14.685± 0.61 ^{d}	10.757± 2.74 ^{e}	31.651± 1.31 ^{a}	31.639± 2.00 ^{a}	31.639± 2.00 ^{a}	31.639± 2.00 ^{a}

Table 11B.

Soil moisture content (%)										
Treatment	0 d	7 d	9 d	11 d	13 d	14 d	R1	R2	R3	R4
NP	31.462± 1.95 [a]	30.941± 1.83 [a]	31.648± 2.14 [a]	31.666± 2.40 [a]	32.105± 2.04 [a]	32.164± 2.85 [a]	30.906± 1.80 [a]	31.416± 1.96 [a]	31.461± 1.94 [a]	31.904± 2.37 [a]
BP	31.391± 2.40 [a]	30.750± 2.39 [a]	31.618± 1.60 [a]	31.893± 2.89 [a]	32.307± 1.47 [a]	32.571± 2.03 [a]	30.785± 2.39 [a]	31.607± 2.20 [a]	31.653± 2.21 [a]	31.898± 2.41 [a]
PP	31.133± 1.98 [a]	31.154± 1.51 [a]	31.190± 2.78 [a]	32.060± 2.07 [a]	32.061± 2.07 [a]	32.602± 2.50 [a]	31.156± 1.51 [a]	32.019± 1.25 [a]	32.065± 1.24 [a]	32.162± 1.33 [a]
UP	31.964± 2.02 [a]	31.699± 1.35 [a]	30.464± 4.36 [a]	31.891± 2.87 [a]	32.907± 1.22 [a]	32.105± 2.04 [a]	31.734± 1.37 [a]	32.053± 1.96 [a]	31.962± 1.98 [a]	32.007± 1.97 [a]
DS	31.811± 2.47 [a]	23.001± 1.77 [b]	19.369± 1.83 [bc]	18.027± 2.46 [bc]	13.693± 1.79 [cd]	10.671± 1.69 [d]	30.530± 1.82 [a]	31.811± 2.17 [a]	31.897± 2.39 [a]	31.984± 2.30 [a]
NP+DS	31.092± 1.11 [a]	23.068± 1.50 [b]	20.060± 1.91 [bc]	18.166± 1.81 [c]	15.229± 1.09 [cd]	11.001± 1.72 [d]	30.842± 2.40 [a]	31.139± 1.16 [a]	31.282± 1.29 [a]	31.044± 1.07 [a]
BP+DS	31.702± 1.43 [a]	22.079± 1.72 [b]	19.981± 2.42 [bc]	18.074± 2.10 [c]	14.279± 2.63 [cd]	11.001± 1.85 [d]	31.287± 1.43 [a]	31.840± 1.41 [a]	32.072± 1.41 [a]	32.119± 1.41 [a]
UP+DS	31.453± 1.96 [a]	22.438± 2.32 [b]	20.060± 1.91 [bc]	18.577± 2.37 [c]	14.864± 0.77 [cd]	11.515± 2.02 [d]	31.604± 1.28 [a]	31.501± 2.01 [a]	31.639± 2.00 [a]	31.591± 1.96 [a]

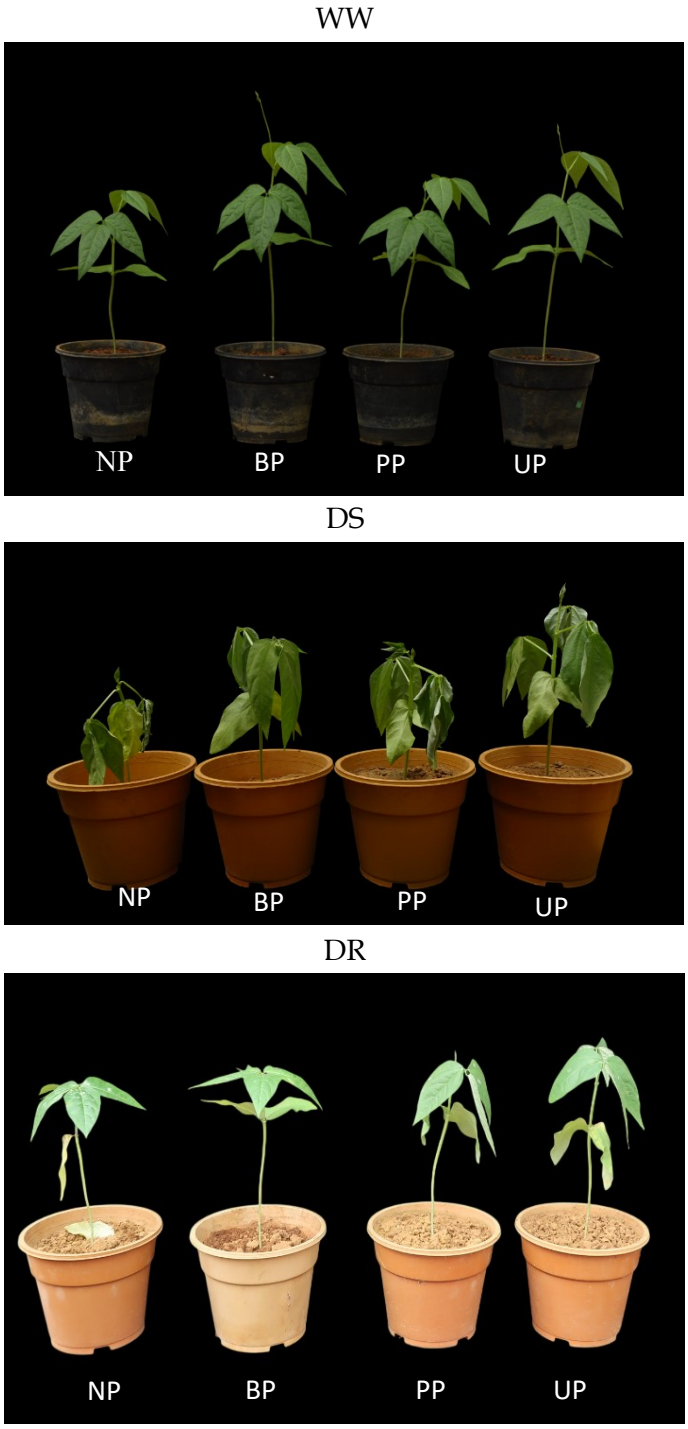

Figure 5 A. Phenotypic changes in non-primed and primed cowpea variety Anaswara grown under well-watered (WW), drought-stressed (DS) and drought recovery conditions (DR). NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants).

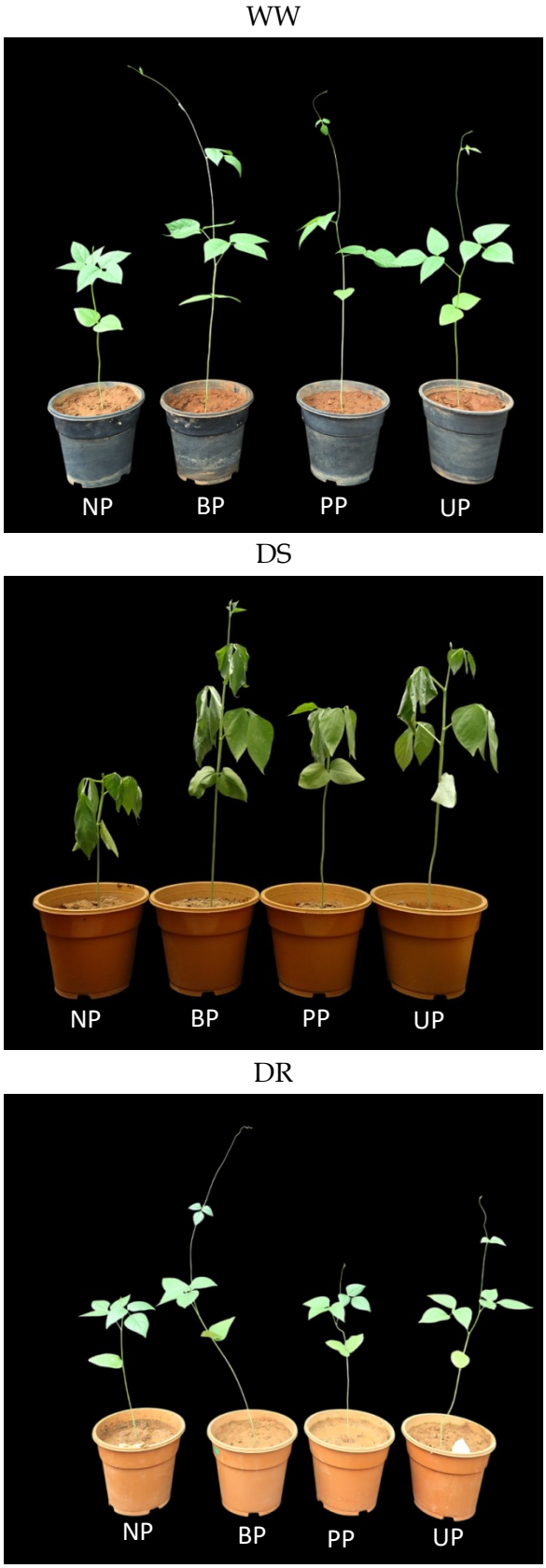

Figure 5 B. Phenotypic changes in non-primed and primed cowpea variety PGCP 6 grown under well-watered (WW), drought-stressed (DS) and drought recovery conditions (DR). NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants).

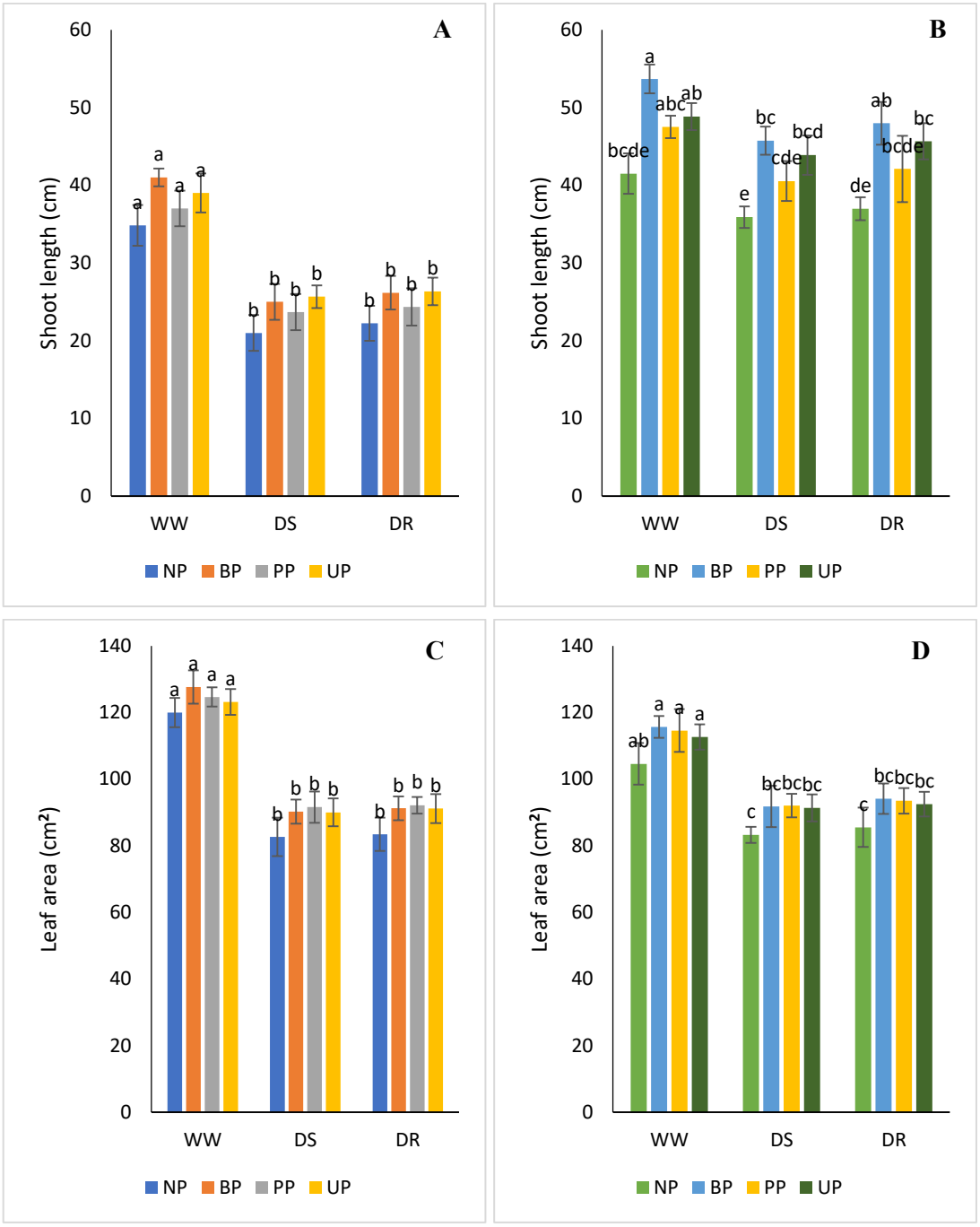

Figure 6. Influence of different seed priming on the shoot length (A-Anaswara and B-PGCP 6) and leaf area (C-Anaswara and D-PGCP 6) of cowpea subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

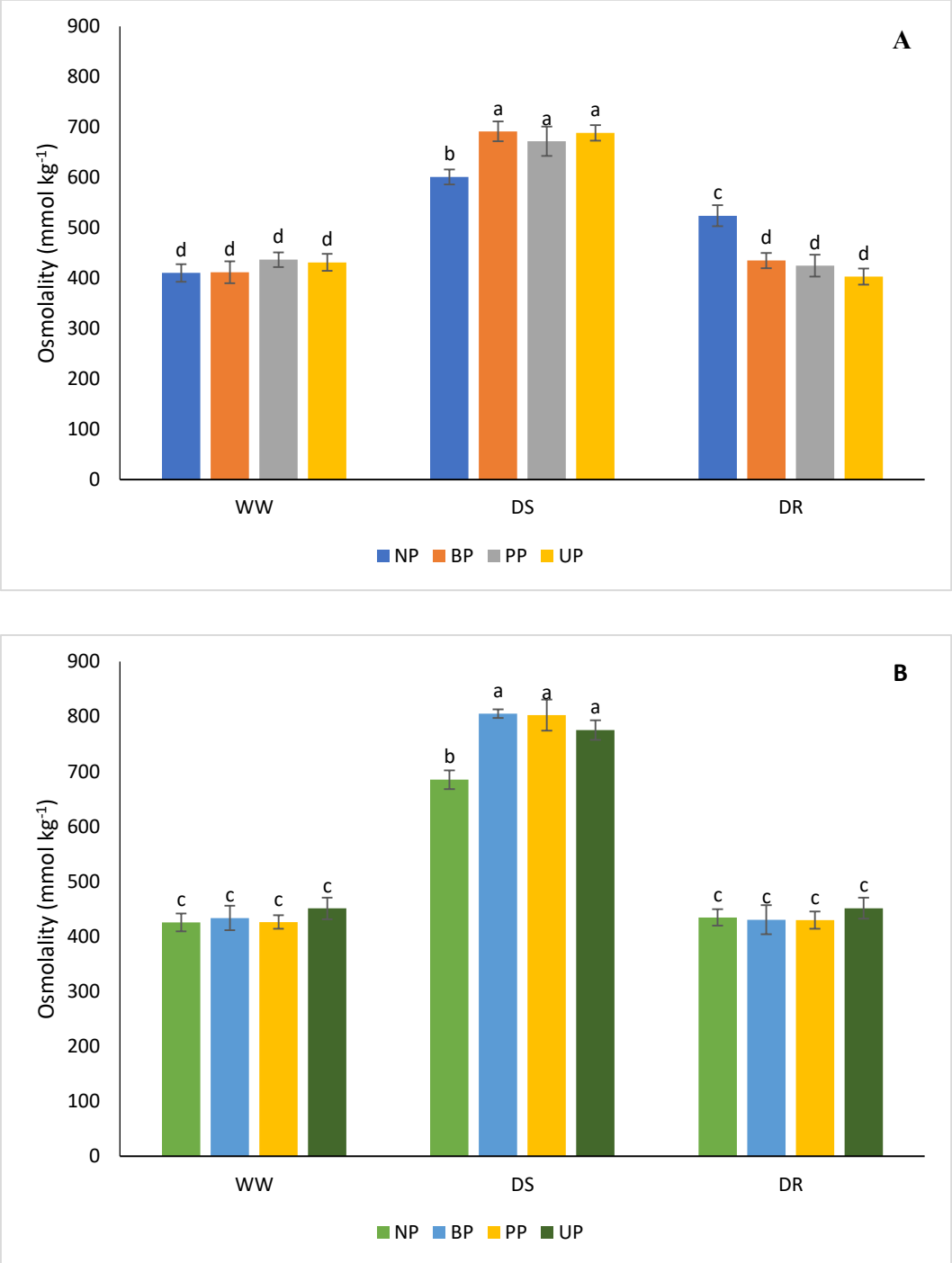

Figure 7. Leaf osmolality of trifoliate leaves of cowpea varieties Anaswara (A) and PGCP 6 (B) as influenced by different seed priming, drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

4.3.5 Osmolytes

4.3.5.1 Total soluble sugars

Total soluble sugars of cowpea leaves increased in response to drought stress. Non-primed plants subjected to drought stress exhibited an increase of 63% and 104% in Anaswara and PGCP 6 respectively than the non-primed plants under well-watered conditions. Priming of PGCP 6 with BABA resulted in the highest level of increase in total soluble sugars, which was 183% higher than the control plants. The second most higher accumulation in total soluble sugars was in PEG primed PGCP 6, which exhibited an increase of 145%. In the case of Anaswara, maximum enhancement was seen in BABA primed plants (143%) followed by PEG primed plants (138%).

The maximum recovery occurred in the case of BABA primed PGCP 6, wherein there was 62% reduction in total soluble sugars than that of the primed drought-stressed plant. As compared to the sensitive variety, tolerant variety exhibited quick recovery with a level equal to that of control. However, the slowest recovery occurred in non-primed sensitive variety (Figure 8).

4.3.5.2 Total free amino acids

Drought exposure significantly increased the total free amino acids content in leaves and higher accumulation was found in tolerant variety as compared with the sensitive variety. The accumulation of total free amino acids in plants emerged from primed seeds were even higher than the plants emerged from non-primed seeds. BABA priming, PEG priming and UV-B priming respectively resulted in 218%, 219% and 164% increase in total free amino acids of Anaswara subjected to drought stress as compared to the control. Whereas the increment was 239%, 222% and 193% in PGCP 6

primed with BABA, PEG and UV-B respectively on being subjected to drought stress.

During recovery from stress, the amino acids content reached to the level of control with the greatest restoration in primed PGCP 6 (56-58%) than the primed Anaswara plants (29-35%). As compared to the non-primed drought-stressed plants, there was a reduction of 20% and 50% respectively in non-primed Anaswara and PGCP 6 during stress recovery (Figure 8).

4.3.5.3 Proline content

There was a significant increase in the proline content in the leaves of both sensitive and tolerant cowpea varieties subjected to drought stress, with tolerant variety exhibiting maximum enhancement. There was an increase of 115% and 102% respectively in tolerant and sensitive cowpea subjected to drought stress and a further enhancement was noted in primed plants. The increment was 154% and 147% in BABA primed tolerant and sensitive cowpea varieties respectively. There was also increase in proline content in PEG and UV-B primed plants, with the highest in PEG primed plants than UV-B primed plants subjected to drought stress.

Proline content significantly reduced in PGCP 6 at the time of recovery from drought, with the maximum reduction of 53% in PEG primed PGCP 6 as compared to the primed drought-stressed plant and this was followed by 51% reduction in the case of BABA and UV-B priming. There was a reduction of 49-55% in the proline content of primed Anaswara upon drought recovery (Figure 9).

4.3.5.4 Total soluble protein

Total soluble protein contents were found to be increased during drought stress in both the sensitive and tolerant varieties. There was an

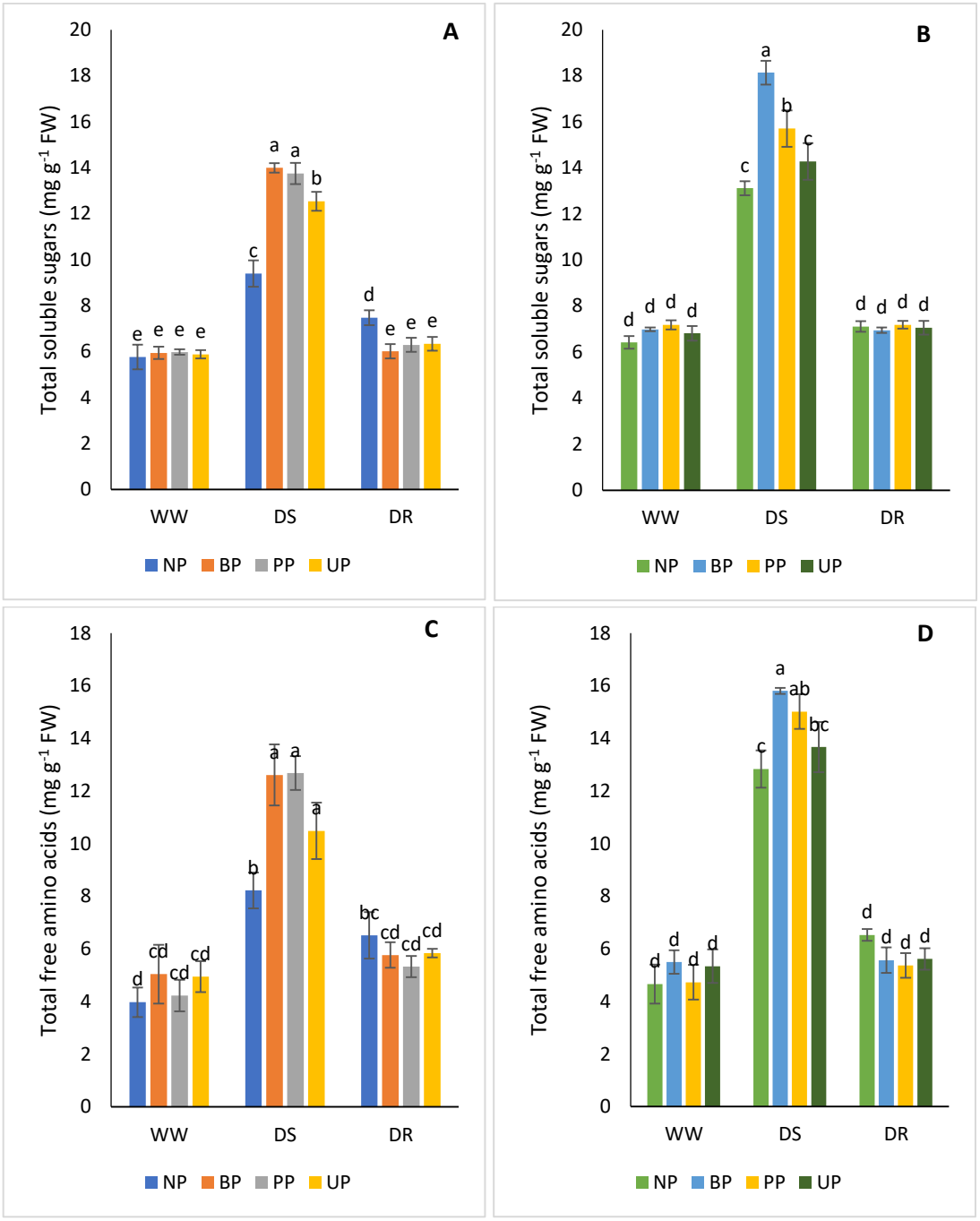

Figure 8. Total soluble sugar content (A-Anaswara and B-PGCP 6) and total free amino acids (C-Anaswara and D-PGCP 6) of trifoliate leaves of cowpea as influenced by different seed priming, drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

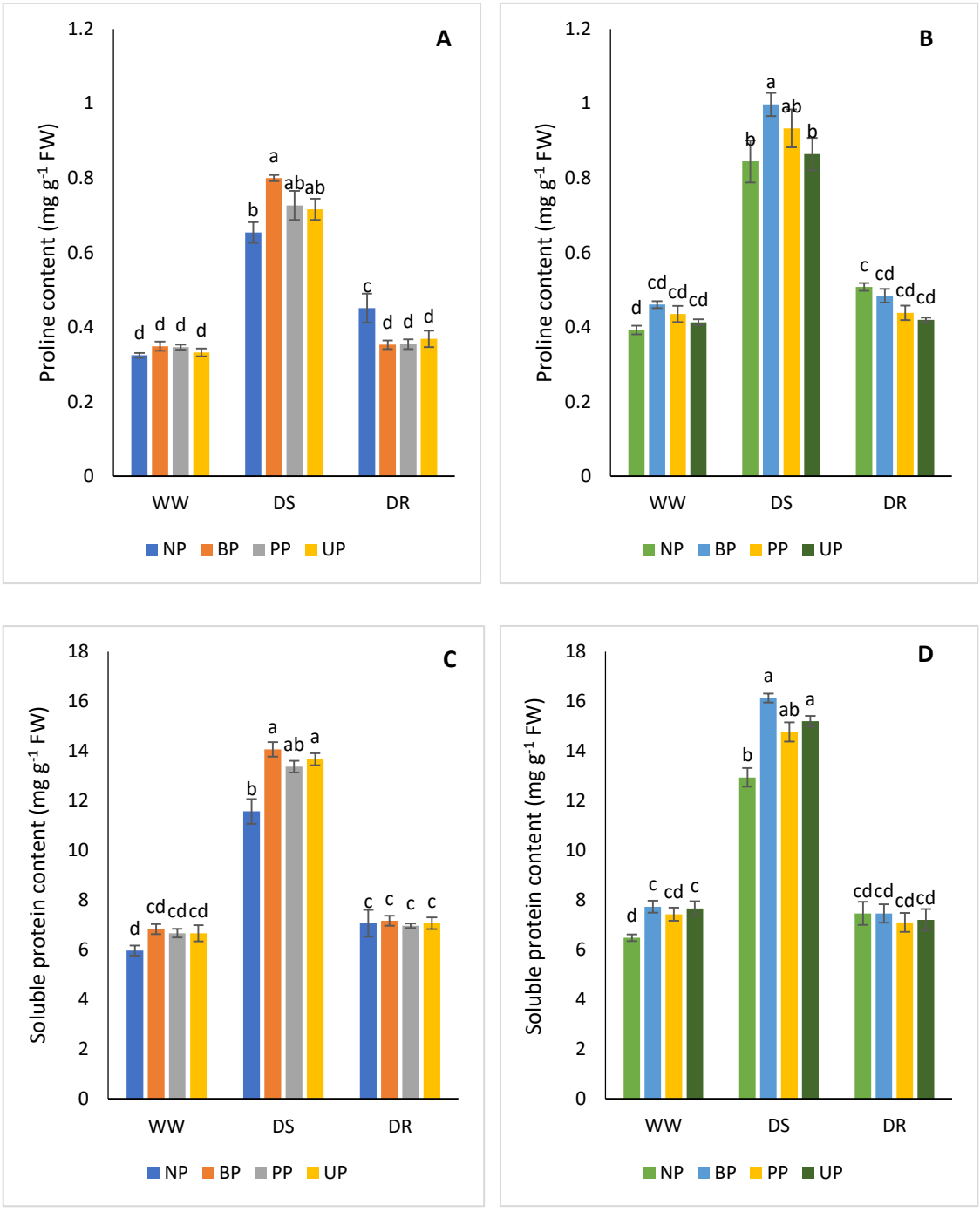

Figure 9. Proline content (A-Anaswara and B-PGCP 6) and total soluble proteins content (C-Anaswara and D-PGCP 6) in trifoliate leaves of cowpea as influenced by different seed priming, drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

increase of 99% and 94% in PGCP 6 and Anaswara respectively upon drought stress exposure. In the case of primed plants, the increase was even higher. The highest increase in total soluble protein was noted in BABA primed PGCP 6 (149%) followed by UV-B primed PGCP 6 (134%) during drought stress.

There was a prominent reduction in the content of total soluble protein during recovery from drought stress. The highest reduction was observed in primed PGCP 6 and as compared to the respective drought-stressed plant there was a reduction of 53-55% in the total soluble protein content, reaching the same level as that of control (Figure 9).

4.3.6 Photosynthetic performance

4.3.6.1 Total chlorophyll content

Total chlorophyll content was reduced in response to drought stress and the reduction was higher in sensitive variety than the tolerant variety. There was a reduction of 48% and 36% in total chlorophyll content of Anaswara and PGCP 6 respectively during drought exposure. Seed priming with BABA, PEG and UV-B radiation improved the total chlorophyll content in both the cowpea varieties and there was only less reduction in chlorophyll under drought stress in the primed plants as compared to the non-primed plants subjected to drought stress. A reduction of 27%, 31% and 38% in Anaswara and a reduction of 9%, 18% and 25% in PGCP 6 was recorded in the case of plants emerged from seeds primed with BABA, PEG and UV-B, respectively on being subjected to drought.

During recovery from drought, there was a significant increase in total chlorophyll content of non-primed and primed plants, with the maximum enhancement observed in primed plants. As compared to the non-primed drought-stressed plants, there was an increase of 79% in BABA

primed, 63% in PEG primed, and 48% in UV-B primed plants of Anaswara during recovery period. In PGCP 6, the increment was 85%, 80% and 62% in BABA, PEG and UV-B primed plants respectively during stress recovery. Whereas, non-primed plants recovered with a lesser increase (26% and 21%) in total chlorophyll content of PGCP 6 and Anaswara respectively (Figure 10).

4.3.6.2 Chlorophyll *a* fluorescence

Multiple chlorophyll *a* fluorescence parameters were examined in the leaves of Anaswara and PGCP 6 to investigate the impact of drought stress treatment on photochemistry. Significant alterations were noted in the Chl *a* fluorescence characteristics of both cowpea varieties relative to the control. Various seed priming treatments enhanced plant vitality, as measured by the performance index based on absorption (PIabs), in both the cowpea varieties. An increase in PIabs compared to control plant was observed under well-watered condition in Anaswara primed with BABA, the increment was 18%. A similar increase in PIabs was observed in the cowpea variety PGCP 6 subjected to BABA and PEG priming and the increase was 25% and 16% respectively. Nonetheless, there was a decline in PIabs during drought stress, with a more rapid and pronounced decrease observed in non-primed Anaswara under drought condition (80%) followed by non-primed PGCP 6 (60%).The reduction in PIabs was lesser in primed plants and the reduction was 61-66% in the case of primed Anaswara during drought stress. A reduction of 33-46% was observed in primed PGCP 6 exposed to drought stress. During the recovery phase, there was a substantial improvement in the PIabs with the maximum restoration observed in primed PGCP 6 (Figure 11 and 12).

The efficiency of the water-splitting complex on the donor side of PSII (Fv/Fo) was reduced under drought stress in both the cowpea varieties.

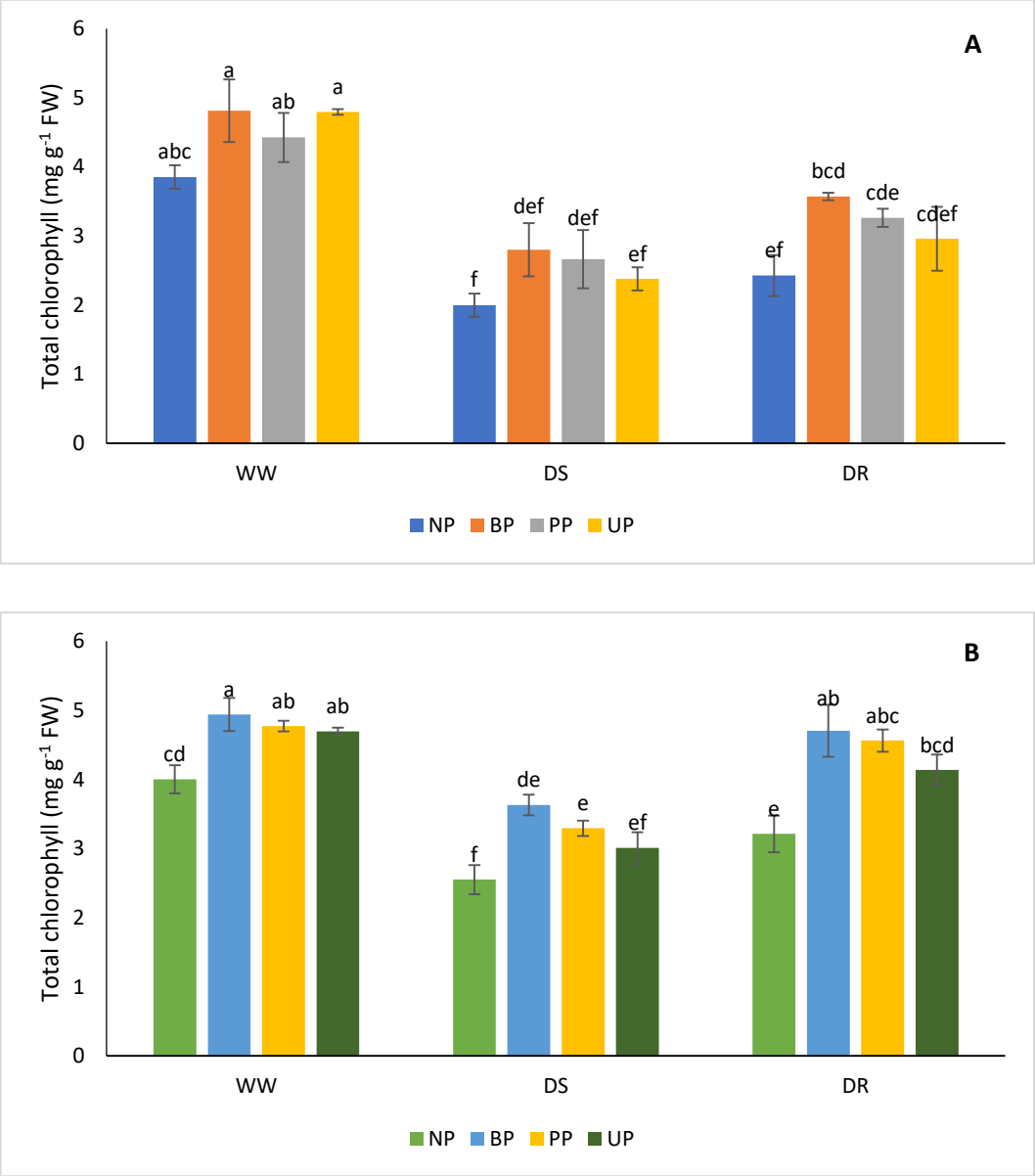

Figure 10. Total chlorophyll content in the trifoliate leaves of cowpea variety Anaswara (A) and PGCP 6 (B) subjected to different priming treatments and exposed to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

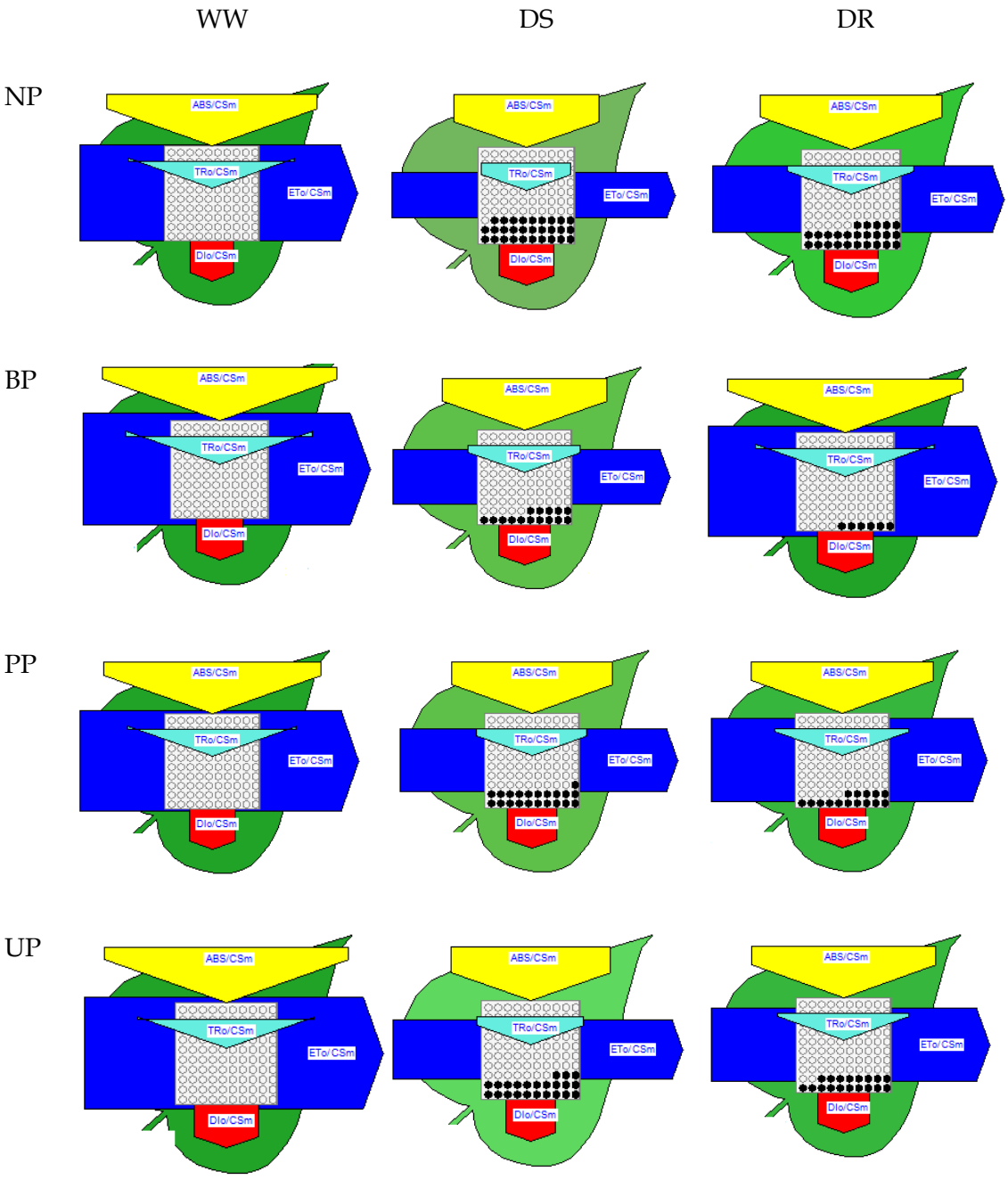

Figure 11 A. Leaf pipeline model showing the proportion of phenomenological energy flux in cowpea variety Anaswara subjected to different priming treatments and exposed to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants).

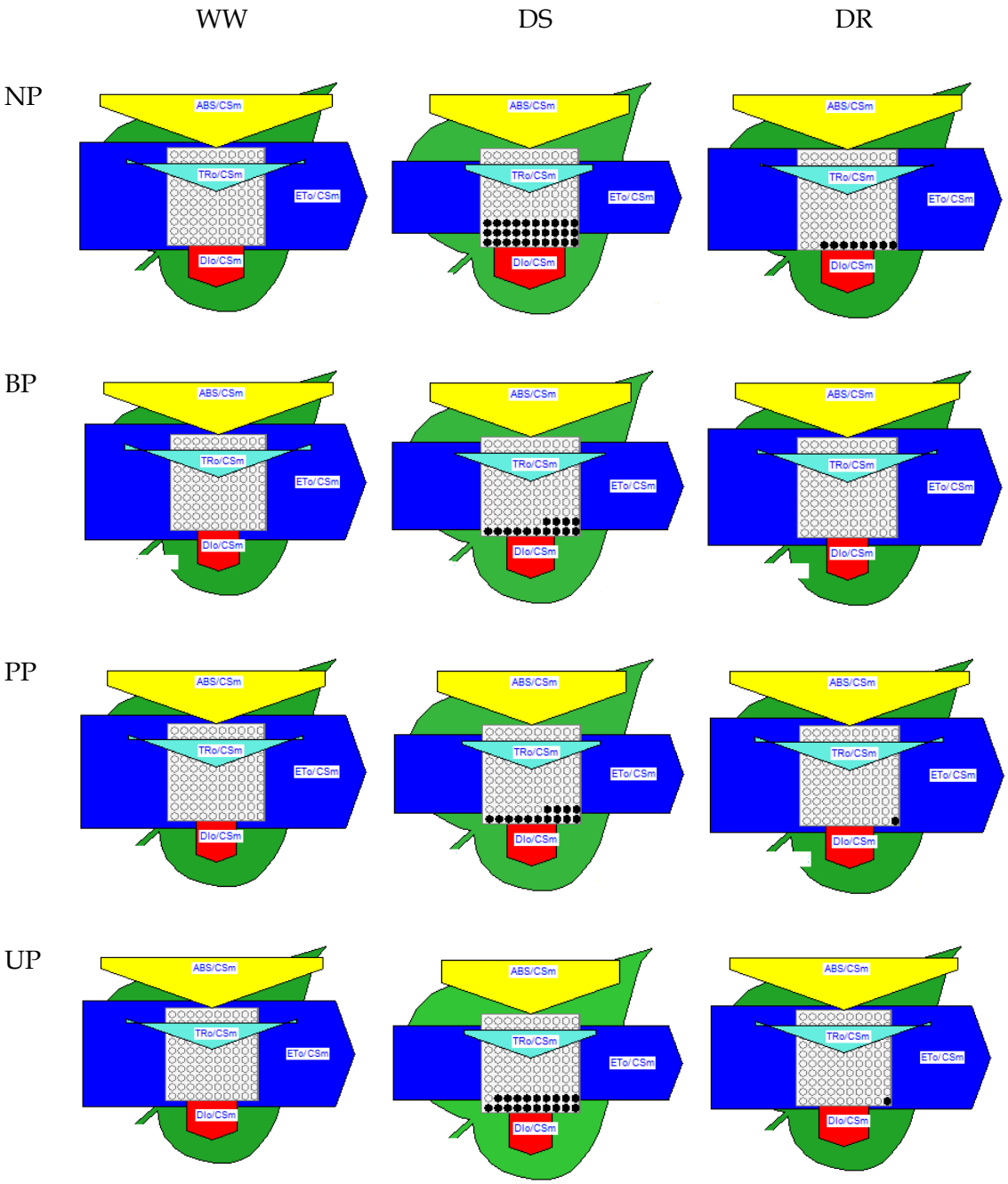

Figure 11 B. Leaf pipeline model showing the proportion of phenomenological energy flux in cowpea variety PGCP 6 subjected to different priming treatments and exposed to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants).

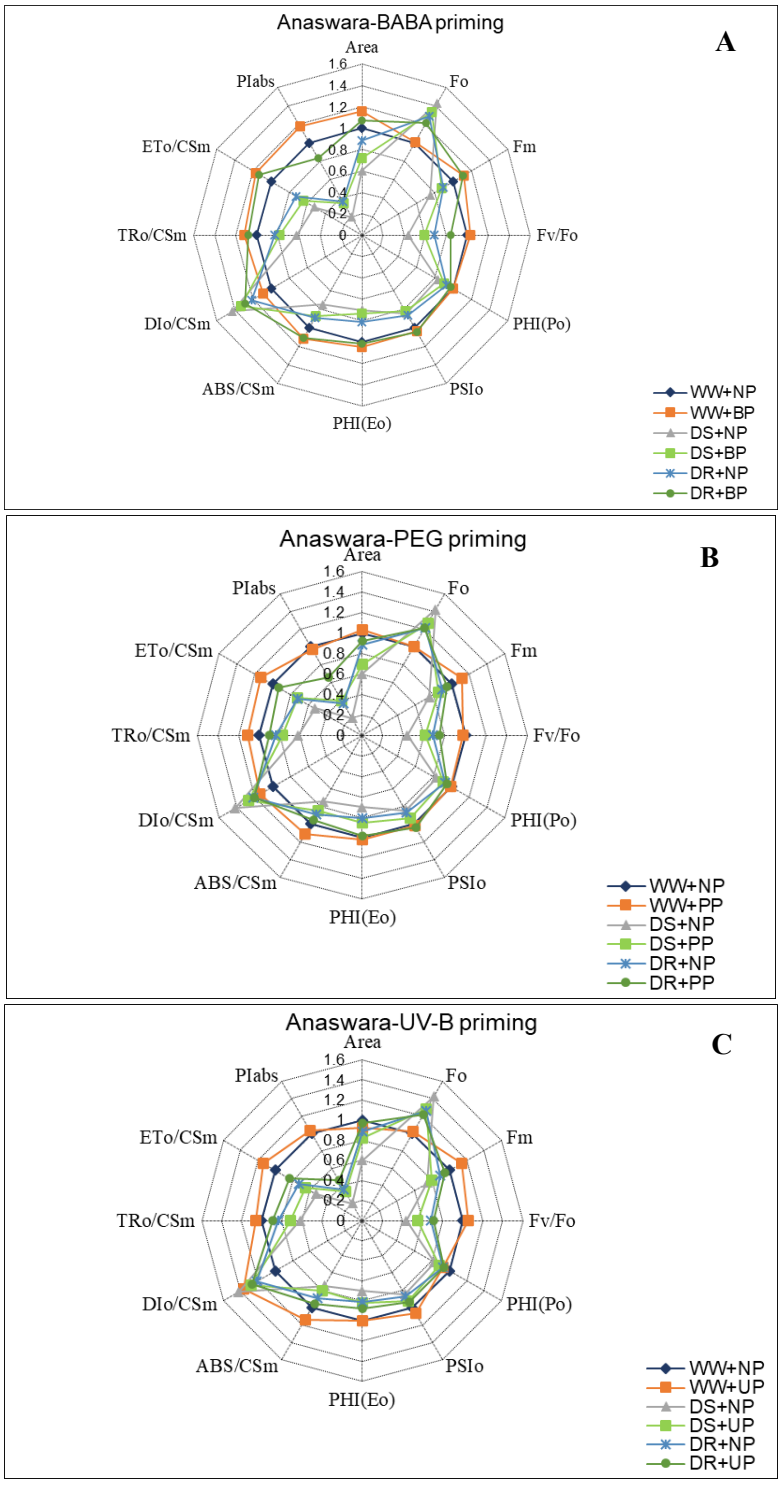

Figure 12 A. Radar plot of cowpea variety Anaswara subjected to drought stress and recovery. (A-BABA priming, B-PEG priming and C-UV-B priming). WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants).

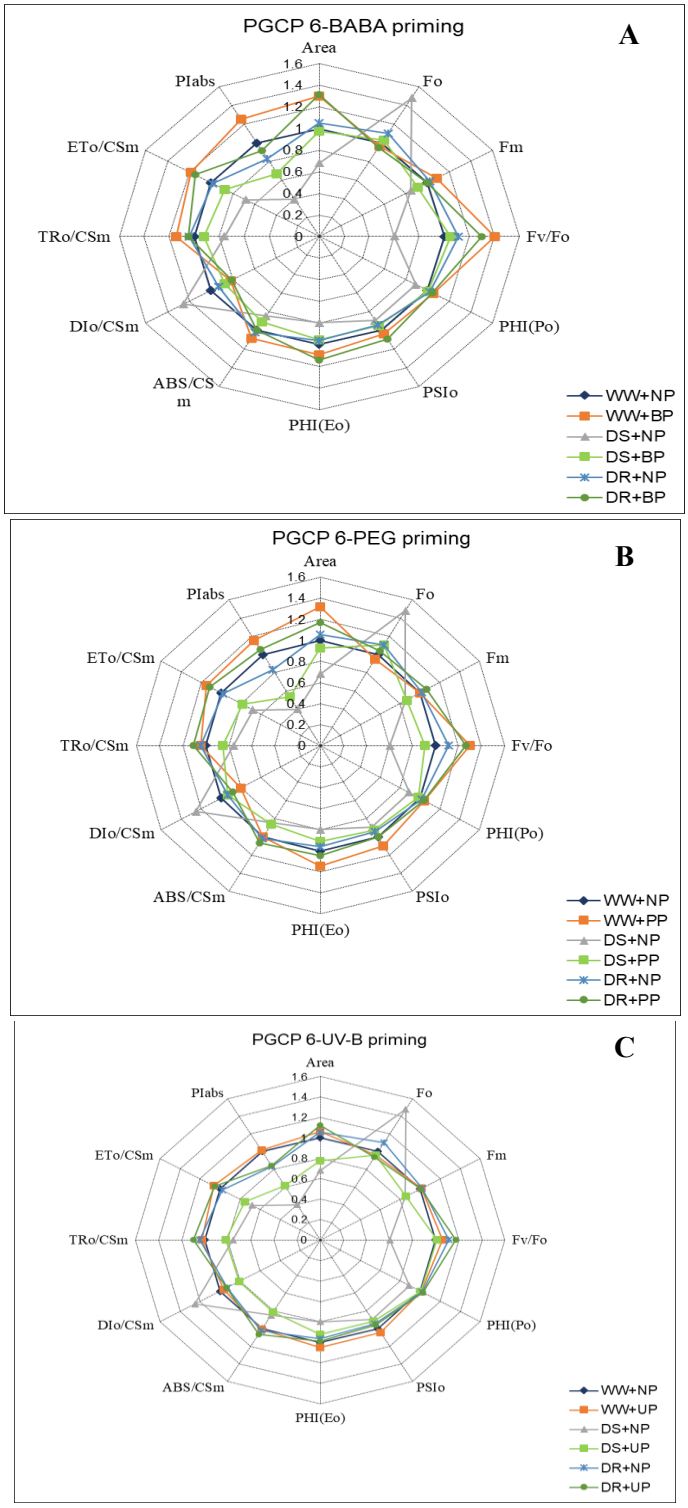

Figure 12 B. Radar plot of cowpea variety PGCP 6 subjected to drought stress and recovery. (A-BABA priming, B-PEG priming and C-UV-B priming). WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants).

Interestingly, there was an elevation in Fv/Fo of primed plants under well-watered conditions, and the decrease in Fv/Fo during drought stress was less pronounced in primed plants compared to non-primed plants. The reduction was 56% and 40% in non-primed Anaswara and PGCP 6 respectively subjected to drought. There was a reduction of 41%, 39% and 44% in variety Anaswara primed with BABA, PEG and UV-B respectively and the reduction was less in the tolerant variety PGCP 6. There was nearly total restoration of Fv/Fo in primed plants upon recovery from stress, with a higher recovery rate observed in the tolerant cowpea (PGCP 6) and it exhibited higher Fv/Fo than the non-primed plant grown under well-watered condition.

Under drought stress, Fo increased by 42% in Anaswara and 48% in PGCP 6, relative to the control plants. Plants treated with BABA, PEG, and UV-B exhibited a lesser increment during drought stress. Nevertheless, a decrease in maximum fluorescence (Fm) was noted during drought stress (25% in Anaswara and 15% in PGCP 6), and notably, this reduction was less pronounced in plants that emerged from primed seeds during drought conditions (13-20% in Anaswara and 9-14% in PGCP 6). Subsequent to the restoration of irrigation, the previously reported values reverted to the levels observed under adequately watered conditions.

Area, the region above the fluorescence induction curve was decreased in drought-exposed plants, with reductions of 40% and 32% seen in Anaswara and PGCP 6, respectively, compared to control plants. In plants primed with BABA, PEG and UV-B, the extent of decrease in area was reduced, with the least reduction observed in PGCP 6 primed with BABA during drought exposure. The reduction in the area was 18-30% in Anaswara emerged from primed seeds compared to the non-primed plants under drought stress. In the case of primed PGCP 6, there was only a

reduction of 2-23% in the area compared to the non-primed plants under drought stress. During recovery from drought stress, non-primed Anaswara exhibited a 11% reduction in area compared to control. While, the reduction in area was fully restored in primed and non-primed PGCP 6. Priming treatments exert a beneficial influence and lead to an increase in the area above the fluorescence induction curve in both the varieties of cowpea with the highest recovery rate observed in the tolerant variety (PGCP 6).

The phenomenological energy flux parameters, such as ABS/CSm, TRo/CSm, and ETo/CSm, were reduced during drought stress treatments. The values were significantly modified throughout drought stress and recovery. It was noted that ABS/CSm, TRo/CSm, and ETo/CSm reduced in primed and non-primed cowpea under drought stress conditions relative to the control. However, a more marked decline was observed in Anaswara exposed to drought (25%, 38% and 47% in ABS/CSm, TRo/CSm, and ETo/CSm respectively). BABA, PEG and UV-B priming significantly enhanced ABS/CSm, TRo/CSm, and ETo/CSm, while reducing energy dissipation (DI) from reaction centres, revealing that the photosynthetic efficiency is superior in primed cowpea than the non-primed cowpea. Upon recovery, the values of all these parameters returned to normal in the primed plants, as same as that of the control plants. Nevertheless, the dissipated energy flux per cross section (DIo/CSm) increased during drought stress in all the treatments and the maximal dissipation energy was observed in the cowpea plants that emerged from non-primed seeds in comparison to plants that emerged from primed seeds (42% in Anaswara and 25% in PGCP 6).

The fluorescence parameters such as PHI(Po), PSIo, and PHI(Eo) showed significant modulations under drought and recovery. PHI(Po), PSIo, and PHI(Eo) considerably reduced in non-primed plants, and the reduction

was 17%, 15% and 30% respectively in Anaswara. The reduction in PHI(Po), PSIo, and PHI(Eo) was 11%, 10% and 20% respectively in non-primed PGCP 6. On the other hand, the decline in these yield characteristics was less pronounced in primed plants exposed to drought conditions. The decline in PHI(Po), PSIo, and PHI(Eo) were reversed upon rewatering, with the most significant recovery noted in primed plants and they reached the same level as that of the control (Figure 11 and 12).

4.3.6.3 Photosystem activities

4.3.6.3.1 PSI activity

The PSI activity increased in the leaves of plants emerged from seeds primed with BABA, PEG and UV-B. The activity of PSI was reduced in response to drought stress in both Anaswara and PGCP 6. There was a reduction of 58% and 42% in non-primed Anaswara and PGCP 6 respectively subjected to drought stress. While in the case of primed plants, the reduction in PSI activity was less, and the reduction was 33%, 39% and 45% in Anaswara primed with BABA, PEG and UV-B respectively. A reduction of 23%, 28% and 35% was recorded in plants of variety PGCP 6 emerged from seeds primed with BABA, PEG and UV-B respectively and subjected to drought stress.

During drought stress recovery, there was a prominent recovery of PSI activities of both the varieties under non-primed and primed conditions. As compared to the control plants, the highest recovery was observed in BABA primed PGCP 6, which recovered to the level same as that of control and the least recovery rate was seen in non-primed Anaswara with a reduction of 42% as compared to the control. While the reduction in PSI activity was 14% in non-primed PGCP 6 during recovery as compared to the respective control (Figure 13).

4.3.6.3.2 PSII activity

There was a slight increase in the PSII activity in primed cowpea plants under well-watered conditions. During drought stress, there was a significant reduction in the activity of PSII. Maximum reduction was noted in non-primed Anaswara exposed to drought i.e., a decrease of 61% as compared to the control. Among the treatments, BABA and PEG priming in PGCP 6 was found to exhibit lesser reduction (37% and 38% respectively) followed by UV-B priming (41%). There was a reduction of 39-47% in PSII activity of primed Anaswara exposed to drought.

PSII activity was restored during rewatering. As compared to the respective drought-stressed plants, there was an increase of 47% in PSII activity of non-primed Anaswara during stress recovery. The highest recovery rate was recorded in primed PGCP 6 plants, reaching the level of control on 4 d. As compared to the non-primed plants under stress, there was an increase of 149%, 139% and 120% in PSII activities of BABA, PEG and UV-B primed PGCP 6 during recovery from stress. In the case of Anaswara, among the three priming treatments, BABA priming contributed maximum towards recovery of PSII activities (118%) than the non-primed drought-stressed Anaswara (Figure 13).

4.3.6.4 Leaf gas exchange parameters

4.3.6.4.1 Net photosynthetic rate (*Pn*)

The leaf gas exchange parameters such as net photosynthetic rate (*Pn*), transpiration rate (*E*), stomatal conductance (*gs*) and intercellular CO_2 concentration (*Ci*) were significantly altered during drought stress and recovery. Exposure of plants to drought stress significantly reduced the net photosynthetic rate in both the cowpea varieties studied. However, the reduction was less in the case of primed plants subjected to drought stress.

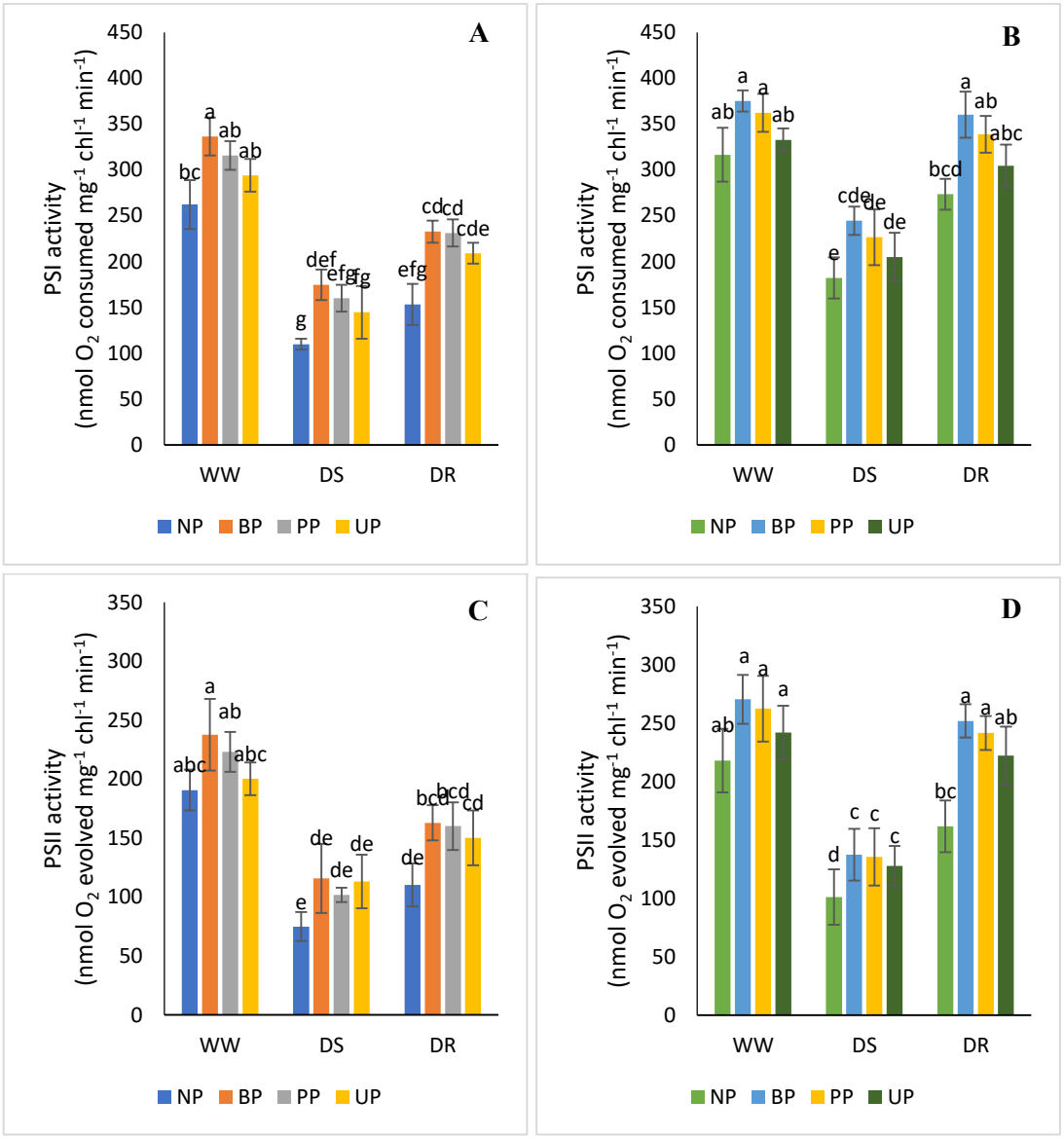

Figure 13. PSI activities (A-Anaswara and B-PGCP 6) and PSII activities **(**C-Anaswara and D-PGCP 6) of cowpea subjected to different priming treatments and exposed to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Minimal reduction was recorded in BABA primed PGCP 6 (28%) followed by PEG priming (31%). While in the case of UV-B primed PGCP 6, there was a 35% reduction in *Pn* as compared to the control. The reduction in *Pn* was 35%, 34% and 41% in BABA, PEG and UV-B primed Anaswara respectively on drought stress exposure.

At the time of recovery from stress, there was a prominent enhancement in *Pn* with a superlative increase in BABA primed PGCP 6, which reached almost same as that of the control. Almost similar recovery pattern was noted in other primed plants also. There was an increase of 101-106% in *Pn* of primed Anaswara during recovery from drought stress. However, there was only 39% and 51% increase in *Pn* of non-primed Anaswara and PGCP 6 respectively upon stress recovery (Figure 14).

4.3.6.4.2 Stomatal conductance (*gs*)

Stomatal conductance (*gs*) was significantly enhanced in plants emerged from primed seeds, with the highest *gs* in BABA primed PGCP 6. In the case of non-primed plants of Anaswara and PGCP 6 exposed to drought stress, *gs* was significantly decreased. The highest reduction was observed in non-primed sensitive variety subjected to drought stress. In plants emerged from primed seeds and subjected to drought, the reduction was less in comparison to the non-primed plants under drought stress. There was a reduction of 54% and 41% in *gs* of non-primed Anaswara and PGCP 6 respectively under drought. Whereas the reduction was least in primed plants and it was 30-32% in PGCP 6 and 30-40% in Anaswara.

There was only negligible reduction in *gs* during drought recovery as compared to the control plants in both the varieties studied. The *gs* value got restored in both sensitive and tolerant varieties and there was an increase of 52 and 60% in *gs* of non-primed PGCP 6 and Anaswara respectively during

recovery. The increase in *gs* was much more (144-155%) in primed sensitive plants at the time of stress recovery as compared to the non-primed plants subjected to drought stress. Primed tolerant variety PGCP 6 reached the same level as that of control during recovery from drought stress (Figure 14).

4.3.6.4.3 Transpiration rate (*E*)

During drought stress, *E* was significantly reduced in Anaswara and PGCP 6, with the maximum reduction recorded in non-primed Anaswara under drought stress as compared to the control and the reduction was 71% and 56% in non-primed Anaswara and PGCP 6 respectively subjected to drought stress. The reduction in *E* was 58-61% in primed Anaswara and 50-52% in primed PGCP 6 subjected to drought stress.

As compared to the respective drought-stressed plants, maximum rate of increase in *E* was observed in Anaswara than PGCP 6 during recovery. The percentage of increase was 168% in non-primed plants and 245%, 261%, 223% respectively in BABA, PEG and UV-B primed Anaswara at the time of stress recovery. In the primed tolerant variety, *E* value recovered to the same level as that of the control (Figure 15).

4.3.6.4.4 Intercellular CO_2 concentration (*Ci*)

Intercellular CO_2 concentration (*Ci*) was significantly decreased in response to drought stress. The maximum reduction in *Ci* was reported in non-primed Anaswara and PGCP 6 (42%). The seedlings emerged from the primed seeds of Anaswara and subjected to drought stress exhibited a lesser reduction of 32-34% in *Ci*, whereas the reduction was only 28-29% in primed PGCP 6 exposed to drought stress.

The rate of increase in *Ci* was higher in primed plants during recovery from drought, which recovered to a value same as that of control.

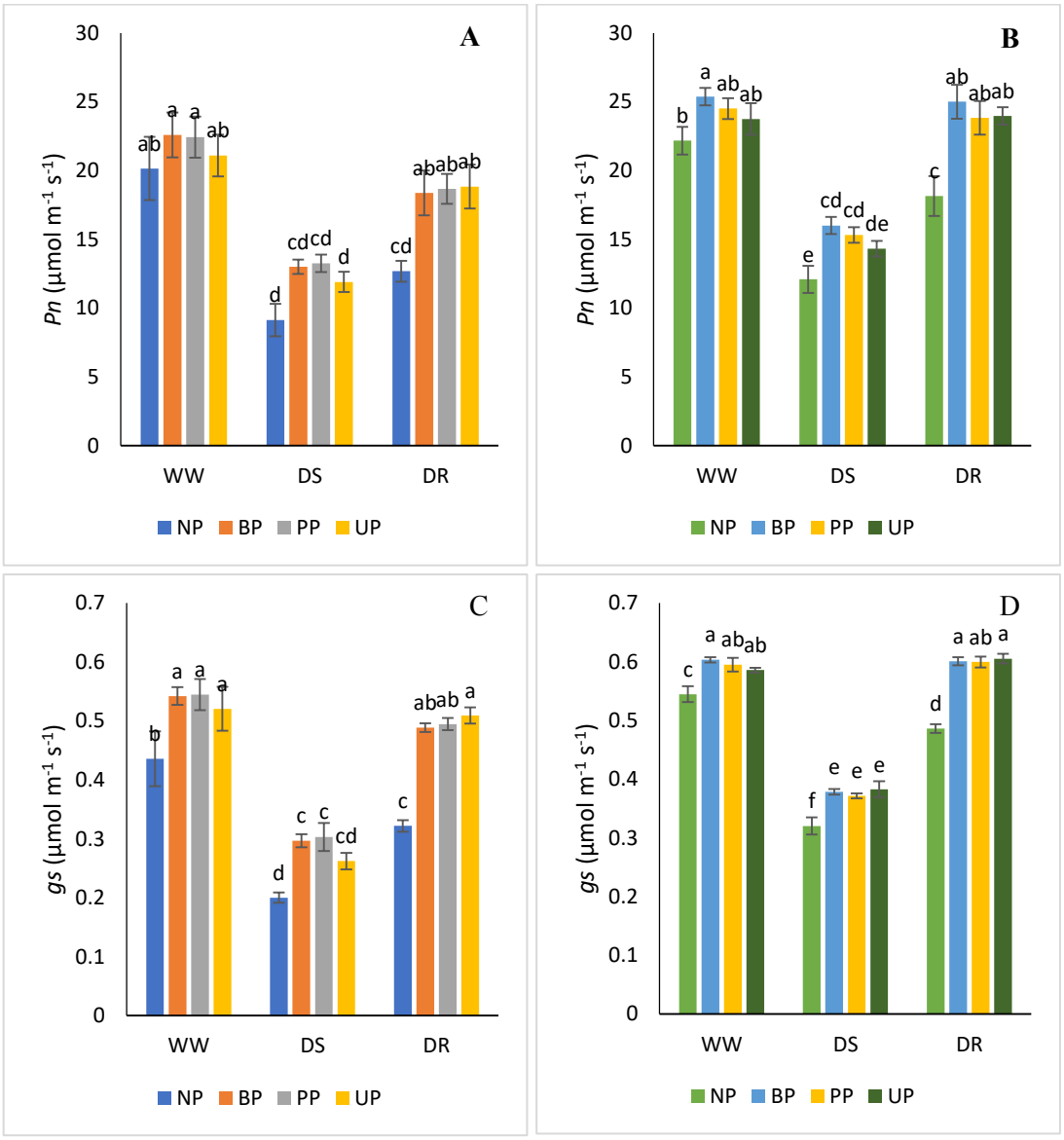

Figure 14. Net photosynthetic rate (*Pn*) (A-Anaswara and B-PGCP 6) and stomatal conductance (*gs*) (C-Anaswara and D-PGCP 6) of cowpea subjected to different priming treatments and exposed to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

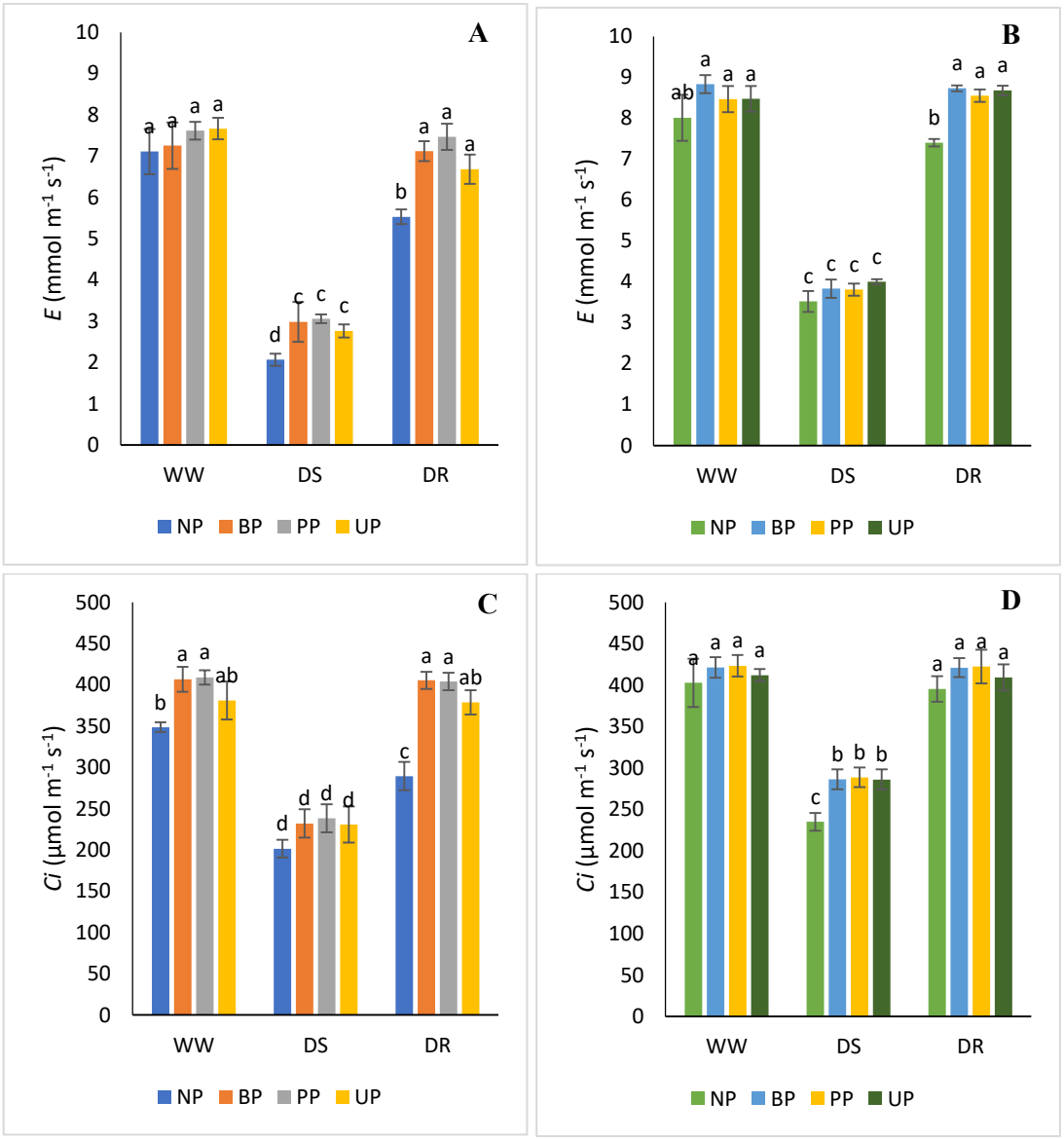

Figure 15. Transpiration rate (*E*) (A-Anaswara and B-PGCP 6) and intercellular CO_2 concentration (*Ci*) (C-Anaswara and D-PGCP 6) of cowpea subjected to different priming treatments and exposed to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

Whereas, non-primed PGCP 6 and Anaswara exhibited only an increase of 68% and 44% respectively during stress recovery (Figure 15).

4.3.7 Leaf micromorphology

4.3.7.1 Scanning electron micrograph of stomata

In control and primed leaves, fully opened stomata were observed in both the cowpea varieties maintained at well-watered conditions. Drought stress resulted in the closure of stomata and a more or less complete closure was noted in non-primed Anaswara subjected to drought treatment. But, in primed Anaswara only partial closing of stomata was observed during drought stress. Priming reduced the reduction in the stomatal diameter of tolerant variety PGCP 6. When compared to the sensitive variety, the tolerant one maintained open stomata on exposure to drought so as to permit proper gaseous exchange (Figure 16).

4.3.7.2 Epicuticular wax

Epicuticular wax content was found to be increased during drought stress and a further increment was noted in UV-B primed plants subjected to drought. The enhancement in epicuticular wax content was 73% in Anaswara and 80% in PGCP 6 under stress. The highest accumulation of epicuticular wax content was found in UV-B primed PGCP 6 (90%) followed by UV-B primed Anaswara (84%) exposed to drought stress. There was no significant reduction in the epicuticular wax content during stress recovery (Figure 17).

FT-IR spectra of epicuticular wax in the leaves of cowpea exhibited important peaks at 1027, 1093, 1600, 2350, 2850, 2900, and 3400 cm^{-1}. Peaks at 1027 cm^{-1} indicates C–O stretching of alcohol, peak at 1093 shows aromatic ring stretching. C=O stretching of carbonyl group is visible in 1696 cm^{-1}. Peaks from 2819 to 3034 region indicates signature of cuticular lipids. CH_2

symmetrical (2826-2871 cm^{-1}) and asymmetrical (2936-2894 cm^{-1}) stretching were also noted in the spectrum. Symmetrical and asymmetrical stretching of methylene groups was strong in UV-B primed and drought-stressed leaves in contrast to control leaves. Peaks in the 3400 cm^{-1} represents O–H stretching vibration of H-bonded hydroxyl groups in polymeric association. Slight peaks at 700 cm^{-1} denotes out of plane ring bending of aromatic ring (Figure 18). Scanning electron microscopic images of the adaxial leaf surfaces also confirms the deposition of wax in response to UV-B irradiation and drought stress. Highest deposition was observed in UV-B primed PGCP subjected to drought stress (Figure 19).

4.3.8 Free radical production

4.3.8.1 Superoxide ($O_2^{\bullet -}$)

Drought stress significantly increased the accumulation of $O_2^{\bullet -}$ in both the sensitive (Anaswara) and tolerant (PGCP 6) cowpea varieties studied and the highest accumulations of $O_2^{\bullet -}$ was noted in the case of non-primed sensitive variety. The increase in $O_2^{\bullet -}$ was 135% and 95% respectively in Anaswara and PGCP 6 subjected to drought. All the three priming treatments reduced the production of $O_2^{\bullet -}$ under drought stress with highest reduction noted in UV-B primed Anaswara and UV-B primed PGCP 6. The increase was only 61%, 79% and 45% respectively in the case of Anaswara emerged from BABA, PEG and UV-B primed seeds and subjected to drought. Whereas in PGCP 6, during drought stress there was only an enhancement of 44%, 38% and 31% in $O_2^{\bullet -}$ content in plants primed with BABA, PEG and UV-B as compared to the control.

Upon recovery through rewatering, the $O_2^{\bullet -}$ content reduced sharply in primed and non-primed cowpea. As compared to the drought-stressed plants, there was a reduction of 35% observed in both the cowpea varieties

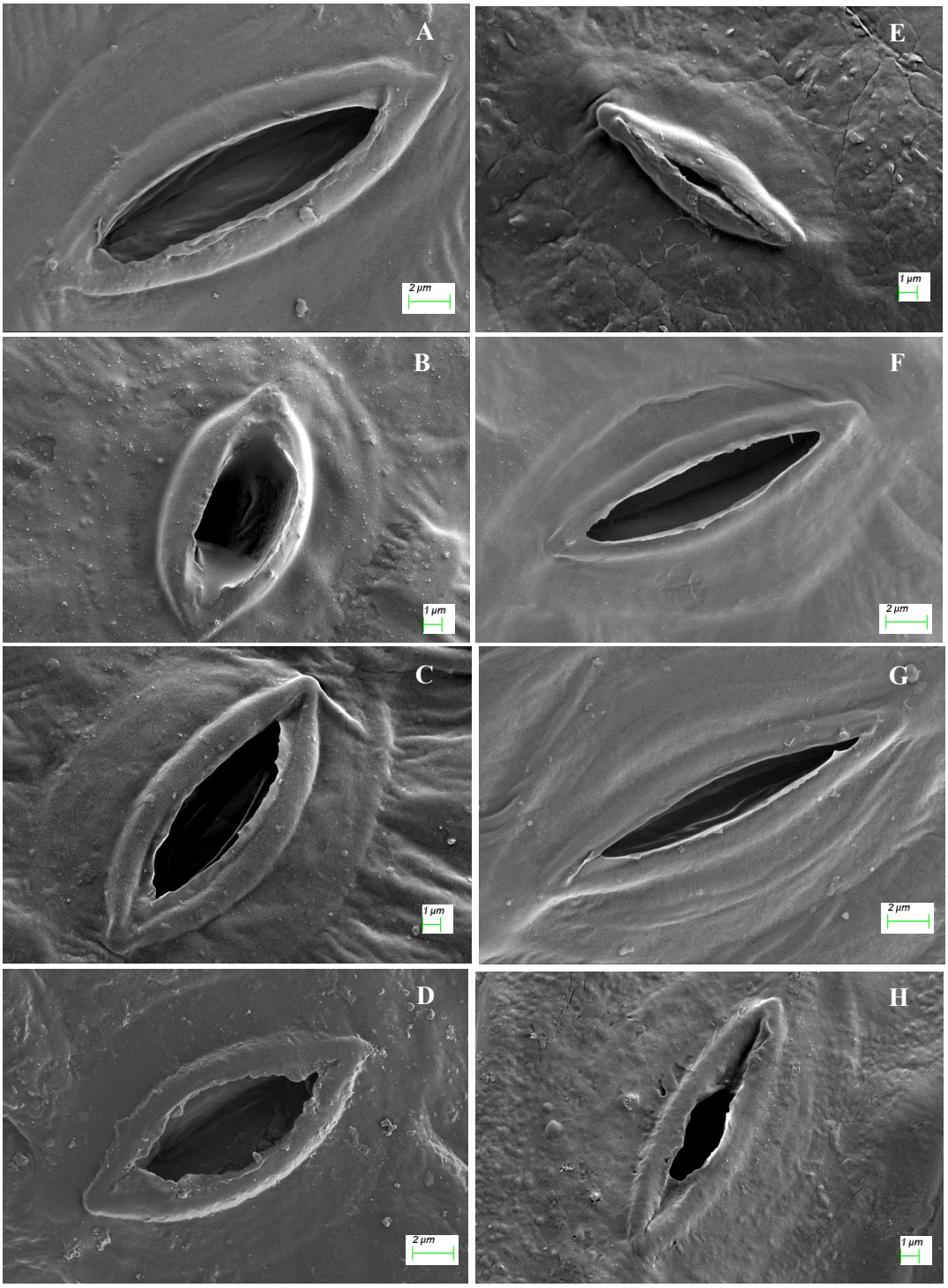

Figure 16 A. Scanning electron micrograph of stomata of trifoliate leaves of cowpea variety Anaswara.

A (Non-primed cowpea under well-watered condition), B (BABA primed cowpea under well-watered condition), C (PEG primed cowpea under well-watered condition), D (UV-B primed cowpea under well-watered condition), E (Non-primed cowpea under drought stress), F (BABA primed cowpea under drought stress), G

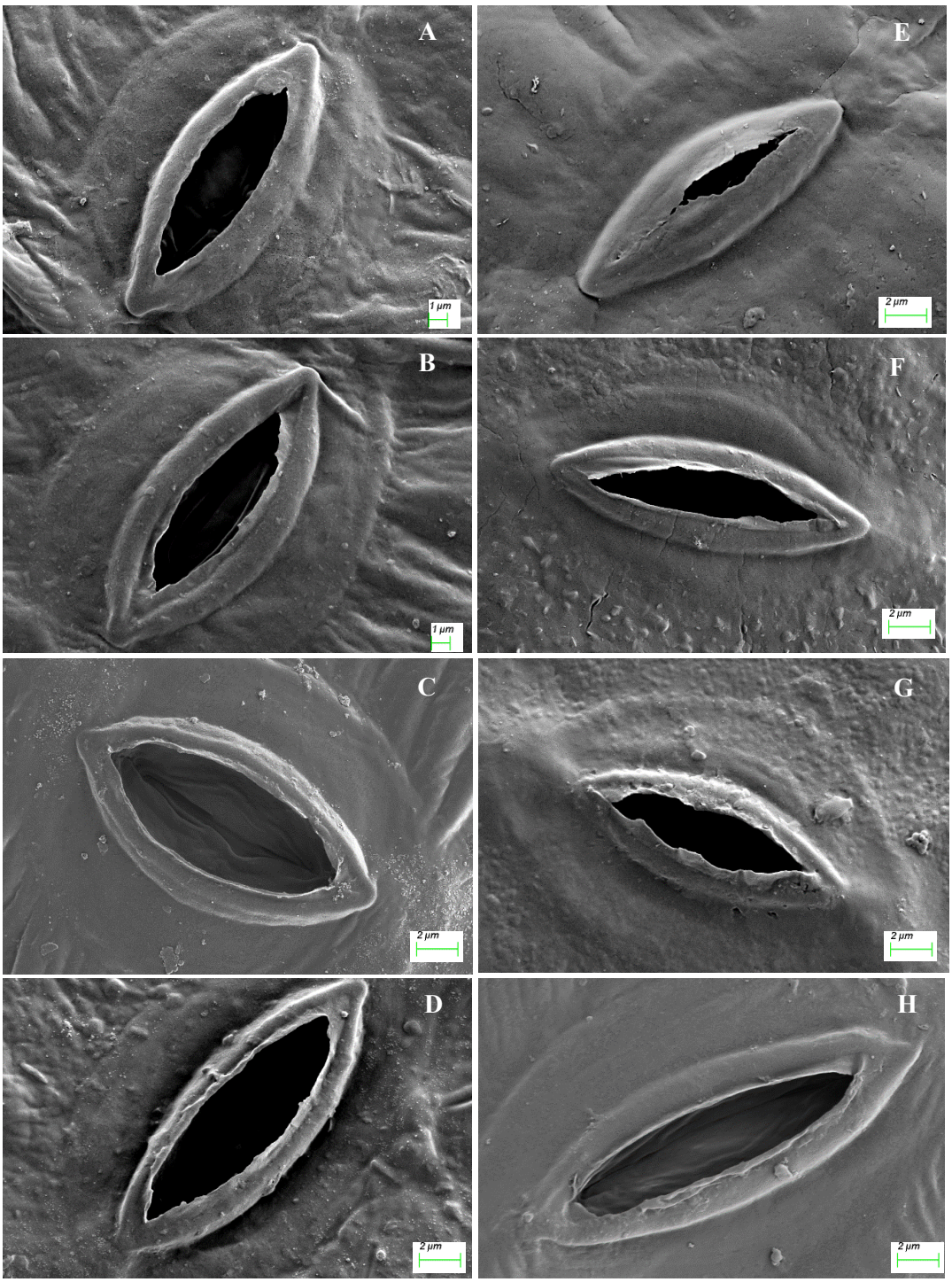

Figure 16 B. Scanning electron micrograph of stomata of trifoliate leaves of cowpea variety PGCP 6.

A (Non-primed cowpea under well-watered condition), B (BABA primed cowpea under well-watered condition), C (PEG primed cowpea under well-watered condition), D (UV-B primed cowpea under well-watered condition), E (Non-primed cowpea under drought stress), F (BABA primed cowpea under drought stress), G (PEG primed cowpea under drought stress) H (UV-B primed cowpea under drought stress).

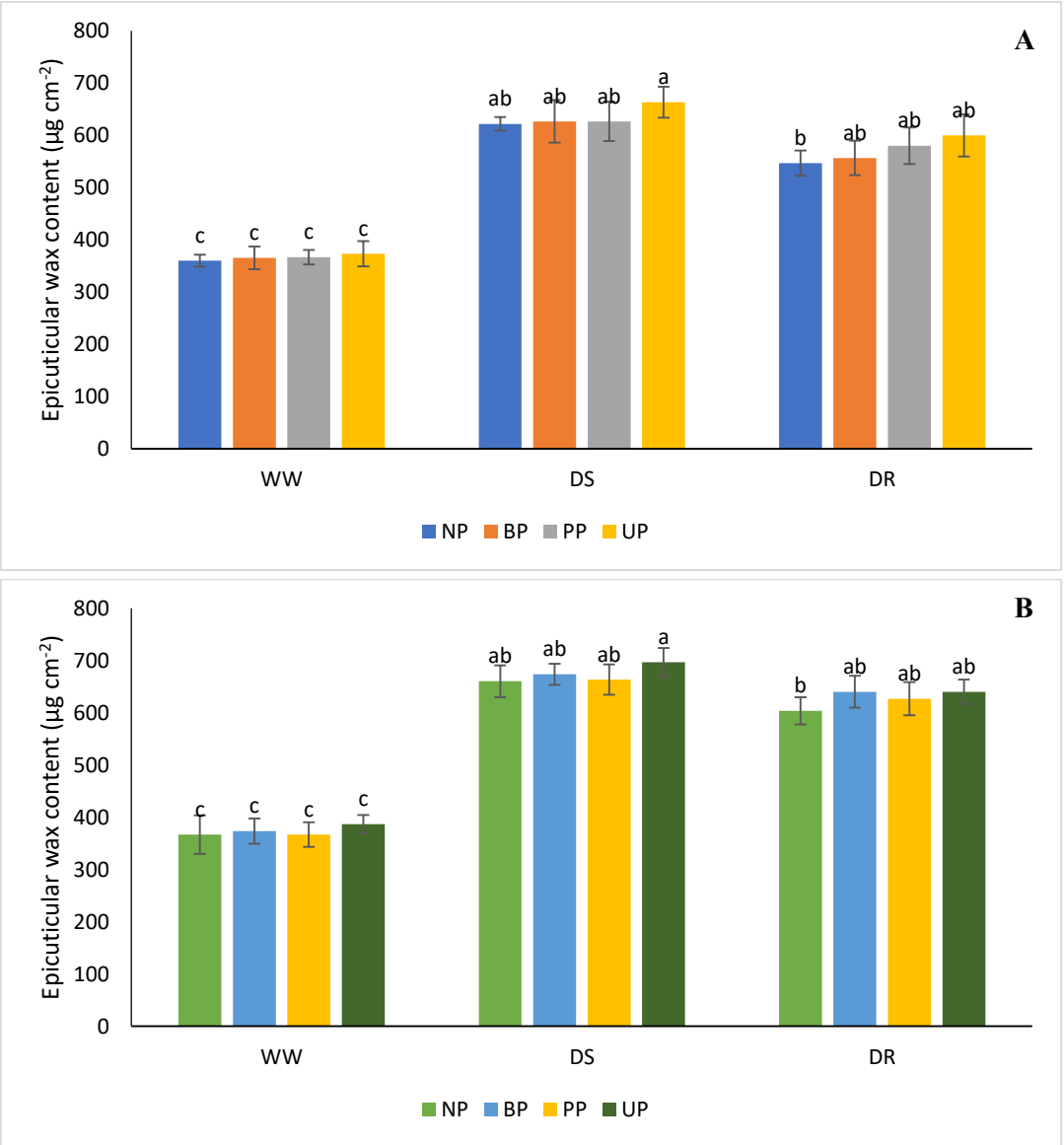

Figure 17. Epicuticular wax content in the trifoliate leaves of cowpea (A-Anaswara and B-PGCP 6) under well-watered, drought-stressed and recovery conditions. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

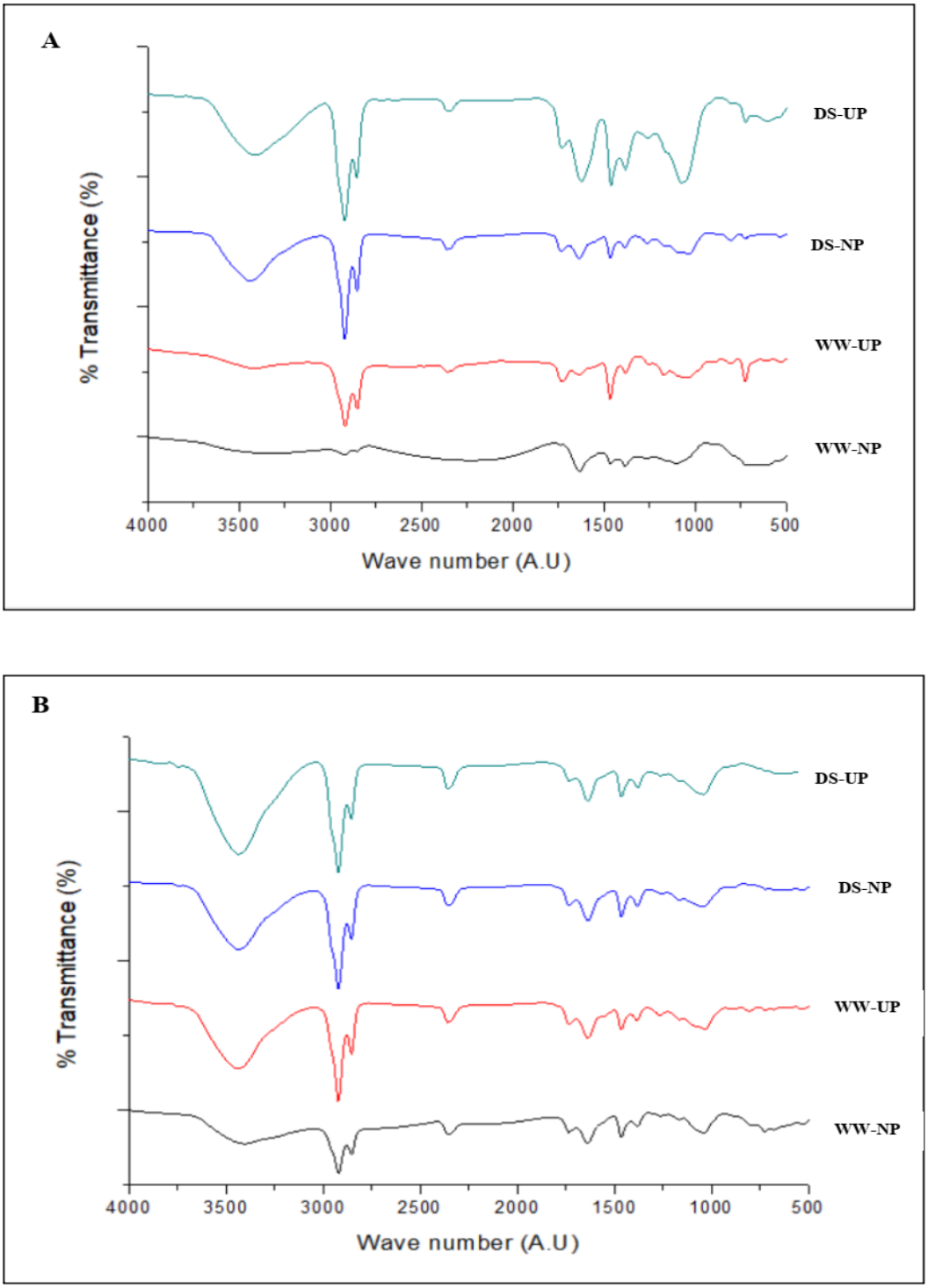

Figure 18. FT-IR spectra of epicuticular wax in the trifoliate leaves of cowpea (A-Anaswara and B-PGCP 6) grown under well-watered and drought-stressed conditions. WW-NP (Non-primed plants maintained at well-watered conditions), WW-UP (UV-B primed plants under well-watered conditions), DS-NP (Non-primed plants under drought stress), DS-UP (UV-B primed plants under drought stress).

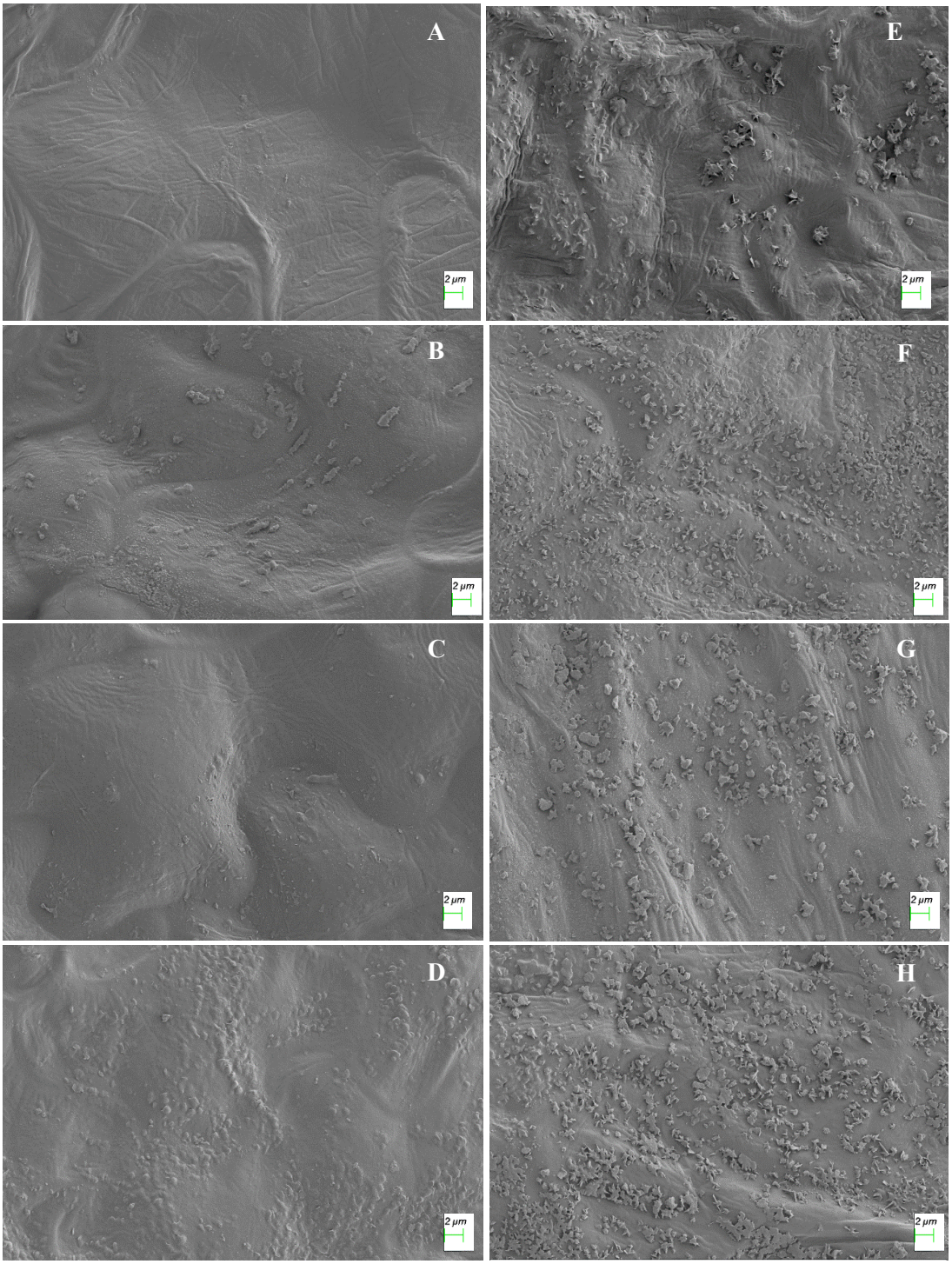

Figure 19. SEM images of adaxial surface of trifoliate leaves of cowpea as influenced by drought stress and UV-B priming. A (Non-primed Anaswara maintained at well-watered conditions), B (UV-B primed Anaswara under well-watered conditions), C (Non-primed PGCP 6 under well-watered condition), D (UV-B primed PGCP 6 under well-watered conditions), E (Non-primed Anaswara under drought stress), F (UV-B primed Anaswara under drought stress), G (Non-primed PGCP 6 under drought stress); H (UV-B primed PGCP 6 under drought stress).

during recovery from drought stress. In all the three priming treatments the reduction in $O_2^{\bullet -}$ was in the range of 60-62% in Anaswara and 52-61% in PGCP 6 during recovery as compared to the non-primed plants subjected to drought stress (Figure 20).

4.3.8.2 Hydrogen peroxide (H_2O_2)

Drought-stressed plants exhibited a higher accumulation of H_2O_2 content, with the highest increase of 173% in non-primed Anaswara as compared to the control. During drought stress, the content of H_2O_2 was increased upto 136% in BABA and PEG primed cowpea variety Anaswara and the increase was 144% in the case of UV-B primed Anaswara. While, in the case of PGCP 6, an increase of 91%, 92% and 105% was observed in BABA, PEG and UV-B priming respectively as compared to the control.

At the time of recovery there was a significant reduction in the content of H_2O_2 in both the non-primed and primed plants than the respective drought-stressed plants. As compared to the sensitive variety, there was a greater reduction in H_2O_2 content of tolerant variety PGCP 6, and the reduction was 52% as compared to the plants under drought stress. But in the case of primed plants, the reduction was even higher. There was a lowering of 71% in BABA and PEG primed and 68% in UV-B primed PGCP 6. When compared to the drought-stressed plant there was a reduction of 38% in H_2O_2 content of non-primed Anaswara during recovery and the reduction was 63-71% in plants emerged from BABA, PEG and UV-B primed seeds (Figure 20).

4.3.8.3 *In situ* localization of $O_2^{\bullet -}$

The leaves of primed and non-primed cowpea grown under well-watered conditions showed identical patterns and colours of staining. However, drought stress treatment resulted in more darkly stained patches,

indicating $O_2^{\bullet -}$ production. The non-primed sensitive variety had more dark blue formazan dots. The appearance of blue colour was less in primed plants, particularly in UV-B primed PGCP 6. During drought recovery, the blue colour decreased, with primed plants showing the greatest reduction (Figure 21).

4.3.9 Stress intensity assessment

4.3.9.1 Membrane stability index (MSI)

The stability of the biomembranes were found to be negatively affected under drought stress in both the cowpea varieties studied. The reduction was more prominent in sensitive variety than the tolerant, and there was a reduction of 41% in non-primed Anaswara and 33% in non-primed PGCP 6. All the priming treatments lowered the reduction in membrane stability index during drought stress. There was only a reduction of 21-25% and 17-19% in primed Anaswara and PGCP 6 respectively.

After stress recovery, the membrane stability index increased and the enhancement was by 26% and 33% in non-primed Anaswara and PGCP 6 respectively. Priming complement, the recovery from stress by reducing membrane damage and as a result there was only lesser reduction in membrane stability of primed plants and it was only 11-14% in Anaswara and 0.2-3% in PGCP 6 as compared to the control. The highest recovery in membrane stability index was observed in primed cowpea, which exhibited an increase of 43-50% in MSI as compared to the non-primed drought-stressed cowpea (Figure 22).

4.3.9.2 Electrolyte leakage (EL)

Drought stress leads to an increase in electrolyte leakage and it was highest in sensitive variety as compared to the tolerant one. The most pronounced increase (87%) was observed in non-primed Anaswara followed

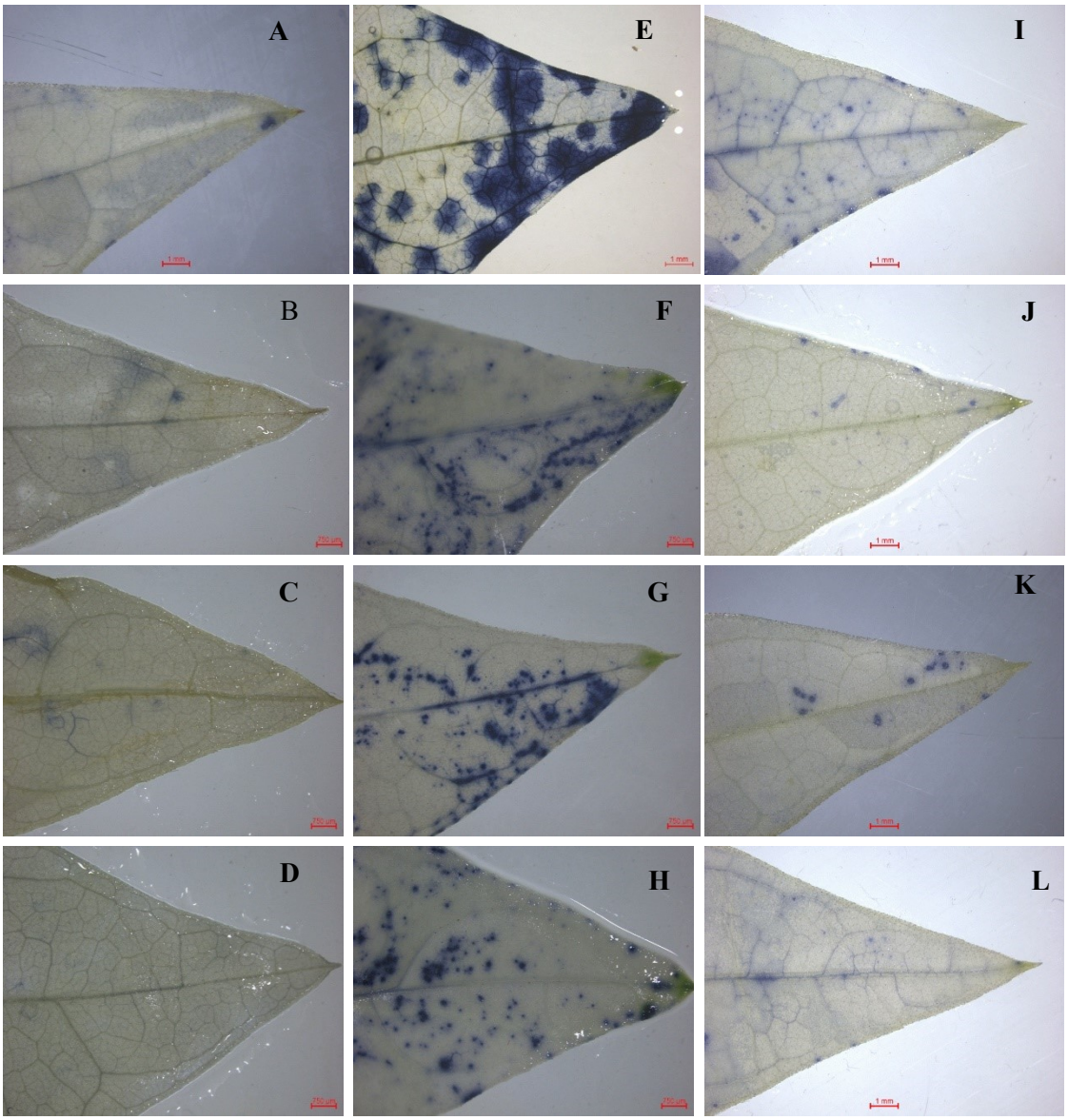

Figure 21 A. NBT staining indicating superoxide accumulation in the leaves of cowpea variety Anaswara.

A (Non-primed cowpea under well-watered condition), B (BABA primed cowpea under well-watered condition), C (PEG primed cowpea under well-watered condition), D (UV-B primed cowpea under well-watered condition), E (Non-primed cowpea under drought stress), F (BABA primed cowpea under drought stress), G (PEG primed cowpea under drought stress) H (UV-B primed cowpea under drought stress), I (Non-primed cowpea during recovery), J (BABA primed cowpea during recovery), K (PEG primed cowpea during recovery), L (UV-B primed cowpea during recovery).

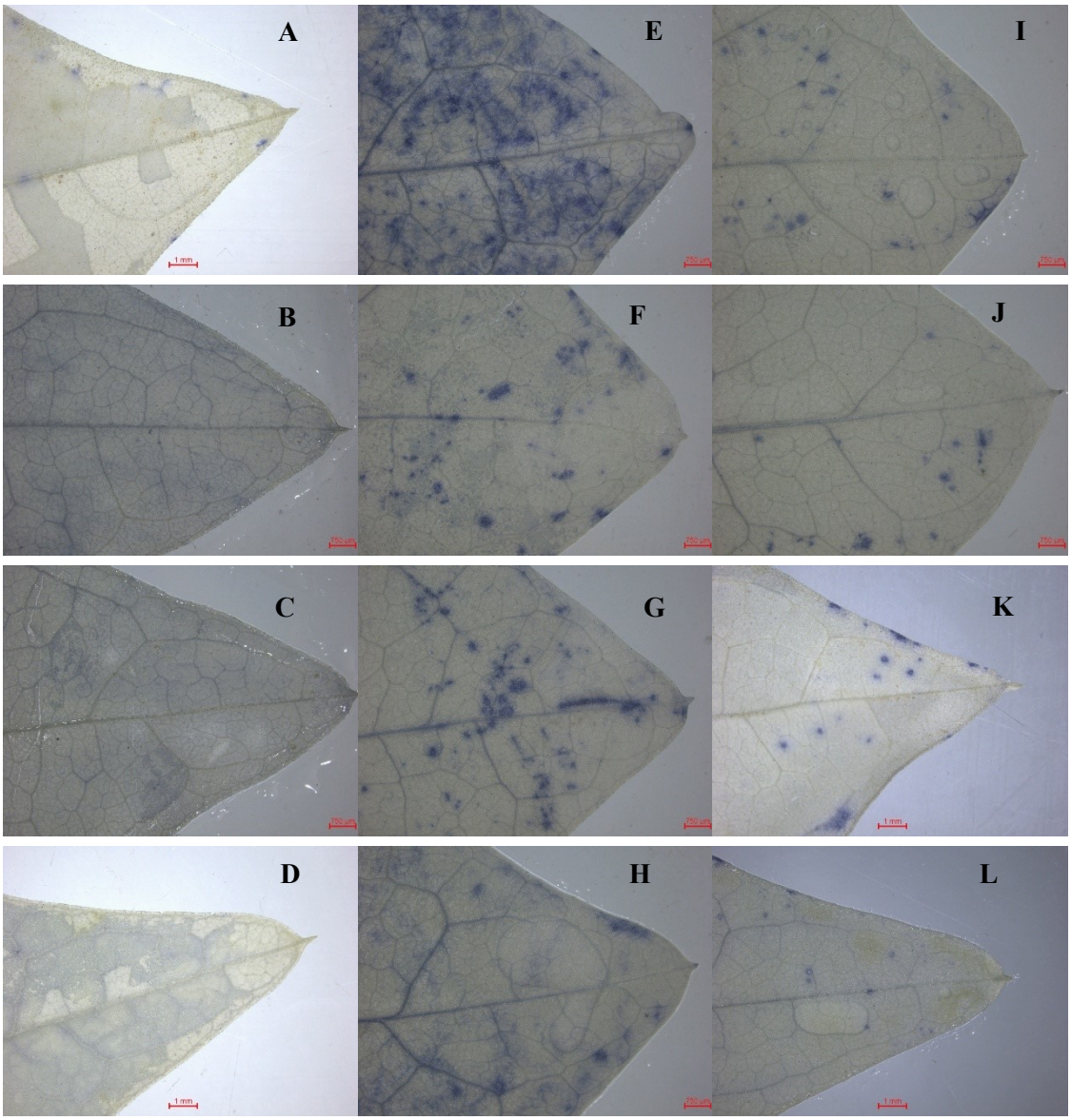

Figure 21 B. NBT staining indicating superoxide accumulation in the leaves of cowpea variety PGCP 6.

A (Non-primed cowpea under well-watered condition), B (BABA primed cowpea under well-watered condition), C (PEG primed cowpea under well-watered condition), D (UV-B primed cowpea under well-watered condition), E (Non-primed cowpea under drought stress), F (BABA primed cowpea under drought stress), G (PEG primed cowpea under drought stress) H (UV-B primed cowpea under drought stress), I (Non-primed cowpea during recovery), J (BABA primed cowpea during recovery), K (PEG primed cowpea during recovery), L (UV-B primed cowpea during recovery).

by non-primed PGCP 6 (70%) subjected to drought stress. The increment was only 55%, 59% and 67% respectively in Anaswara subjected to BABA, PEG and UV-B priming and subjected to drought. There was less increase in EL% in primed PGCP 6, with the least increment in BABA (41%) followed by PEG (43%) and UV-B primed (48%) cowpea.

During recovery, there was a notable decrease in electrolyte leakage levels in both non-primed and primed plants compared to the drought-stressed plants. Anaswara and PGCP 6 exhibits a more significant reduction in electrolyte leakage percentage, with a decrease of 27-29% relative to plants subjected to drought stress. However, for primed plants, the reduction was even more pronounced, and it was respectively 40%, 44%, and 39% in BABA, PEG, and UV-B primed Anaswara. In comparison to the non-primed drought-stressed plant, there was a reduction of 41% in PGCP 6 plants that emerged from BABA and PEG primed seeds followed by a reduction of 36% in plants that emerged from UV-B primed seeds (Figure 22).

4.3.9.3 Lipid peroxidation (MDA content)

There was a significant enhancement in the malondialdehyde content of both the cowpea varieties during drought stress. There was an increase of 127% in non-primed Anaswara and 101% increase in non-primed PGCP 6 subjected to drought stress. In comparison to the non-primed plants, there was only lesser increase in the MDA content of primed plants subjected to drought stress. The increment was only 61%, 75% and 83% in PGCP 6 and 73%, 86% and 103% in Anaswara primed with BABA, PEG, and UV-B respectively and exposed to drought stress.

As compared to the drought-stressed plants, the content of MDA significantly reduced during recovery from drought stress. The highest

reduction was observed in BABA primed PGCP 6 (42%) followed by PEG primed PGCP 6 (40%) (Figure 22).

4.3.10 Free radical scavenging system

4.3.10.1 Enzymatic antioxidants

4.3.10.1.1 Superoxide dismutase (SOD)

The activity of SOD was highly varied during drought stress and recovery. There was an enhancement of 75% and 85% respectively in the activity of SOD in the leaves of non-primed Anaswara and PGCP 6 subjected to drought. Further enhancement of 129-170% and 162-179% was noted in primed Anaswara and PGCP 6 respectively exposed to drought. The maximum augmentation of 179% was observed in UV-B primed PGCP 6 upon drought stress exposure.

At the time of recovery, the activity of SOD was reduced in both the varieties studied. In both the primed and non-primed plants of PGCP 6, SOD activity reached a level near to that of the control, with the highest recovery noted in primed plants. Similarly, in the variety Anaswara also the SOD activity of primed plants reached almost to the same level as that of the control (Figure 23).

4.3.10.1.2 Catalase (CAT)

The [redacted]e leaves of cowpea plants exposed to drought stress increased and the increase was much higher in the primed plants subjected to drought. Highest activity of CAT was observed in the BABA primed PGCP 6 and there was an increase of 305% in the CAT activity. Primed Anaswara plants subjected to drought stress also had high CAT activity and the increase was 245-283%. While, non-primed PGCP 6

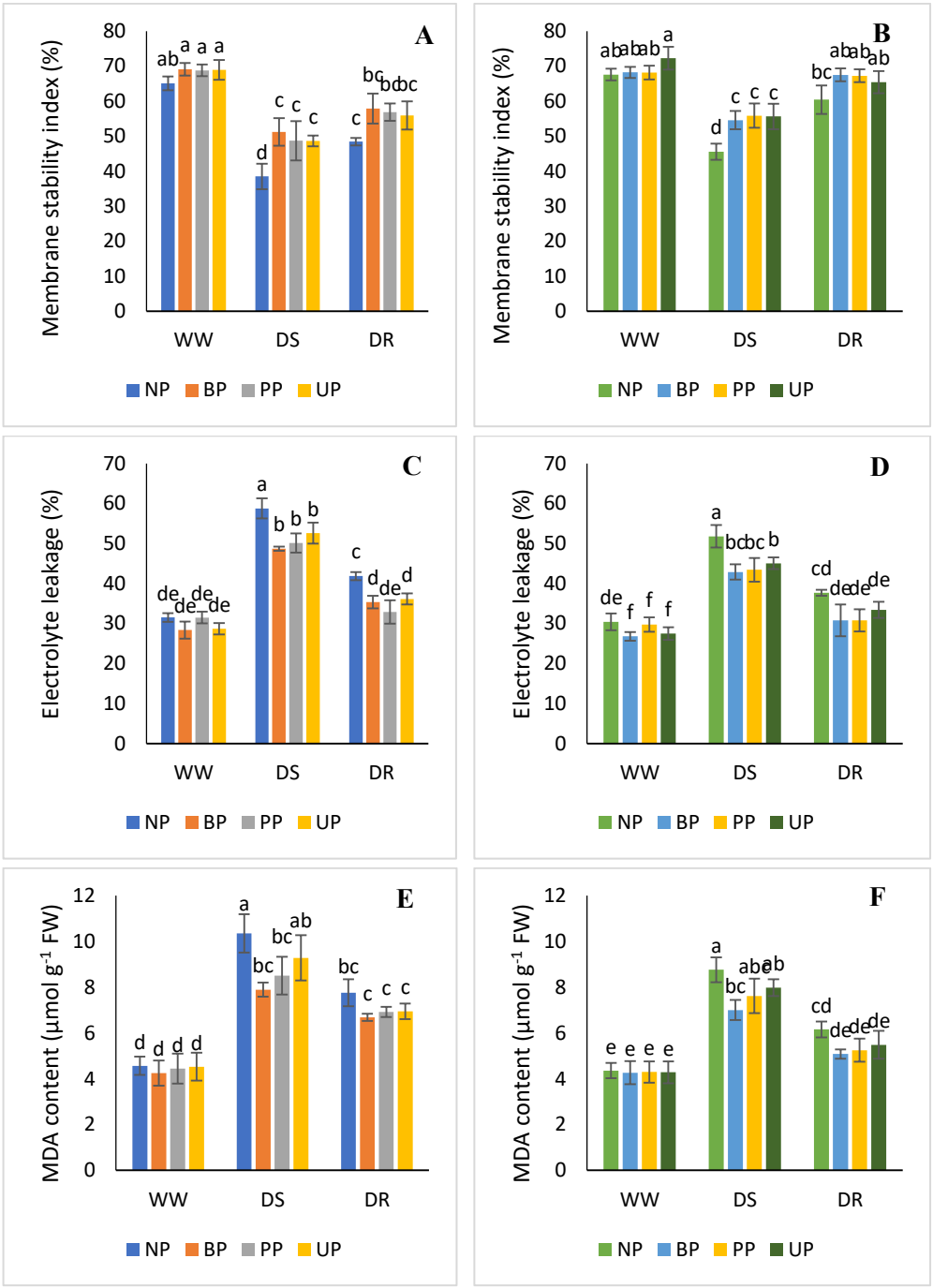

Figure 22. Membrane stability index (A-Anaswara and B-PGCP 6), Electrolyte leakage (C-Anaswara and D-PGCP 6) and MDA content (E-Anaswara and F-PGCP 6) in the trifoliate leaves of cowpea as influenced by different priming and subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

subjected to drought stress had an increase of 254% and in the case of non-primed Anaswara it was 214% during drought stress exposure.

The activity of CAT decreased during stress recovery from drought with a reduction of 26% in Anaswara and 64% in PGCP 6. During stress recovery, the CAT activity in plants subjected to priming treatments in the variety PGCP 6 reached a level same as that of the control (Figure 23).

4.3.10.1.3 Ascorbate peroxidase (APX)

The APX activity was significantly modulated during drought stress and recovery in cowpea plants. Exposure of cowpea to drought prominently augmented the activity of APX, yet the effects varied among the varieties and treatments. There was an increase of 232% and 266% in non-primed Anaswara and PGCP 6 respectively under drought stress as compared to the respective control plants. In leaves, the maximum APX activity was found in the drought-stressed PEG primed plants of PGCP 6 (367%) followed by BABA primed PGCP 6 (361%). While, in the variety Anaswara, maximum APX activity was recorded in the BABA and PEG primed plants subjected to drought stress (354% and 352% respectively) followed by UV-B primed plants (302%).

Upon stress recovery there was a significant reduction in the activity of APX, with the highest decrease observed in PGCP 6. Non-primed PGCP 6 exhibited a reduction of 63% in APX activity at the time of rewatering, while non-primed Anaswara had a reduction of 50% as compared to the plants subjected to drought stress. The level of APX activity reached the level of control in both the non-primed and primed plants of PGCP 6. As compared to the respective control plants under stress, primed PGCP 6 exhibited a reduction of 76-77% in APX activity and primed Anaswara had a reduction of 67-71% upon recovery (Figure 24).

4.3.10.1.4 Guaiacol peroxidase (GPOX)

There was an increase in the GPOX activity in the leaves of primed plants as compared to the non-primed plants of both the cowpea varieties studied. During drought stress exposure there was higher increment in the activity of GPOX. As compared to the variety Anaswara, variety PGCP 6 exhibited higher GPOX activity during drought stress exposure. There was an increase of 146% and 175% in non-primed Anaswara and PGCP 6 respectively during drought stress. There was a further enhancement in the GPOX activity of the primed plants under stress and it was 230-256% in primed Anaswara and 245-260% in primed PGCP 6.

The GPOX activity reduced upon recovery from drought stress with the maximum recovery observed in primed plants of PGCP 6, which reached equal to that of the control. As compared to the drought-stressed plants, there was a reduction of 41% and 22% in the GPOX activity of non-primed PGCP 6 and Anaswara respectively during drought recovery (Figure 24).

4.3.10.2 Non-enzymatic antioxidants

4.3.10.2.1 Ascorbate content

An enhancement in ascorbate levels due to drought stress was observed in both the sensitive and tolerant cowpea varieties studied. Plants raised from seeds primed with BABA and PEG and then subjected to drought stress exhibited elevated levels of ascorbate than the non-primed plants under stress. BABA priming increased ascorbate content by 240% in the sensitive variety and by 283% in tolerant variety under drought stress. Likewise, PEG priming and UV-B priming enhanced ascorbate accumulation, with an increases of 240% in ascorbate content of PEG primed Anaswara followed by 181% increase in UV-B primed Anaswara. While the

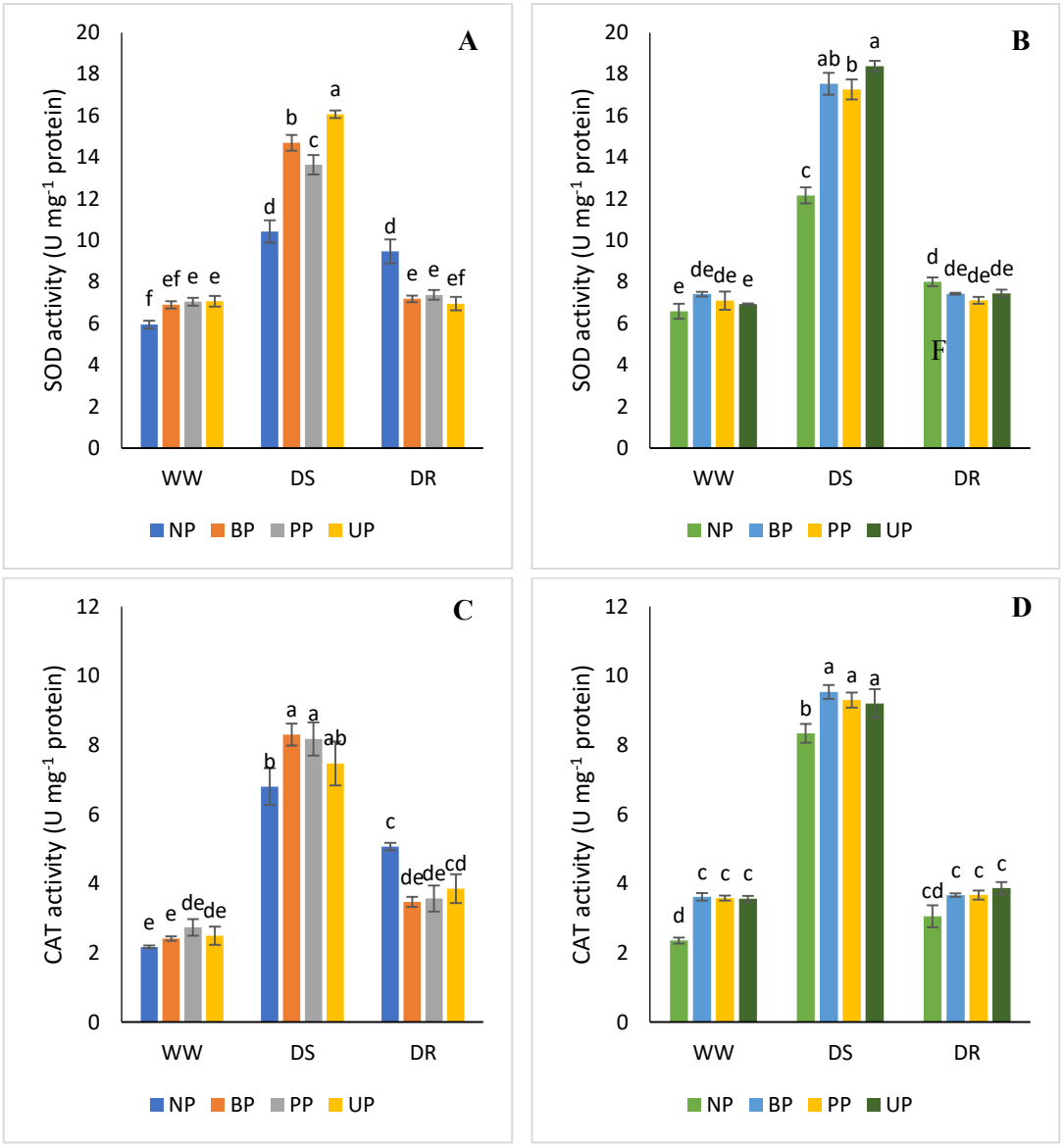

Figure 23. SOD (A-Anaswara and B-PGCP 6) and CAT (C-Anaswara and D-PGCP 6) activity in the trifoliate leaves of cowpea as influenced by different priming and subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

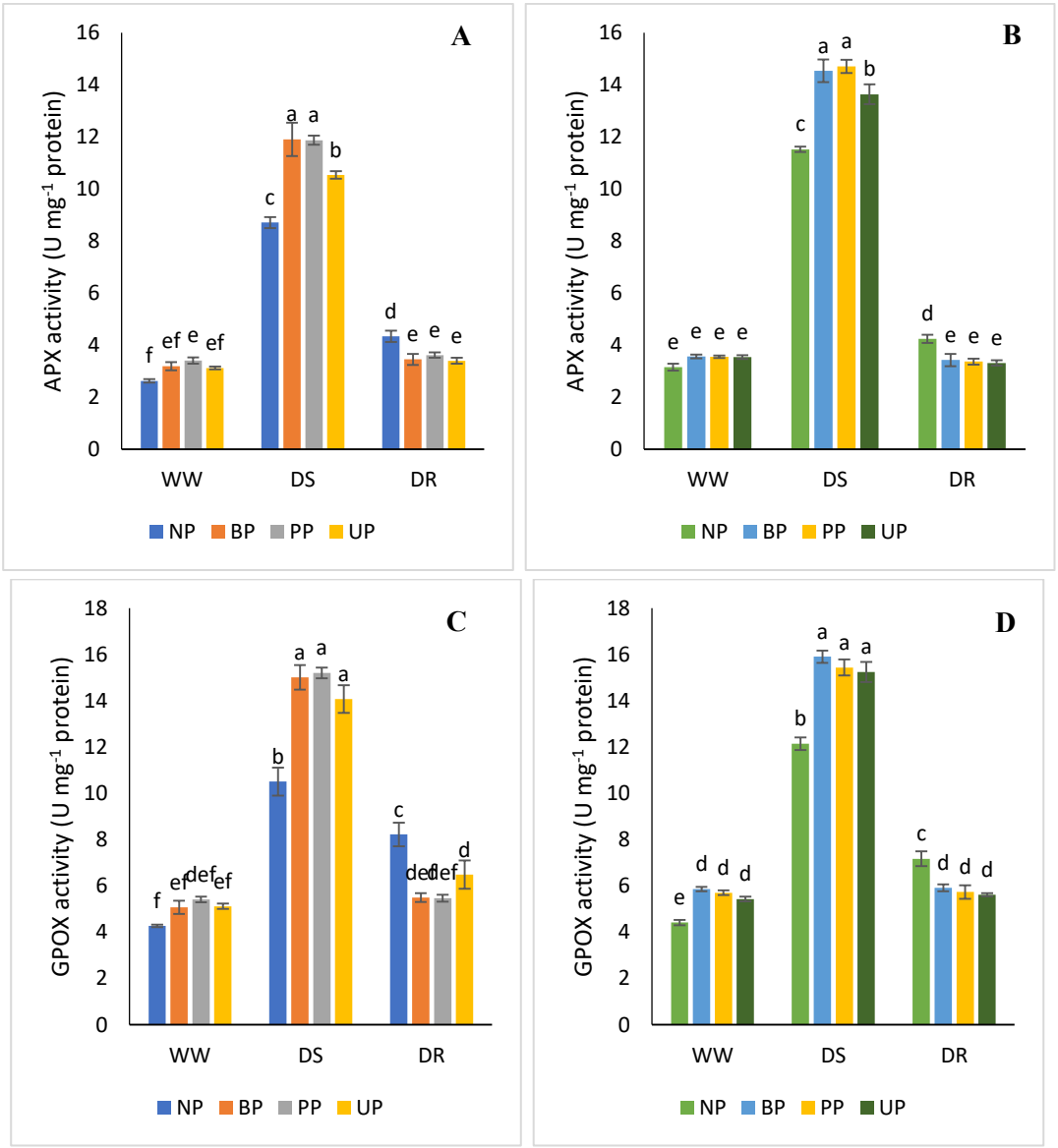

Figure 24. APX (A-Anaswara and B-PGCP 6) and GPOX (C-Anaswara and D-PGCP 6) activity in the trifoliate leaves of cowpea as influenced by different priming and subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

enhancement was 291% and 275% in PEG and UV-B primed PGCP 6 respectively.

Plants subjected to post drought recovery showed a greater reduction in ascorbate content than the plants under drought stress. There was a reduction of 40% and 63% in Anaswara and PGCP 6 respectively than the drought-stressed plants. The highest reduction was observed in PEG primed PGCP 6 (72%) followed by BABA (69%) and UV-B (68%) primed PGCP 6. Similar pattern was observed in the case of primed Anaswara also but with lesser reduction (52-64%) upon recovery from drought stress (Figure 25).

4.3.10.2.2 Glutathione content

Drought stress elevated the glutathione contents in both the cowpea varieties studied. The highest increment was noted in the tolerant variety PGCP 6 (178%) than the sensitive variety Anaswara (127%). All the priming treatments significantly enhanced the glutathione accumulation, with the highest increase of 247% in BABA primed PGCP 6 followed by PEG primed PGCP 6 (242%). In the case of variety Anaswara, maximum enhancement of glutathione content was noted in the case of BABA priming (184%).

At the time of stress recovery, the glutathione content significantly reduced in both the varieties. BABA and PEG primed PGCP 6 exhibited the maximum reduction in glutathione content as compared to the respective primed plants subjected to drought stress. There was a reduction of 64% in glutathione content of both BABA and PEG primed PGCP 6 during drought recovery. Primed Anaswara exhibited a reduction of 49-50% upon recovery (Figure 25).

4.3.11 Secondary metabolites

4.3.11.1 Anthocyanin content

A significant increase in anthocyanin content was noted in BABA and UV-B primed varieties of cowpea under non-stressed conditions. Whereas, in the case of primed and non-primed plants under stressed condition, a drastic enhancement in anthocyanin accumulation was observed in both varieties, the increase was 62% and 71% in sensitive and tolerant varieties respectively. The increase was even higher in primed sensitive (88-106%) and in primed tolerant variety (118-141%). The highest accumulation of anthocyanin was observed in UV-B primed plants of both the varieties.

During stress recovery, there was a reduction of 25% and 34% in anthocyanin content of non-primed sensitive and tolerant varieties respectively. The reduction was further increased in BABA (51%) and PEG (48%) primed plants of Anaswara followed by BABA primed PGCP 6 (44%). Least reduction was observed in PEG (31%) and UV-B primed (36%) PGCP 6 (Figure 26).

4.3.11.2 Flavonoids content

A radical increase in the content of flavonoid was observed in drought-stressed plants of Anaswara (160%) and PGCP 6 (167%). However, in primed plants the flavonoid content was significantly enhanced than the non-primed plants subjected to drought. There was an increase of 199%, 173% and 203% in PGCP 6 primed with BABA, PEG and UV-B respectively. The increase was more prominent in the primed sensitive variety and it was 184-255%.

In the case of plants subjected to recovery after drought, there was only a slight reduction in flavonoid content than that of the respective plants exposed to drought. There was a slight reduction of only 9-18% in primed

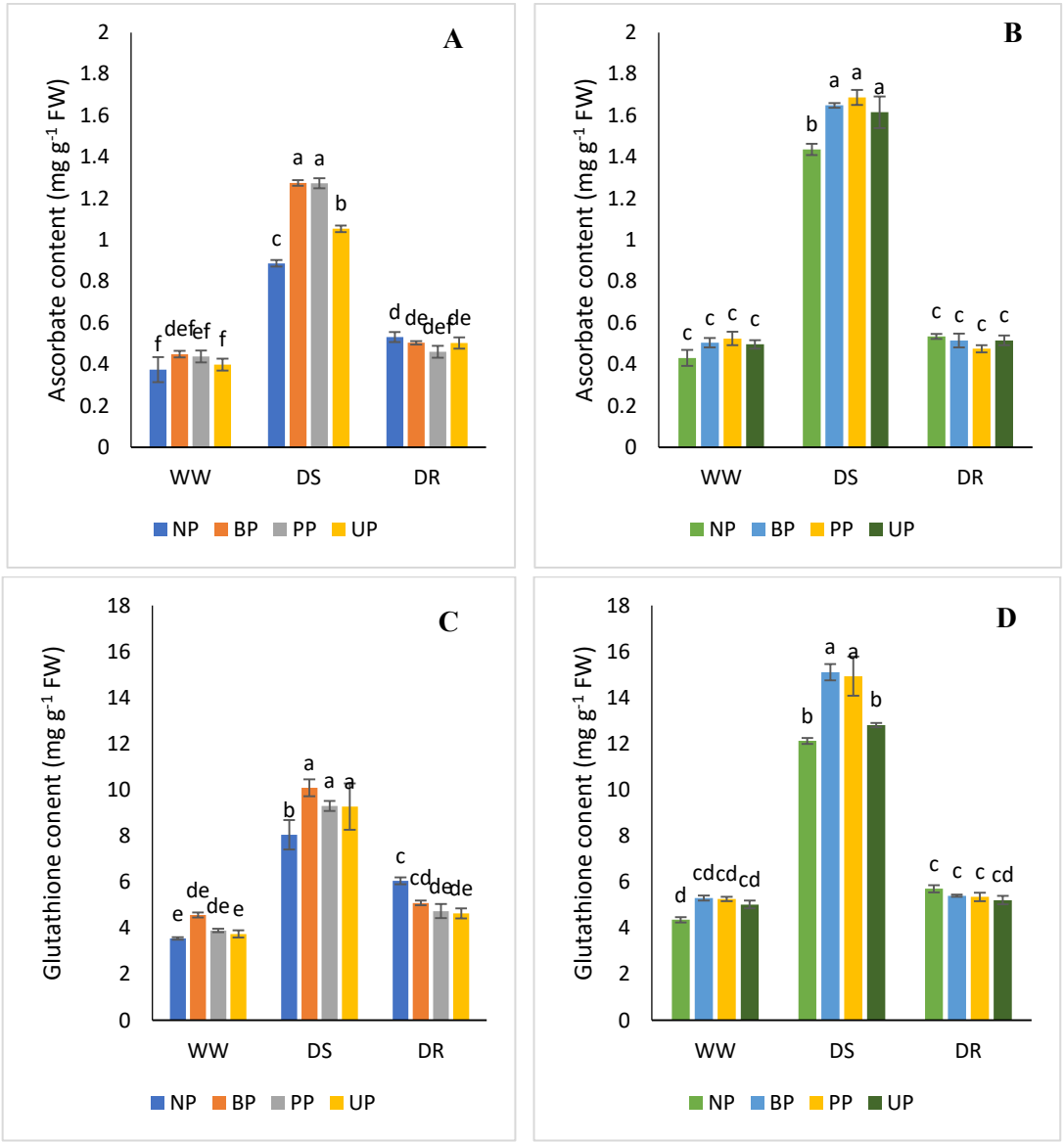

Figure 25. Ascorbate (A-Anaswara and B-PGCP 6) and glutathione (C-Anaswara and D-PGCP 6) content in the trifoliate leaves of cowpea as influenced by different priming and subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

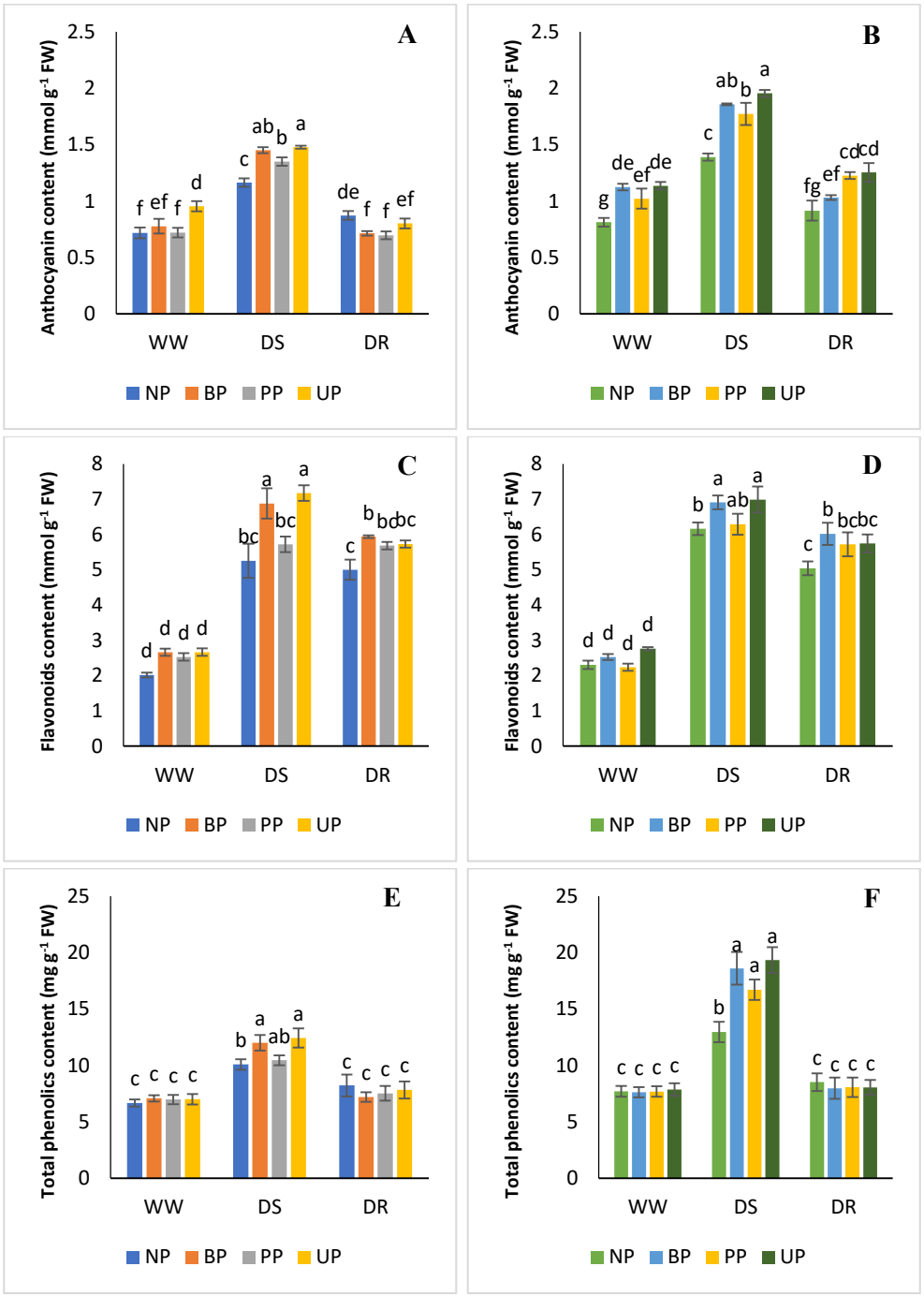

Figure 26. Anthocyanin (A-Anaswara and B-PGCP 6), flavonoids (C-Anaswara and D-PGCP 6) and phenolics (E-Anaswara and F-PGCP 6) content in the trifoliate leaves of cowpea as influenced by different priming and subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

PGCP 6 during stress recovery and there was no prominent reduction in flavonoid contents of non-primed plants of both varieties on 4 d of recovery (Figure 26).

4.3.11.3 Total phenolics content

The total phenolics content of cowpea was higher in primed plants subjected to drought stress (51% in Anaswara and 68% in PGCP 6) compared to non-primed plants under well-watered condition. There was an increase of 80%, 57% and 87% in BABA, PEG and UV-B primed Anaswara respectively subjected to drought. Whereas the increase was 141%, 116% and 151% in PGCP 6 subjected to BABA, PEG and UV-B priming respectively.

The amount of total phenolics reached almost similar level as that of the control upon recovery in primed PGCP 6. Likewise, in primed Anaswara subjected to recovery from drought stress, the content of total phenolics was significantly reduced following the resumption of watering. The highest reduction was observed in UV-B (58%) and BABA primed PGCP 6 (57%), followed by a reduction of 51% in PEG primed PGCP 6 as compared to the respective primed plants subjected to drought stress (Figure 26).

4.3.12 Root and root nodule study

4.3.12.1 Root length

Root length was slightly increased in plants subjected to drought stress and the increase was by 20% in Anaswara and by 28% in PGCP 6; an increase of 43% and 30% was noted in BABA primed PGCP 6 and Answara respectively under drought condition. Priming alone does not trigger root elongation in cowpea and no measurable increase in root length was noted in primed plants under well-watered condition. However, an enhancement in lateral root growth was seen in plants subjected to priming treatments. Under drought stress, lateral root growth was diminished in both the

varieties studied. But the reduction was less in the case of primed plants. There was reduction in the root length of plants during recovery from drought stress (Figure 27 and 28).

4.3.12.2 Nodule number and nodule size

Drought stress significantly reduced the root nodule number and nodule size in Anaswara and PGCP 6. The nodule size varied between the varieties and among the treatment. Nodule number reduced by 48% in Anaswara and 47% in PGCP 6 during drought, compared with the non-primed plants under well-watered conditions. Nodule number increased in plants subjected to BABA and UV-B priming but not exposed to drought. The increase was 53% and 67% respectively in BABA and UV-B primed PGCP 6. Whereas there was an increase of 40% and 50% in BABA and UV-B primed Anaswara. The reduction in nodule number observed in plants subjected to drought was lowered through priming treatment. The reduction was only 20-33% in PGCP 6 and 5-20% in Anaswara.

There was a prominent difference in the size of root nodule of cowpea grown under well-watered and water stressed conditions. As compared to the control plants, nodule size of plants subjected to drought treatment reduced by 61% and 50% in Anaswara and PGCP 6 respectively. Only a slight increase in nodule size was noted in primed plants under well-watered condition and it was not significant. The beneficial effect of priming with respect to nodule size was significant under drought stress and the reduction was only 23-38% in PGCP 6 and 43-48% in Anaswara (Figure 27 and 29).

4.3.12.3 Elemental analysis of root nodules

Drought stress significantly affected the N, Mo and Fe content in the root nodules of cowpea varieties studied. However, the concentration of

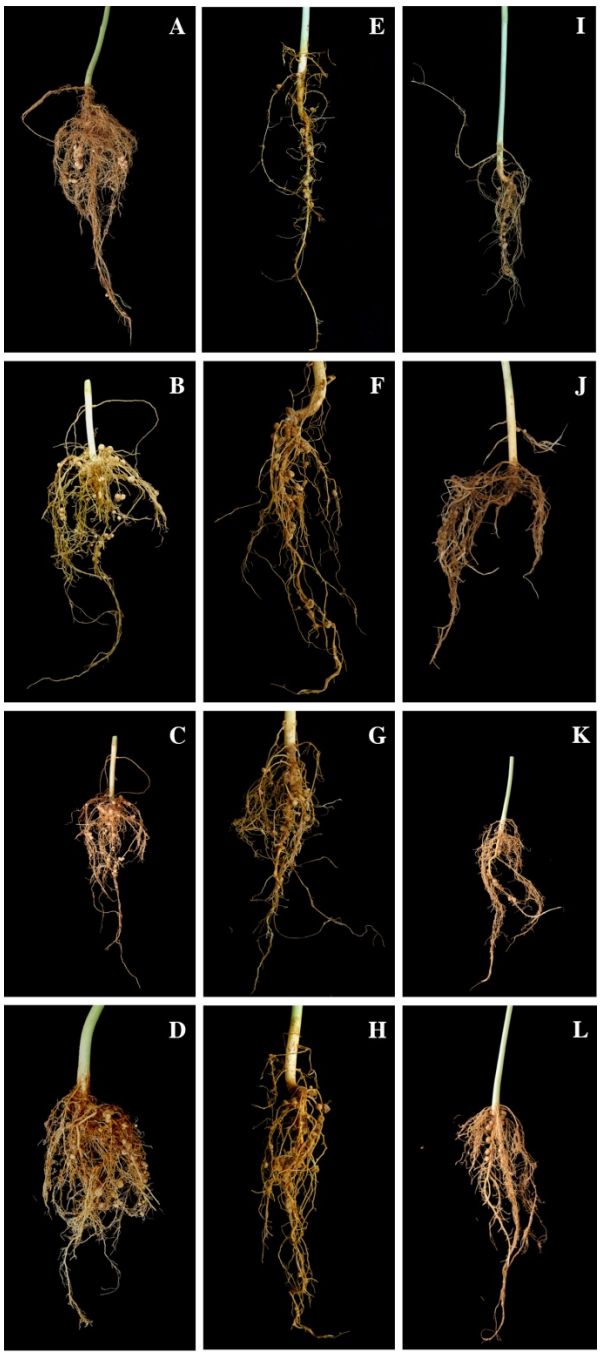

Figure 27 A. Root architecture and nodulation characteristics of cowpea as influenced by drought stress and priming in the variety Anaswara.

A (Non-primed cowpea under well-watered condition), B (BABA primed cowpea under well-watered condition), C (PEG primed cowpea under well-watered condition), D (UV-B primed cowpea under well-watered condition), E (Non-primed cowpea under drought stress), F (BABA primed cowpea under drought stress), G (PEG primed cowpea under drought stress) H (UV-B primed cowpea under drought stress), I (Non-primed cowpea during recovery), J (BABA primed cowpea during recovery), K (PEG primed cowpea during recovery), L (UV-B primed cowpea during recovery).

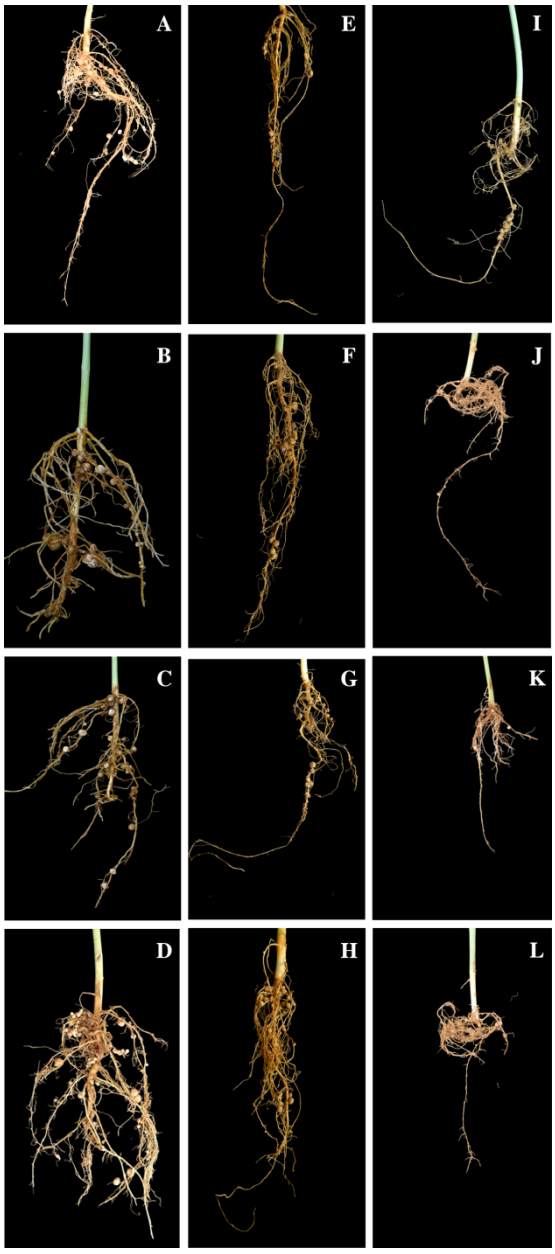

Figure 27 B. Root architecture and nodulation characteristics of cowpea as influenced by drought stress and priming in the variety PGCP 6.

A (Non-primed cowpea under well-watered condition), B (BABA primed cowpea under well-watered condition), C (PEG primed cowpea under well-watered condition), D (UV-B primed cowpea under well-watered condition), E (Non-primed cowpea under drought stress), F (BABA primed cowpea under drought stress), G (PEG primed cowpea under drought stress) H (UV-B primed cowpea under drought stress), I (Non-primed cowpea during recovery), J (BABA primed cowpea during recovery), K (PEG primed cowpea during recovery), L (UV-B primed cowpea during recovery).

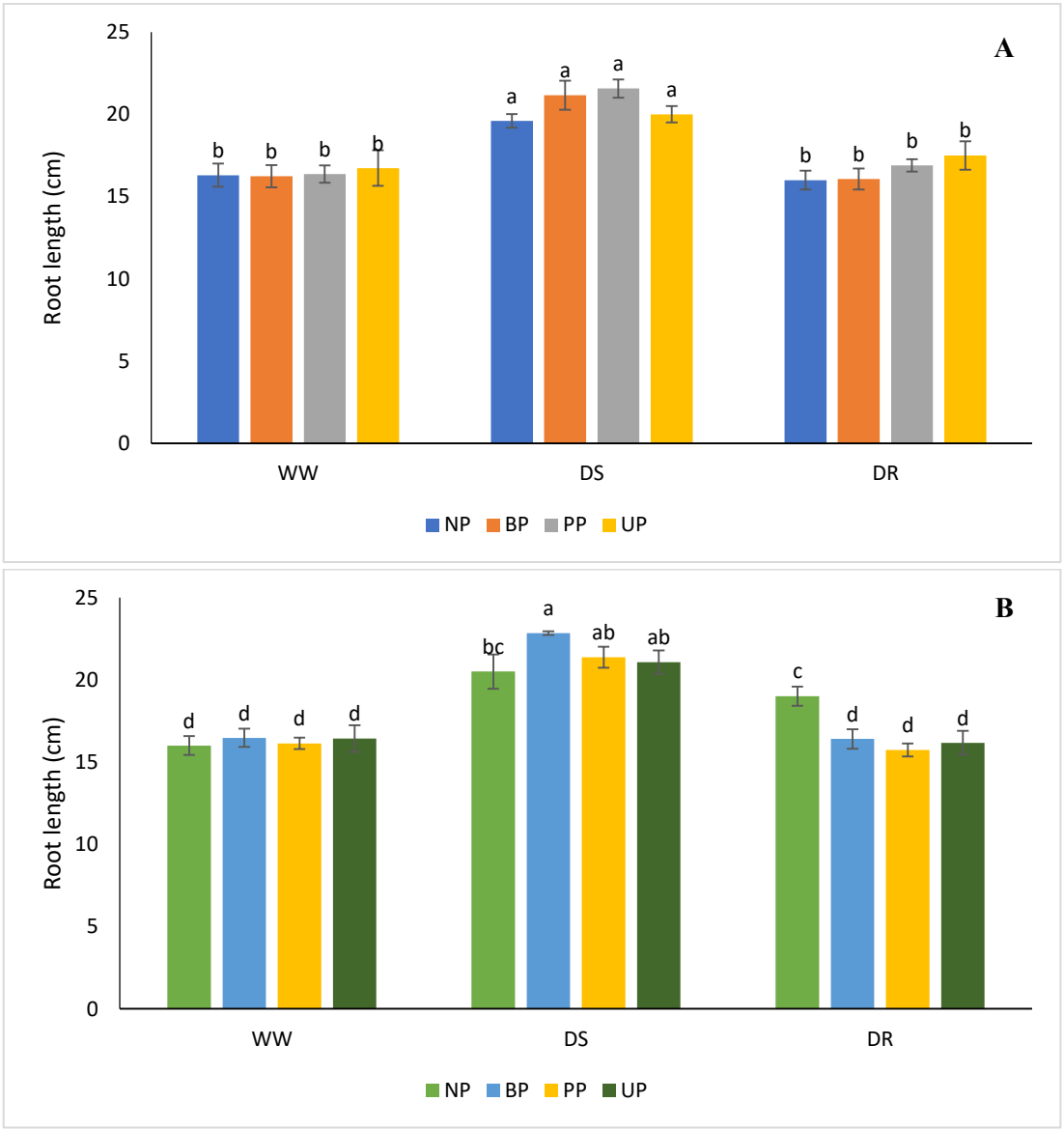

Figure 28. Root length of cowpea variety Anaswara (A) and PGCP 6 (B) as influenced by different priming and subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

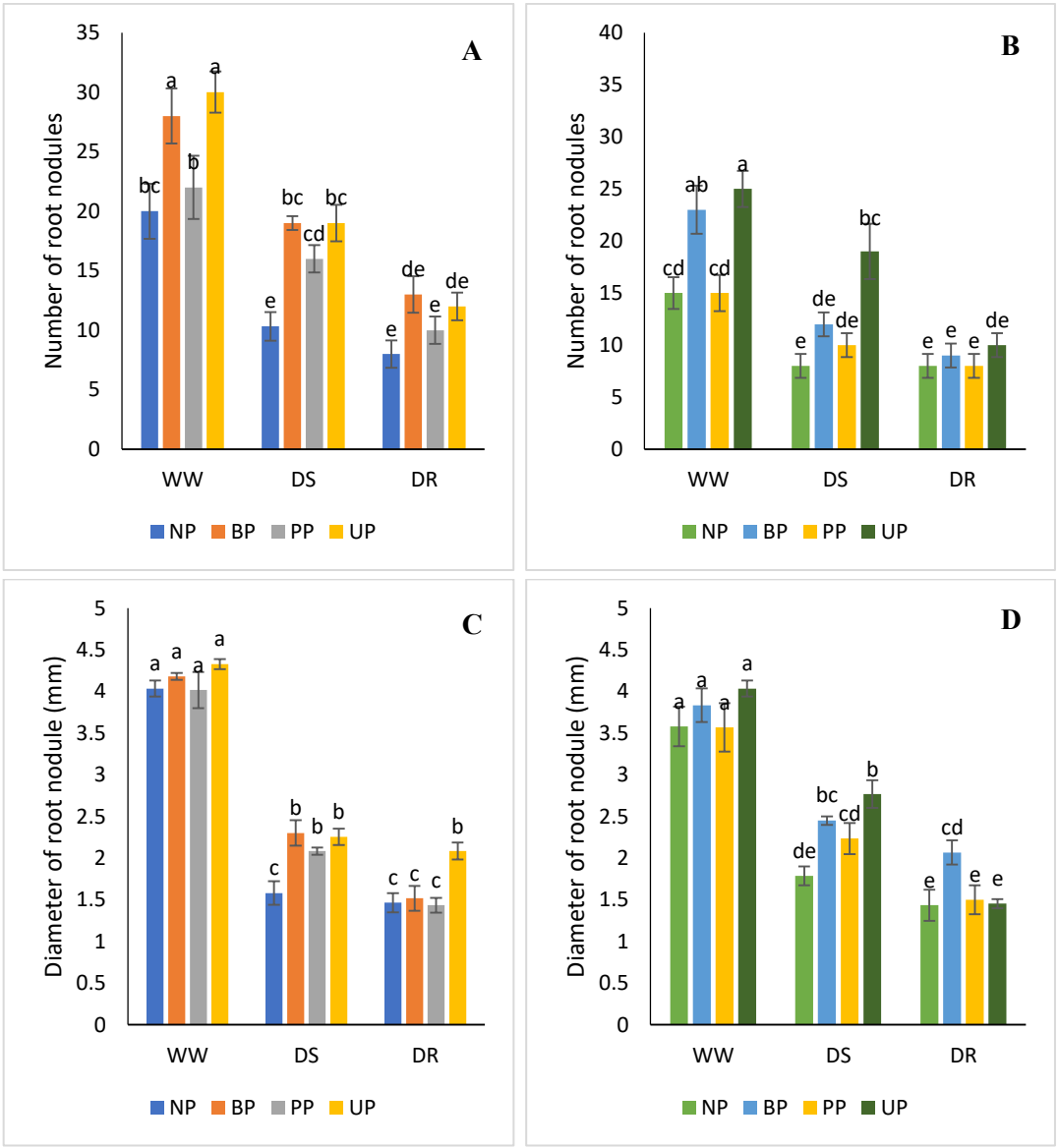

Figure 29. Root nodule number (A-Anaswara and B-PGCP 6) and nodule size (C-Anaswara and D-PGCP 6) of cowpea as influenced by different priming and subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

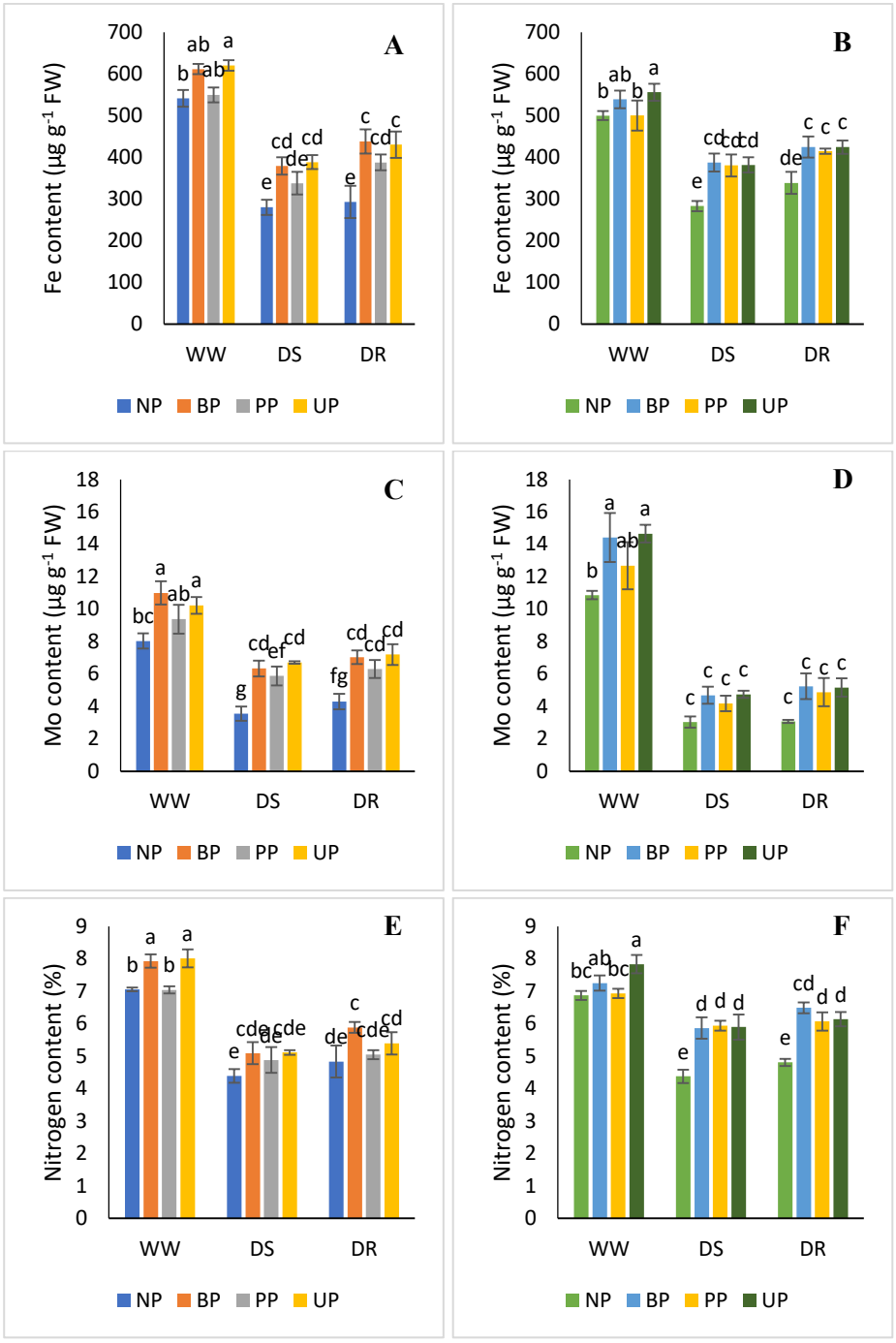

Figure 30. Iron (A-Anaswara and B-PGCP 6), molybdenum (C-Anaswara and D-PGCP 6) and nitrogen content (E-Anaswara and F-PGCP 6) of cowpea as influenced by different priming and subjected to drought stress and recovery. WW (well-watered condition), DS (Drought-stressed condition), DR (drought recovery), NP (Non-primed plants), BP (BABA primed plants), PP (PEG primed plants), UP (UV-B primed plants). Values are the means±SE. Alphabetical letters represent significant difference between treatments performed using Duncan's multiple range tests at $p \leq 0.05$.

these elements slightly increased in primed plants as compared to the non-primed plants. The reduction in N was highest in non-primed Anaswara (38%) and PGCP 6 (36%) subjected to drought, but the drop was less in the primed plants on encountering stress. There was a reduction of only 14-15% in N content of primed PGCP 6 and a reduction of 28-31% in primed Anaswara. In Anaswara, the reduction in Fe was 48%, and that of PGCP 6 was 43% under drought stress. As compared to the non-primed plants, the reduction was less in primed plants subjected to drought stress, with the least reduction in primed PGCP 6 (23%). Compared to the plants that emerged from non-primed seeds, the concentration of Mo significantly increased in plants that emerged from primed seeds. The increase was 37% in BABA primed Anaswara and 35% in UV-B primed PGCP 6. During drought stress, a significant reduction in Mo was found in both Anaswara (56%) and PGCP 6 (72%), and the decline was less in the case of primed plants subjected to drought stress. The reduction in the N, Fe and Mo content was less at the time of recovery from drought stress (Figure 30).

4.3.13 Gene expression studies of dehydrins

The gene expression levels of *Vu400*, *Vu500*, *Vu600*, *Vu700* and *Vu800* dehydrins were analysed in both sensitive and tolerant cowpea varieties emerged from primed and non-primed seeds and grown under well-watered, drought-stressed and recovery conditions. The expression level of *Vu400* gene was downregulated during drought stress in primed and non-primed Anaswara plants. During recovery from drought, the phenomenon of downregulation in the expression of *Vu400* remained the same in non-primed, BABA primed and PEG primed Anaswara. But the expression of *Vu400* in UV-B primed Anaswara during recovery was same as that of the control. No change was observed in the relative expression of *Vu400* in primed tolerant variety (PGCP 6) grown under well-watered condition as

compared to control. However, upon imposing with drought stress, the increased expression of *Vu400* was observed in non-primed and primed cowpea plants. The highest expression was observed in leaves of BABA primed (37-fold) cowpea followed by PEG primed cowpea (19-fold) plants. The expression levels of *Vu400* reached the same level as that of the control during recovery phase in the leaves of non-primed and primed cowpea plants (Figure 31).

Vu500 gene was downregulated in Anaswara variety under well-watered, drought-stressed and recovery conditions with an exception of increased expression in non-primed plants subjected to drought stress (2.6-fold). However, increased *Vu500* gene expression was observed in PEG primed and UV-B primed primed PGCP 6 grown under well-watered condition. Upon drought stress, there was upregulation in the expression of *Vu500* and the highest expression was observed in BABA primed PGCP 6 (52-fold). The induction in gene expression was higher by 17-fold in non-primed PGCP 6 and 5.95-fold in PEG primed and 3.783 fold in UV-B primed PGCP 6. In the recovery phase, expression levels of *Vu500* in both non-primed and primed PGCP 6 was equivalent to those of the control plants.

The expression of *Vu600* increased in Anaswara during drought stress only in the case of non-primed plants. While, it was downregulated in all the primed plants subjected to drought stress. Similar pattern was observed during recovery also wherein the expression was reduced in all the cases except in PEG primed plants, which exhibited no change in the relative expression. In PGCP 6, there was an over expression of *Vu600* in all the primed plants under well-watered conditions. The expression was higher in BABA primed plants (25-fold) followed by PEG primed PGCP 6 (20-fold) under well-watered condition. During drought stress, the expression levels increased by 2.83-fold in non-primed plants, while the expression levels

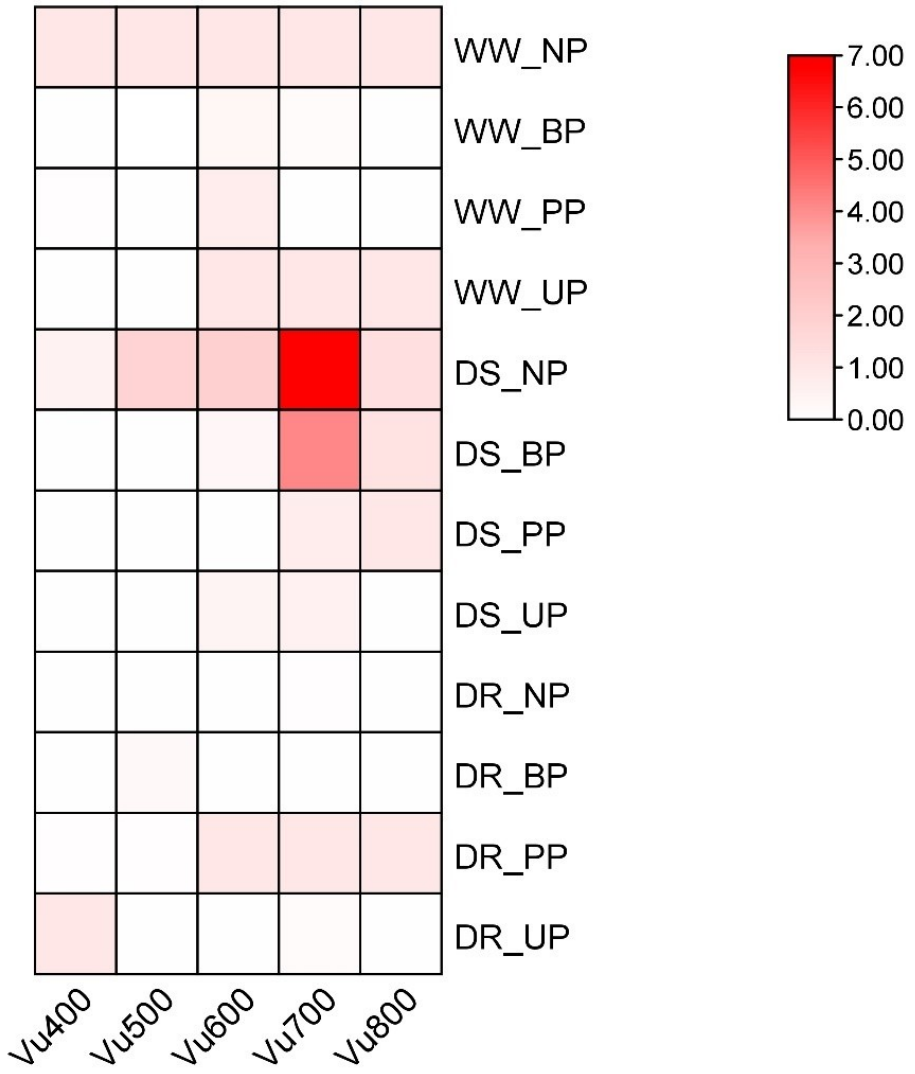

Figure 31 A. Expression analysis of dehydrin gene (*DHN*) in Anaswara subjected to different priming, drought stress and recovery.

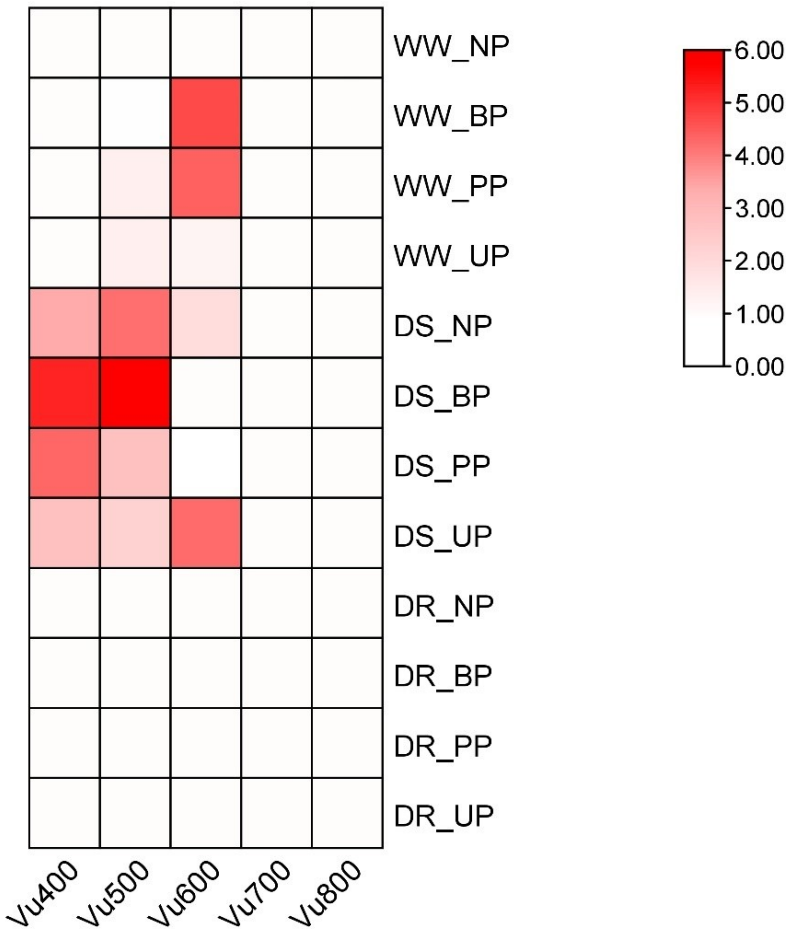

Figure 31 B. Expression analysis of dehydrin gene (*DHN*) in PGCP 6 subjected to different priming, drought stress and recovery.

were unchanged in BABA primed plants and downregulated in PEG primed plants and upregulated in UV-B primed plants (18-fold). During recovery from stress, relative expression of *Vu600* in both non-primed and primed plants reverted to the level as that of control.

During drought stress there was a prominent increase in the expression of *Vu700* in non-primed Anaswara (108-fold) and the expression was increased in BABA primed Anaswara (17-fold) also. During recovery, the expression was decreased in leaves of non-primed, BABA primed and UV-B primed plants, but remained same as that of the control levels in PEG primed Anaswara. *Vu700* was not expressed in tolerant cowpea under any circumstances and/or treatments. An induction of *Vu800* in non-primed and BABA primed Anaswara was observed during drought stress. While the expression remained unchanged in PEG primed, it got downregulated in UV-B primed Anaswara. There was downregulation in the expression of *Vu800* in non-primed, BABA primed and UV-B primed Anaswara during recovery but the expression remained the same level of control in PEG primed Anaswara. There was no expression of *Vu800* in both non-primed and primed PGCP 6 during well-watered, drought and recovery conditions (Figure 31).

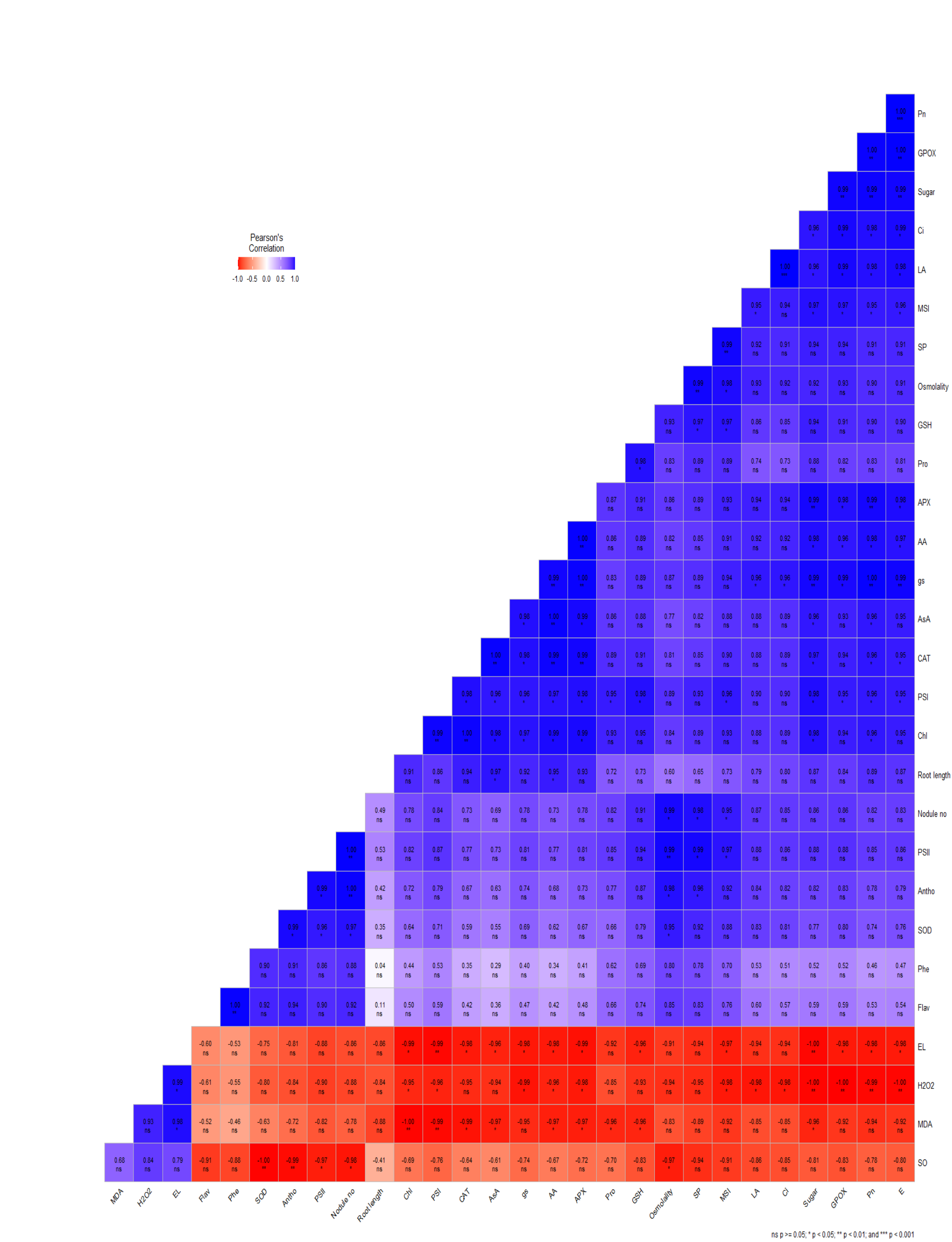

Figure 32. The correlation matrix heatmap of leaves of cowpea variety Anaswara subjected to drought stress showing the values of Pearson correlation coefficient between parameters. The positive values are represented in blue colour and negative in red colour.

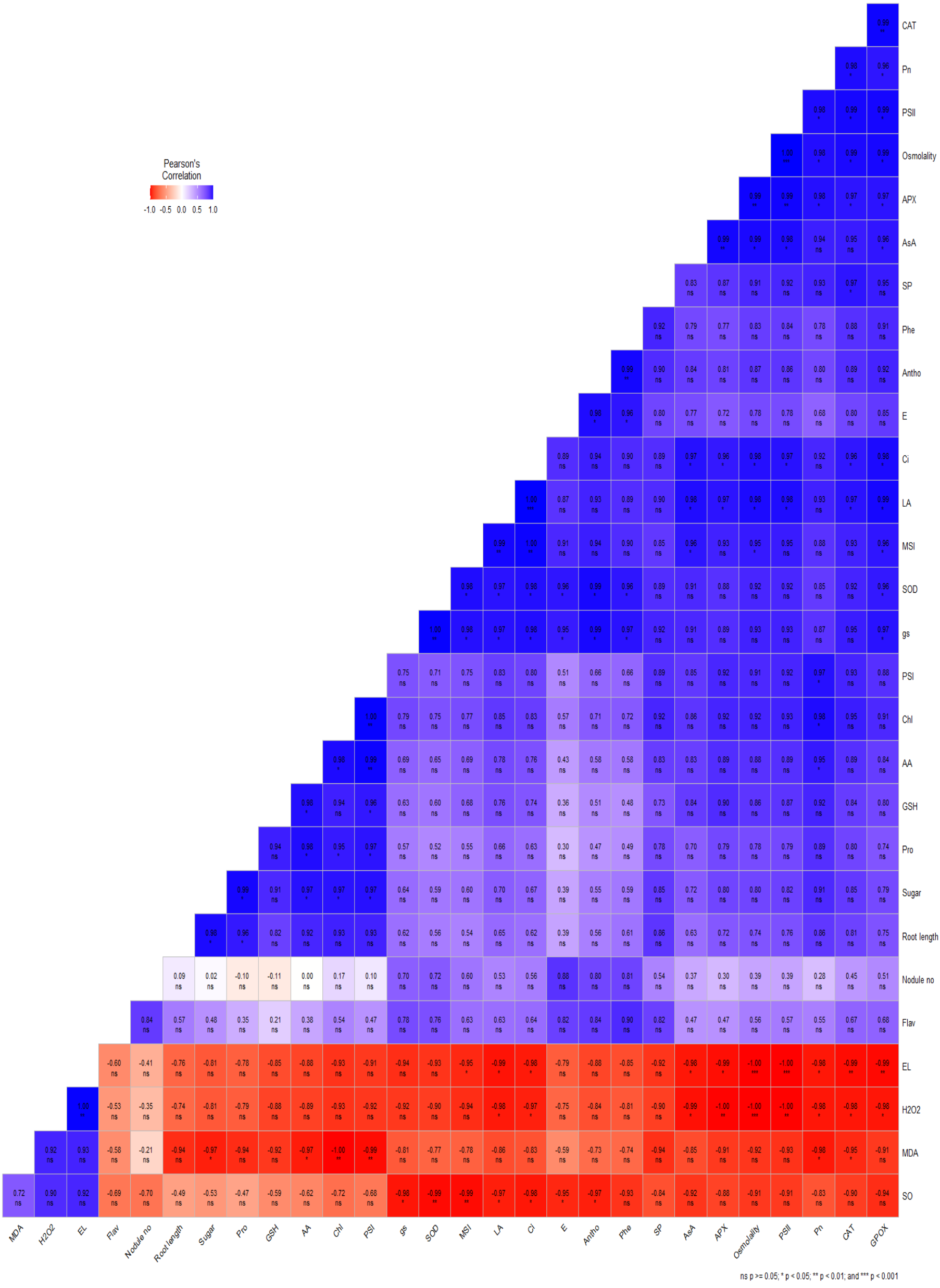

Figure 33. The correlation matrix heatmap of leaves of cowpea variety PGCP 6 subjected to drought stress showing the values of Pearson correlation coefficient between parameters. The positive values are represented in blue colour and negative in red colour.

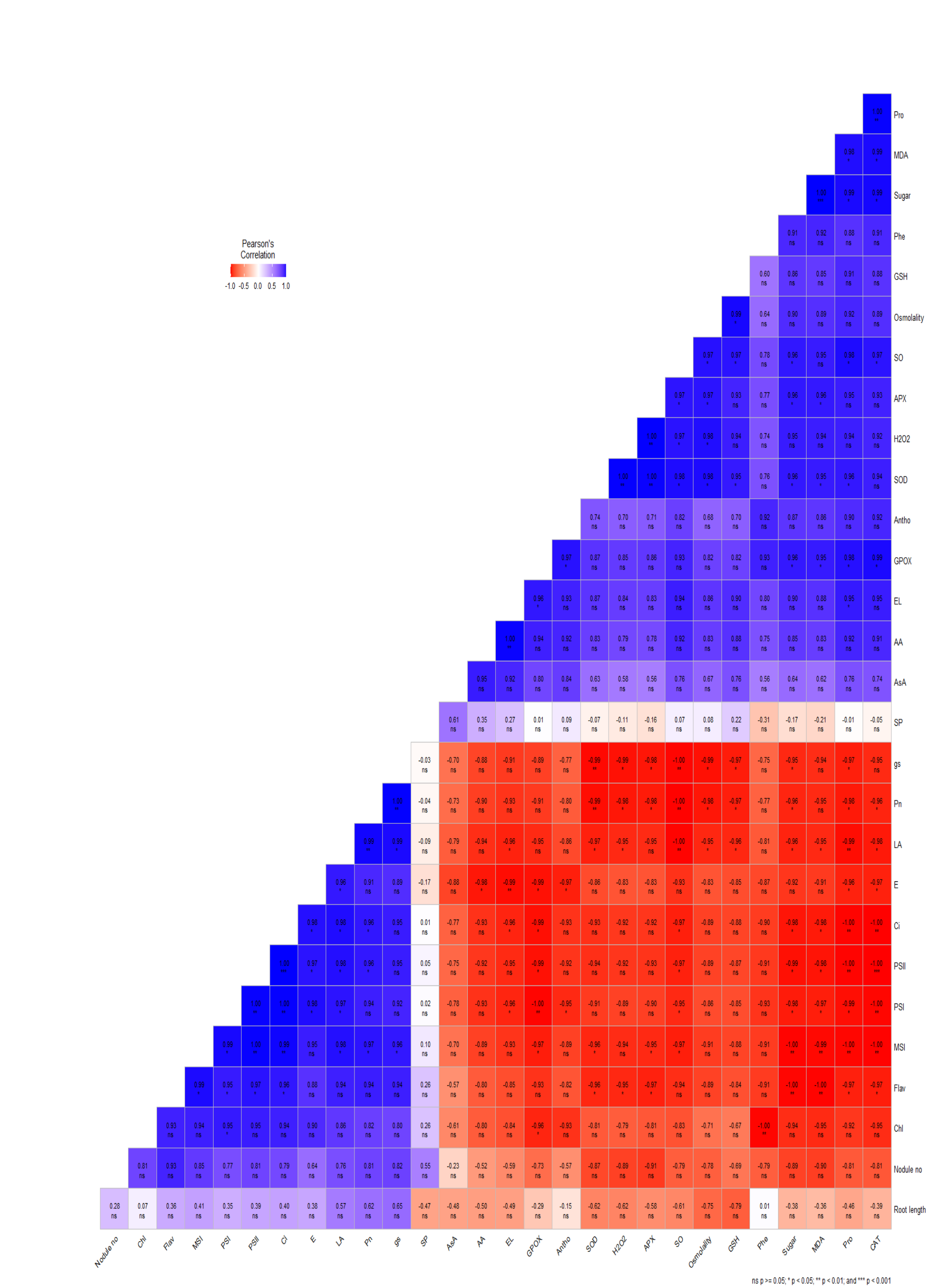

Figure 34. The correlation matrix heatmap of leaves of cowpea variety Anaswara during recovery from drought stress showing the values of Pearson correlation coefficient between parameters. The positive values are represented in blue colour and negative in red colour.

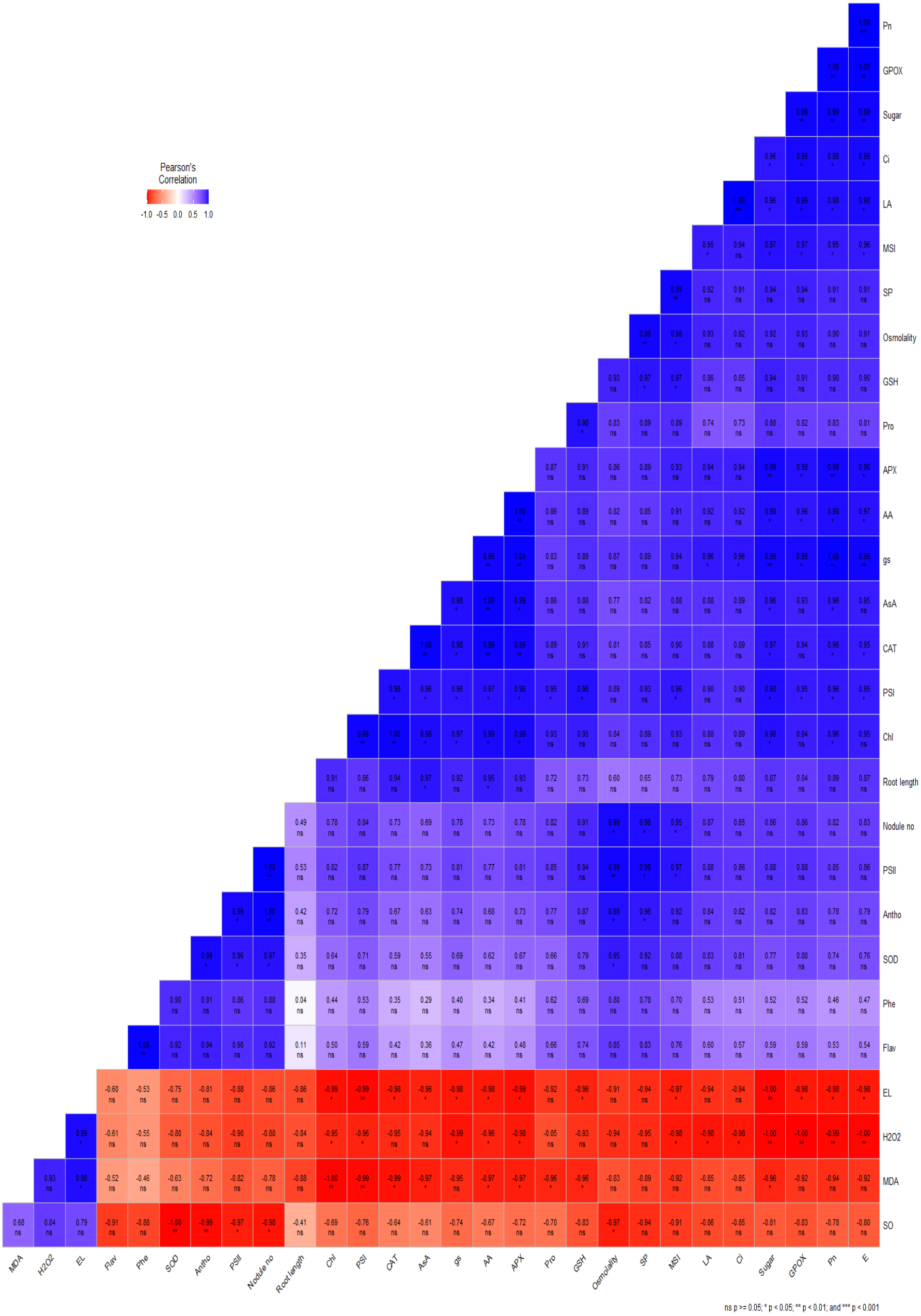

Figure 35. The correlation matrix heatmap of leaves of cowpea variety PGCP 6 during recovery from drought stress showing the values of Pearson correlation coefficient between parameters. The positive values are represented in blue colour and negative in red colour.

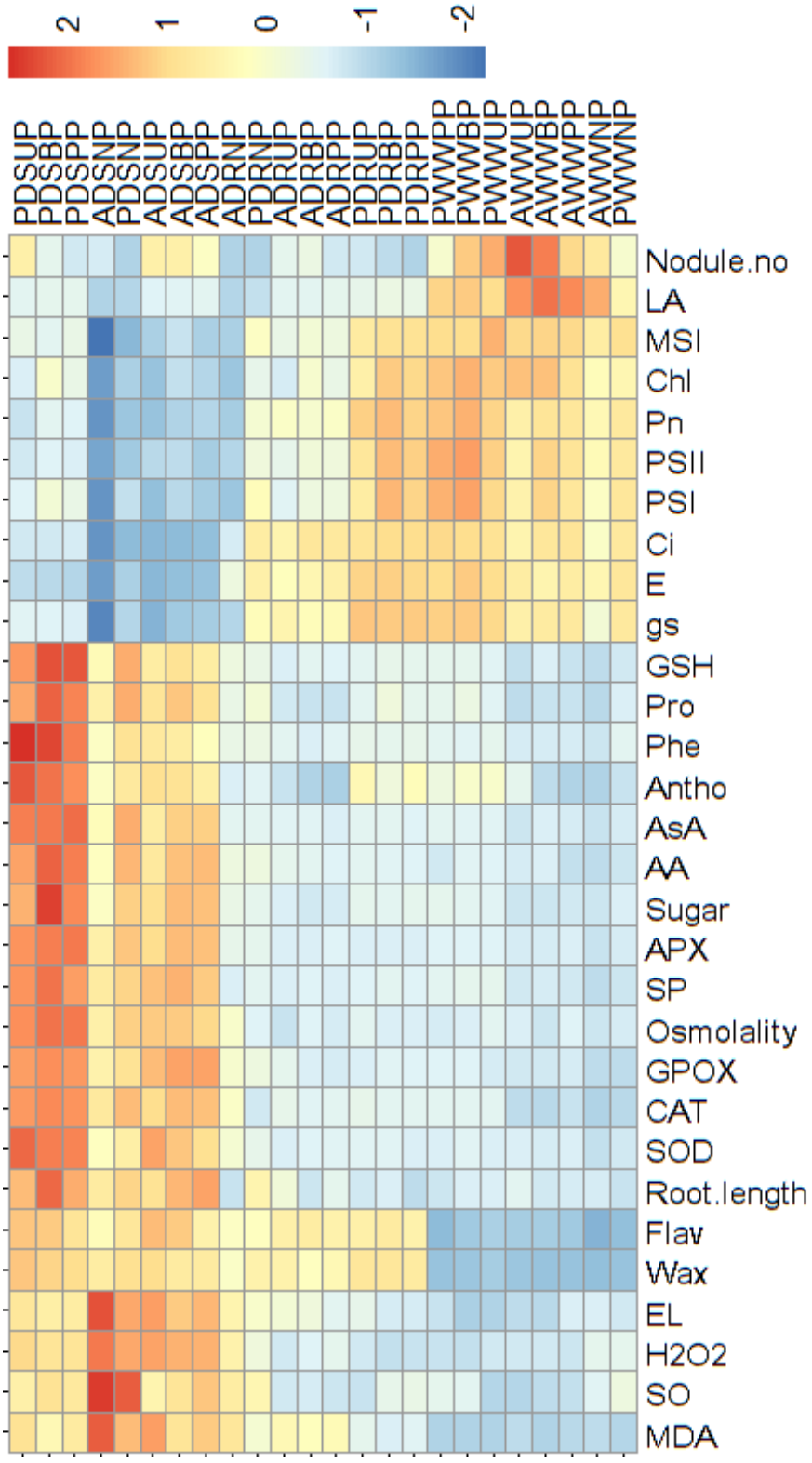

Figure 36. Heatmap of cowpea varieties Anaswara and PGCP 6 subjected to different priming treatments and exposed to drought stress and recovery.

5. DISCUSSION

Drought is a meteorological word that refers to a period of prolonged absence of precipitation, resulting in reduced soil moisture, often coupled with elevated evaporative demand, resulting in continual water loss through transpiration. It is regarded as the most prevalent catastrophic disaster in numerous places globally and has a devastating impact on the world (Raza et al., 2019). Approximately half of the global land experiences insufficient water availability. The demand for water is projected to rise by 55%, whereas presently 25% of cities worldwide are experiencing water scarcity (Tanveer et al., 2023). Drought stress can disrupt normal plant growth, stomatal conductance, photosynthetic efficiency, and ion homeostasis, while also inducing oxidative damage through the excessive accumulation of reactive oxygen species (ROS), which hinders normal plant development (Farooq et al., 2009; Matos et al., 2010). *Vigna unguiculata* (cowpea), is a protein-rich warm-weather, semi-arid legume crop, yet its growth and performance is extremely reliant on the availability of water. Hence, developing a strategy to enhance the drought tolerance potential of cowpea is of immense importance.

There are reports on the positive impact of seed priming for alleviating the adverse effects of drought stress on plant growth and performance. Seed priming makes plants more robust by augmenting various morpho-physiological and molecular parameters such as improved growth, photosynthetic performance, antioxidant system, accumulation of stress protective proteins and higher expression of stress related genes, which aids in developing stress resilience. Thereby, it is expected that primed plants will recover from drought stress more quickly as it is less severely affected by drought.

5.1 Screening of varieties for the determination of stress-imparting concentrations of PEG 6000

Polyethylene glycol (PEG) was utilised in the initial phase of the studies that imitate the dehydration effects of soil dryness. PEG is regarded as an ideal regulator of water potential, it has significant water absorption ability, thereby dehydrate cells and mimic drought stress (Murillo-Amador et al., 2002; Bukan et al., 2024). The growth phase in cowpea varieties subjected to PEG stress was evaluated based on shoot length, fresh weight, dry weight, and total chlorophyll content. The shoot length of cowpea seedlings continued to increase, and signs of stress started to show up on 8 d of germination. After 8 d, the rate of increase in growth parameters were very less and in majority of cowpea varieties leaf chlorosis was noted from 9 d onwards, which increased with stress exposure and this may be due to the limitation of growing in bottles with limited resources. Hence 8 d of germination was selected as the optimal growth phase to study the effect of PEG stress in cowpea grown in culture bottles, grown under controlled conditions. All the seeds of four cowpea cultivars were exposed to different concentrations of PEG stress and a comprehensive analysis of morphological and physiological characteristics was conducted on control and PEG treated seedlings, distinguishing the difference between PEG tolerant and PEG sensitive varieties.

In the current study, among all the varieties of cowpea studied, maximum decrease in growth traits as well as chlorophyll pigments was observed in the seedlings of Anaswara subjected to PEG stress and the least reduction was seen in the variety PGCP 6. Hence, among the four cowpea varieties studied, Anaswara and PGCP 6 were selected as the drought sensitive and drought tolerant varieties respectively. The selection of stress imparting concentration of PEG was also done through the analysis of fresh

weight, dry weight, shoot length and total chlorophyll content of cowpea seedlings. Seedling growth difference of varieties under different PEG stress concentrations depends on the tolerance potential of the varieties. From six different concentrations of PEG, around 50% reduction in the above described parameters was observed in the variety Anaswara exposed to 15% PEG and variety PGCP 6 exposed to 20% PEG. Beyond 15% PEG stress, the growth of variety Anaswara was significantly reduced because it is more susceptible to PEG induced drought stress. However, PGCP 6 is tolerant towards PEG stress up to 20% and above this the growth was prominently decreased.

The decrease in shoot length and biomass resulted from inhibited cell division and suppressed cell expansion and proliferation due to decreased turgor pressure (Jaleel et al., 2009). Furthermore, plants experiencing stress are susceptible to reduced growth as a result of altered plant hormone levels (Verma et al., 2016). Typically, stress-induced buildup of ROS can initiate the DNA damage response (DDR) pathway, leading to cell cycle arrest; the cell cycle inhibitor gene SMR5 is consistently activated in response to heat, drought, or elevated light conditions (Yi et al., 2014). Another reason for the reduction in biomass under PEG stress conditions was due to the reduced content of photosynthetic pigments and their functions, which possibly resulted from an increase in the activity of chlorophyll-degrading enzyme, chlorophyllase and, consequently, the loss in plant biomass and it was observed in several plant species like green gram (Jisha and Puthur, 2014), peanut (Shivakrishna et al., 2018), soybean (Basal et al., 2020) etc. The maximum reduction in growth occurs when the plants are more susceptible to the stress and in the present study, variety Anaswara exhibited prominent reduction in shoot length, fresh weight and dry weight under PEG stress indicating its susceptibility towards PEG induced drought stress. Whereas

PGCP 6 exhibited the least reduction among all the varieties studied, which denotes the innate tolerance potential of the cowpea variety PGCP 6.

The reduction of chlorophyll content was identified as a common indicator of oxidative stress. The decrease in chlorophyll content, resulting from the suppression of chlorophyll biosynthesis and the activation of its destruction by the enzyme chlorophyllase, and may constitute a photoprotection mechanism that lessens light absorption by lowering chlorophyll levels (Taïbi et al., 2016). PEG stress adversely affected the chlorophyll content in the cowpea varieties Anaswara, Bhagyalakshmi, Kanakamony, and PGCP 6, with a 50% loss in pigments observed at 15% PEG in Anaswara and Bhagyalakshmi. But, in the case of Kanakamony and PGCP 6, a 50% drop in photosynthetic pigments was observed upon exposure to 20% PEG. The findings reveal that the maximum pigment degradation induced by PEG stress occurred in the variety Anaswara followed by Bhagyalakshmi and at the same time the tolerant variety PGCP 6 had lesser reduction in photosynthetic performance owing to their improved growth and performance even under stressful environment. The overall growth parameters suggested that variety PGCP 6 is more tolerant towards PEG stress and the variety Anaswara is the most sensitive among the four cowpea varieties selected for the present study.

5.2 Standardisation of seed priming duration and concentration/dosage of BABA, Hydro, PEG and UV-B priming

PEG stress negatively affects seedling growth; however, due to the effects of seed priming treatments, the reduction in growth parameters, such as shoot length and dry weight and total chlorophyll content in primed seedlings was less pronounced than in non-primed seedlings. The increased shoot and root length in plants emerged from primed seeds is attributed to modifications in germination processes, including a decrease in the lag time

between imbibition and radical emergence (Waqas, 2019). Moreover, in primed seedlings, elevated respiration rates and enhanced ATP synthesis may facilitate optimal seedling growth under stressful conditions (Chen and Arora, 2013). Among the cowpea varieties, the highest growth performance was observed in primed PGCP 6 seedlings under PEG stress conditions, whereas the lowest was recorded in Anaswara seedlings. This indicates that priming treatments enhance the stress tolerance capacity in the tolerant cowpea variety more significantly than in the sensitive cowpea variety, hence increasing the resilience of tolerant plants and at the same time imparting stress tolerance to the sensitive variety. This finding is consistent with the results of Sen et al. (2021), wherein they reported that UV-B priming enhanced PEG stress tolerance in rice, with a greater improvement observed in the tolerant variety compared to the sensitive variety.

The reduction in shoot length, fresh weight, dry weight and total chlorophyll content in cowpea exposed to PEG stress was alleviated due to seed priming effect of hydropriming, PEG priming, BABA priming and UV-B priming. Previous studies reported the beneficial role of priming to mitigate drought stress in various plant species (Bibi et al., 2024; Decsi et al., 2024; Ma et al., 2024; Shoaib et al., 2024). Optimization of priming time and concentration/dosage is critical for efficient priming to enhance the stress tolerance of plants (Chen and Arora, 2011). The priming concentration/dosage was chosen as the mild priming stimulus that resulted in highest growth enhancement. Concentrations/dosages above this cause inhibitory effects, while concentrations/dosages below the priming concentration have no significant effects on plants. The priming concentration differed, as seen by the contrasting stress tolerance potential across the selected cowpea varieties. The priming period for both the cowpea varieties were established at 6 h, as exceeding this duration resulted in the leaching of solutes from the seeds. Consistent with our findings,

previous works have also demonstrated that soaking seeds for more than 6 h exerts an inhibitory impact on *Vigna radiata* due to the leaching of solutes from the seeds (Jisha and Puthur, 2018).

The BABA priming resulted in maximum seedling performance in the case of Anaswara and PGCP 6 when provided with 1.5 mM for 6 h. Previously it was discovered that 1 mM BABA priming for 6 h was effective against PEG stress (Jisha and Puthur, 2016). Similarly, the positive effect of BABA for enhancing the drought tolerance were observed in several plants such as *Arabidopsis* (Jakab et al., 2005), *Brassica napus* (Mohamadi et al., 2017), *Capsicum annuum* (Abdulbaki et al., 2024) etc. BABA, rather than being a synthetic exogenous molecule, is also a natural product of plants, with endogenous levels of BABA rapidly elevating in response to stress (Thevenet et al., 2017). BABA is known to be involved in stress defensive responses in plants by regulating gene expression and the activity of antioxidant enzymes, as well as elevating the expression levels of certain stress-related genes (Ma et al., 2022). It was found that, BABA treatment induces GABA, which is also a stress-responsive metabolite, accumulating in response to a variety of environmental stimuli. Although its role in plants is still debated, it has been proposed that GABA helps with osmoregulation and protects against oxidative stress (Quero et al., 2015). In response to oxidative stress, GABA undergoes metabolism through the GABA shunt pathway, facilitating the production of succinate, which bypasses the actions of succinyl-CoA ligase and α-ketoglutarate dehydrogenase, two enzymes within the tricarboxylic acid (TCA) cycle that are susceptible to oxidative stress (Bouché and Fromm, 2004).

During hydropriming, the highest improvement in the growth and performance of cowpea was in the seedlings emerged from seeds of both varieties when soaked in water for 6 h. Previous research by Jisha and

Puthur (2018) demonstrated that hydropriming improved the PEG-induced drought stress tolerance of rice and green gram seedlings, with varying effects across different varieties. Hydropriming for 6 h was effective in the case of green gram in tolerating PEG stress (Jisha and Puthur, 2014; 2018), This align with the present finding also, because 3 h of priming duration was insufficient to bring the maximum growth performance of cowpea through the controlled hydration that was achieved through the soaking of seeds and at the same time higher duration (9 h) caused deleterious effects due to the leaching of the solutes from the seeds and hence it was confirmed that the optimum priming period for cowpea was 6 h. Research indicates that hydropriming enhances the overall phenotypic characteristics of plants, regardless of stress exposure. Hydropriming of seeds may induce or modify specific physiological mechanisms within the seed, which are subsequently transferred to the seedlings, thus enhancing their stress-resilience potential. Hydropriming was found to be effective in enhancing the photosynthetic pigment content and photosystem activities of the seedlings (Jisha and Puthur, 2018). Also, hydropriming enhanced faba bean germination and seedling emergence in the field, with the extent of the response being depended on environmental conditions, particularly evident under restricted soil moisture (Damalas et al., 2019). Numerous studies have substantiated that hydropriming accelerated growth traits across various crop species, including germination index, germination duration, and seedling dry weight (Umair et al., 2010; Farooq et al., 2019).

In the case of PEG priming, soaking the seeds of Anaswara in 10% PEG for 6 h, and PGCP 6 in 15% PEG for 6 h provided the better growth traits and tolerance to PEG stress. PEG is a widely utilised osmopriming agent that mitigates the adverse effects of abiotic stressors. When seeds are primed with PEG, the water supply is regulated at a level insufficient for complete germination, yet adequate to initiate a sequence of metabolic

responses associated with the onset of germination. Furthermore, PEG induces modest osmotic stress in plants, hence enhancing their tolerance to subsequent stress exposure. Consequently, in the current study PEG primed cowpea plants exhibited tolerance towards PEG-induced stress. Generally, PEG primed plants exhibit beneficial effects on seed germination, seedling establishment, and yield; however, these advantages vary based on factors such as plant species and type of stress (Zhang et al., 2015; Lei et al., 2021). Previous studies found that priming of seeds with 14%, 20% PEG 6000 markedly enhanced germination parameters and antioxidant activity in different plants under conditions of water stress (Zhang et al., 2015; Uddin et al., 2021).

In the tolerant variety PGCP 6, the priming dosage of UV-B was 3.6 kJ m^{-2} s^{-1} to confer PEG stress tolerance, whereas it was 1.8 kJ m^{-2} s^{-1} in the sensitive variety. The advantageous impact of modest doses of UV-B in providing tolerance to PEG stress was demonstrated by Sen and Puthur (2020) and Thomas and Puthur (2019). In these findings also researchers observed that the lowest dosage of UV-B brought priming effect in sensitive variety than the higher dosage that was beneficial for the tolerant variety. Low levels of UV-B radiation enhance the growth, production of secondary metabolites, increase the accumulation of compatible solutes, boost antioxidant activity, improve pigment composition, and upregulate the expression of stress-responsive genes, thereby augmenting the drought tolerance potential of plants and facilitating stress acclimatization (Thomas and Puthur, 2020; Luo et al., 2022). The enhancement of growth parameters in cowpea seedlings emerged from UV-B primed seeds resulted from increased cell division, cell elongation, cell expansion, and higher seedling vigor.

The variation in the priming concentration/dosage of PEG and UV-B in two varieties of cowpea is due to their differential stress tolerance level. As Anaswara is more sensitive to stress, even mild priming concentration (10%) of PEG is enough to impart mild stress in Anaswara and hence trigger the innate immune system. While, in PGCP 6 as it is a tolerant variety, it required higher concentrations of PEG (15%) to exhibit maximum advantageous effects on growth. Same was observed in the case of UV-B dosage also wherein the irradiation of cowpea seeds with 1.8 kJ m^{-2} s^{-1} of UV-B radiation was suitable for bringing the maximum growth performance and stress tolerance in Anaswara and higher concentration had inhibitory effects. Whereas in PGCP 6, eventhough lower dosages brought about priming effects, the highest increment was noted in 3.6 kJ m^{-2} s^{-1}, it is because of their inherent tolerance potential. In summary, sensitive and tolerant cowpea varieties showed higher tolerance to PEG stress after BABA, PEG, UV-B, and hydropriming. Eventhough hydropriming improved the growth of cowpea, it was less effective as compared to other priming treatments, in improving the PEG induced drought stress tolerance potential of cowpea. Therefore, for the further study hydropriming was excluded and BABA, PEG and UV-B priming treatments were performed to examine the drought stress tolerance potential and recovery kinetics of cowpea grown in soil.

5.3 Drought stress and recovery kinetics study under greenhouse conditions

5.3.1 Relative water content

The relative water content (RWC) of leaves is a key physiological trait directly associated with soil water content, serving as a major indicator of water stress in leaves. Previous research has revealed that the relative water content reduced in plants subjected to water stress (Arafa et al., 2021; Khan

et al., 2024). In the present work, RWC significantly decreased under drought stress, while significantly increased during the recovery phase in Anaswara and PGCP 6. As a result of reduced RWC due to drought stress, the leaves exhibited wilting and leaf rolling (Ghahfarokhi et al., 2015). Numerous researchers have indicated that a reduction in RWC resulting from drought stress correlates with stomatal closure, which is subsequently induced by the accumulation of abscisic acid (ABA) synthesized in guard cells and roots (Chaves et al., 2003; Khan et al., 2007).

Comparative analyses of sensitive and tolerant varieties have demonstrated that RWC can be used as a marker to effectively differentiate between drought tolerant and drought sensitive plants. The comparison of RWC between drought tolerant and drought sensitive beans (*Phaseolus vulgaris*) facilitated the differentiation of ecotypes (Rosales et al., 2013). In the model organism *Arabidopsis*, various ecotypes could be differentiated based on the variations in RWC in response to mild drought conditions. Likewise plant populations acclimatized to arid environments can be identified through direct assessment of RWC (Mckay et al., 2003) and the midday RWC decrease is a metric that facilitates the differentiation of some cowpea ecotypes (Kumar et al., 2008; Zegaoui et al., 2017). In accordance with these findings, the current research demonstrated that the cowpea variety PGCP 6 is a drought tolerant variety, as it exhibited a lower decline in RWC when subjected to drought stress compared to the sensitive variety, implying superior drought tolerance capability of PGCP 6; as RWC serves as a physiological indicator to distinguish between tolerant and susceptible types (Zegaoui et al., 2017).

In the current work primed plants exhibited improved RWC than the non-primed plant during drought exposure and this is aligning with the findings of Khalequzzaman et al. (2023). The decrease in RWC can lead to a

reduction in a number of other parameters associated with plant growth. Seed priming with BABA, PEG, and UV-B significantly improved RWC in cowpea plants subjected to drought stress. The rise in RWC can be related to the potential roles of BABA, PEG, and UV-B in preserving osmotic balance through improved osmolyte synthesis, which helps to maintain greater RWC in primed plants. The priming-mediated osmoticum maintenance is described in detail at 5.3.3. This finding is consistent with the findings of Sen and Puthur (2021), wherein they found that UV-B primed rice plants sustain elevated RWC by accumulating osmolytes. In accordance with the findings, Zhang et al. (2015), observed a notable reduction in the RWC of leaf, root, and stem under drought stress. But it was observed that, seed priming with PEG significantly improved the RWC of leaves, roots, and stems under all soil moisture levels (Zhang et al., 2015). Study of Rivas et al. (2016) revealed that the drought sensitivity of the sensitive cultivar correlated with its leaf water status, exhibiting lower RWC compared to the tolerant cultivar under moderate drought conditions. This is due to the larger leaf area of sensitive cultivar than the tolerant cultivar, suggesting that the total volume of water transpired by the sensitive cultivar was likely greater, resulting in lower RWC. It was also shown that certain genotypes maintained a high RWC by stomatal closure and a reduction of leaf area (Anyia and Herzog, 2004). But in contrast, in this study, even though the leaf area reduction was less in PGCP 6 as compared to Anaswara during drought stress, it maintained higher RWC than Anaswara, indicating that there was no significant correlation between leaf area and RWC in these cowpea varieties.

Restoration of the RWC is the vital process that occurs during the recovery from drought stress. Rivas et al. (2016) found that, during recovery phase, leaf water potential rose concurrently with RWC and soil moisture content (SMC) in both tolerant and sensitive cultivars of cowpea. Concomitantly, in the current study also, the SMC rose during rewatering,

resulting in an increase in RWC and it was also found that the tolerant plants recovered faster because of the faster regaining of RWC. So, from the finding it can be conclude that RWC is a reliable indicator to study the drought stress tolerance and the recovery kinetics of cowpea plants.

5.3.2 Phenotypic traits

The present study revealed that the overall growth of cowpea plants were adversely affected during drought stress. Inhibition of shoot growth during drought stress decreases the plant's metabolic demands and reallocates metabolites for the production of compounds that are necessary for osmotic adjustment to protect the plant from stress effects. Growth inhibition may occur due to the reduction of cell turgor resulting from insufficient water supply to the developing cells. Cellular water availability is reduced because of inadequate hydraulic conductivity from roots to leaves, resulting from stomatal closure. A reduction in hydraulic conductance reduces nutrient transport to the shoot, however it also inhibits embolism in the xylem, potentially serving as an adaptive response (Bhargava and Sawant, 2013). In cotton plants, especially in sensitive cultivars, plant height is a reliable indicator of inhibition of growth under drought stress. Plant height either remained constant or exhibited a little decrease in drought-tolerant genotypes (Singh et al., 2021). Additionally, wilting of leaves, chlorosis of older leaves, and premature senescence were noted in drought-stressed plants, resulting in a decrease in both the number of leaves per plant and total leaf area (Dubey et al., 2022; Mahmood et al., 2022). During drought stress, the reduction in plant height and leaf area was less in PGCP 6 as compared to Anaswara and the reduction of shoot length and leaf area are the key morphological alterations under drought conditions, which can serve as morphological markers for selecting resistant genotypes (Toscano et al., 2019).

The current study substantiates the advantageous effect of different priming treatments on the overall growth and performance of cowpea subjected to drought stress and subsequent recovery. Research has demonstrated the efficacy of BABA priming in enhancing plant height and leaf area in *Capsicum annuum* L. (Abdulbaki et al., 2024) and its effectiveness in mitigating drought stress (Jakab et al., 2005). This may result from the comprehensive metabolic alterations in the seedlings, triggered by the priming of the seeds with BABA. In another work, seed priming with BABA enhanced the seedling vigour of pearl millet (Shailasree et al., 2001) and increased the fresh weight and length of seedlings (Jisha and Puthur, 2016).

Likewise, PEG-primed plants exhibited beneficial effects on seed germination, seedling establishment, and yield; however, these advantages vary based on factors such as crop species and type of stress. It was observed that seed priming with PEG significantly enhanced the seedling vigor index of *Vigna radiata* under drought stress (Uddin et al., 2021). Likewise, Sen et al. (2022) determined that an optimal UV-B dosage of 4 kJm^{-2} enhanced the drought tolerance potential of rice. In a different study, basil plants (*Ocimum basilicum*) were subjected to UV-B light (222.6 μWm^{-2} for 3 hours each morning for two weeks), resulting in a significant increase in leaf area (Chang et al., 2009). In the study, 1.8 kJ m^{-2} s^{-1} and 3.6 kJ m^{-2} s^{-1} were found to be efficient in improving morphological characteristics, including shoot length and leaf area in both sensitive and tolerant varieties of cowpea, respectively. This outcome aligns with prior studies, indicating that modest doses of UV-B enhance drought stress tolerance and mitigate adverse morphological effects in stressful environments (Hamid and Jawaid, 2011).

Consistent with the previous research, cowpea plants showed improvement in performance upon recovery from drought stress. There

were no visible symptoms of leaf wilting in the recovered plants. But there was no significant regrowth within the 4 d of recovery and this is in accordance with the findings of Ahmed et al. (2022), wherein some genotypes exhibited regrowth only a few days after recovery; nevertheless, their regrowth was minimal. It was found that, upon rehydration, the plant growth resumed at a level same as that of control plants in the wild species of sesame. The complete recovery of the wild species following rewatering after drought indicates its resilience capacity (Jeyaraj et al., 2024). Earlier investigations indicate that plants capable of enduring dehydration of their vegetative tissues, particularly those classified as resurrection plants, can regain their full metabolic activity following rehydration (Dinakar and Bartels, 2013; Oliver et al., 2020).

5.3.3 Osmotic adjustments

Maintaining optimal water conditions is essential for plant survival in arid areas. The buildup of nontoxic, compatible solutes in plant cells for osmotic adjustment (OA), is a significant characteristic linked to the maintenance of higher cell turgor potential and water retention during drought stress (DaCosta and Huang, 2006.). Osmolality was observed to increase during drought stress, with a further enhancement in primed plants subjected to drought (Aswathi et al., 2023). In the present study, osmolality was found to be increased during drought stress, with a maximum enhancement observed in the tolerant cowpea. Maintenance of osmoticum was mainly attained through the priming mediated accumulation of osmolytes. Osmolality decreased in both the cowpea varieties during recovery from drought stress with a greater reduction observed in PGCP 6. The alleviation of drought stress through rewatering was associated with a reduction in osmolality (Sen and Puthur, 2021). Osmotically active solutes associated with osmotic adjustment (OA) comprise amino acids (e.g.,

proline), ammonium compounds (e.g., glycine betaine), sugars (e.g., glucose, fructans, sucrose), polyols (e.g., mannitol), inorganic ions (e.g., potassium), and organic acids (e.g., malate) (Zhang et al., 1999; Chaves et al., 2003). Osmotic adjustment-induced turgor maintenance, facilitates the cell elongation under water deficit. Besides turgor maintenance, the accumulation of these solutes is linked to the preservation of membrane and protein structures, protection against oxidative damage, and enhanced structural stabilization under water deficit conditions. OA may also facilitate drought avoidance as indicated from its correlation with improved root development and soil water extraction (Nguyen-Queyrens and Bouchet-Lannat, 2003). Leaf OA exhibits a favourable correlation with the drought tolerance of multiple species. Besides it's drought tolerance potential, OA also aids in the recovery from drought stress (Chaves et al., 2003). Elmi and West (1995) proposed that OA-enhanced turgor maintenance augmented the viability of meristematic and leaf elongation zones in tall fescue, crucial for survival and recovery after drought stress.

The present study indicates that drought stress exposure led to an elevation in total soluble sugars content in cowpea, facilitating improved osmotic adjustment of dehydrated cells. A similar finding was noted in the research conducted by Hakimi et al. (1995), wherein they discovered a positive correlation between the buildup of sugar and the RWC as well as the ability of the plant to withstand stress. Sugar accumulation was higher in response to all three priming treatments, indicating its potential significance in developing osmoticum. Seed priming with BABA, PEG, and UV-B enhanced the levels of free amino acids, proline, soluble sugars, and soluble proteins in rice, maize, and mung bean (Zhang et al., 2015; Jisha and Puthur, 2016; Thomas and Puthur, 2019). Osmolyte buildup induced by priming, especially sugars, was crucial in enhancing osmotic stress tolerance in cowpea by preserving cell turgor pressure. Sugars safeguard cells from

osmotic stress via two ways. In dehydration, hydroxyl groups of sugar may replace water for preserving hydrophilic interactions in biomembranes and plant cell proteins. Also, by the formation of hydrogen bonds, sugars inhibit protein denaturation under conditions of water stress. Secondly, sugars play a crucial role in vitrification, which is the development of a biological glass within the cytoplasm of dehydrated cells (Mohammadkhani and Heidari, 2008).

Soluble sugars are crucial in plant metabolism as hydrolytic products, serving as substrates in biosynthetic processes, energy sources, and playing a role in sugar sensing and signalling mechanisms. Elevation in the contents of sucrose and/or reducing sugars have been reported in different plants under drought (Chaves et al., 2003). The availability of sugar and its delivery to various plant tissues serve numerous critical functions: including an osmotic role, providing energy to cells that respond to stress, and supporting the growth of sink tissues. Moreover, they influence sugar-sensing mechanisms that govern the expression of genes associated with photosynthesis, respiration, maturation and storage (Koch, 2004). It was also suggested that sugar flux may function as a signal for metabolic regulation (Mohammadkhani and Heidari, 2008). In line with these findings, the present study also revealed the accumulation of total soluble sugars in cowpea complementing drought stress tolerance. On the contrary, the research conducted by Uddin et al. (2021) revealed that sugar, proline, and protein levels drastically reduced with prolonged drought stress during the vegetative phase in mungbean. This could be resulting from the collapse of metabolic functions, leading to death of the plant.

The present work demonstrates that drought stress lead to an increased production of total free amino acids. The buildup of amino acids induced by priming may initiate the synthesis of stress-related proteins (Sen

et al., 2022). In addition, plants accumulate free amino acids in response to stress for osmotic adjustment. Osmotic stress induces augmented amino acids buildup due to the priming effects of BABA, PEG and UV-B, facilitates improved water absorption and thereby aid in alleviation of drought stress. The accumulation of certain amino acids in plants, including γ-amino butyric acid (GABA) and proline during drought stress, can mitigate drought induced damage by scavenging harmful ROS or preserving cellular water balance and membrane stability through osmotic adjustment (Farooq et al., 2009). Study of Chapman et al. (2022) found that, active amino acid assimilation and the ensuing post drought regrowth may have been influenced by the accumulation of nitrogen rich glutamic acid, proline, and GABA. The findings suggest that proline or GABA were most successful in increasing the drought stress tolerance of creeping bentgrass. Hence, endogenous amino acids, such as GABA, proline, alanine, and glutamic acid, can be utilised as components to enhance drought stress tolerance and post stress recovery in cool-season turfgrass species, as well as biomarkers for the selection of drought-tolerant plants. BABA treatment was reported to increase transcripts associated with proline biosynthesis in plants (Bengtsson et al., 2014; Quero et al., 2015). Previous study demonstrated that the exogenous application of BABA (1 mM) through the rooting media assisted *Vicia faba* plants in sustaining RWC, and thus alleviating the detrimental effects of drought stress. The rise in RWC mainly results from a net accumulation of proline and soluble sugars in BABA primed plants, highlighting the role of BABA in adjusting the osmotic potential of plant cells (Abid et al., 2020).

Augmented proline buildup resulting from drought stress is recognised as an adaptation associated with stress tolerance, playing a crucial role in osmotic regulation. This present study indicates that drought stress exposure led to an elevated proline content in seedlings of cowpea

emerged from primed seeds, facilitating improved osmotic adjustment in dehydrating cells. In the study of Uddin et al. (2021) in mung bean it was found that the osmoprimed plants exhibited significant buildup of osmolytes, particularly in those subjected to a 7 d drought. These osmolytes including proline and carbohydrates contribute positively towards enhancing plant resilience to drought stress. Seed priming enabled the seedlings to sustain balanced osmolality within the cells, aiding them to withstand osmotic stress without impairing normal metabolic functions. According to the current study, seed priming facilitated the accumulation of proline, total soluble sugars, and total free amino acids, hence enhancing tolerance through the regulation of ROS detoxification and the maintenance of osmotic balance in cowpea, with a more pronounced effect in the tolerant variety (PGCP 6) compared to the sensitive variety (Anaswara).

Under extreme drought conditions, proline levels increased significantly due to higher biosynthesis, suggesting its potential role as an osmoprotectant and downregulated during the recovery period (Hayat et al., 2012). Proline metabolic reactions to drought and recovery are directly correlated with the severity of drought. Consequently, the significant buildup of proline is likely a crucial mechanism for fast recovery from severe drought (An et al., 2013). Besides its known functions in enhancing plant resilience during drought and serving as energy, carbon, and nitrogen sources for recovery, significantly accumulated proline can also facilitate the repair of plant damage by up-regulating antioxidant activity during recovery from severe drought stress (Jurkonienė et al., 2023). In agreement with the previous findings, proline content increased during drought stress in both the cowpea varieties studies with a higher increase noted in primed PGCP 6 subjected to drought indicating its innate tolerance potential and subsequently the maximum recovery was also observed in the same. Among the priming treatments, BABA priming exhibited the highest increment in

proline contents of both Anaswara and PGCP 6. The accumulation of proline is mostly due to its synthesis from glutamic acid and ornithine, catalyzed by Δ1-pyrroline-5-carboxylate synthase (PCS) and ornithine-δ-aminotransferase (OAT), respectively, and its breakdown, which is catalyzed by proline dehydrogenase (PDH) (Verbruggen and Hermans, 2008). The research by Wang et al. (2023b) revealed that BABA treatment significantly increased the expression of PCS and OAT genes while reducing the gene expression of PDH, hence augmenting proline content levels. From the present result, it can also be found that there is an increase in proline and reduction in chlorophyll contents in cowpea at the time of drought stress. This may be because glutamate is a common precursor for both proline and chlorophyll synthesis (Jawahar et al., 2019).

The elevation of total soluble proteins under drought stress may be due to the de novo synthesis of stress proteins driven by augmented amino acid biosynthesis. Azevedo et al. (2010) proposed that the augmented buildup of proteins may be attributed to either increased de novo synthesis or the inhibition of protein breakdown. Concomitant with our finding, Lyon et al. (2016) noted that the drought-responsive proteins in *Medicago truncatula* returned to baseline levels after rewatering, whereas several novel root and shoot proteins emerged after rewatering. There was an increase in total soluble proteins of Anaswara and PGCP 6 (129 and 134% respectively) upon seed priming with UV-B, and it can be correlated with the findings of Thomas et al. (2019), wherein they have reported that the heat shock protein (*HSP*) gene substantially expressed in UV-B primed rice seedlings under stress conditions. The accumulating HSP will be contributing towards the increase in protein content of seedlings exposed to UV-B priming. Furthermore, the HSPs would inhibit protein breakdown during stress, resulting in enhanced protein accumulation in UV-B primed plants exposed

to stress. There was a positive correlation between protein content and osmolality in Anaswara during drought stress ($r=0.993$ and $p\leq0.01$).

Conforming with the present findings, various researchers have found that BABA priming activates particular biochemical and physiological pathways connected to defence responses and the expression of stress-related proteins during stress exposure and it was also found that, pre-exposure to priming agents such as BABA/GABA induced the expression of certain mRNA binding protein precursors, as evidenced by the protein profile of leaves from primed potato plants (Arasimowicz-Jelonek et al., 2013). These RNA-binding proteins regulate several developmental and defensive pathways, likely by modulating multiple molecular processes such as splicing, polyadenylation, RNA stability, and RNA export through chromatin modification (Lorkovic, 2009). This aligns with the present study, wherein the highest protein accumulation was noted in the plants emerged from BABA primed seeds. Proteins produced under stress conditions may also serve as a nitrogen reservoir that is re-utilized during post stress recovery and plays a crucial role in osmotic adjustment (Parida et al., 2007), this facilitates the quick recovery of primed plants at the time of rewatering.

5.3.4 Photosynthetic performance

Drought stress drastically reduced the chlorophyll content in cowpea with a higher reduction observed in non-primed sensitive variety. The probable causes for the decrease in chlorophyll content may be due to the inhibition of chlorophyll biosynthesis enzymes and/or accelerated breakdown of pigment molecules. In accordance with this, El-Samad et al. (2011) observed chloroplast degradation and instability of pigment-protein complexes under osmotic stress. Moreover, it was revealed that the decrease in chlorophyll content was attributed to the generation of ROS, resulting in lipid peroxidation of membranes and subsequent chlorophyll degradation

(Shivakrishna et al., 2018). In the current work, least reduction in chlorophyll content during drought was noted in BABA primed PGCP 6 and this can be correlated with the efficient detoxification of ROS by antioxidants in these plants. Additionally, previous studies reported that BABA treatment induced the production of GABA and GABA can be converted into succinate through the action of γ-amino butyrate transaminase and succinate semialdehyde dehydrogenase. Succinate participates in the tricarboxylic acid cycle to sustain the carbon-nitrogen cycle in plants, referred to as the GABA metabolic bypass. Studies reported that the activation of the GABA shunt allows GABA to yield metabolic intermediates such as succinate, essential for the synthesis of chlorophyll precursors. This facilitates enhanced photosynthetic efficiency, supported by elevated chlorophyll content (Salah et al., 2019). As compared to the non-primed plants of Anaswara and PGCP 6, the reduction in total chlorophyll content of PEG and UV-B primed plants were also less during drought stress. Similar role of priming for improved photosynthetic performance by increasing the photosynthetic pigments were reported by many researchers (Arafa et al., 2021; Uddin et al., 2021).

At the time of recovery there was a prominent enhancement in the total chlorophyll contents in both the cowpea varieties with a prominent increase noted in PGCP 6. This improvement can be due to the reactivation of the chlorophyll biosynthesis, which significantly augments light energy absorption and utilization in leaves of cowpea, hence facilitating the photosynthetic recovery. The maximum rate of chlorophyll restoration was observed in BABA primed PGCP 6 (85%). This could be because, the chlorophyll biosynthesis may not be significantly disrupted during drought in BABA primed plants and could recoup easily from stress. From the findings it is clear that, as the stress-induced damage was reduced in primed plants, effective recovery could happen following rewatering.

5.3.4.1 Chlorophyll *a* fluorescence

Chlorophyll fluorescence parameters is a useful marker for evaluating the impacts of stressors on primary photosynthetic chemistry (Strasser et al., 2000; Kalaji et al., 2016). Measurements of chlorophyll *a* fluorescence provide a simple, non-invasive, cost-effective, and rapid approach for analyzing light-dependent photosynthetic activities in plants (Kalaji et al., 2016). The impact of drought stress in cowpea was studied by assessing various chl *a* fluorescence parameters such as minimum fluorescence (Fo), maximal fluorescence (Fm), Area (area over fluorescence induction curve), Fv/Fo (the efficiency of water splitting complex), and energy flux parameters such as absorption flux per cross section (ABS/CSm), trapped energy per cross section (TRo/CSm) electron transport flux per cross section (ETo/CSm), and dissipated energy flux per cross section (DIo/CSm). A prominent reduction in Fm, Fv/Fo, ABS/CSm, TRo/CSm, ETo/CSm and PIabs were noted in cowpea plants subjected to drought stress. Likewise, PHI(Po), PSIo, and PHI(Eo) were considerably reduced during drought stress. However, the impact was less pronounced in plants emerged from seeds primed with BABA, PEG, and UV-B.

Reduction in the area above the fluorescence induction curve indicated the impact of drought stress and recovery on the photosynthetic apparatus and is linearly correlated with the pool size of electron acceptors, such as plastoquinones, located on the reducing side of photosystem II. The plants exposed to drought stress exhibited a reduced photosynthetic rate due to the obstruction of electron transfer from the reaction center to the electron acceptor pool (Strasser et al., 2004). There was an increase in Fo and a decrease in Fm in cowpea plants exposed to drought stress. The Fm value decreased in cowpea exposed to drought stress, with the most significant drop observed in the non-primed sensitive cowpea. A phenomenon leading

to the elevation in Fo, potentially attributable to the incomplete oxidation of the reduced plastoquinone acceptor (Q_A^-), caused by the impediment of electron flow through PSII, or the dissociation of light-harvesting Chl a/b protein complexes from the PSII core complex. The reduction of Fm may be linked to processes involving reduced activity of the water-splitting enzyme complex and maybe concurrent cyclic electron transport within or surrounding PSII (Zlatev, 2009). The least reduction in Fm was observed following BABA, PEG, and UV-B priming, attributed to the effective harvesting and transferring of trapped energy to the Z pathway, hence enhancing the photosynthetic rate.

Multiple abiotic challenges can inflict significant damage on the photosynthetic apparatus, with PSII being the most susceptible component that endures the primary impact of these stresses. Excessive production of ROS impairs the photosynthetic machinery, particularly PSII, leading to photoinhibition due to an imbalance in the photosynthetic redox state and the suppression of PSII repair (Sasi et al., 2018). The decline in chlorophyll content, and partial disintegration of the thylakoid membrane lead to diminished PSII activity under stress circumstances in cowpea. In plants experiencing drought stress, the activity of PSII was significantly diminished due to the decreased oxygen evolution, which was associated with the substantial accumulation of ROS in thylakoid membranes, hence impeding oxygen evolution at the oxygen-evolving complex (OEC) of PSII, which can get disintegrated even at the onset of drought stress (Mahajan and Tuteja, 2005; Gupta, 2020). The current findings indicated that seed priming influenced the primary photochemistry by improving the efficiency of the water-splitting complex at the donor side of PSII (Fv/Fo). A substantial augmentation in the Fv/Fo ratio, was noted in with BABA, PEG, and UV-B primed plants of both the cowpea varieties. The enhancement in the efficiency of Fv/Fo on the donor side of PSII may be ascribed to the priming,

which likely improved the PSII complex's efficacy in conducting primary photochemistry. Previous findings also indicated the beneficial role of priming for improved Fv/Fo. It was found that glutamic acid primed plant demonstrated a prominently elevated Fv/Fo ratio, corroborating the observation that glutamic acid treatment resulted in substantially increased biomass and chlorophyll (Quan et al., 2022). The present results also revealed that the elevated Fv/Fo in the primed plant is due to the increased chlorophyll content and improved photosynthetic efficiency of primed cowpea.

During drought stress exposure, all photosynthetic yield parameters were reduced in both the cowpea varieties, although primed plants were less adversely affected. The decrease in quantum yield (PHI(Eo)) was associated with the suppression of electron transfer to PQ (Li et al., 2010). PIabs is the comprehensive measure of absorption, trapping, and conversion of enegy during the electron transport processes. This parameter is very sensitive to most environmental stress conditions (Oukarroum et al., 2007; Strasser et al., 2000). Drought stress has been reported to inactivate the reaction centers and diminish the performance index. The study indicates that the decline in PIabs is attributed to a reduction in the density of active reaction centers, alongside an increase in dissipated energy flux and antenna size. Moreover, the decline may also result from reductions in energy flux and electron transport per cross section. This signifies that the reduction of quantum yield [PHI(Eo)] caused by drought is also linked to the suppression of electron transfer from Q_A^- to Q_B to PQ. At the same time the electron transfer was least affected in primed plants signifying the role of seed priming in protecting the photosynthetic machinery from oxidative damage caused by drought stress.

The more pronounced reduction in ABS due to drought stress indicates the reduced energy absorption efficiency of PSII in cowpea under drought stress conditions. Drought stress results in the inactivation of active reaction centres in cowpea plants or reduced the frequency of active reaction centers in a cross-sectional area, resulting in a lower ABS/CSm ratio. Together with this, the reduction in energy absorption by chlorophyll molecules is attributed to a decrease in the size of the PSII antenna or a structural alteration in the antenna LHC component (Tongra et al., 2011). It influences the photochemistry of cowpea by decreasing the rates of photon trapping (TR) and electron transport (ET).

Drought-stressed cowpea plants had higher DIo/CSm due to inefficient use of absorbed light energy for electron transport in their reaction centers. DIo/CSm increases when the number of inactive reaction centers increases and electron transport decreases due to a drop in the functional Q_A pool (Kalaji et al., 2011; Faseela et al., 2020). The inactive reaction centres fail to effectively capture photons; hence, there is an elevation in the quantity of untrapped photons, which subsequently results in the increase of DIo/RC (Mathur et al., 2013). Seedlings derived from BABA, PEG and UV-B primed seeds exhibited enhanced energy absorption, photon trapping, and electron transport, indicating an augmentation in the quantity and/or functionality of reaction centres, even during drought stress and hence the DIo/CSm was less. In agreement with the findings, various priming strategies have demonstrated potential in this regard, as seen by studies with *Pisum sativum* and *Triticum aestivum*, among others (Szafranska et al., 2016; Ambreen et al., 2021).

Tolerant cultivars exhibited superior responses compared to sensitive ones by protecting the photosynthetic apparatus through diminished ROS generation and enhanced antioxidant mechanisms and osmotic equilibrium.

The research by Sen and Puthur (2020) corroborates these findings, indicating that seed priming in rice enhances the tolerance capacity of tolerant varieties more significantly than that of sensitive varieties by effectively scavenging ROS and thus safeguarding biomembranes and photosynthetic performance under drought stress, session 5.3.12 provides a detailed explanation. Seed priming markedly diminishes photoinhibition and safeguards chloroplast functionality in cowpea subjected to drought stress, hence promoting rapid recovery upon rehydration. BABA, PEG, and UV-B priming facilitates the swift restoration of photosynthetic efficiency in cowpea, providing necessary support for the tolerance strategy. Seed priming can substantially alter the size of antennae in cowpea seedlings during recovery, enhancing energy absorption and utilization, hence increasing the photosynthetic rate and accelerating recovery upon re-watering. Consistent with the finding, prior research revealed that, UV-B and halopriming, facilitates the swift restoration of photosynthetic efficiency in rice seedlings upon recovery, providing convincing support for the tolerance strategy (Sen and Puthur, 2021).

5.3.4.2 Photosystems activities

The activities of photosystem I and II in cowpea plants were reduced during drought stress, due to the generation of ROS in response to drought, that adversely affects chlorophyll and chloroplast structures. The significant damage to PSI and PSII, including disruptions in the electron transport system in cowpea, clearly indicates an increased rate of $O_2^{\bullet-}$ radical generation during drought stress. ROS attack on the D1 protein of PSII and its subsequent degradation results in photoinhibition (Saha et al., 2022). The current study is consistent with the previous findings, and the PSII activity was affected more than the PSI activity, which could be attributed to D1 protein damage occurred during drought exposure. There was a significant

negative correlation between PSII activity and H_2O_2 in PGCP 6 (r=-0.999 and p≤0.01) exposed to drought. Also, during drought stress there was negative correlation between PSII activity and $O_2^{\bullet-}$ (r=-0.971 and p≤0.05) and PSI activity and H_2O_2 in Anaswara (r=-0.963 and p≤0.05). Previously researchers have reported that the photosynthetic genes were downregulated in drought-stressed plants. The expression of chlorophyll genes *OsPsbD1* and *OsPsbD2* was downregulated in rice subjected to drought stress (Teng et al., 2014). The transcriptional regulation of chloroplast genes is crucial for plant resistance to environmental stress, as it mediates chlorophyll biosynthesis and influences metabolism, including Chl synthetase (*CHLS*), Mg-protoporphyrin IX methyltransferase (*MPM*), ferrochelatase (*FC*), pchlide oxidoreductase (*PO*), protoporphyrinogen oxidase (*PPO*), and the D, H, and I subunits of magnesium chelatase (*MCD*, *MCH*, and *MCI*) (Liu et al., 2012; Niedzwiedzki et al., 2016).

Current finding has shown that the priming of cowpea seeds with BABA, PEG and UV-B enhances photosystem I and II functions. In accordance with this, the study of Wang et al. (2018) revealed that the chloroplast *psbA* and *psbD* transcription increased during drought stress in wheat primed with 5-aminolevulinic acid, which improved photosynthesis. Alternatively, Thomas and Puthur (2020) found that seed priming with UV-B stimulated the production of photo-protective compounds like anthocyanin, which enhanced ROS scavenging and plant defense. In accordance with this, the current study found that seed priming increased the accumulation of anthocyanin and flavonoids, which detoxify ROS and protect photosystems from oxidative damage. BABA, PEG, and UV-B priming enhanced the photosystem activity of both cowpea varieties subjected to drought, with tolerant varieties exhibiting better responses compared to sensitive ones by protecting the photosynthetic apparatus through reduced ROS production, improved antioxidative mechanisms, and

better osmotic balance. Seed priming markedly reduced the photoinhibition and safeguards chloroplast functionality in cowpea during drought stress, hence promoting the rapid recovery of plants upon rehydration.

5.3.4.3 Infra-red gas exchange parameters

The initial response of most plants to drought stress is the partial closure of stomata, which may become entirely closed under severe drought conditions, depending upon the plant species. Pirasteh-Anosheh et al. (2016) found that tolerant species modulate stomatal opening to facilitate efficient carbon absorption and enhance water use efficiency. The current work aligns with this observation, revealing only partial stomatal closure in PGCP 6, under drought conditions, which correlates with the drought tolerance of this variety. The beneficial effect of priming on stomatal control during stressful conditions has been established by extensive prior studies. Priming mitigates the significant decline in leaf water potential induced by water stress and allows the stomata to remain partially open even in adverse conditions. The open stomata facilitated an enhancement in light-saturated net CO_2 assimilation rate (*Pn*), stomatal conductance (*gs*), and intercellular CO_2 (*Ci*) in primed plants (Thomas et al., 2020). This aligns with our findings wherein positive regulation of stomatal opening and enhancement of net photosynthetic activity occurs as a result of priming. There was a complete closure of stomata in the non-primed sensitive variety Anaswara so as to prevent water loss, as indicated by reduced stomatal conductance alongside a corresponding decline in *Pn* and *Ci* in stress sensitive plants (Henry et al., 2019).

Complete restoration of all gas exchange and fluorescence indices were observed after four days of recovery in cowpea. Nevertheless, on the initial day following the alleviation of drought stress, assimilation rates exhibited only a partial recovery despite the presence of ambient internal

CO_2, indicating a potential non-stomatal limitation of photosynthesis in cowpea (Souza et al., 2004). According to Rivas et al. (2016), under drought stress, the leaf gas exchange and chlorophyll fluorescence parameters reduced more rapidly in the sensitive cultivar than in the tolerant cultivar. Following 48 h of rehydration, the stressed plants of both cultivars failed to attain the maximum rates of carboxylation, and electron transfer facilitating RuBP regeneration, and overall photosynthetic performance. Nonetheless, the tolerant cultivar restored all photosynthetic parameters more rapidly than the sensitive cultivar following 60 h of rehydration. This finding indicate that the tolerant cultivar sustained superior photochemical activity and leaf gas exchange even during prolonged water deficit compared to the sensitive cultivar, potentially mitigating stress impacts on the photosynthetic apparatus and enhancing recovery capacity (Rivas et al., 2016). The present works aligns with this and there was maximum recovery in the primed tolerant cowpea variety (PGCP 6). Osmolyte accumulation (OA) in plant cells causes a reduction in cell osmotic potential, resulting in the maintenance of water absorption and cell turgor pressure, which may help to support physiological processes such as stomatal opening, photosynthesis, and cell expansion (Seraj and Sinclair, 2002) This is consistent with our observations, in which osmolyte buildup was higher (previously elaborated in session 5.3.3) and stomata remained partially open even under drought stress in these plants. This promotes better gaseous exchange and improved photosynthesis during drought stress, which eases the recovery process.

5.3.5 Epicuticular wax

The cuticle is a lipid coat in plants composed of cutin and intra- and epicuticular waxes. Epicuticular wax constitutes the outermost hydrophobic layer of the cuticle and comprises a complex of very long chain fatty acids

and their derivatives, which include hydrocarbons, alcohols, wax esters, ketones aldehydes, terpenes, and flavones. The composition of this layer differs among plants, organs, and cells, and is depending upon developmental stages or environmental conditions (Jenks et al., 2000; Laskos et al., 2021). Cuticular waxes safeguard the aerial plant parts against excessive water loss and ultraviolet radiation (Bourdenx et al., 2011). It functions as a protective sunscreen in plants, offering a shielding capacity in leaves to reduce excess water loss.

The cowpea variety PGCP 6 exhibited increased wax deposition, which may confer improved tolerance to drought stress. Studies revealed that, wax accumulation under drought stress is associated with the tolerance mechanisms of plants (Xue et al., 2017). PGCP 6, being tolerant, exhibited a greater accumulation of wax content compared to sensitive variety Anaswara subjected to drought stress, hence enhancing its ability to mitigate water loss more efficiently. This finding was corroborated by previous studies, which indicated that increased deposition of epicuticular wax on leaf surfaces of several plants protects against excessive water loss by transpiration and serves as a significant factor in drought tolerance (Wijewardana et al., 2016). The buildup of epicuticular wax was higher in plants emerged from UV-B primed seeds, subjected to drought stress and there was an increase of 84% and 90% in UV-B primed Anaswara and PGCP 6 respectively exposed to drought. This is in conformity with the observations of Fukuda et al. (2008), wherein they found increased deposition of wax in the cotyledons subjected to medium dose of UV-B. Priming of seeds with BABA and PEG did not significantly enhance wax deposition on the leaves of either variety, indicating that wax production is a rapid response to drought stress (Premachandra et al., 1991), and BABA and PEG priming do not further enhance the rate of wax synthesis in cowpea.

Cuticular waxes have a complex chemical composition and a tubular or planar shape which vary with the plant species. Plant cuticular waxes consist of long chain aliphatic groups, including hydrocarbons (mostly n-alkanes), fatty acid esters and fatty alcohols, primary and secondary alcohols, aldehydes and free fatty acids (Koch et al., 2006). FTIR spectra of epicuticular wax in the leaves of UV-B primed plants showed peaks in the range of 3000-2850 cm^{-1} and 1740-1720 cm^{-1}, and it was same as the observations in rice exposed to UV-B priming (Thomas and Puthur, 2020). Significant peaks at 1027, 1093, 1600, 2350, 2850, 2900, and 3400 cm^{-1}, indicative of C–O stretching of alcohols, aromatic ring stretching, and C=O stretching in carbonyl groups, and were seen in UV-B primed plants exposed to drought stress conditions. The peaks ranging from 2819 to 3034 signify the presence of a cuticular lipid region. In comparison to the control, additional absorption peaks were observed mostly in UV-B primed and drought-stressed cowpea plants. Similar to observation of this study, Thomas and Puthur (2019) identified additional absorption peaks in the epicuticular wax of rice, predominantly in leaves of rice subjected to UV-B primed stressed conditions and non-primed UV-B stress conditions. They found four distinct peaks in the epicuticular wax from seedlings emerged from UV-B primed seeds and subjected to PEG stress, within the range of 3000–2850 cm^{-1}.

In another study conducted by Khambatta et al. (2021), significant asymmetric vibration (CH_2) at 2930 cm^{-1} and symmetric vibration (CH_2) at 2848 cm^{-1} were detected, and in line with this there were peaks at these regions corresponding to the symmetrical and asymmetrical stretching of methylene groups and it was strong in UV-B primed and drought-stressed leaves in contrast to control leaves. The presence of aromatic compounds in the wax is indicated by the peak at 1093 cm^{-1}, suggesting the accumulation of the same in the epicuticular wax of cowpea. Liu et al. (2020) studied

Arabidopsis leaf cuticular wax and suggested that, FTIR spectroscopy is a valuable tool for understanding the cuticle chemistry under drought stress and thereby helps to identify tolerance response of plants towards drought. They found changes in the integrated area of symmetrical and asymmetrical CH_2 peaks, CH_3 peak and ester carbonyl peak. In the present study modifications in FTIR peaks indicates the changes in wax composition associated with UV-B priming and drought stress in both cowpea varieties.

Epicuticular wax blooms are found on leaf surfaces in various patterns and chemical compositions, depending on the plant type. In this study, SEM examination revealed visible differences in the density of epicuticular wax crystals of cowpea produced under well-watered and drought-stressed conditions. It was found that the drought tolerant variety had higher wax crystal density on its adaxial surfaces. In line with this, study of Willick and co-workers (2018), observed the highest wax crystals on the leaves of tolerant wheat. Cuticular wax can maintain high water potential, PSII activity, photosynthetic rate and grain yield in glaucous cultivars compared to non-glaucous cultivars during drought stress. This suggests that leaf cuticular wax content can be a useful selection criterion for developing drought-tolerant cultivars (Guo et al., 2016). FTIR spectra and SEM analysis offered additional evidence supporting the accumulation and biochemistry of epicuticular wax in cowpea leaves under drought stress, indicating that the increased wax accumulation in PGCP 6 enhanced its stress tolerance potential relative to the sensitive variety.

5.3.6 Oxidative stress indicators

A small part of the oxygen within the plant is converted into ROS, notably superoxide radical ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH$), as a result of aerobic metabolism within various cellular organelles, including chloroplasts and mitochondria. The

regulatory network of enzymatic and non-enzymatic antioxidant mechanisms maintains ROS within plant cells at non-damaging levels. Under stress conditions, the generation rate of ROS escalates drastically, beyond the capacity of antioxidant scavengers, triggering an oxidative burst that impacts biomolecules and disrupts cellular redox equilibrium. ROS function as a double-edged sword; when present below a certain threshold, they facilitate redox signalling pathways that promote plant growth, development, and stress acclimatisation. Hence, the generation of ROS in plant cells exhibits both harmful and advantageous consequences (Sachdev et al., 2021).

Exposure to drought stress results in the accumulation of ROS in several plant species, which are harmful to plants (Alam et al., 2022). This increased accumulation of toxic byproducts causes membrane damage, DNA fragmentation, enzyme inhibition, and programmed cell death (Quan et al., 2008; Verma et al., 2019). The current study shown that plants subjected to drought stress exhibited a rise in H_2O_2 and $O_2^{\bullet-}$ levels, leading to higher electrolyte leakage. Nonetheless, a decrease in ROS levels and EL% was observed in primed plants, indicating that a clear system for scavenging ROS is functioning in these plants and thus reducing electrolyte leakage. Prior research on rice has indicated a decrease in lipid peroxidation following UV-B and halopriming. This resulted from the priming induced augmentation of ROS scavenging enzymatic and non-enzymatic antioxidants (Sen et al., 2022). Similar increase in antioxidative process is functional here too, which is detailed in 5.3.7 and 5.3.8.

The current study shows that the Anaswara variety exhibited the highest accumulation of H_2O_2 and $O_2^{\bullet-}$, when subjected to drought, likely due to its sensitive nature. Stress-induced production of H_2O_2 and $O_2^{\bullet-}$ leads to lipid and protein peroxidation of biological membranes, resulting in

increased MDA content in cowpea, which was more pronounced in Anaswara compared to the tolerant variety PGCP 6. The excessive production of ROS such as H_2O_2, $O_2^{\bullet-}$ and hydroxyl radicals (OH^-) can disrupt normal metabolism by causing chlorophyll degradation, membrane lipid peroxidation, protein carbonylation, and inactivation of –SH containing enzymes during stress (Basu et al., 2010). Pronounced reduction of $O_2^{\bullet-}$ during drought stress was observed in UV-B primed plants of Anaswara and PGCP 6, this can be attributed to the higher activity of superoxide dismutase, which detoxifies superoxide radicals. Whereas the accumulation of H_2O_2 was least in BABA and PEG primed plants of both the varieties, which implies the effective detoxification of H_2O_2 via the robust antioxidant system in primed plants.

Augmented ROS levels in plants exposed to drought stress causes oxidative cellular damage that disrupts redox potential. Excess H_2O_2, $O_2^{\bullet-}$ produced alter the integrity of membrane, resulting in enhanced peroxidation of proteins and lipids, which leads to increased membrane fluidity and electrolyte leakage. Elevated electrolyte leakage indicates oxidative damage to membrane lipids and macromolecules such as DNA, RNA and proteins. Nonetheless, BABA, PEG, and UV-B priming reduced the rate of lipid peroxidation as the production of H_2O_2, $O_2^{\bullet-}$ under drought stress was less in both the cowpea varieties. Furthermore, seedlings derived from primed seeds can sustain cellular redox equilibrium, facilitating faster recovery.

According to previous studies, BABA priming decreased the electrolyte leakage, malondialdehyde content, and ROS generation, which in turn decreased oxidative stress-induced cell membrane damage (Mohamadi et al., 2017; Abdulbaki et al., 2024). In line with this there was a significant reduction in lipid peroxidation rate and electrolyte leakage in cowpea plants

emerged from BABA primed seeds with the highest reduction observed in PGCP 6. Priming protects biological membranes from drought stress, resulting in fewer injuries to cellular structures and higher photosynthetic efficiency by protecting photosynthetic machinery, which results in improved photosystem activity and a higher net photosynthetic rate, as discussed previously in session 5.3.4. Likewise, seed priming with PEG and UV-B significantly mitigated the detrimental impacts of drought, as demonstrated by the improved membrane integrity indicated by decreased electrolyte leakage and reduced levels of H_2O_2, $O_2^{\bullet -}$, and MDA.

Retention of membrane stability and integrity under abiotic stress is a fundamental component of stress tolerance in plants. The membrane stability index (MSI) is a physiological metric extensively utilised to assess stress tolerance. Oxidative damage disrupts membrane integrity, resulting in enhanced ion conductivity (İşeri et al., 2014; Khoshbakht et al., 2018). Diverse stress conditions impact membrane stability via lipid peroxidation, resulting in the generation of peroxide ions and malondialdehyde (MDA). Consequently, variations in MDA concentration and electrolyte leakage serve as reliable markers of membrane structural integrity under stress conditions (Sanchez-Reinoso et al., 2014). In line with this studies many other researchers have found that drought stress adversely impacts membrane integrity and electrolyte leakage in borage leaves and durum wheat (Ahmadizadeh, 2011; Dastborhan and Ghassemi-Golezani, 2015). In this work it was found that seed priming protects the biological membrane from oxidative stress induced by drought stress by effectively scavenging ROS and as a result least increment in MDA was observed in BABA primed plants of both the varieties.

ROS accumulation was shown to be reduced during recovery from drought stress and thus the MDA and electrolyte leakage, and this could be

due to the support of the activity of enzymatic and non-enzymatic antioxidants. As compared to the non-primed plants, the ROS accumulation reduced significantly in primed plants indicating their faster recovery. Similarly, rewatering decreases the rate of ROS-induced protein peroxidation in rice seedlings, with a more rapid effect observed in UV-B and haloprimed seedlings, leading to swift recovery from stress (Sen and Puthur, 2021). There was a significant positive correlation between H_2O_2 and electrolyte leakage ($r=0.997$ and $p\leq0.01$) and negative correlation between $O^{\bullet-}$ and MSI ($r=-0.997$ and $p\leq0.01$) of PGCP 6 during drought exposure. In Anaswara, H_2O_2 was positively correlated with electrolyte leakage ($r=0.985$ and $p\leq0.05$), and negatively correlated with MSI ($r=-0.979$ and $p\leq0.05$). As per Upadhyaya et al. (2008), the tolerant cultivar of tea (*Camellia sinensis*) exhibited reduced ROS levels and enhanced antioxidant recovery upon rewatering after drought stress, hence indicating greater recovery capability. In line with this, the present study indicated that decreased ROS levels in tolerant cowpea during recovery from drought stress facilitated a rapid restoration of physiochemical activities upon rewatering, especially in plants emerged from primed seeds and there was higher membrane stability index and lower electrolyte leakage and lipid peroxidation rate in primed cowpea.

5.3.7 Enzymatic antioxidants

Antioxidant enzymes, such as superoxide dismutase, catalase and peroxidase (POD), play a crucial role in cellular defence and prevention against oxidative damage. These enzymes are extensively present in plant cells and are crucial in the detoxification of ROS from chloroplasts (Wu et al., 2019). Peroxidases exhibit reducing action at their active sites via cysteine residues. These enzymes lack a prosthetic group and facilitate the reduction of H_2O_2, peroxynitrite, and other organic hydroperoxides to their respective alcohols (Uddin et al., 2021). Superoxide dismutase (SOD) is a

metalloenzyme family member found universally in all aerobic species. During environmental stress, SOD serves as the primary defence against damage caused by ROS. SOD facilitates the removal of $O_2^{\cdot-}$ by converting them into O_2 and H_2O_2 (Fatima and Ahmad, 2004; Liochev and Fridovich, 2007). The study of Uddin et al. (2021) have shown that peroxidase and SOD activity considerably enhanced under drought stress in all osmoprimed plants compared to non-primed plants.

In the present study the activities of antioxidant enzymes in cowpea derived from primed and non-primed seeds were assessed under both unstressed and stressed conditions to elucidate the mechanism of priming-induced detoxification of ROS. The activity of SOD was higher in UV-B primed PGCP 6 during drought stress, and this correlates with the highest reduction of $O_2^{\bullet-}$ in UV-B primed plants, as the SOD accelerated the conversion of $O_2^{\bullet-}$ into H_2O_2. A strong negative correlation between SOD and $O_2^{\bullet-}$ was observed in drought-stressed plants of Anaswara ($r=-0.998$, $p\leq0.01$) and PGCP 6 ($r=-0.993$, $p\leq0.01$). This is in conformity with the findings of Thomas et al. (2020), wherein they found that UV-B primed seedlings exhibited a modest increase in the expression of SOD, CAT, and APX in the absence of stress conditions. This phenomenon is attributed to the baseline activation of antioxidant enzymes induced by UV-B priming, highlighting the significance of UV-B priming in the initiation of enhanced expression of genes encoding antioxidant enzymes. It was also reported that the increase in $O_2^{\cdot-}$ due to supplementary UV-B radiation induced de novo synthesis of SOD to preserve the metabolic stability of rice plants (Wang et al. 2015). This could explain the increased SOD induction in UV-B primed cowpea varieties in the current study. Highest increase of SOD activity (179%) was recorded in UV-B primed PGCP 6 followed by an increase of 170% in UV-B primed Anaswara, which facilitated the effective scavenging of superoxide radicals in primed plants subjected to drought. In line with

the current study, prior research have found increased expression of the *Cu/Zn SOD* gene in UV-B primed plants under both stress and non-stress conditions (Thomas et al., 2020), highlighting the significance of priming for the activation of antioxidant gene expression and a subsequent reduction in the content of ROS.

Plants possess H_2O_2-metabolizing enzymes, including CAT, APX, some peroxiredoxins, glutathione/thioredoxin peroxidases, and glutathione sulfo-transferases. Nevertheless, the most prominently recognised enzymes are CAT and APX, as the former predominantly exists in peroxisomes and does not need a reductant for the catalyzation of a dismutation process. Specifically, APX exhibits a greater affinity for H_2O_2, converting it to H_2O in chloroplasts, cytosol, mitochondria, and peroxisomes, as well as in the apoplastic space, employing ascorbate as a specific electron donor (Sofo et al., 2015). Prior research has indicated that diverse seed priming techniques enhance the antioxidant enzyme activities in various crops subjected to drought stress. Osmopriming improved the activities of GPOX and CAT, decreased malonyldialdehyde (MDA) levels, and reduced electrolyte leakage under drought stress in alfalfa (Mouradi et al., 2016). Likewise, PEG priming enhanced the antioxidant activity of SOD, CAT, APX and GPOX in sorghum under drought conditions (Zhang et al., 2015). Studies found that seed priming with UV radiation markedly enhanced the activities of SOD, CAT, and APX in lettuce (Ouhibi et al., 2014), wheat (Badridze et al., 2015), and rice (Thomas and Puthur, 2019). In the present work, the activity of CAT, APX and GPOX was higher in primed cowpea exposed to drought stress, indicating a more significant function in the detoxification of H_2O_2. It is concomitant with the earlier finding which implies that, APX, glutathione peroxidase (GPX), and CAT are the primary enzymes that detoxify the hazardous H_2O_2 (Apel and Hirt, 2004). In comparison to catalase, which also metabolises H_2O_2, APX exhibits a higher affinity for H_2O_2, characterised by a

Km in the micromolar range, that is at least one hundred times greater. Consequently, despite the minimal amounts of APX, its strong affinity for H_2O_2 enables it to finely regulate ROS (De Gara et al., 2010). In the current work, the highest activity of APX was observed in BABA and PEG primed plants owing to their robust role in the detoxification of H_2O_2. A significant negative correlation between H_2O_2 and APX in PGCP 6 ($r=-0.998$ and $p\leq0.01$) and in Anaswara ($r=-0.978$ and $p\leq0.05$) under drought stress corroborate the findings. Similarly, there was a strong negative correlation between H_2O_2 and GPOX ($r=-0.999$ and $p\leq0.01$) in Anaswara and PGCP 6 ($r=-0.982$ and $p\leq0.05$) subjected to drought stress.

Seed priming enhanced the enzymatic antioxidant system in cowpea plants as a result of the initial mild stress encountered during the seed priming method. In primed seedlings, the amount of ROS exhibited a negative correlation with the activities of SOD, CAT, and APX, indicating the efficient scavenging of ROS produced during drought stress. The drought tolerance of a particular cultivar is depended upon the functionality of its antioxidative mechanisms (Basu et al., 2010). Enhanced activity of ROS-scavenging enzymes and elevated antioxidant levels associated with stress-induced oxidative stress tolerance were identified as traits of tolerant cultivars (Sreenivasulu et al., 2000). In accordance with this there was higher activity of enzymatic antioxidants in the tolerant variety (PGCP 6) than the sensitive variety (Anaswara).

Upon rewatering, the activities of SOD, CAT, GPOX and APX were downregulated in cowpea, as their role turns to be minimal due to the swift recovery of primed cowpea plants from stress. Previous works reported similar findings, noting the downregulation of the enzymatic antioxidants in rewatered plants due to a reduced requirement for the elimination of ROS (Wu et al., 2012; Jeyaraj et al., 2024).

5.3.8 Non-enzymatic antioxidants

Non-enzymatic antioxidants safeguard cells from damage by directly detoxifying ROS through the donation of electrons or hydrogen, or by reducing substrates for antioxidant enzymes. These include ascorbate, glutathione, tocopherol, carotenoids and phenolic compounds such as polyphenols and flavonoids (Gill and Tuteja, 2010). Ascorbate is the most prevalent and thoroughly researched antioxidant in plants (Arrigoni and de Tullio, 2000) and is regarded as a potent ROS scavenger due to its capacity to donate electrons in several enzymatic and non-enzymatic activities. It safeguards PSII against donor side photoinhibition by functioning as an alternate electron donor in leaves with an inactive oxygen-evolving complex (Tóth et al., 2011). It also serves as a cofactor for violaxanthin de-epoxidase, thereby dissipating excess excitation energy from chloroplasts during photoinhibition. Glutathione (GSH) is a tripeptide produced in the chloroplast and cytosol that scavenges singlet oxygen and H_2O_2, and oxidised to glutathione disulphide (GSSG) while functioning as an antioxidant and redox regulator (Sofo et al., 2010). GSH directly or indirectly participates in the detoxification of ROS in plant cells. In addition to these functions, GSH is involved in the detoxification of methylglyoxal, phytochelatins synthesis, interactions with plant hormones and other signalling molecules, and its redox status initiates signal transduction, while also serving as a cofactor in other metabolic activities (Hossain et al., 2022). Likewise, studies have found that plant phenolic compounds can also reduce ROS accumulation and serve as metal chelators, while also working synergistically with other physiological antioxidants like ascorbate or tocopherol to enhance their biological effects. The antioxidant activity of phenolics in plants under stress conditions will enhance with an increase in the quantity of free hydroxyl groups and the conjugation of side chains to the aromatic rings (Bergmann et al., 1994).

This study demonstrated that non-enzymatic antioxidants increased during drought stress in Anaswara and PGCP 6. The increased accumulation of these non-enzymatic antioxidants facilitates the detoxification of ROS, thereby significantly contributing to the regulation of lipid peroxidation and electrolyte leakage through the preservation of membrane stability. There was a significant negative correlation of ascorbate with H_2O_2 ($r=-0.988$ and $p\leq0.05$), and electrolyte leakage ($r=-0.981$ and $p\leq0.05$) and positive correlation between ascorbate and MSI ($r=0.964$ and $p\leq0.05$) in PGCP 6 subjected to drought. In Anaswara, there was a negative correlation between electrolyte leakage with ascorbate and glutathione content ($r=-0.964$ and $p\leq0.05$) under drought. The activity of different enzymes within the AsA-GSH cycle were significantly influenced by seed priming, leading to enhanced accumulation of ascorbate and glutathione levels (Dusart et al., 2019; Sen and Puthur, 2020). Seed priming further strengthened the activities of non-enzymatic antioxidants in cowpea, with a greater increase of glutathione in BABA primed (247%) PGCP 6 followed by PEG primed (242%) PGCP 6 subjected to drought stress. The increase in ascorbate content was greater with PEG priming (291%) in PGCP 6, however it was also more with BABA priming (240%) in Anaswara. The improved action of antioxidants lowered MDA content, enhances growth, improves ROS scavenging, and increases tolerance to drought stress more effectively in primed cowpea plants compared to non-primed plants.

5.3.9 Secondary metabolites

Higher accumulation of anthocyanin, flavonoids and phenolics were recorded in cowpea under drought stress with the highest accumulation observed in UV-B primed plants. Secondary metabolites are distinctive to each species and are frequently synthesised during particular growth phases (Morshedloo et al., 2017). The synthesis of secondary metabolites in response

to adverse stimuli and environmental stresses is seen as a defensive strategy to maintain equilibrium (Sun and Fernie, 2024). The accumulation of secondary metabolites during periods of stress functions as an adaptive strategy to mitigate the detrimental impacts of drought conditions (Alhaithloul et al., 2020). Under UV-B exposure, it has been found that compounds that absorb UV-B and protect against UV radiation are accumulated in plants (Jenkins, 2009; Fabón et al., 2012), and this could be the possible reason for the increased contents of anthocyanin, flavonoids and phenolics in UV-B primed cowpea.

An optimal growth of plants under fluctuating environmental conditions necessitates a balanced production of secondary metabolites. Secondary metabolites provide multiple activities, including control of enzyme activity, signalling, and defence mechanisms. Drought stress induces the production of secondary metabolites and it facilitate antioxidant mechanisms to eliminate ROS during drought stress. Secondary metabolites mitigate ROS to save plants from oxidative injuries during drought stress. Flavonoids are secondary metabolites produced via the phenylpropanoid pathway that aid in the mitigation of ROS. Flavonoids also functions as signalling molecules and detoxifying agents in plants, playing a crucial role in stress tolerance (Samanta et al., 2011). The existence of antioxidant flavonoids in the chloroplast indicates their role as scavengers of singlet oxygen and stabilisers of the chloroplast outer membrane (Agati et al., 2012). In keeping with this, it can be found that the elevated levels of flavonoids in the primed cowpea plants protect the chloroplast from oxidative stress and thereby improving the photosynthetic performance.

Anthocyanins has a photoprotective and antioxidative function in plants, accumulating in response to diverse environmental challenges that facilitate normal plant life (Zheng et al., 2021). The aromatic hydroxyl

groups and ortho-dihydroxyl group in anthocyanin can suppress free radical chain reactions and hydroxyl radicals. Anthocyanin accumulation is depending upon H_2O_2 generation and has been demonstrated to rise in response to elevated ROS production (Kou et al., 2019). The detoxification of long lived ROS like H_2O_2 is taken care by anthocyanin and the detoxification occurs in the vacuole. While transient ROS, such as $O_2^{\bullet -}$ are swiftly protonated in the cytoplasm to form the hydroperoxyl radical or dismutated by SOD to H_2O_2, both of which are electrically neutral and can easily permeate the vacuole to be scavenged by anthocyanins (Neill and Gould, 2003). In line with the above, this study demonstrated that primed plants exposed to drought stress exhibited enhanced flavonoids and anthocyanin accumulation, ensuring effective ROS scavenging and an improved photosynthetic rate.

Similarly, many researchers discovered that the total phenolic content augmented during drought stress, and there was an additional rise observed in primed plants exposed to drought conditions. In agreement with this, Mahmood et al. (2024) discovered that melatonin priming enhanced the overall phenolics content in sunflower. On the other hand, under PEG stress, the phenolic content reduced in all the seedlings emerged from primed seeds (Srivastava et al., 2010). The current study shown that drought stress conditions elevated total phenolic content in cowpea, aiding the plant in managing the stress. Seed priming facilitated the accumulation of total phenolics in seedlings for more effective tolerance towards drought stress with the highest increase in BABA and UV-B primed plants. The elevated phenolics content during drought stress serves as a reliable indicator of drought stress tolerance, as these secondary metabolites are vital to growth and development and also function as components of the cell wall. It was found that the increased amount of cell wall-bound phenolics improved cell wall tightness and reduced leaf water loss (Hura et al., 2013). Rewatering

results in a reduction of phenolic buildup in cowpea, with a more rapid effect observed in primed plants. The rapid decrease in anthocyanin and phenolics content in primed plants is a significant evidence of substantial detoxification of ROS produced during the drought period. However, the flavonoid content persisted in cowpea plants even after stress recovery, and it has got significance and effectiveness in the commencement of symbiotic association, as flavonoids are released into the rhizosphere and identified by suitable *Rhizobium* sp. for effective association and nodule formation (Cooper, 2004; Wasson et al., 2006).

5.3.10 Root and root nodule traits

In higher plants, root development is regulated by hormones and environmental factors. Cowpea has a stronger drought tolerance than other legumes and is extensively farmed in semi-arid places, and as a semi-arid crop. Therefore, this legume should have prominent root system for extracting more water even from water deficit soil and can withstand drought stress. This study shows that the selected varieties, Anaswara and PGCP 6, exhibit a robust root system under well-watered conditions, enabling them to extract more water from the deeper layers of soil. However, when the stress persisted, there was a significant reduction in lateral root growth in the sensitive and tolerant varieties of cowpea, with a greater decrease observed in non-primed plants subjected to drought stress. Still there was no much reduction in the lateral roots of primed plants of both the varieties subjected to drought stress. Similar to our findings, a more pronounced decline in lateral growth of roots was observed at 25% field capacity in sesamum, and following rehydration, lateral root development resumed in both the species (Jeyaraj et al., 2024). Earlier findings discovered that the lateral root suppression has been observed as an adaptive response

that promotes the growth of the primary root, facilitating water extraction from deeper soil layers (Xiong et al., 2006).

During drought stress there was an increase in the root length of both the primed and non-primed plants and the increment was slightly higher in the tolerant variety (PGCP 6). ABA accumulated fast in roots under water stress and the enhanced accumulation of ABA towards the root tip was essential for maintaining root elongation by its regulatory activities in ion homeostasis, osmotic adjustment and cell wall extensibility during water shortage (Sosnowski et al., 2023), In addition, ABA might play a function in controlling IAA transport in the root apex to maintain root elongation and root hair formation under water stress. In the study of Hu et al. (2018), the higher IAA and ABA contents offered the tolerant cultivar with better capability to build a longer root system to take up more water than sensitive cultivar under water deficit. Similarly, studies of Tanveer et al. (2023) also revealed that root length of plants increased during drought stress and the present finding is in conformity with the former. During recovery phase following drought, the restoration of the hydrological balance in plants and soil enables the reestablishment of normal state similar to the well-watered condition, (Fang and Xiong, 2015), and as a result regeneration of fine roots occurred. Additionally, there was a decrease in root length of cowpea during rewatering, likely due to the redirection of energy towards the repair of damaged tissues and the resumption of normal physiological processes in the shoots.

Biological nitrogen fixation serves as the primary source of nitrogen (N) input in ecosystems, generating an estimated 50-70 Tg (Tg=10^{12} g) of organic N annually in agricultural systems (Herridge et al., 2008). The symbiotic association between rhizobia and legume roots enhances nodulation and, consequently, nitrogen fixation (Schwember et al., 2019).

Under well-watered condition, the variety Anaswara had higher nodule number and it may be supported by the greater leaf area leading to enhanced photosynthesis and higher rate of sucrose translocation to nodules. Likewise, BABA and UV-B primed plants had a better root nodulation in terms of the number and size of nodules, as well as the elements that support root nodulation, than the non-primed plants. Under drought stress, the number and size of nodules decreased in cowpea with a drastic reduction in non-primed plants and the reduction was less in primed plants due to stronger photosynthate allocation into their nodules. There was a strong positive correlation between PSII activity and number of root nodules in Anaswara subjected to drought ($r=0.998$ and $p\leq0.01$). Galvez et al. (2005) discovered that sucrose synthase activity in pea nodules reduced concurrently with nitrogenase activity under water stress. They hypothesized that lower nitrogen fixation could be due to a limited carbon flux. Thus, enhanced sucrose synthase activity in nodules of plants grown from primed seeds may contribute to increased nodule biomass (Kaur et al., 2006).

In the present work, the highest number of nodules was noted in UV-B primed plants of Answara and PGCP 6. Previous studies demonstrated that near-UV radiation augmented nodulation and symbiotic N_2 fixation in *Pisum sativum* by two fold and eight fold, respectively. This augmentation resulted from the stimulation of nitrogenase activity by UV light, corroborated by elevated levels of ATP and substrates serving as reducing power sources (Shiozaki et al., 1999). A potential explanation for the augmented number and size of nodules may also be the effect of UV-B on the expression of nod genes or flavonoid biosynthesis genes (Liu and Murray, 2016). Modest UV-B exposure to leaves enhanced the synthesis of UV-B absorbing molecules, which were then translocated from shoots to roots. The transmission of this signal to the root enhances nodulation during

UV-B exposure (Pinto et al., 2002). This aligns with our findings, which indicate that increased flavonoid accumulation was observed in UV-B primed cowpea, potentially serving as a stimulus for bacterial colonisation around the root (detailed in 5.3.9). Furthermore, UV-B priming enhanced lateral root development, hence expanding potential sites for rhizobial invasion and infection, consistent with the observed improvements in root architecture and greater nodulation in chickpea (Shahzad et al., 2010).

The nitrogen contained in the root nodules of leguminous plants serves as a crucial source of mineral nitrogen for the soil ecosystem (Wardle and Greenfield, 1991). Highest nitrogen content was observed in BABA and UV-B primed plants grown under well-watered condition and the nitrogen content in the root nodules of cowpea reduced during drought conditions. Research indicates that drought stress diminishes nitrogen fixation by inhibiting nitrogenase activity. The decrease in photosynthesis, which produces ATP as the energy source for nitrogen fixation, is the possible reason. The highest reduction in photosynthetic parameters in non-primed plants during drought stress can be correlated with the reduced nitrogen content in these plants. Iron and molybdenum are other important elements that are crucial for nitrogen fixation. Iron has a crucial role in the establishment, development, and functioning of symbiosis; therefore, iron shortage negatively impacts the initiation and growth of root nodules (Brear et al., 2013). Findings of this study suggest that the decrease in nodule size and quantity, along with the diminished Fe concentration in nodules, adversely impacts nitrogen fixation during drought stress. A rise in nodule iron concentrations was observed to correlate favourably with the rate of nitrogen fixation in *Phaseolus vulgaris* nodules (Slatni et al., 2008). Beneficial role of seed priming for improving nodulation was reported by Jangir et al. (2020), wherein the treatment of chickpea seeds with nano FeS_2 led to an increased number of nodules and a subsequent rise in the concentrations of

Fe and Mo in the roots. Increased molybdenum concentration indicates higher nitrogenase activity, as it serves as a structural component of the enzymes nitrogenase and nitrate reductase, which are crucial for symbiotic nitrogen fixation and absorption (Banerjee and Nath, 2022). In line with this, our findings also recorded higher Mo content in BABA and UV-B primed plants. Establishing that seed priming has a positive role to improve the overall functioning of root and root nodules under conditions of drought stress. During recovery from stress, cowpea plants exhibited slight recovery in terms of N, Fe and Mo content present in the root nodules following four days of rehydration. It was also noticed that new root nodules formed after recovery in Anaswara and PGCP 6. Similar to the present observation, previous research work also found the development of new root nodules at the time of rewatering in pea plants (Couchoud et al., 2020).

5.3.11 Dehydrins gene expression analysis

Dehydrins are known for their essential role in conferring drought resistance to different plant species (Liu et al., 2017). The current study was conducted to analyze the expression profiles of *Vigna unguiculata* dehydrin genes (*VuDHN*) in sensitive and tolerant cowpea cultivars upon different priming treatments under drought stress and recovery conditions. In the tolerant variety (PGCP 6), only three *DHN* genes (*Vu400*, *Vu500* and *Vu600*) were induced during drought stress, and the other two *DHN* genes (*Vu700* and *Vu800*) were not drought responsive. The induction of *Vu400*, *Vu500* and *Vu600* gene expression in PGCP 6 could possibly have a role in protection of plants from drought induced damage. This is due to their significant hydrophilicity, comprising of a substantial number of charged and polar amino acids, a minimal fraction of hydrophobic, non-polar residues. Along with this, the elevated levels of antioxidant amino acids in dehydrins, including lysine, histidine, and glycine, can neutralise ROS by

oxidative modification (Liu et al., 2017). During recovery, the expression of these *DHN* genes in both primed and non-primed plants reverted to the control level. This may be due to the mitigation of the drought induced effects during drought and attaining the normal physiological state.

Vu400 was non-inducible under well-watered condition in PGCP 6. However, its expression was increased during drought stress with a maximum enhancement in BABA primed cowpea (37-fold) suggesting the importance of BABA in increasing the expression of this gene in leaves of cowpea variety PGCP 6 under drought stress, thereby imparting enhanced drought tolerance. In conformity, a prior study demonstrated that exogenous BABA markedly enhanced the expression of genes such as *DHN*, *LEA*, *MYB*, *NAC*, *WRKY* associated with both ABA-dependent and ABA-independent signalling (Abid et al., 2020). BABA-induced expression of *DHN* may enhance drought tolerance by promoting membrane stability and water retention in the plant (Yu et al., 2018). In agreement to this, the higher expression of *Vu400* and *Vu500* (52-fold) are likely to contribute towards the increased drought stress tolerance potential of PGCP 6.

During well-watered condition, there was a downregulation in the expression of *Vu400* in Anaswara subjected to priming treatments. Likewise, there was a decreased expression of *Vu400* during drought stress in all the primed and non-primed Anaswara. The expression got reduced during stress recovery in all the treatments except in UV-B primed Anaswara which has the same expression level of the control. There may be a delay in the activation of stress-protective genes in sensitive plants, and this absence of early activation likely hinders the sensitive varieties from the impacts of drought stress and recovery from it. In line with, there was a downregulation in the photosynthetic gene expression during stress exposure in the leaves of sensitive rice variety, while tolerant plants showed

upregulation. Accordingly, only activation of a restricted number of genes were observed in the sensitive plants (Razzaque et al., 2019). *Vu500* and *Vu600* were activated exclusively in non-primed Anaswara during drought stress, suggesting that priming did not facilitate the induction of these genes, but downregulated the expression.

There was an induction of *Vu600* in BABA and PEG primed PGCP 6 under well-watered conditions, signifying the response in these plants due to priming which help them to counteract the later stress, possibly by developing stress memory. In accordance with, previous study conducted by Razzaque and co-workers (2019) found the activation of *Os05g01270.1*, *PPIase* (2-peptidyl-prolyl cis-trans isomerase) in tolerant progenies of rice under non-stressed condition, indicates the preparedness of these plants to encounter a stress at a later course. In PGCP 6, the induction of *Vu600* in UV-B primed plants under well-watered condition was less as compared to other priming treatments. But during drought exposure there was higher expression of *Vu600* in UV-B primed PGCP 6. It reveals that, UV-B priming has the potential to activate the function and keep it in alert mode, with expression levels increasing only when the situation requires it (Sen et al., 2021). In line with these, previous reports also found that the expression of stress-responsive genes such as *LEA* and *HSP* induced during PEG stress in UV-B primed plants and augmented the tolerance potential (Thomas et al., 2019).

There was no change in the relative expression of *Vu700* and *Vu800* in PGCP 6, this constitutive expression indicated that these genes were not drought inducible in PGCP 6, however the constitutive expression aids to the drought tolerance of PGCP 6. Similarly, it was reported that the *peudhn1* gene was constitutively expressed in *Populus euramericana* Dorskamp clone, and this was associated to the drought stress tolerance (Caruso et al., 2002).

However, in the sensitive variety (Anaswara), a higher expression of *Vu700* was seen in non-primed plants and BABA-primed plants subjected to drought. Taken together, the study revealed that not all *DHN* genes are inducible during drought stress. Therefore, a holistic investigation of the physiological and molecular mechanisms is necessary to underpin the drought tolerance mechanism of cowpea based on the differential expression pattern of dehydrin genes (*DHN*).

5.3.12 Beneficial effects of seed priming is more prominent in tolerant cowpea variety than the sensitive cowpea during drought stress

Seed priming with BABA, PEG and UV-B augmented the drought stress tolerance capacity of tolerant cowpea varieties and induces drought tolerance to sensitive varieties. The degree of priming-induced tolerance was greater in the tolerant variety. This may be attributed to its inherent tolerance potential, which is further boosted by priming stimulus. The priming stimulus simultaneously confers stress tolerance to the sensitive variety by triggering the defense response. In the drought tolerant variety PGCP 6, the antioxidant system exhibited more activity compared to the senstive variety Anaswara, facilitating the efficient detoxification of ROS. The maintenance of a balance between ROS and antioxidants enhances the redox state, mitigates membrane damage caused by osmotic stress, and stabilizes biomembranes. This, in turn, positively influences the photosynthetic performance of cowpea plants under stress conditions. Together with this, PGCP 6 exhibited a greater buildup of osmolytes, which aids in maintaining cell turgor, enhanced stomatal conductance, and improves the net photosynthetic rate under drought stress. Consistent with this, prior research has demonstrated that the inherent tolerance potential of tolerant varieties responds more favourably than that of stress sensitive varieties (Thomas and Puthur, 2020; Sen et al., 2021). Priming is more

effective in invigorating the less expressed stress tolerance traits, which is normally active in a tolerant variety and thus alleviate the stress induced damage. Hence it was proven that priming is a strategy that can enhance the tolerance of a tolerant variety and convert a sensitive variety into a more tolerant one. The present study demonstrated that priming more effectively diminished the severity of stress-induced damage in the tolerant variety compared to the sensitive variety, resulting in a more rapid recovery from drought following rewatering in the tolerant cowpea.

5.3.13 Trans-priming effect of BABA and cross-tolerance to drought stress

Most of the time, stress occurs as a multifaceted phenomenon. An initial stress stimulus sets off shared defenses that make it easier to withstand other stresses of similar nature. Plants have the benefit of being able to overcome multiple stressors in a better way because of the synergistic co-activation of the stress responses (Hossain et al., 2016). In the present study, it was found that the priming treatments equips the cowpea plants to react more swiftly and efficiently to subsequent drought stress. BABA priming entails trans-priming, whereby the priming stimuli and the preceding stress differ in nature, yet they may foster cross-tolerance towards stress responses in cowpea. It is because BABA acts as a signalling molecule in plants and prepares plants for improved tolerance to abiotic and biotic stress by initiating a complex phytohormone signalling network involving abscisic acid, jasmonic acid, salicylic acid, and ethylene (Virág et al., 2024).

Various investigators often suppose that BABA priming activates particular biochemical and physiological pathways connected to defence responses and the expression of stress-related proteins at the outset of stress (Jakab et al., 2005). BABA activates multiple transcription factor families, including *WRKY*, which in turn stimulates the ICS1 and PBS3 promoters within the salicylic acid signalling pathway. *MYC* and *AP2/ERF* govern

separate branches of the jasmonic acid signalling pathway, with *MYC* co-regulated by abscisic acid and *AP2/ERF* by ethylene (Vos et al., 2013). As these genes are also involved in drought signalling they can bring about cross-tolerance towards drought stress responses. This could be the possible reason for the augmented drought tolerance of cowpea subjected to BABA priming. There are many other reports revealing the cross stress tolerance of BABA. BABA priming was demonstrated to be efficacious in enhancing chilling tolerance (Wang et al., 2023b), mitigating heat stress (Quan et al., 2022), alleviating drought stress (Shaw et al., 2016), salinity and PEG stresses (Jisha and Puthur, 2016). It was also found that the BABA primed plants exhibited faster recovery in terms of growth, relative water content, stability of the membrane, photosynthesis etc. In line with it was found that, in crab apple seedlings, enhanced cell wall rigidity and inhibition of lignin production were proposed as potential mechanisms contributing to BABA-primed drought tolerance (Macarisin et al., 2009). This mechanism aids in minimising water loss from internal tissues. As the intensity of drought stress is reduced in primed cowpea plants, a rapid recovery is achieved during the recovery phase. Consequently, the BABA-primed Anaswara and PGCP 6 demonstrated the highest recovery relative to other priming treatments.

5.3.14 Seed priming speed up the processes of recovery from drought stress

Alleviation of the intensity of drought stress through osmolyte accumulation, maintenance of better tissue water status, increased antioxidant activity, improved photosynthetic performance, and the upregulation of *DHNs* expression due to seed priming, altogether contributed towards the faster recovery of cowpea plants from drought stress. Plant recovery kinetics are determined by the severity of the drought

and the extent of stress-induced damage (Rivas et al., 2016). Less damage from drought means faster and more consistent recovery (Chen et al., 2016). In the present work, it was found that seed priming increased osmolyte accumulation in cowpea, with BABA primed PGCP 6 showing the greatest increment. This aids the plant in retaining a higher relative water content, which in turn mitigates the impact of drought. This is consistent with the fact that BABA primed plants showed the quickest recovery after the removal of drought stress. During drought stress, primed plants exhibited increased activity of both enzymatic and non-enzymatic antioxidants, with the most significant enhancement observed in tolerant plants subjected to priming treatments. This facilitated the efficient scavenging of ROS and lessened ROS-induced membrane damage, resulted in reduced electrolyte leakage and malondialdehyde content and facilitated faster recovery. This indicates that the decrease in lipid peroxidation in primed plants may be ascribed to priming mediated membrane recovery (Kathiravan et al., 2024). Consistent with the study, prior research indicated that nanosilicon priming accelerates the recovery of barley plants from drought, with post-drought recovery linked to the modulations in chlorophyll content, osmolyte and metabolite profiles, cellular injury and membrane stability indices, and the activity of antioxidant enzymes (SOD, CAT, APX and POD) (Ghorbanpour et al., 2020). In the current findings higher photosynthetic recovery was observed in the case of primed tolerant plants and this could be due to the maintenance of higher RWC in these plants. Because, there exists a strong correlation between leaf water potential and the rate and speed of recovery of photosynthesis, stomatal conductance and transpiration (Miyashita et al., 2005). There was upregulation in the expression of *DHN* genes in plants subjected to drought stress and priming and the augmented expression of these stress-responsive genes in plants confer benefits during the recovery phase after stress.

6. SUMMARY AND CONCLUSIONS

The legume crop *Vigna unguiculata* (L.) Walp., known as cowpea, was used for the present research work. Seeds of four different cowpea varieties viz. Anaswara, Bhagyalakshmi, Kanakamony, and PGCP 6 were procured from Kerala Agricultural University (KAU) in Thrissur and the Regional Agricultural Research Station (RARS) in Pattambi, Kerala, India. The study was conducted mainly in two phases. The first phase involved the selection of the concentration of polyethylene glycol (PEG 6000) which imparted 50% retardation in growth of cowpea. For the imparting of PEG stress, healthy, randomly selected cowpea seeds were surface sterilized and were allowed to germinate in culture bottles containing distilled water (control) and various concentrations of PEG 6000 (0%, 5%, 10%, 15%, 20%, and 25%). The culture bottles were maintained in a plant growth chamber under controlled conditions. Growth parameters and total chlorophyll contents were analyzed on 8 d old cowpea seedlings. The shoot length, fresh weight, dry weight, and total chlorophyll content of the seedlings decreased as the PEG concentration increased and the concentration of PEG, which imparted ~50% reduction in growth and total chlorophyll contents were selected as the stress imparting concentration of PEG. Among the varieties studied, Anaswara was more sensitive to PEG stress, which showed 50% growth retardation at 15% PEG. In contrast, variety PGCP 6 had maximum tolerance to PEG stress and exhibited ~50% reduction in growth parameters at 20% PEG stress. Therefore, the priming treatments were done in these two varieties (Anaswara and PGCP 6) with contrasting stress tolerance potential to augment the innate stress tolerance potential. To maximise seedling performance, standardised the concentrations/dosages and duration of priming treatments, including BABA priming, hydropriming, PEG priming, and UV-B priming in the varieties Anaswara and PGCP 6.

For BABA priming, the seeds of sensitive and tolerant varieties were subjected to different concentrations of BABA (0, 0.5, 1.0, 1.5, 2, 2.5 mM) for durations of 3, 6, and 9 h, respectively. Hydropriming was conducted by soaking the seeds in distilled water for 3, 6, and 9 h. For osmopriming, sterilized seeds were immersed in polyethylene glycol solution (PEG 6000) of different concentrations (5, 10, 15, 20, and 25%) for durations of 3, 6, and 9 h. Subsequent to the priming treatments, seeds were rinsed thrice with distilled water and then dried to their initial moisture content. For UV-B priming, surface sterilized seeds were exposed to low doses of UV-B radiation using Philips TL 20W/01 RS narrowband UV-B tubes. After priming of seeds, both the primed and non-primed seeds were transferred to culture bottles packed with absorbent cotton and soaked with double distilled water (control), as well as polyethylene glycol 6000 (stress). These were incubated in a plant growth chamber under controlled conditions of light, temperature and relative humidity, maintaining a photoperiod of 14/10 h. The growth and biochemical characteristics were documented in 8 d old seedlings. Preliminary screening revealed that seed priming with BABA, PEG, and UV-B resulted in the best growth performance of cowpea, so these three priming treatments were used to examine drought stress tolerance and recovery kinetics.

In the next phase of the work, a pot study was conducted to evaluate the effect of BABA, PEG and UV-B priming techniques on drought stress tolerance potential and recovery kinetics of cowpea. For the study, primed and non-primed seeds of cowpea were grown in the greenhouse under controlled conditions at the Department of Botany, University of Calicut. Pot was filled with 2 Kg of soil with known properties. The experiments were performed in a completely randomized factorial design. Three weeks old plants with fully developed trifoliate leaves were subjected to two different watering regimes. Controlled plants were maintained under well-watered

conditions, while drought stress was induced by withholding irrigation until the leaf relative water content (RWC) declined to 50%. Non-primed sensitive variety (Anaswara) was subjected to progressive drought stress by withholding watering until day 7 (7 d), and the primed sensitive variety was subjected to drought stress up to day 9 (9 d). While, non-primed tolerant variety (PGCP 6) was progressively stressed up to day 11 (11 d) and primed tolerant variety up to day 14 (14 d). Following the stress, the seedlings were rehydrated for four days for recovery from drought stress and analysis was carried out to assess the kinetics of recovery.

Key findings from the current investigations are summarised below:

- Among the four cowpea varieties, PGCP 6 had the highest tolerance to PEG, while Anaswara exhibited the lowest. Therefore, these two varieties were selected for further study. Stress sensitive variety Anaswara exhibited 50% growth retardation at 15% PEG; whereas, 50% growth retardation was at 20% PEG for the tolerant variety PGCP 6. The establishment of seedlings is a critical stage in the life cycle of plants. PEG impede and reduce seedling establishment rates by decreasing water potential in seed and seedling tissues, significantly affecting plant growth. The decrease in biomass under PEG stress conditions was attributed to a reduced content of photosynthetic pigments, likely resulting from an elevated activity of the chlorophyll-degrading enzyme chlorophyllase, leading to reduction in the photosynthesis rate, which ultimately reflected in plant biomass. Moreover, stress-induced reactive oxygen species (ROS) impair cell membranes, resulting in an increased rate of membrane lipid peroxidation and cell organelle damage.

- Seed priming enabled seedlings to sustain balanced osmolality within the cells, so as to mitigate osmotic stress without impairing normal

metabolic functions. The selected priming concentration of BABA for Anaswara and PGCP 6 was 1.5 mM for a duration of 6 h. The hydropriming period for both the varieties was 6 h. PEG priming concentration for Anaswara was 10% PEG for 6 h, while for PGCP 6, it was 15% PEG for 6 h. The UV-B priming dosage imparted priming effect was 1.8 kJ m^{-2} s^{-1} for Anaswara and 3.6 kJ m^{-2} s^{-1} for PGCP 6. Among the priming treatments, BABA, PEG and UV-B priming were more effective against PEG-induced drought stress. So these priming treatments were selected for the evaluation of drought stress tolerance and recovery kinetics of plants grown under polyhouse conditions.

- There was a progressive reduction in the relative water content in sensitive and tolerant cowpea varieties subjected to drought. Compared to the non-primed plants subjected to drought, primed ones under drought stress exhibited a lesser reduction in RWC%. Non-primed sensitive variety (Anaswara) reached 50% RWC on day 7 of drought stress (7 d), and the primed plants took 9 days (9 d). Whereas the tolerant cowpea variety (PGCP 6) attained 50% reduction in RWC on day 11 (11 d) and the primed PGCP 6 plants reached 50% RWC on day 14 (14 d). Rewatering led to the restoration of RWC, with the non-primed tolerant variety (PGCP 6) achieving initial RWC levels on 3 d after rewatering. The leaf RWC of primed PGCP 6 recovered completely within 2 d of rewatering, while the sensitive variety Anaswara returned to initial RWC levels by 4 d, although primed plants recovered by 3 d.

- The soil moisture content (SMC) was 31-32% at well-watered conditions and there was a gradual decline in the percentage of soil moisture content during the progress of drought stress (DS). On 7 d of

imposing drought (7 d), the soil moisture content was 22-23%, followed by 19-20% on 9 d after drought stress exposure (9 d). The soil moisture content reached 18% on 11 d of stress (11 d), 14-15% on 13 d and 10-11% on 14 d of drought treatment (14 d). Upon recovery, SMC returned to a level same as that of the control soil. On the first day of recovery itself, the SMC was between 30% and 32%.

- There was a significant reduction in shoot length and leaf area of both varieties during drought stress condition. As compared to the control plants, the reduction was more prominent in sensitive variety. A significant reduction of 40% was observed in the variety Anaswara followed by 13% reduction in shoot length of PGCP 6. The reduction in shoot length was less in the primed plants subjected to drought stress than the non-primed plants under drought stress. Similarly, the reduction in leaf area was less in tolerant variety (20%) than the sensitive variety (31%). Also, there was lesser reduction in the leaf area of primed plants as compared to the non-primed plants subjected to drought stress. There were no prominent changes in the shoot length and leaf area of primed and non-primed cowpea after the recovery from drought stress, but there was a slight increase in the shoot length of tolerant variety.

- Osmolality was increased significantly when the plants were exposed to drought stress, with the highest osmolality in the primed tolerant variety. The maximum enhancement was noted in BABA and PEG primed PGCP 6 (89%), followed by UV-B primed PGCP 6 (82%). There was an increase of 64-68% in leaf osmolality of primed sensitive variety (Anaswara). During recovery, the leaf osmolality was reduced as compared to the drought stressed plants in both the cowpea varieties studied. The highest reduction was observed in the tolerant

variety during the recuperation from drought stress. The rise in RWC can be related to the potential roles of BABA, PEG, and UV-B in preserving osmotic balance through improved osmolyte synthesis/accumulation, which helps to maintain greater RWC in primed plants.

- Total soluble sugars, total free amino acids, proline and total soluble protein of cowpea leaves increased in response to drought stress. The accumulation of these osmolytes in plants emerged from primed seeds were even higher than the plants emerged from non-primed seeds. The augmented buildup of osmoprotectants protects the biomembranes, organelles and cytosolic enzymes, and it has been established that the accumulation of osmolytes under stress conditions impedes plant growth as photosynthate is redirected towards the synthesis of these solutes. The accumulation of proline and total amino acids also functions as a source of nitrogen and carbon, facilitating faster recovery from stress. During recovery from stress, the contents reached to the level of control with the greatest restoration in primed tolerant variety (PGCP 6) indicating the quick revival potential from stress.

- Total chlorophyll content was reduced in response to drought stress and the reduction was higher in sensitive variety than the tolerant variety. There was a reduction of 48% and 36% in total chlorophyll content of Anaswara and PGCP 6 respectively during drought exposure. Reduction in chlorophyll content was attributed to the generation of ROS, resulting in lipid peroxidation of membranes and subsequent chlorophyll degradation. Seed priming with BABA, PEG and UV-B radiation improved the total chlorophyll content in both the cowpea varieties and there was only less reduction in chlorophyll

under drought stress in the primed plants as compared to the non-primed plants subjected to drought stress. During recovery from drought, there was significant increase in total chlorophyll content of non-primed and primed plants, with the maximum enhancement observed in BABA primed PGCP 6. This improvement can be due to the reactivation of the chlorophyll biosynthesis, which significantly augments light energy absorption and utilization in leaves of cowpea, hence facilitating the photosynthetic recovery.

- Chlorophyll *a* fluorescence parameters serves as an effective indicator for assessing the impact of drought stress on primary photochemistry. The parameters such as the maximum efficiency of water splitting complex (Fv/Fo), absorption energy flux per cross section (ABS/CSm), trapping flux per cross section (TRo/CSm), and electron transport flux per cross section (ETo/CSm) and performance index on absorption basis (PI_{abs}) drastically reduced in the plants subjected to drought stress as compared with the control plants. In plants subjected to drought stress, the activity of PSII was markedly reduced, linked to the considerable accumulation of ROS in thylakoid membranes, hence hindering oxygen evolution at the oxygen-evolving complex (OEC) of PSII. Drought stress results in the inactivation of active reaction centres in cowpea plants or reduce the frequency of active reaction centers in a cross-sectional area, resulting in a lower ABS/CSm ratio. Along with this, the diminished energy absorption by chlorophyll molecules is ascribed to a change in the size of the PSII antenna or a structural modification in the antenna LHC component. It affects the photochemistry of cowpea by reducing the rates of photon trapping (TR) and electron transport (ET). The reduction was less in primed plants, indicating an increase in the quantity and/or functionality of reaction centres, even during

drought stress, and they exhibited maximum recovery during rewatering.

- The activities of photosystems (PSI and PSII) was reduced in response to drought stress. The extensive damage to PSI and PSII, along with distruption in the electron transport chain in cowpea, unequivocally signifies an elevated accumulation of $O_2^{\bullet-}$ radicals during drought stress. The attack of reactive oxygen species on the D1 protein of PSII and its ensuing degradation leads to photoinhibition. But, the reduction was less in primed cowpea plants. The reduction in PSI activity was least in BABA primed PGCP 6 subjected to drought stress, and the highest recovery was also observed in the same. Maximum reduction in PSII activity was noted in non-primed Anaswara exposed to drought i.e., a decrease of 61% as compared to the control. Among the treatments, BABA and PEG priming in PGCP 6 was found to exhibit lesser reduction in PSII ctivity under drought stress. The highest recovery rate was recorded in primed PGCP 6 plants, reaching the level of control on 4 d of rewatering.

- The leaf gas exchange parameters such as net photosynthetic rate (*Pn*), transpiration rate (*E*), stomatal conductance (*gs*) and intercellular CO_2 concentration (*Ci*) were significantly reduced during drought stress. However, the reduction was less in the case of primed plants subjected to drought stress. Minimal reduction of *Pn* was recorded in BABA primed PGCP 6 (28%) followed by PEG priming (31%). Priming alleviates the significant decline in leaf water potential induced by water stress and allows the stomata to remain partially open even in adverse conditions of drought. The open stomata facilitated an enhancement in light-saturated net CO_2 assimilation rate (*Pn*), stomatal conductance (*gs*), and intercellular CO_2 (*Ci*) in

primed plants. At the time of recovery from stress, there was a prominent enhancement in *Pn* with a superlative increase in BABA primed PGCP 6, which reached almost the level same as that of the control.

- Epicuticular wax content on the leaf surface was found to be increased during drought stress. It safeguards the aerial plant parts against excessive water loss. The highest accumulation of epicuticular wax content was found in UV-B primed PGCP 6 (90%) followed by UV-B primed Anaswara (84%) exposed to drought stress. Significant peaks at 1027, 1093, 1600, 2350, 2850, 2900, and 3400 cm^{-1}, indicative of C–O stretching of alcohols, aromatic ring stretching, and C=O stretching in carbonyl groups, were seen in UV-B primed plants exposed to drought stress conditions. The peaks ranging from 2819 to 3034 signify the presence of lipid content in cuticular wax. There was no significant reduction in the epicuticular wax content during stress recovery even on 4 d.

- Drought stress significantly increased the accumulation of $O_2^{\bullet-}$ and H_2O_2 in both the sensitive (Anaswara) and tolerant (PGCP 6) cowpea varieties and the highest accumulation of $O_2^{\bullet-}$ was noted in the case of the non-primed sensitive variety. This increased accumulation of toxic byproducts causes membrane damage, DNA fragmentation, enzyme inhibition, and programmed cell death. The least increment in $O_2^{\bullet-}$ was observed in UV-B primed PGCP 6. At the time of recovery, there was a significant reduction in the content of $O_2^{\bullet-}$ and H_2O_2 in both the non-primed and primed plants than the respective drought stressed plants. As compared to the sensitive variety, there was a greater reduction in tolerant variety PGCP 6, indicating that a

clear antioxidation system for scavenging ROS is functioning in these plants.

- The stability of the biomembranes was found to be negatively affected under drought stress in both the cowpea varieties studied. The reduction was more prominent in the sensitive variety than the tolerant, and there was a reduction of 41% in non-primed Anaswara and 33% in non-primed PGCP 6. All the priming treatments lowered the reduction in membrane stability index during drought stress. After stress recovery, the membrane stability index increased and the enhancement was by 26% and 33% in non-primed Anaswara and PGCP 6 respectively.

- Drought stress leads to an increase in electrolyte leakage and it was highest in sensitive variety as compared to the tolerant one. The most pronounced increase was observed in non-primed Anaswara (87%) followed by non-primed PGCP 6 (70%) subjected to drought stress. There was less increase in EL% in primed PGCP 6, with the least increment in BABA (41%) followed by PEG (43%) and UV-B primed (48%) cowpea. During recovery, there was a notable decrease in electrolyte leakage levels in both non-primed and primed plants compared to the drought-stressed plants.

- There was a significant enhancement in the malondialdehyde content of both the cowpea varieties during drought stress. There was an increase of 127% in non-primed Anaswara and 101% increase in non-primed PGCP 6 subjected to drought stress. In comparison to the non-primed plants, there was only lesser increase in the MDA content of primed plants subjected to drought stress. Seed priming protects the biological membranes from oxidative stress induced by drought stress by effectively scavenging ROS and as a result least increment in

MDA was observed. The content of MDA significantly reduced during recovery from drought stress. The highest reduction was observed in BABA primed PGCP 6 (42%) followed by PEG primed PGCP 6 (40%), indicating the restoration of membrane integrity upon rewatering.

- Antioxidant enzymes, such as superoxide dismutase, catalase and peroxidase (POD), play a crucial role in cellular defence and prevention against oxidative damage. These enzymes are extensively present in plant cells and are crucial in the detoxification of ROS from chloroplasts. Seed priming significantly augmented the tolerance mechanism in cowpea by enhancing antioxidant activities. Significant increase of enzymatic antioxidants such as SOD, CAT, APX, GPOX was observed in primed cowpea exposed to drought. The activity of SOD was higher in UV-B primed PGCP 6 during drought stress, and this correlates with the highest reduction of $O_2^{\bullet -}$ in UV-B primed plants, as the SOD accelerated the conversion of $O_2^{\bullet -}$ into H_2O_2. Also, CAT, APX and GPOX activity was higher in BABA and PEG primed plants, with highest increment of APX activity, and it detoxifies H_2O_2. Upon rewatering, the activities of SOD, CAT, GPOX and APX were downregulated in cowpea, as their role turns to be minimal due to the swift recovery of primed cowpea plants from stress.

- Non-enzymatic antioxidants such as ascorbate and glutathione increased during drought stress. Ascorbate is the most prevalent and thoroughly researched antioxidant in plants and is regarded as a potent ROS scavenger due to its capacity to donate electrons in several enzymatic and non-enzymatic activities. It safeguards PSII against donor side photoinhibition by functioning as an alternate electron donor in leaves with an inactive oxygen-evolving complex.

Ascorbate content was highest in BABA and PEG primed plants under drought stress. Glutathione (GSH) is a tripeptide produced in the chloroplast and cytosol that scavenges singlet oxygen and H_2O_2, and oxidised to glutathione disulphide (GSSG) while functioning as an antioxidant and redox regulator. The increased accumulation of these non-enzymatic antioxidants facilitates the detoxification of ROS, thereby significantly contributing to the regulation of lipid peroxidation and electrolyte leakage through the preservation of membrane stability. This owes to the faster recovery of primed plants at the time of rewatering.

- A radical increase in the content of flavonoid, anthocyanin and total phenolics were observed in drought stressed plants of Anaswara and PGCP 6. Secondary metabolites provide multiple activities, including control of enzyme activity, signalling, and defence mechanisms. The increase of flavonoid was more prominent in the primed sensitive variety than the tolerant variety. Elevated levels of flavonoids in the primed cowpea plants protect the chloroplast from oxidative stress and thereby improving the photosynthetic performance. In the case of plants subjected to recovery after drought, there was only a slight reduction in flavonoid content than that of the respective plants exposed to drought. Maximum increase in anthocyanin and phenolics content were observed in UV-B primed plants and were reduced during recovery. The rapid decrease in anthocyanin and phenolics content in primed plants is a significant evidence of substantial detoxification of ROS produced during the drought period. However, the flavonoid content persisted in cowpea plants even after stress recovery, and it has got significance in the effective establishment of symbiotic association.

- Root length was slightly increased in plants subjected to drought stress and the increase was by 20% in Anaswara and by 28% in PGCP 6; an increase of 43% and 30% was noted in BABA primed Answara and PGCP 6 respectively under drought condition. ABA accumulated fast in roots under water stress and the enhanced accumulation of ABA towards the root tip was essential for maintaining root elongation by its regulatory activities in ion homeostasis, osmotic adjustment and cell wall extensibility during water shortage. This enable them to extract more water from the deeper layers of soil. Under drought stress, lateral root growth was diminished in both the varieties studied. However, the reduction was less in the case of primed plants. But, there was a decrease in root length of cowpea during rewatering, likely due to the redirection of energy towards the repair of damaged tissues and the resumption of normal physiological processes in the shoots.

- Drought stress significantly reduced the root nodule number and nodule size in Anaswara and PGCP 6. The nodule size varies between the varieties and among the treatments. Nodule number reduced by 62% in Anaswara and 61% in PGCP 6 during drought, compared with the non-primed plants under well-watered conditions. Nodule number increased in plants subjected to BABA and UV-B priming but not exposed to drought. The reduction in nodule number observed in plants subjected to drought was lowered through priming treatment, probably due to increased photosynthesis and higher photosynthate allocation to the nodules. A potential explanation for the augmented number and size of nodules may be the effect of UV-B and BABA either on the expression of nod genes or flavonoid biosynthesis genes. Priming enhanced the synthesis of UV-B absorbing compounds,

which were then translocated from shoots to roots, which can act as a signal for nodulation.

- Drought stress significantly affected the nitrogen (N), molybdenum (Mo) and iron (Fe) content in the root nodules of cowpea varieties studied. The reduction in N was highest in non-primed Anaswara (38%) and PGCP 6 (36%) subjected to drought, but the drop was less in the primed plants on encountering stress. Drought stress reduced nitrogen fixation by inhibiting nitrogenase activity. The decrease in photosynthesis, which produces ATP as the energy source for nitrogen fixation could be the possible reason. The highest reduction in photosynthetic parameters in non-primed plants during drought stress can be correlated with the reduced nitrogen content in these plants. There was a significant reduction in the iron and molybdenum content during drought stress in both primed and non-primed cowpea. Fe and Mo are important elements that are crucial for nitrogen fixation. Iron has a crucial role in the establishment, development, and functioning of symbiosis; therefore, iron shortage negatively impacts the initiation and growth of root nodules. The findings also recorded higher Mo content in BABA and UV-B primed plants, establishing that seed priming has a positive role to improve the overall functioning of root and root nodules under conditions of drought stress. During recovery there was no much pronounced enhancement in the content of N, Fe and Mo in the nodules.

- RT-qPCR analysis was used to assess the expression of dehydrin genes of cowpea during well-watered, drought stressed and recovery conditions. In the tolerant variety (PGCP 6), only three *DHN* genes (*Vu400*, *Vu500* and *Vu600*) were induced during drought stress, and the other two *DHN* genes (*Vu700* and *Vu800*) were not drought

responsive. The expression of *Vu400* was increased during drought stress with a maximum enhancement in BABA primed cowpea (37-fold) suggesting the importance of BABA in increasing the expression of dehydrin genes in leaves of cowpea variety PGCP 6 under drought stress. However, there was a decreased expression of *Vu400* during drought stress in all the primed and non-primed Anaswara and this necessitates further investigation. *Vu500* and *Vu600* were activated exclusively in non-primed Anaswara during drought stress, suggesting that priming did not facilitate the induction of these genes. There was an induction of *Vu600* in BABA and PEG primed PGCP 6 under well-watered conditions, signifying the unique response in these plants due to priming which help them to counteract the later stress by developing stress memory. There was no change in the expression of *Vu700* and *Vu800* in PGCP 6, and this constitutive expression indicates that these genes are not drought inducible in PGCP 6, but expressed throughout.

- Seed priming with BABA, PEG and UV-B augmented the drought stress tolerance capacity of tolerant cowpea varieties and induces drought tolerance to sensitive varieties. The degree of priming-induced tolerance was greater in the tolerant variety. This may be attributed to its inherent tolerance potential, which is further boosted by priming stimulus. Alleviation of drought stress through osmolyte accumulation, maintenance of better tissue water status, increased antioxidant activity, improved photosynthetic performance, and the upregulation of *DHNs* expression due to seed priming altogether contributed the faster recovery of cowpea plants from drought stress.

- BABA priming was found to more effective in alleviating drought stress in cowpea variety Anaswara and PGCP 6. BABA priming

entails trans-priming, whereby the priming stimuli and the preceding stress differ in nature, yet they may foster cross-tolerance towards stress responses in cowpea. It is because BABA acts as a signalling molecule in plants and prepares plants for improved tolerance to abiotic and biotic stresses by initiating a complex phytohormone signalling network involving abscisic acid, jasmonic acid, salicylic acid, and ethylene. BABA priming activates particular biochemical and physiological pathways connected to defence responses and the expression of stress-related proteins. As the intensity of drought stress is reduced by rewatering in primed cowpea plants, allowing for a rapid recovery during the recovery phase. Consequently, the BABA-primed Anaswara and PGCP 6 demonstrated the highest recovery relative to other priming treatments.

RECOMMENDATIONS

The effect of BABA, PEG and UV-B priming on the drought tolerance potential and the recovery kinetics of *V. unguiculata* was studied in detail, and the following recommendations are put forth from the present study for future research.

- To conduct omics approach (genomics, transcriptomics and proteomics analysis) in cowpea subjected to priming treatments as they will provide a significant understanding of the mechanisms behind plant responses to drought stress and recovery.
- Transgenerational study to understand that whether the stress memory developed during priming is persistent over generations and aids in recovery from stress.
- Study of epigenetic mechanisms involved in drought stress memory and its regulation during recovery.
- Identify the BABA mediated signalling involved in drought tolerance response in cowpea and to explore the mechanism behind the cross talk.
- Underpinning the role of UV-B priming and BABA priming for the better root nodulation by analyzing the genes and signalling pathways involved in root nodulation.

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PUBLICATIONS AND PRESENTATIONS

Articles

- **Aswathi, K. R.**, Sen, A., and Puthur, J. T. (2023). Comparative study of cis-and trans-priming effect of PEG and BABA in cowpea seedlings on exposure to PEG-induced osmotic stress. *Seeds*, 2(1), 85-100.

- **Aswathi, K. R.**, Kalaji, H. M., and Puthur, J. T. (2022). Seed priming of plants aiding in drought stress tolerance and faster recovery: a review. *Plant Growth Regulation*, 97(2), 235-253.

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- Veena, M., Sameena, P. P., Sarath, N. G., Noble, L., **Aswathi, K. R.**, Amritha, M. S., Johnson Riya, Joy M. Joel, K. S. Anjitha, and Puthur, J. T. (2023). Revelations on photosystem II, thermoluminescence, and artificial photosynthesis: a retrospective of Govindjee from fundamentals to applications. *Physiology and Molecular Biology of Plants*, 29(9), 1225-1238.

- Sherin, G., **Aswathi, K. R.**, and Puthur, J. T. (2022). Photosynthetic functions in plants subjected to stresses are positively influenced by priming. *Plant Stress*, 4, 100079.

- Janeeshma, E., Johnson, R., Amritha, M. S., Noble, L., **Aswathi, K. R.**, Telesiński, A., Kalaji, H. M., Auriga, A., and Puthur, J. T. (2022). Modulations in chlorophyll a fluorescence based on intensity and spectral variations of light. *International Journal of Molecular Sciences*, 23(10), 5599.

- Shackira, A. M., Sarath, N. G., **Aswathi, K. R.**, Pardha-Saradhi, P., and Puthur, J. T. (2022). Green seed photosynthesis: What is it? What do we know about it? Where to go?. *Plant Physiology Reports*, 27(4), 573-579.

Book chapters

- Aswathi, K. P. R., Jisha, K.C., Veena, M., Sen, A., Sarath, N.G. and Puthur, J.T., 2025. GABA Priming Induced Modulations in the Redox Homeostasis of Plants under Osmotic Stress. In: *GABA in Plants: Biosynthesis, Plant Development, and Food Security*, **Singh**, S., **Tripathi**, D. K., **Singh**, V. P. (Eds.), Wiley, pp.173-187. ISBN:9781394217786

- Sarath, N. G., Prakash, C. A., Janeeshma, E., Aswathi, K. R., Shackira, A.M., Sebastian, D.P. and Puthur, J. T. (2024). Molecular and Physiological Attributes Regulating Phytoremediation Potential of Plants. In: *Phytoremediation and Biofortification,* **Nand K. Singh**, **Afzal**, S. and Aftab, T. (Eds.), Apple Academic Press, pp. 159-182. ISBN: 9781774914083

- Shackira, A. M., Sarath, N. G., Veena, M., Johnson, R., Jeyaraj, S., K. P. Raj Aswathi and Puthur, J. T. (2023). Resistance Identification and Implementation: Genomics-Assisted Use of Genetic Resources for Breeding against Abiotic Stress. In: *Cereal Crops,* Shah, T., Texeira Filho, M. C. M., Nie, L. and Amir, R. (Eds.), CRC Press, pp. 141-156. ISBN: 9781003250845

- T. T. Dhanya Thomas, **K. P. Aswathi Raj**, M. S. Amritha, Jos T. Puthur (2022). Photosynthetic Response of Crop Plants Under UV Stress. In: *Photosynthesis and Respiratory Cycles during Environmental Stress Response in Plants,* Aryadeep Roychoudhury (Ed.), Apple Academic Press, pp.141-162. ISBN: 9781003315162

- E. Janeeshma, Akhila Sen, **K. P. Raj Aswathi**, Riya Johnson, Om P. Dhankher, Jos T. Puthur (2022). Reclamation and Phytoremediation of Heavy Metal Contaminated Land. In: *Bioenergy Crops: A Sustainable Means of Phytoremediation,* Puthur J. T., Dhankher O. P. (Eds.), CRC Press, Taylor and Francis Group, pp. 187-203. ISBN: 13-9780367489137

Papers Presented

- Aswathi Raj K. P. and Puthur, J. T. (2024). 'Dehydrin isoforms exhibit differential expression in *vigna unguiculata* under drought stress and recovery' in the International Phytotechnology Conference (IPC-18) on 'Phytotechnologies for Sustainable Environment and Food Safety' organized by Department of Botany, University of Calicut, October 22 – 24, 2024.

- Aswathi Raj K. P. and Puthur, J. T. (2023). 'BABA priming: A green vaccination technique for developing climate resilient cowpea in the National Seminar on 'Green Economy: Concepts and Perspectives' organized by Govt. College Madappally, October 26-27, 2023.

- Aswathi Raj K. P. and Puthur, J. T. (2023). 'BABA priming: An ecofriendly and efficient method for improving the drought tolerance potential of cowpea' in the International Seminar on 'NEW HORIZONS IN PLANT

SCIENCES (NHPS 2023)-Emergent and Innovative technologies in Plant Sciences' organized by University of Kerala, March 21-23, 2023.

- Aswathi Raj K. P. and Puthur, J. T. (2023). 'Holistic improvement in drought tolerance potential of cowpea subjected to UV-B priming' in the International Conference on 'Current Trends and Future Prospects of Plant Biology (CTFPPB-2023) and 14th Plant Sciences Colloquium' organized by University of Hyderabad, February 23-25, 2023.

- Aswathi Raj K. P. and Puthur, J. T. (2022). 'Priming induced redox homeostasis in cowpea seedlings subjected to PEG stress' in the International Conference on 'Physiological and molecular mechanisms for abiotic stress tolerance in plants' jointly organized by University of Calicut and Indian Society for Plant Physiology (ISPP), October 26-28, 2022.

- Aswathi Raj K. P. and Puthur, J. T. (2020). 'Enhancement in major metabolites content of cowpea seeds induced by β-amino butyric acid (BABA)' in the International Webinar on 'Plant Functional Biology' organized by Sir Syed College, Thaliparamba, Kannur, June 05-07, 2020.

Best paper awards

- Received Best paper award in the National Seminar on "Green Economy: Concepts and Perspectives" organized by Govt. College Madappally, Kozhikode, October 26-27, 2023.