Physiological and Biochemical Studies on Drought Tolerance in Black Pepper (*Piper nigrum* L)

Cultivars

THESIS SUBMITTED TO THE UNIVERSITY OF CALICUT

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DOCTOR OF PHILOSOPHY

By

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CERTIFICATE

I hereby certify that the thesis entitled "Physiological and Biochemical studies on drought tolerance in Black Pepper (*Piper nigrum* L.) Cultivars" contains the results of bonafide research work done by Ms.Vasantha at National Research Centre for Spices, under my supervision and guidance. I further certify that this thesis or part of it has not been submitted to any University for the award of any other degree or diploma.

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Place : Calicut

Date : 08.11.96

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DECLARATION

I hereby declare that the thesis entitled "Physiological and Biochemical studies on drought tolerance in Black Pepper (*Piper nigrum* L.) Cultivars" contains the results of bonafide Research work done by me at NRCS, Calicut under the supervision of Dr.A.Ramadasan, Principal Scientist (Plant Physiology) NRCS, Calicut. Further this thesis or part of it has not been submitted to any University for the award of any other degree or diploma.

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Introduction

INTRODUCTION

Indegenous to India black pepper is one of the oldest and best known spices in the world. Black pepper (Piper nigrum L.) belongs to the family PIPERACEAE. It is a liane of perennial habit. The fruits are berries formed in spikes and contains oleoresins and essential oils which impart the characteristic pungency and flavour for which it is highly valued. Black pepper vines start yielding from the second year onwards, however, the yield stability is attained in the 4th or 5th year in most of the popular cultivars.

Optimum conditions for higher productivity of black pepper are:

Rain fall : 125-250 cm annually and well distributed.
Temperature : 10-40°C
Altitude : Sea level upto 1500 m.

Pepper vines thrive extremely well in the above conditions. Most of the popular cultivars yield upto 20-25 years.

Pepper is mainly cultivated in India, Brazil, Indonasia, Malaysia, Thailand and Sri Lanka. India's contribution in the world production of black pepper is 32% and that makes her one of the largest pepper producers (Anon 1990).

Pepper is a rainfed crop of humid tropics. In India, Kerala accounts for 96% of area and production of this crop. However,

productivity per unit area is poor compared to Malaysia. The low productivity is attributed to low planting density (500-600 Vines/ha) in homested garden, cultivation of low yielding cultivars and lack of proper scientific management.

The rainfall pattern in Kerala is however, different; the monsoon starts by June and ends by November followed by dry spell for about 5-6 months. The potential evaporation during the dry period is about 5.4 mm per day (Sadanandan 1991). Drought is regarded as one of the major constraints in increasing the productivity of black pepper (Ramadasan 1987).

The traditional drought management programme comprises of mulching and growing cover crops like Calpagonium around the basins. In practice, the above management practices may fail to protect the crop from severe drought situations and also when sensitive cultivars are grown. Therefore, growing drought tolerant cultivars, assumes importance as the occurrence of drought is a regular feature in Kerala.

The most important phenophase of black pepper sensitive to moisture stress is the flowering phase Which commences in May-June. The delayed monsoon postpones the flowering process. After the new flushing and flowering, the rainfall should be continous till the fruit development, orelse the productivity is reduced drastically. Long spells of dry periods are detrimental for the crop (Anon 1982).

With the scope of improving the productivity of black pepper, the study on characterisation of drought tolerance was takenup at National Research Centre for Spices, Calicut. The objectives of the present work is to:

* Study various parameters and shortlist characters related to drought tolerance.
* Develop drought index for screening large germplasm materials based on the above characters.
* Identify drought tolerant cultivar from among the cultivars studied.
* Understand the drought tolerance mechanism in black pepper.

Review of Literature

REVIEW OF LITERATURE

The numerous response of plants to moisture stress generally vary with the severity as well as the duration of the stress. Only the most sensitive processes are altered by a very mild stress. As the stress increases, these changes intensify and additional processes become affected in accordance with their relative sensitivities to the stress. If the stress is prolonged there is more time for the initial effects to lead to secondary and tertiary responses(Bradford and Hsiao 1982). Research is needed to reduce the chances of crop failure by improving and updating the crop and soil management, develop cultivars to withstand drought and achieving basic understanding of the effects of drought stress on plants.

Research on crop and soil management through agronomic pratices is a short-term means of reducing the effects of drought, while developing drought tolerant line would fetch permanant solution particularly for traditional drought prone areas. Selection of drought tolerant lines is tried at various levels in crop species. The most common approaches are:

* Selecting for yield stability over dry areas and years. As this approach underlines importance of yield criterion it often has the drawback of missing drought tolerant lines which are poor yielders. e.g., most of the wild types of wheat are drought tolerant lines and poor yielders.(Sinha 1988).

* Selecting directly for performance in controlled drought stress nurseries: The development of field techniques for direct screening for drought resistance is more difficult than screening for pest or disease resistance. A repeatable screening in the field can help in obtaining consistant differences among cultivars over years (O' Toole and Chang 1979).

* Selecting for physiological or biochemical characteristics directly related to field tolerance. This is theoritically more rapid and effective than the first two approaches. It offers the possibility of working directly with a small no., of characters (whose inheritance can be determined), rather than a complex procedure.

Drought resistance /tolerance is the term used to cover a range of mechanisms whereby plants withstand periods of dry weather. With most agricultural crops the seed is the economic yield and mechanisms that maintain productivity and increase in reproductive efficiency under drought are important (Turner1979). By contrast in pasture system mechanisms that maintain leaf production and plant persistence through periods of moisture stress are more important (Turner and Beggs 1978). In perennial and tree crops persistance overrides production mechanisms (Jones, Turner and Osmand 1981). The range of resistance available in a particular crop is influenced by genetic and environmental factors.

Moisture stress has been a major selective force in plant evolution and ability to cope with drought situations is an important determinant of natural distribution of plants and of crop distribution and productivity (Fischer and Turner 1978). Understanding of the mechanisms that confer adaptation to dry environments thus holds much theoritical and practical value. Plant adaptations to such environments can be expressed at four levels:
phenological or developmental, morphological, physiological and metabolic (Hanson 1980).

Phenological responses to stress:

Cell Division:

The growth and development of a plant depends basically on continuing cell division, on the progressive initiation of tissues and organs and on the differentiation and enlargement of cells until the characteristic form of the plant is realised (Slatyer 1973a). It has often been stated that cell division appears less sensitive to moisture stress than cell enlargement (Vaadia et. al., 1961 Salter and Goode 1967 Slatyer 1967 and Hsiao 1973).Evidence for this view is given by the observation that cell number is frequently of the same general order in plants exposed to moisture stress compared with controls, although cell size is greater in the latter and by the phenomena

of more rapid growth on recovery from stress compared with controls(Gates 1955 a,b). This could result from cell division continuing during stress, though at a reduced rate and thus providing an opportunity for a relatively rapid resumption of growth when stress is removed (Slatyer 1973b).

Cell enlargements :

The sensitivity of cell enlargement to water deficits in some species has been demonstrated by the work on maize. Leaf enlargement declined rapidly at leaf water potential below -2 bars and ceased at potentials of -7 to-9 bars (Boyer 1970a; Acevado et.al.,1971). Reduction in leaf enlargement and the declined rates of leaf expansion with depleting soil moisture has been well documented in several works (Karamonas et.al.1982, Tanguiling et.al., 1987, passioura 1988, Boyer 1988,Kemp et.al.,1989 Hay and Janette 1988, Joly and Hahn 1989, Randall and Sinclair 1989, Kallarackal et.al.1990).

In general, there is a rapid and more gradual decline in the rates of cell enlargement as water stress develops. Passioura (1988) attributes the declined leaf expansion rate to the signals given by root system in drying soils in wheat. The exceptional sensitivity of leaf enlargement was first shown by Boyer (1968,1970a) who showed that leaf enlargement was first reduced to 25% of the control or less when leaf water potentials decreased to -4 bars in maize, soybean and sunflower.

One of the most important consequences of the sensitivity of leaf enlargement to small moisture stresses is a marked reduction in leaf area. Leaf growth is generally more sensitive to water stress than other physiological and biochemical parameters. Reduction in leaf area means reduced photosynthetically active surface. The loss in leaf area and reduced photosynthetic activity when taken together represent potentially large loss of photosynthate for crops. Consequently, water deficiencies decrease productivity.

Physiological response to stress:

Plant water status: plant water status is the quantification of the condition of water in a plant relative to its requirement. It is best characterised by a combination of its physicochemical availability for plant functions, amount present and movement through the system (Taylor 1968, Taylor and Slatyer 1961). Physicochemical avilability relates to absolute availability or energy aspects of plant water status, whereas amount and movement seem to relate more to logistical aspects although amount play subtle roles in maintaining functional structure not related directly to energy status (Barrs 1968). Water potential is the physicochemical availability of the water to participate in plant functions and determines the tendency for net water movement within the system (Taylor 1968; Slatyer and Taylor 1960).

The conceptual development of water movement along the soil plant atmosphere continuum, the development of a thermodynamic framework for total water potential and its components and relatively simple methods of measuring total water potentials have led to crop growth processes being correlated with total water potential. Leaf water potential varies greatly depending upon the the type of plant and upon environmental conditions. For mesophytic plants leaf water potential ranges from nearly 0.0 Mpa for well watered plants having very low transpiration rates to values of -3 Mpa or lower when desiccated nearly to the point of death (Kaufmann 1981).

Hsiao et.al.(1976) outlined a no.of plant responses to water stress which occur well before desiccation become lethal. Most responses(e.g. cell growth, photosynthesis, enzyme activities etc.,) are affected by leaf water potential reductions of less than 1.5 Mpa. Passive plant control of desiccation itself occurs when stomatal closure results from reduced leaf water potentials. Stomatal closure occurs at potentials as high as 0.6.Mpa in Vicia faba(Kassam 1975). In contrast complete stomatal closure may not occur unless leaf water potential is below -2.5 Mpa in citrus (Kaufmann and Levy 1976) and below -3.o Mpa in cotton (Brown et.al.1976)

Variations also exists in the lowest leaf water potential at which different plants survive. Sanchez-diaz and Kramer

(1971) observed desiccation injury in corn at a leaf water potential of -1.3 Mpa. A soil water potential of -1.0 Mpa may be considered a mild drought for woody species but a devastating treatment for herbaceous plant.

Water release curves and drought resistance: A relationship between drought resistance and the slope of the water release curve (RWC against water potential) has been noted for photosynthetic tissues (Jarvis and Jarvis 1963, Connor and Tunstall 1968). A smaller slope of the water release curve is usually taken to indicate higher drought resistance. Since a large potential gradient for water uptake results from a given change in the tissue water content. A large value of osmotic potential at full turgor, a low tissue elasticity and high ability to accumulate solutes as tissue water contents decline, each contribute to small slope of the water release curve. It is obvious that evaluation of drought resistance on the basis of water release curves, is unlikely to be meaningful if different life forms or species differeing in drought resistance mechanisms are compared.

Control of water potential: Diurual variations in leaf water potential in fruit trees are similar to those in other specise and are explicable in terms of the mechanisms explained elsewhere (Jone et.al.,1985).There are marked diurnal changes in leaf water

potential with minimum values of between -1.0 and -2.5 Mpa usually occuring in the early afternoon at the time of highest transpiration rates (Kriedemann and Barrs 1981; Chalmers et.al.1983).Surprisingly, there is little difference between the minimum water potential achieved in well watered humid and arid environments (Levy and Syvertsen 1981). This indicates an effective physiological control of leaf water potential largely by means of control of transpiration rate under conditions of high evaporative demand (Schulze et.al.1974 and Jones 1983a). Leaf water potential has been used as an index for drought tolerance in coconut(Rajagopal et.al. 1988).

Stomatal resistance and transpiration responses to moisture stress

Stomatal closure provides a mechanism for reducing water loss. The response of stomata to leaf water potential and leaf turgor is well recognised (Turner 1974 a,b) in the past decade. The sensitivity of stomata to vapour pressure deficit may provide an important mechanism for restricting water loss in the midday when atmospheric humidities are low, while maintaining some photosynthetic activity at times of day when humidities are higher (Cowen and Farguher 1977).

Begg and Turner (1976) showed that stomata do not close until a threshold value of leaf water potential or leaf turgor pressure is reached. Subsequent work has shown in some cases no

threshould response is observed, with stomatal conductance decreasing linearly or almost linearly with leaf water potential or leaf turgor pressure (Jones and Rawson 1979; Schulze and Hall 1982: Sobrado and Turner 1983b).

It is useful to distinguish between the water transpired by the leaves and rest of the parts by a crop. Only the transpired water is involved in the flux dependent lowering leaf water potential. This can be shown by the fact that treatments(such as wilting) that reduce the proportion of water lost by transpiration from a crop act directly to raise tissue water potential.

Stomata of several crop species are sensitive to environment tending to close in dry air(Schulze et.al.1972; West and Gaff 1976; Hall et.al.1976). Stomatal response to humidity and temperature generally act to minimise the effect of changing environment and hence leaf water potential via feed back and feedforward control (Jones 1983b). This is therefore an important mechanism acting to maintain favourable tissue water potential even in severely desiccating environment. The degree of stomatal closure can vary among species (Davies and Koslowski 1974) for eg., Citrus stomata may reopen more slowly than stomata in several temperate tree species. Good stomatal control of leaf water potential over a range of evaporative demand has also been reported for citrus (Levy 1980a).

Hygen (1953) has pointed out 3 distinct phases of water loss from detached leaves 1. a constant rate phase when open stomates exercise little control over water loss, 2.a decreasing rate phase when stomatal closure progressively reduces transpiration and a phase when closed stomates limit water loss to the cuticular route.

Transpiration and net photosynthesis decreased as water stress increased in Douglasfir (Fry and Walker 1964). Unirrigated coconut palms showed reduced rates of transpiration compared to various irrigation treatments (Rajagopal et.al.,1989). Johnson et.al.,(1974) has shown the linear declining of transpiration and photosynthesis with the flag leaf water potential.

Desiccation and heat tolerance tests have been correlated with drought tolerance in several works (Havaux et.al.,1988: Premachandra and Shimada 1988: Hanna oblog and Alina Kacperska 1981: Venkataramana et.al., 1983: Premachandra et.al.,1989). As these tests are based on membrane thermo/desiccation stability the tolerant genotypes are expected to leach out fewer solutes and ions in the leachate as damage to the membrane would be less and higher solutes and ions in the case of sensitive types (Tonuthi & Giulivo 1987). Applicability of these tests in the case of perennial and tree crops largely relies on its confirmation with field tolerance.

BIOMASS PARTITIONING: Reduction of growth in terms of dry matter, net assimilation rate, and leaf area has been reported in many crop species (Morton and Watson 1948: Baker and Musgrave 1964: Lehane and Staple 1962). In tomato Gates (1957) has shown the influence of low soil moisture in reducing the total dry weight. Partitioning in the stem was maximum while leaf and roots got lesser photosynthates. Dry weight of leaf lamina also decreased in moisture stressed plants compared to control plants. This has a direct bearing on specific leaf weight under stress. Silvius et.al.,(1977) using radio carbon study pointed out the efficient distribution of drymatter and photosynthates in soybean under moisture stress. Steinberg et.al., (1990) showed the dry matter partitioning with respect vegetative parts in peach trees subjected to moisture stress. Effect of moisture stress and nitrogen stress in dry matter distribution and water use efficiency was reported in wheat (Heitholt 1989). Regulation of root/shoot ratio under moisture stress was studied in soybean (Creedeman 1989),& Sweet potato (Clarence Johnson Jr. 1991). Increases in roots relative to shoots have often been observed when water is limiting (El Nadi et.al., 1969: Pearson 1966). While the change may be attributed mostly to reduction in shoot growth, there are instances when water stress effected an increase in the absoluts root biomass (Hsiao and Acevedo 1974, Sharp and Davies 1975). Poor partitioning of the dry matter has

been reported for moisture stressed coconut palm (Rajagopal et.al.,1988). The alterations in the leaf thickness and photosynthesis to moisture stress was reported in soybean varities (Sachie Kishitani and Tsunoda 1982). Indira and Kabeerathumma (1986) reported reduced specific leaf weight as moisture stress intensified.

METABOLIC RESPONSES TO MOISTURE STRESS: Metabolic responses of plants to moisture stress can be viewed in two different ways: as derangement that result from stress induced leisions at vulnerable sites in metabolism, or as potentially adaptive changes that reflect ordered operation of metabolic regulatory mechanisms and which favours the performance of the plant as a whole during or after stress (Stewart and Hanson 1980: Wyn Jones 1979).

Osmotic adjustment during moisture stress is based on cellular metabolic changes associated with the accumlation of organic solutes and with increases in and maintenance of, cellular ion gradients as well as with solute translocation within the plant (Raven et.al.,1979: Turner and Jones 1980), Generally, a range of solutes accumlate during osmotic adjustment in both fully expanded and growing tissues. The solutes include inorganic ions (K^+, Cl^-, NO_3^-), organic anions, soluble carbohydrates, amino acids and quarternary ammonium compounds (Acevedo et.al., 1979:Boyer and Mayer 1979 : Jones et.al., 1980 : Munns et.al., 1979: Raven et.al., 1979 : Thornley 1977).

An increase of the free proline content in the leaf tissues is noticed in many mesophytic plants during moisture stress. Proline accumulation is favoured by high leaf carbohydrate status and also by illumination. Various experimental methods of water stress imposition can elicit proline accumulation in leaf tissues of young plants (Blum and Ebercon 1976: Hanson et.al., 1977 : Huang and Cavalieri 1979: Iwai et.al.,1979: Munns et.al., 1979: Singh et.al.,1973 : Stewart 1978, 1981 : Rajagopal et.al., 1977; Parameshwara et.al.,1988. Chanan Itai et.al., (1988) showed a high correlation of proline accumulation with stomatal regulation. They concluded that elevated levels of proline under moisture stress may play a role in stomatal regulation. Increased levels of proline under moisture stress is reported in several crops Viz., coffee (Venkataramanan and Ramaiah 1986); Sweet potato (Indira and Kabeerathumma 1986).

It has been advocated that this is advantageous to the plant in coping with drought and that proline accumulation be used as an indicator in selecting for drought tolerance in crop breeding (Singh et.al.,1973). Recent work however, suggests that the opposits may be true; proline accumulation is indicative of the stress damage (Hanson et.al., 1977,1979,Stewart and Hanson 1980). Proline acumulation generally begins only after water deficit has become severe enough to prevent growth and cause stomatal closure (Mc.Micheal and Elmore 1977).

Water stress may have both qualitative and quantitative effects on plant constituents. Probably the most direct effects are on carbohydrates through the inhibition of photosynthesis. Woodhams and Kozlowski (1954) noted the rapid conversion of starch to sugars in tomato and bean plants. Increased accumulation of soluble sugars has been reported in several studies (Drossopoulos et.al.,1987: Garg et.al., 1981 Cortes and Sinclair 1987: Fanjul and Rosher 1984).

Increased levels of alkaloids and phenolic compounds under moisture stress were reported (Salch et.al.,1978; Kubota et.al.,1988). Reports are varied on the accumulation of lutin in citrus and Nicotiana sp. (Salch et.al., 1978).

Plant Pigments : There is a relationship between the severity of water stress and the extent and reversibility of structural and functional damage (Nir 1969; Hsiao 1973; Crevecoeur et.al.,1976).As moisture stress increases, the structural changes become more pronounced and following extreme loss of the fresh weight in higher plants (usually exceeding 50-60%) the changes are irreversible. Alberte et.al.,(1977) showed the loss of chlorophyll from maize leaves upon water stress which resulted in chlorophyll content falling to almost 60% of control 8 days after irrigation. Balakumar et.al.,(1988) reported reduction in chlorophyll proportional to carotenoids in cotton and sorgham subjected to moisture stress. However, Benes and Houpis (1989)

reported that chlorophyll reduction showed no significant relation between pigment levels and moisture stress.

Chlorophyll stability index has been used for invitro screening for drought tolerance in cocoa (Ravindran & Menon 1981). Chlorophyll fluresence has been shown to have utility in identifying heat and drought tolerant plants (Havaux et.al.,1988).

Enzymes :Enzyme activities and enzyme systems are very sensitive to moisture stress as water forms the site for enzyme functions. Therefore, moisture stress at cellular level affects the enzyme structures as well as activities. The following observations have been generally noted in enzyme activitiers under moisture stress by (Glenn W. Todd 1960):

* Severe water deficits generally cause an overall decrease in enzyme level.

* Levels of enzymes involving hydrolysis or degradation usually either remain same or increase but they do not decrease until fairly severs desiccation taken place.

* Levels of some enzymes involved in synthesis are decreased and levels of others increase as a result of water deficit.

Common enzymes studied under moisture stress include Nitrate reductase, RUBP carboxylase, PEP carboxylase, sucrose synthetase, acid phosphatase, peroxidase etc., Among the lot, nitrate

reductase has been studied to a greater extent as it is sensitive to even mild stress (Huffaker et.al.,1970). Since proline accumulation is one of the major changes in the nitrogen metabolism of water stressed plants, the relationship between nitrate reductase and proline accumulation was examined in several crop species (Sinha and Rajagopal, unpublished). There was a sharp decline in enzyme activity in response to water stress in wheat, barley,sorghum,maize,brassica and safflower. Reduction in nitrate reductase activity to the tune of 75-87 % is reported for drought stressed cotton (Ganesan et.al.1988). Vyas et.al.(1988) reported improved activity of enzymes Viz., nitrate reductase,glutamine synthatase, glutamate dehydrogenase in prestressed sesame. Diurnal course of activity was maintained at lower levels in stressed plants compared to unstressed wheat (Rajagopal et.al.,1977). A highly positive correlation between leaf water potential and NRA was reported in sugarcane (Venkataramana et.al.1987).

Reports on the effect of moisture stress on the activity of acid phosphatase are varied. Vieira de silva (1968 &69), Takaoki (1968) have shown increased activity in the soulble fraction. Increased activity is shown in crops like cotton and swiss chard (Nir and Poljakoff-mayber 1966: Vieira de silva et.al., 1974). Thakur (1991) reported increased activity during water stress and treatment with triacontanol and mixtalol.

Peroxidase is the most common oxido-reductase that gets affected when the metabolic changes due to environment occurs. In maize the increased peroxidase activity due to moisture stress upto permenant wilting point is reported by Petinov and Malysheva (1960). Smirnoff and Colombe (1988) showed the drought influence on this enzyme. Increased peroxidase activity has been reported for wheat seedings (Li and Liang 1988). Zbiec et.al.,(1989) reported similar activities in several crop species.

Work on Black pepper:

Research reports on moisture stress is scarce in black pepper. Pepper yield has been correlated with rainfall (Sadanandan, 1986). Well distributed rains during May and June enhances higher spike intensity and berry set. The most sensitive phenophase of black pepper to moisture stress is flowering phase. The delayed monsoon postpones flowering also. However, after new flusing and flowering the rainfall should be continuous till the fruit development or else the productivity is drastically reduced (Anonymous 1982). Long spells of dry periods are unfavourable for the crop. Purseglove et.al., (1981) highlighted the necessity of adequate moisture availability for fruiting of pepper.

Report of Vijayakumar et.al., (1982) does not provide conclusive result on the effect and response of black pepper

cultivars to moisture stress. Chlorophyll degradation due to moisture stress is reported by Kurup and Vijayakumar (1987). Reduction in chlorophyll end carotenoids pigment has been reported for higher temperature treatments(Vasantha et.al., 1989) Chlorophyll/ carotenoids ratio has been suggested for its possible utility in screeing work. Vasantha et.al., (1990) reported the response of physiological parameters Viz., stomatal resistance, transpiration rate and leaf water potential to depleting soil moisture content and used them for screening popular cultivars for moisture stress.

Diurnal course of activity of nitrate reductase in the flag leaf of Panniyur-1 black pepper was reported by (Raju & Rajagopal, 1989). Proline accumulation has been reported in the leaf discs subjects to moisture stress induced by PEG, in black pepper (Thomas et.al.,1990).

The reports available on drought studies in black pepper are all isolated and not systematic. Response at cellular, tissue level and whole plant level is necessary to indicate the importance of character that regulate and outline drought tolerance mechanism. It is with this objective the present work was initiated.

Materials & Methods

MATERIALS AND METHODS

Brief note on methodology:

Inducing moisture stress in plant systems is a complex procedure as soil moisture levels are influenced by various other environmental factors. Careful steps for artificially regulating moisture supply in the substrate need to be taken in order to determine the effects of water stress on plant growth and development. Methods for regulating water deficits in plant tissues are perhaps some of the most difficult of all environmental variables to control experimentally because of the dynamic nature of water in the plant and its surrounding substrate(Krizek, 1985). In order to conduct a thorough study of water relations of a particular plant genotype, it is essential to investigate the relationships between water poential of the root medium,plant water potential, plant growth,transpiration rate, stomatal activity and plant survival(Jarvis,1963).

The easiest and most frequently used method, especially, under field conditions is withholding irrigation till desired results are achieved. Any screening; maintaining similar soil/edaphic conditions, where natural adaptations occur in plant system to moisture stress, is more meaningful as it gives a chance for repeatable testing in the field.

Outline of the experiment and treatment

The experiment was conducted during the months of Jan-Mar, 1990, in a semipermanant waterproof shed at National, Research Centre for Spices, Calicut. Earthern pots (12") were filled with forest soil, Rooted cuttings of sixty numbers of each of six popular cultivars were obtained from germplasm Nursery of NRCS, farm Peruvannamuzhi.

The plants were allowed to establish for about four weeks. The cuttings were trained on bamboo poles of 1.5m height. Moisture stress was imposed by withholding irrigation to a set of plants (30 nos) while the other set was irrigated regularly so as to maintain the soil moisture at field capacity (FC). The experiment was concluded when wilting was noticed in majority of plants of any of the cultivars.

Materials : Black pepper cultivars used for the study include Aimpiriyan (856), Arakulam munda (1467), Kalluvally (880), Karimunda (51), Narayakodi (965), Panniyur -1.

The general morphological, yield and quality characters of these popular cultivars are as follows(Ravindran & Nair 1984: Ravindran & Babu 1988):

Aimpiriyan: It is a popular cultivar of wynad area of Kerala, a good yielder and produce pepper of high quality having oleoresin of 15% piperine 4.7% and essential oil 2.6%. The leaves are

large,spikes medium to long with thick setting. The name is derived from the fact that the berries are arranged in five rows on the spikes.

Arakulam munda: A moderately good and regular bearer and comes to maturity earlier to most of other cultivars. The spikes are medium-long,berries bold and heavy. This yields 9.8% oleoresin,4.4% piperline and 4.7% essential oil.

Kalluvally: A promising north Kerala cultivar, hardy and regular yielder. Leaves medium, ovate and elliptic. Regular bearer and reportedly tolerant to moisture stress and diseases. More than one cultivar is known by this name and some of these are rather poor yielders. They donot seem to be as hardy as the name indicates and found to differ in quality aspects also (Oleoresin ranges from 8.4-10.9%: piperine 4.2-5.4% essential oil 0.4-3.2%).

Karimunda: It is apopular cultivars of Kerala, good and regular yielder. It is characterised by small ovate to elliptical leaves with short medium long spikes and high setting, Karimunda is relatively more tolerant to water stress and has good quality: oleoresin 11%, piperine 4.4% and essential oil 4%.

Narayakodi: A popular cultivar of central part of Kerala Karimula exhibits considerable variations in growth and productivity

It has relatively short spikes and thick setting of berries. It"s quality attributes are oleoresin 10.85% piperine 5.4% and essential oil 4%.

Panniyur-1: A hybrid cultivar and a high yielder. It is a vigorously growing climber with large leaves, long spikes and good setting. The pepper is of medium quality having oleoresin 9.5%, piperine 3.6% and essential oil 3.5%. It is found to give excellent yield when trailed on coconut palms of about 30 years or more, and expecially in the open without shade.

General observations and sampling intervals:

Agrometeorological data viz., relative humidity (RH%), temperature and photosynthetically active radiation were recorded on all sampling dates. Youngest fully matured leaf was used for all physiological and biochemical parameters. For expansion growth study youngest opened leaves were tagged and observations were recorded.

For all physiological and biochemical studies the sampling was done on every fifth day.

Observations on leaf expansion growth were recorded on alternate days.

Morphological and biomass observations were recorded when the experiment were concluded.

Growth and Biomass observations:

Youngest opened leaves were tagged (six numbers for each cultivar for each of the treatments) for leaf expansion growth. Observations on leaf length and leaf width were recorded for the

same leaves on alternate days to the nearest mm. Leaf area was estimated for these leaves as per the method of Shivasankar et.al.,(1986). Leaf expansion rate (mm/day) and leaf area development (mm2/day) were calculated for each of the treatment and varieties.

Leaf discs of 1mm diameter (20 no., replicated six times) were oven dried to a constant weight and specific leaf weight was determined using the formula

$$SLW = \frac{\text{Leaf weight}}{\text{Leaf area}}$$

and expressed as mg/mm2.

When the experiment was concluded the following morphological observations were made; root length, shoot length to the nearest cm.,root volume and leaf number.

For Biomass partioning study six randomly selected plants of each cultivar under each treatment were uprooted carefully without loosing feeder roots, washed throughly and plant parts viz.stem,leaves and roots were separated and oven dried (at 80°C). Dry weight was recorded for different plant parts(g) Root shoot ratio was calculated using the formula

$$R/S = \frac{\text{root weight}}{\text{Shoot weight}}$$

Physiological parameters:

Physiological parameters studied include soil moisture content (SMC),stomatal diffusive resistance (rs), transpiration rate (t), leaf temperature (°C), leaf water potential(ψL) and relative water content (RWC).

Soil samples were taken at 15 cm depth Soil moisture content was determined by conventional gravimetric method and expressed in percentage throughout the experiment.

Agrometerological data viz. RH%, leaf temperature and photosynthetically active radiation were recorded with steady state porometer of LICOR, Model L1 1600, Lincoln, Nebraska USA. Leaf stomatal diffusive resistance and transpiration rate were recorded on the abaxial surface of the leaves using the same equipment.

The functioning of the equipment is based on the measurement of diffusion of water vapour from the sub-stomatal cavities through the stomata. Dry gas is passed over an enclosed leaf at a known flow rate and the humidity of the gas is measured. Of all the methods used to measure stomatal resistance,diffusion parameters provide the most promising approach to quantitative measurements.

The stomatal response curve was drawn for the depleting soil moisture content and this facilitated the determination of

critical moistue content (CMC) (Vasantha et.al.-1990). Stomatal resistance was expressed as S cm-1, transpiration rate in µg/cm2/S-1 and photosynthetically active radiation as µmole, S-1,M-2.

Leaf water potential

The establishment of plant water status on a sound thermodynamic basis by the introduction of the concepts of water potential (Slatyer and Taylor 1960) and possibility of its measurement by thermocouple psychrometry and the pressure chamber techinique lead to the adoption of total water potential as the major measure of plant water status. Because of the difficulty in measuring water pontential of other plant parts, leaf water potential has become the primary index of crop water status.

The pressure chamber described by Scholander et.al.,(1964 & 65) is the most popular method used to measure water potential. The method consists of increasing the pressure around a single leaf or leafy shoot until sap from the xylem appears at the cut end of the shoot. Leaf water potential was determined using pressure chamber techinique with plant water status console model 3005 of Soil Moisture Equipment Corporation, U.S.A. The leaf water potential was expressed as - bars.

Relative water content:

Relative water content is a direct measure of tissue water

content and best measure among the water content measurements. It indicates water content relative to the maximum possible (100% relative turgidity or Zero water deficit) and it therefore, easily relates the degree of water deficit.

Leaf discs (20 Nos. of 2 cm diameter) were floated in distilled water for 2 hours immediately after recording fresh weight.After incubation turgid weight was recorded. The samples were then oven dried to constant weight and dry weight was recorded. RWC was determined using the following formula:

$$RWC = \frac{Fr.\ Wt - Dr.Wt}{Tw.\ Wt - Dr.Wt} \times 100$$

and expressed in percentage.

Bio chemical parameters:

For all assays youngest fully matured leaf was used. All absorbance measurements were recorded with SICO, GL(UV-VIS) Spectrophotometer.Incubations wherever, apporpriate was done with B.O.D. Incubator (Calton with a temperature range from 5-50°C).

Weighing was done with an electronic balance(SARTORIUS - Germany). chemicals used were of standard analytical grade.

Plant pigments:

Plant pigments viz. chlorophylls and Carotenoids were extracted and estimated as per Weybrew (1957).

One g of leaf tissue was extracted with 95% Ethanol to which 0.5 gm of calcium carbonate (to neutralise plant acids) and 0.5 gm of polyvinly pyrrolidone (to remove phenolics) was added. The extract was filtered and the residue was extracted with acetone and filtered. The filtrates were combined. This was repeated till the residue became colourless (pigment free).

The combined extrace was then transferred to a separating funnel and peroxide free ether added to it. After shaking, the pigments were forced into the ether layer by flushing with dist water. The ether layer was then collected and made upto 40 ml with ether and absorbance were recorded at 665,649,642.5,485,474 and 470 nm. The readings were computed with the following formula to calculate pigment content.

Tot. chl = $5566.5\ A_{(649)}$

Chl.a = $1994.5\ A_{(665)} - 173.4\ A_{(642.5)}$

Chl.b = $3528\ A_{(642.5)} - 607\ A_{(665)}$

Total Car = $982.1\ A_{(475)} - 0.0255$ chl.(a) $- 0.255$ chl.(b)

Caro. = $2518.2\ A_{(485)} - 1198.5\ A_{(470)} - 0.0298$ chl.(a) $+ 0.3356$ chl.(b)

Xan = $2026.1\ A_{(470)} - 2288.6\ A_{(485)} + 0.0036$ chl(a) $- 0.6518$ chl (b)

The pigment content was expressed as mg/g-1 fr.wt.

Proline content:

Free proline estimation was done as per Bates et.al.,(1973). Leaf Sample of 500 mg was homogenised with 10 ml of 3%

sulphosalicylic acid. Homogenate was filtered and the filterate was used for the assay. 2 ml of filtrate was added to 2 ml of acid Ninhydrin (1.25 gm Ninhydrin dissolved in 30 ml of glacial acetic acid). The tubes containing the mixture was incubated for 1 hr at 100°C in a water bath. After the incubation period the tubes were then transferred to an ice bath to teriminate the reaction. To each of the tubes 4 ml toluene was added and shaked thoroughly to bring the chromophore to toluene layed using a test tube saker for 15 to 20 seconds. The pigmented toluene layer was then separated in a seperating funnel and absorbance of the chromophore toluene layer was recorded at 520 nm. A standard curve was prepared using quantities of authentic proline and the proline content in the sample was calculated using the formula:

$$\frac{\mu g \text{ proline} \times \text{ml toluene} \times 4 \times 5}{115.5 \times \text{fr.wt.}} = \mu \text{mole of proline/g}$$

Total sugars:

For total sugar estimation 100 mg of leaf tissue was homogenised with 10 ml ethanol and the homogenate filtered. The filtrate was then passed through magnesium oxide column to remove plant pigments. The extract thus obtained was used for estimation of Sugars (Dubois et.al.1951). Total sugars was estimated using anthrone reagent. To one ml of extract 4 ml of anthrone reagent was added (2 gm. of Anthrone dissolved in 1L of

conc. $H_2 SO_4$). The tubes were placed in boiling waterbath for one minute and cooled in running water. Absorbance was recorded at 625 nm. A standard curve was prepared using known amount of glucose and the sugar content of the samples was calculated from the standard curve.

Total phenols

Total phenol content was estimated as per Bray and Thorp (1954). To 1 ml of alcohol extract 1 ml of Folin-ciocalteu's reagent was added followed by 2 ml of 20% sodium carbonate solution. The blue resultant solution was centrifuged to remove the prcipitate and made up to 25ml and the absorbance was recorded at 650 nm. A standard was prepared using catechol and phenolic content was calculated from the standard curve and expressed as mg/g as equivalent of catechol.

Nitrate reductase:

Nitrate reductase activity was estimated spectrophotometrically using sulphanilamide and N- (1.naphthyl) ethylene diamine dihyrochloride reagents, as per Hageman and Hucklesby (1971). About 200 mg of leaf tissue was cut into 2 to 3 mm pieces and suspended in 5 ml of 0.1 M Phosphate Buffer pH 7.0 containing 0.1 M KNO3. The tubes were kept in desiccator and vaccum infiltrated for 3 minutes. It was then incubuated for 2 hours at 35°C. At the end of the incubation period the extract was filtered through activated charcoal (to remove pigments) and the filtrate was used for assay of enzyme activity.

Enzyme activity assay:

To 0.4 ml of extract was added 0.2 ml of 1% sulphanilamide in 3NHCL and 0.2 ml of 0.2% N.napthyl ethylene diamine dihydrochloride. After 20 minutes 4 ml of distilled water was added and the absorbance was measured at 540 nm. A standard curve was prepared using known amount of Pottasium nitrite and nitrate formed in the sample was calculated from standard curve. The NR activity was expressed as μ moles of Nitrate formed/hr/g.(fr.wt).

Peroxidase activity:

Peroxidase was extracted and assayed as per Ujwal Kumar (1982). Leaf tissue of 100 mg was homogenised in 10 ml (50mm) phosphate buffer PH 6.0, containing 1MNacl and polyvinyl pyrrollidone. The homogenate was centrifuged for 15 mts. at 10000 rpm at 5°C. The supernatent was used for enzyme activity.

Enzyme activity assay:

To 4.5 ml of citrate buffer (10 mm) PH 5.5 containing 0.5% Guaicol (substract) was added 0.25 ml of 0.1% hydrogenperoxide and the absorbance adjusted to 0. To this cuvette was added 0.25 ml of enzyme extract, shaked throughly and increase in absor bance was recorded for every 30 seconds for 3 minutes. The velocity of reaction was calculated from the linear portion of

the curve (at 470 nm) and peroxidase activity was expressed as unit activity/hr/g of tissue (1 unit of peroxidase activity is defined as the amount of enzyme required to cause an absorbance of 0.1 per minute at 470 nm).

Acid phosphatase activity:

Acid phosphatase activity was assayed as per Jones (1969).one hundred mg leaf tissue was homogenised with 0.85% Nacl in an ice bath. The homogenate was filtered and centrifuged at 10000 Rpm for 20 minutes at 4°C. The supernatant was used for enzyme activity assay.

Assay of enzyme activity:

To 1 ml of extract, 1 ml of substrate solution was added (p.nitrophenyl phosphate, 5mg/10ml) 0.1 M Magnesium chloride 1ml; 0.1 M Acetate buffer pH 4.8 30ml). The mixture was kept in an incubator at 37°C for 30 minutes. After incubation 5 ml of 0.1N NaOH was added and the resultant yellow colour was read at 410 nm. A standard curve was prepared with known amount of p.nitrophenol and p.nitrophenol content of the samples were calculated from the standard curve. Acid phosphatase activity was expressed as μmoles of p.nitrophenol formed/hr/g (fr.wt).

Statistical analysis was done for all the characters studied using randomised complete block design. Individual characters

significance and interaction of various factors were worked out in computer using Irristat program. Correlation matrix for all the twenty four characters was prepared using the above programme.

Indexing of characters

Characters which has shown very high correlation with soil moisture content were shortlisted for indexing for drought tolerance. The indexing is based on stomatal resistance. eg. stomatal resistance was taken in x axis and each of the other characters selected in y axis. Half-max line was drawn for both stomatal resistance and respective characters selected. The half-max lines divided the graph into four blocks. The cultivars studied were fitted to whichever block it belongs. Block numbering and scoring was done as follows:

eg. Stomatal resistance vs leaf water potential

Character	Block	Score
High Stomatal resistance and high leaf water potential	I	1
High stomatal resistance and low leaf leaf water potential	II	2
Low Stomatal resistance and high leaf water potential	III	3
Low stomatal resistance and low leaf water potential	IV	4

The scoring is in the ascending order from block I. The lowest score for each of the character is the most preferred.

Screening of promising lines:

Five promising genotypes (viz. Acc. no.1495,KS69,KS88 Panchami and Acc no.931 were screened in pot culture experiment. Moisture stress was imposed by withholding watering to a set of plants. The methodology was similar to previous experiment. Observations on short listed characters viz., stomatal resistance, transpiration rate, leaf water potential, relative water content,specific leaf weight,proline content,total sugars and nitrate reductase activity,were recorded at four days interval till the plants of anyone of the genotypes showed wilting symptoms. Each of the above parameters were recorded as explained in the first experiment using standardised methodology.

Indexing methodology proposed in the first experiment was used to classify the genotypes for drought tolerance.

Field experiment: The two promising lines (for drought tolerance) viz Acc.no.1495 and KS69 were planted in the field (12nos each) to test their field tolerance. Recommended agronomic practices were followed while planting and irrigation was given in the first year (1991) during summer months, for the

establishment of plants. In the following years the plants were maintained as rainfed crop. Physiological parameters were recorded, at monthly intervals included, stomatal resistance, transpiration rate,leaf water potential and soil moisture content. Results are presented in graphic mode.

Results

RESULTS

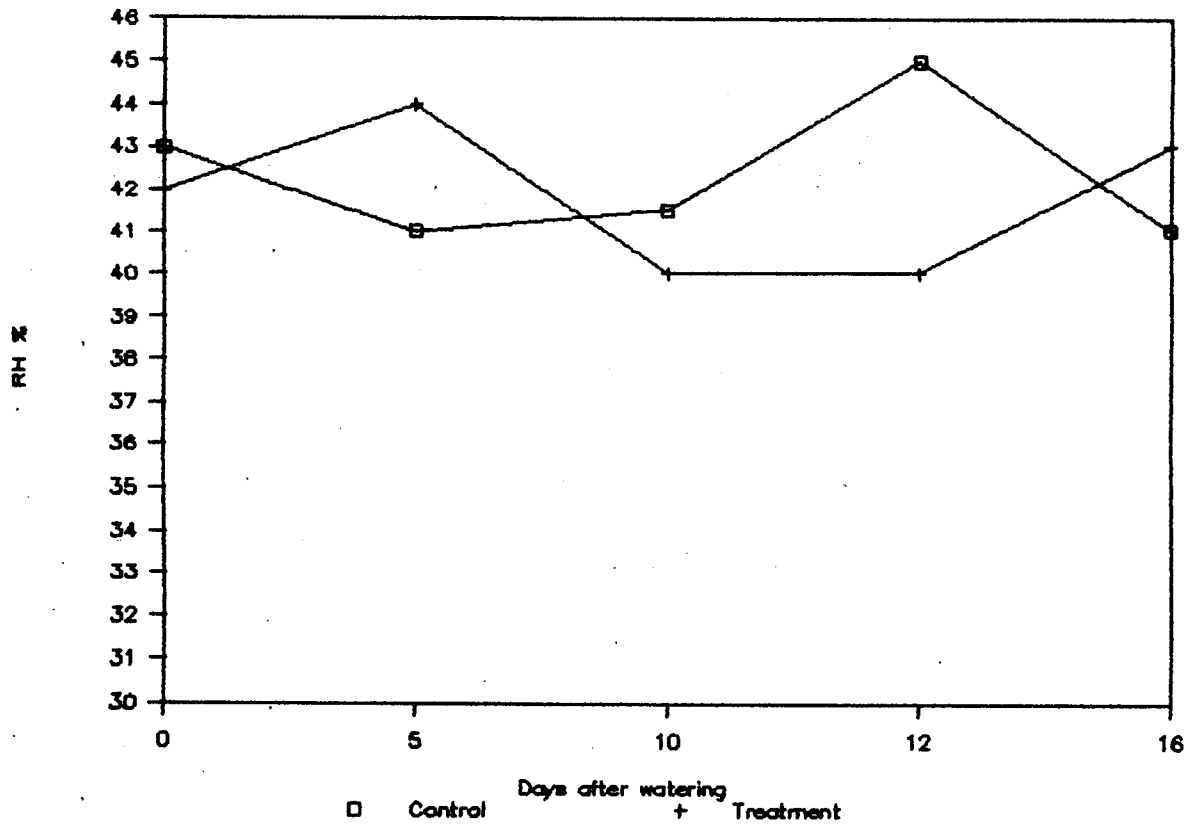
Relative Humidity (RH%), temperature and radiation inteception during the experiment

Many crop species experience detrimental effects during some part of their life cycle due to soil and atmospheric drought (Hall 1981). The important aspect of moisture stress studies is the agroclimatic conditions viz., Relative humidity (RH%), temperature (°C) and radiation interception, prevailed during the experiment. Several of the plant responses are influenced by climatic factors especially under soil moisture stress. Higher temperature and radiation interception coupled with lower relative humidity (RH%) increased the intensity of moisture stress in coconut (Rajagopal et.al, 1989) and Cocoa (Balasimha & Rajagopal 1986).

Relative Humidity (RH%)

Relative humidity (%) recorded on different sampling dates both for control and stress treatment is presented in Fig.1. Relative humidity ranged from 40-45% in control and moisture stress treatment indicating the higher evaporative damand irrespective of the treatment. Variations between treatments and different dates of sampling were insignificant. The lower RH% recorded increased the intensity of soil moisture stress through higher evaporative demand of the atmosphere.

Fig.1 : Relative humidity (%) during different sampling dates



Temperature

Temperature recorded of different sampling dates both for control and moisture stress ranged from 29-33 ° C during the experiment (Fig.2). The higher temperature increased the evaporative demand thereby intensifying soil moisture stress. Temperature variations were little for treatment as well as different dates of sampling.

Radiation Interception

Radiation interception recorded for control and moisture stressed plants on different sampling dates is presented in Fig.3. Photosynthetically active radiation (PAR) ranged from 1300-1400 (µmole/S/m2). Variations due to treatment as well as different dates of sampling were negligible with regard to radiation interception.

Higher radiation interception and higher temperature coupled with low relative humidity creates an atmosphere,demanding higher evaporation which in turn intensifies the soil moisture stress (Rajagopal et.al. 1989). Daily pan evaporation during the experimental period was about 4.5 to 5.2 mm (unpublished data). This indicates the higher intensity of moisture stress prevailed during the experiment.

Fig.2 : Temperature recorded during different sampling dates

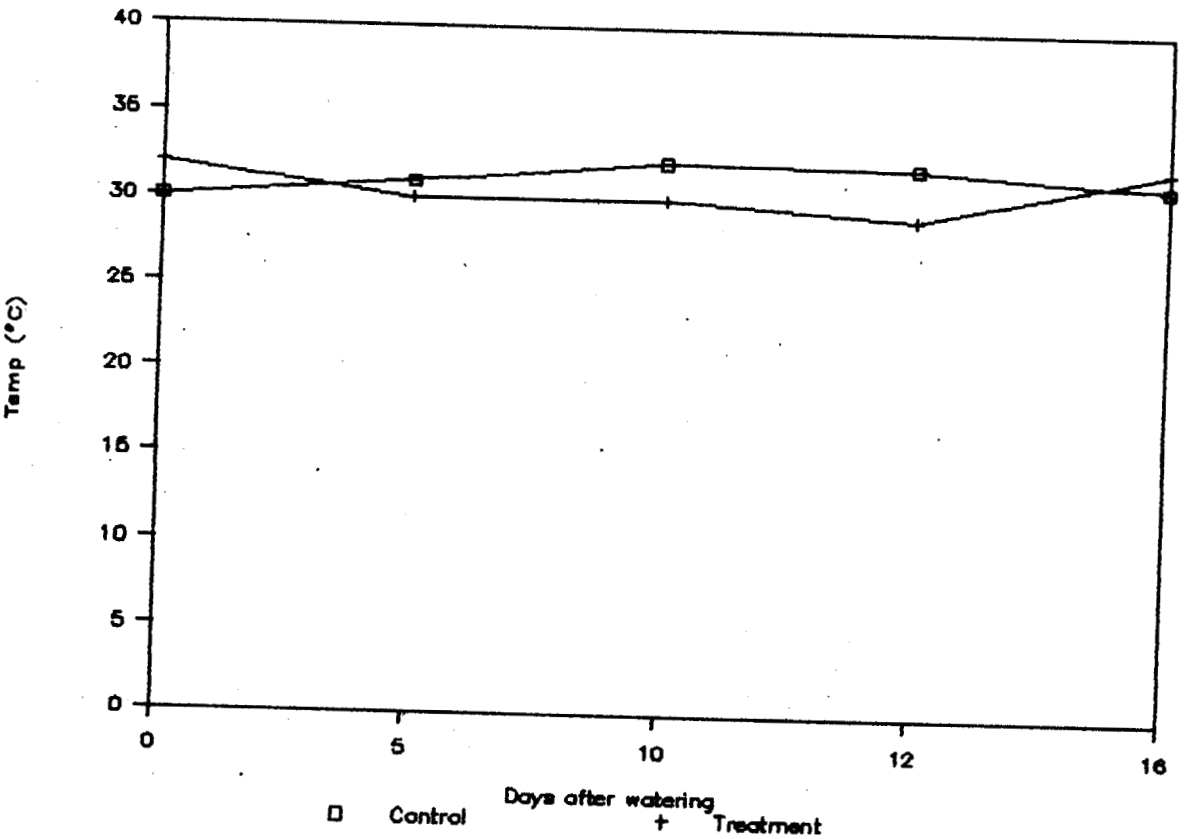
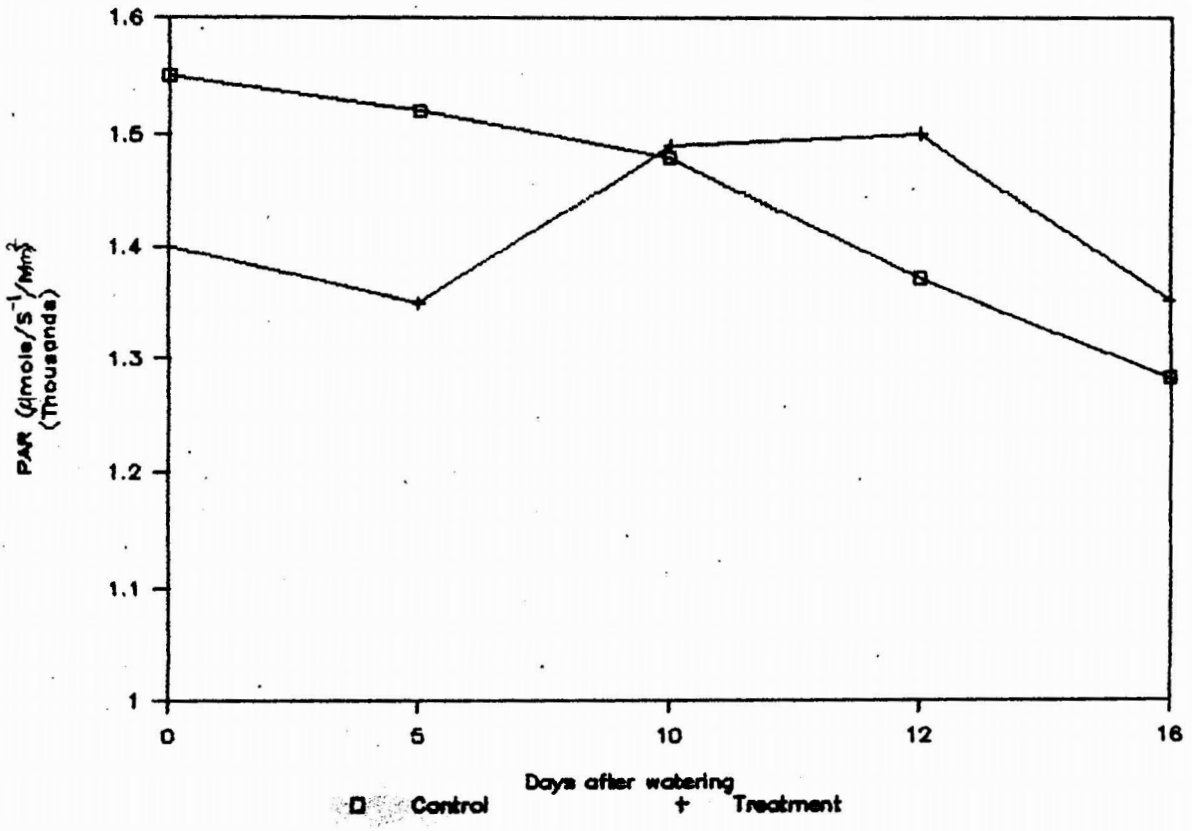


Fig.3 : Photosynthetically active radiation during different sampling dates



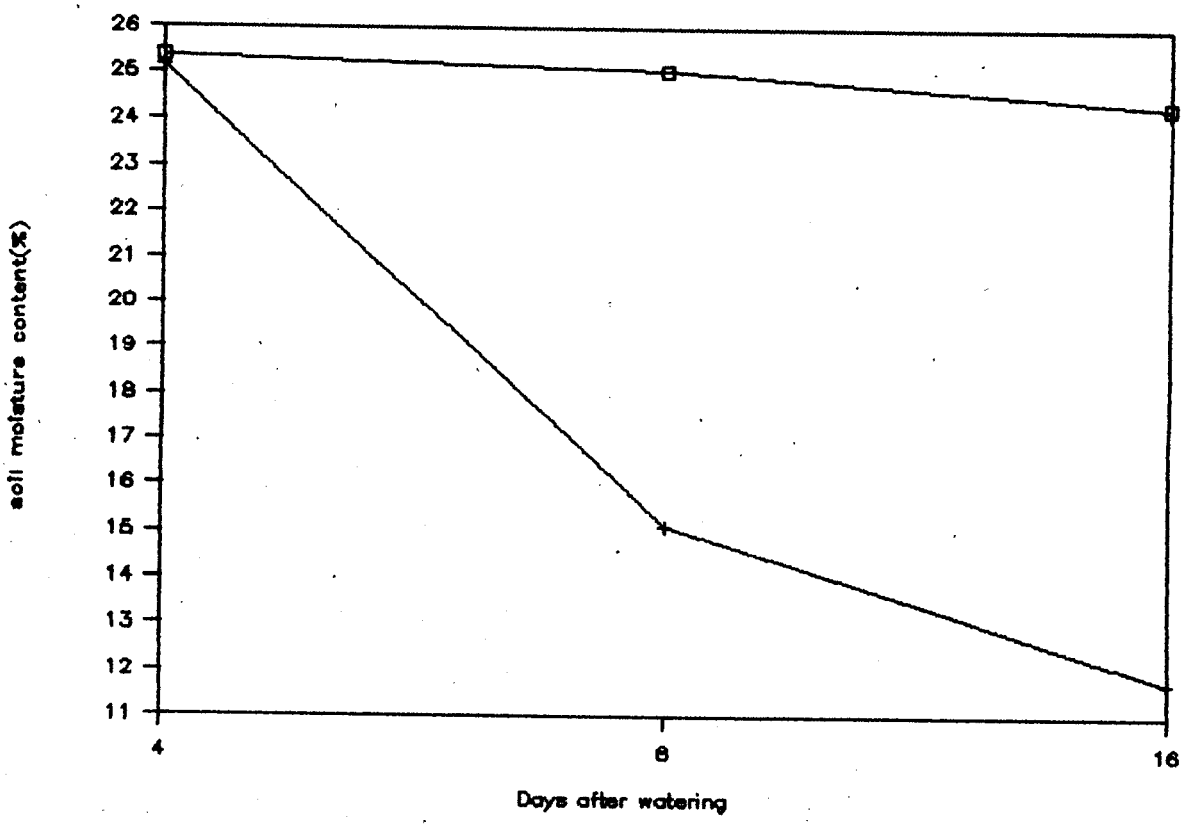
Soil moisture depletion pattern in relation to stress development :

The intensity of soil moisture depletion is high when atmospheric drought exists alongwith soil drought (Rajagopal et.al. 1989.). This highlights the importance of environmental influence on production of moisture stress and soil moisture depletion pattern through soil plant-atmosphere continuum.

Soil moisture depletion pattern :

The field capacity of the soil ranged from 24-26%. Soil moisture depletion pattern for both control and moisture stress tretment is presented in Fig.4. Soil moisture depleted by 56% in stress treatment in about 20 days after watering and the decline was sharp. Soil moisture content on different sampling dates is presented in table 1. In control plants the soil moisture varied from 24-26% while in moisture stress treatment the soil moisture content varied from 24.0 to 10.4 % from, 0 days till the last sampling. The soil moisture content declined moisture stress reached clitical limits (visual wilting symptoms observed) at about twenty days after Watering was withheld in the treatment plants. Interaction between cultivars and treatments (control & M.S) was significant only at the last sampling date, while at first and second sampling stages soil moisture content did not differ significantly between treatments or cultivars.

Fig.4 : Soil moisture depletion pattern



□Control + Treatment

Table 1. Soil moisture content on different sampling dates.

S.NO.	CULTIVAR	SOIL MOISTURE CONTENT (%)					
		CONTROL			MOISTURE STRESS		
		1	2	3	1	2	3
1.	Aimpiriyan	25.93	25.68	24.97	25.53	15.30	12.53
2.	Arakulam munda	24.81	24.34	23.98	24.32	14.97	10.69
3.	Kalluvally	25.55	24.99	24.34	25.15	14.16	10.43
4.	Karimunda	25.50	26.00	25.42	25.33	15.41	12.37
5.	Narayakodi	24.68	24.67	24.52	25.04	15.56	11.15
6.	Panniyur-1	25.69	25.87	25.47	25.62	15.15	13.37
		NS	NS	NS	NS	NS	*

NS : Not Significant, * Singnificant at 5% level : CD : 1.79

Interactions :

Cultivar x Stages = Not significant
Cultivar x Treatment = Not significant
Stages x Treatment = Significant at 5% level L.S.D. =0.85

1, 2 and 3 Corresponds 0, 10th and 16th day after watering

Cultivars differed in soil moisture extraction capacity and also in various responses to depleting soil moisture content. cv. Kalluvally recorded lowest soil moisture content of 10.4% in the last sampling date while cv. Panniyur-1 recorded highest Soil Moisture Content of 13.4% and showed wilting symptoms.

Growth responses under moisture stress:

The most sensitive response of plants to moisture stress is the developmental/growth response, as the growth of a species entirely depend on the moisture supply(Passiouia and Anne gardner 1990, Kallarackal et. al. 1990, Kemp et.al. 1989). Among growth parameters leaf expansion rate and leaf production rate are important as these determine the canopy structure and crop stand. Leaf production reduced when the moisture supply becomes a limiting factor. Poor biomass production and partitioning has been attributed to moisture stress in coconut (Rajagopal et. al. 1989).

Leaf expansion rate :

Leaf expansion rate both length and widthwise is presented in Figs.6 and 7. The leaf expansion rate declined with the onset of moisture stress in all the cultivars studied. The expansion rate was negligible from 5th day (after commencement of Moisture Stress treatment) onwards and ceased completely thereafter on 8th

Fig.6 : Leaf expansion rate (Lengthwise) under moisture stress

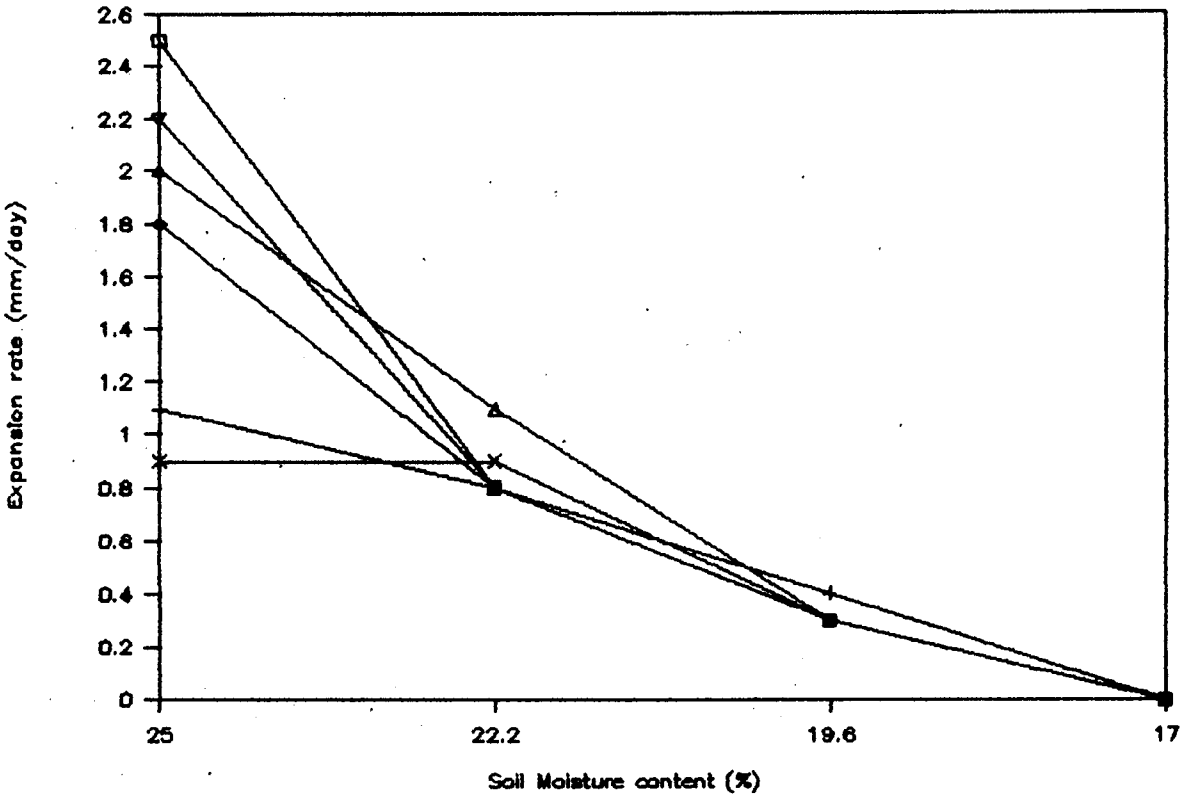
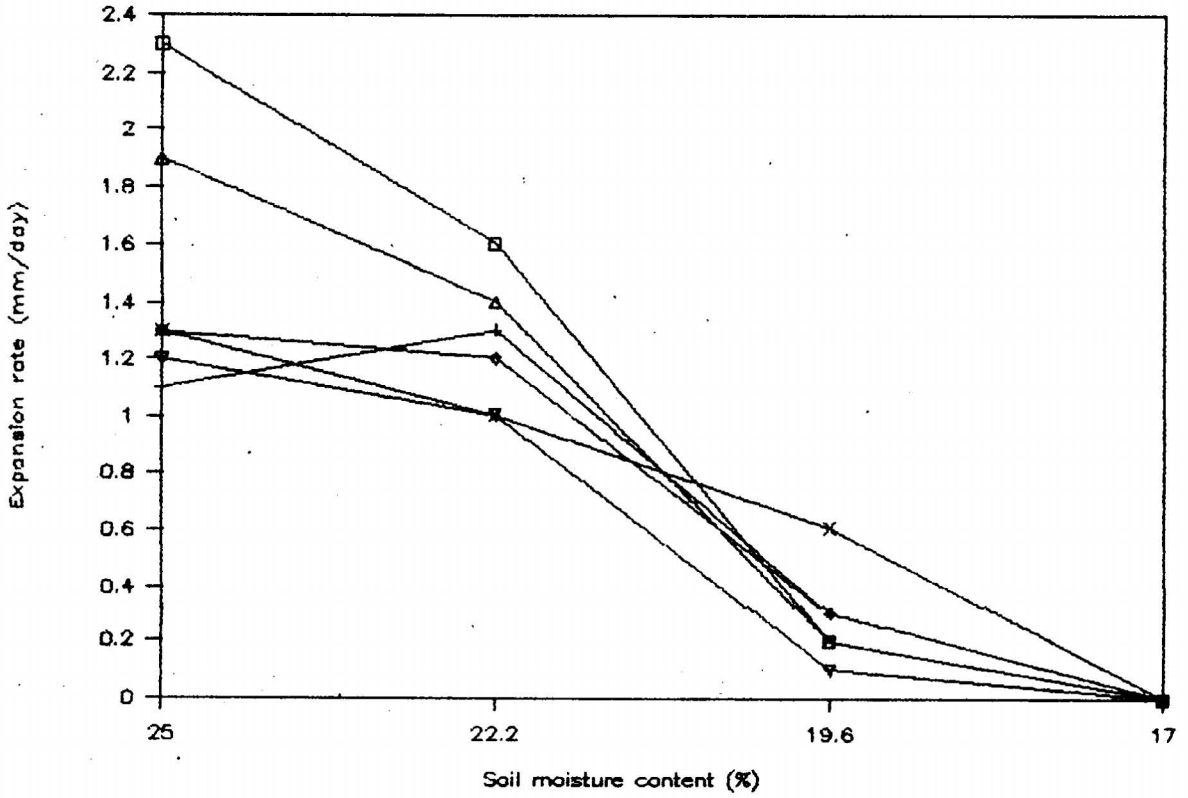


Fig.7 : Leaf expansion rate (widthwise) under moisture stress



day. The leaf expansion rate was very low when the soil moisture content was 19.5% and as the soil moisture content declined to 17% the leaf expansion ceased. This indicates the sensitivity of leaf expansion growth to even mild stress. Leaf area increment (mm^2/day) is presented in table 2, which shows similar trend. The decline in leaf expansion rate is reflected upon the leaf area increment. The decline in leaf area increment was sharp and leaf area increment ceased as leaf expansion ceased.

Morphological characters :

Morphological characters viz., root length, shoot length and leaf No. did not correlate with moisture stress (table 15). Root length, shoot length and leaf No., for control and stress treatment for the cultivars studied is presented in table 3. Root length varied from 21.4 to 32.0 cm in control plants and from 21.8 to 35.7 cm in moisture stressed plants. Root length did not show significant difference due to moisture stress. The lowest and highest root length recorded for control was in cv. Aimpiriyan (21.4 cm) and cv. Kalluvally (32 cm) respectively.

In moisture stress treatment the lowest root length was recorded in cv. Panniyur -1 and highest in cv. Kalluvally. Shoot length ranged from 38.5 - 51.4 cm in control plants and from 21.8 to 64.6 cm in stressed plants. The lowest shoot length in both control and moisture stress treatment was recorded in cv.

Table 2. Leaf area Increment as influenced by moisture stress

S.NO.	CULTIVAR	LEAF AREA INCREMENT mm^2 / day							
		CONTROL				MOISTURE STRESS			
		25.00	25.06	24.23	24.8%	22.20	19.60	17.0	14.2
1.	Aimpiriyan	8.8	5.3	4.7	3.2	4.7	0.8	-	-
2.	Arakulam munda	3.0	5.1	5.0	2.7	4.0	1.2	-	-
3.	Kalluvally	4.1	4.8	5.1	5.0	3.4	0.8	-	-
4.	Karimunda	5.8	3.5	1.9	1.9	3.9	0.9	-	-
5.	Narayakodi	3.7	2.0	3.1	2.7	3.0	1.5	-	-
6.	Panniyur-1	6.2	2.8	2.5	6.3	3.3	0.5	-	-

* Cultivar X Treatment X Stages interaction significant at 5% level by DMRT

L S D : 1.8

Panniyur -1 (38.5 & 21.8 cm respectively). The highest shoot length for control was recorded in Cv. Arakulamunda (57.4 cm) and for moisture stress treatment in cv. Kalluvally (64.6 cm). Leaf nos. varied from 5.8 to 12.2 in control plants highest being in Cv. Arakulamunda and lowest in Cv. Karimunda & Panniyur -1. In moisture stress treatment leaf no. varied from 4.2 to 11.0, highest being in Cv. Kall uvally and lowest in Panniyur -1. Genotypic variation as well as treatment variations were not statistically significant.

Biomass allocation :

Biomass partitioning into stem, leaf and root is presented in table 4. Dry matter allocation towards leaf was highest followed by stem. Stem weight varied from 3.7 to 5.2g in control, highest being in Cv. Arakulamunda and lowest in Cv. Aimpiriyan. In moisture stress treatment stem weight ranged from 3.4 to 5.4g highest in Kalluvally and lowest in Cv. Panniyur -1. Cultivar x treatment interaction was statistically significant at 5% level for stem weight. Dry matter content of leaves varied from 4.1 to 5.8g in control plants, highest being in Cv. Arakulammunda and lowest in Cv. Karimunda. In moisture stressed plants the biomass allocation towards leaves varied from 3.8 to 5.6g, highest being in Cv. Kalluvally and lowest in Cv. Karimunda. Cultivar x treatment interaction was significant (at 5% level) with

TABLE 4 : Biomass partitioning in different cultivars subjected to moisture stress

S.NO.	CULTIVAR	Root (g)		Stem (g) *		Leaf (g) **	
		C	M S	C	M S	C	M S
1.	Aimpiriyan	3.32	3.40	3.72	3.80	4.22	4.18
2.	Arakulam munda	3.80	3.94	5.16	5.18	5.84	5.38
3.	Kalluvally	4.08	4.48	4.64	5.36	5.42	5.60
4.	Karimunda	3.18	3.16	3.74	3.58	4.14	3.84
5.	Narayakodi	3.92	3.64	4.62	3.80	5.40	4.24
6.	Panniyur-1	3.32	3.20	3.80	3.38	4.64	3.96

Interaction

* Cultivar x Treatment significant at 5% level by DMRT - L S D:0.52

** Cultivar x Treatment significant at 5% level by DMRT - L S D:0.60

respect to leaf biomass allocation. Specific leaf weight and root/shoot ratio recorded for various cultivars are presented in table 5. specific leaf weight decreased in all the cultivars in the last stage of sampling. Specific leaf weight varied from 5.0 to 6.6 mg/cm^2 in control plants while in treatment plants it ranges from 2.9 to 4.7 mg/cm^2. Specific leaf weight showed a significant and positive correlation with depleting soil moisture content (r=0.695)

Root/Shoot ratio varied from 0.39 to 0.43 in control plants and 0.39 to 0.45 in stress treated plants. It remained more or less the same with slight increase in some of the cultivars. Root/Shoot ratio showed negative correlation with depleting moisture content, though not highly significant. However, cultivars variation due to moisture stress treatment was not significant.

The results recorded on growth responses show the influence of depleting soil moisture content at the root region on general biomass production, allocation and expansion growth. However, the results do not warrent the utilisation of the above parameters for indexing for drought tolerance, as leaf expansion is very sensitive to even mild stress and has not expressed variations due to genotypes and biomass partitioning among

TABLE 5 : Specific leaf weight and Root/Shoot ratio as affected by moisture stress*

S.NO.	CULTIVAR	Specific Leaf Weight		Root / Shoot Ratio	
		C	M S	C	M S
1.	Aimpiriyan	5.21	4.56	0.43	0.43
2.	Arakulam munda	5.04	2.87	0.35	0.39
3.	Kalluvally	6.62	4.22	0.41	0.41
4.	Karimunda	6.51	4.72	0.41	0.43
5.	Narayakodi	6.05	3.01	0.40	0.45
6.	Panniyur-1	5.47	3.16	0.39	0.44

* Values are means of six replications

cultivars were not significantly different.

Stomatal resistance, transpiration rate, leaf water potential and relative water content (water release curve.)

Physiological parameters viz. Stomatal resistance, transpiration rate, leaf water potential and relative water content have been shown to respond not only to, soil moisture stress but also to atmospheric drought(Rajagopal et.al.1989). Stomatal response to humidity, temperature aids the plant to minimise the effect of changing atmosphere. Hence, stomatal regulation is an important mechanism to maintain turgidity. The degree of stomatal closure with the detection of stress varies with crop species.

Stomatal resistance:

Stomatal resistance showed a highly significant and negative correlation with depleting moisture content ($r = -0.894$). Stomatal resistance recorded on different sampling dates for

control and moisture stressed plants is presented in table 6. In control plants, the stomatal resistance ranged from 1.12 to 1.63 $S.cm^{-1}$, throughout the experiment and was not significantly different among the cultivars or different dates of sampling. In moisture stressed plants, stomatal resistance varied from 1.41 to 20.66 (S,cm-1) from o day till the conclusion of the experiment (the experiment was concluded when majority of plants of a cultivar wilted). Cultivars difference was highly significant (At 1% level) during 2nd and 3rd sampling dates in moisture stress treatment. Further, treatment x stages interaction was highly significant.

Transpiration rate:

Transpiration rate recorded for the cultivars on different sampling dates both in control and stress treatment is presented in table 7. The transpiration rate ranged from 12.77 to 13.09 (µg cm2, s1) in control plants while in moisture stress treatment the range was from 0.26 to 13.21 (µg, cm2, s1) during entire period of the experiment. In moisture stressed plants the highest transpiration rate record during last stage of sampling was 2.16 (Cv.Aimpiriyan) and lowest was 0.26(µg, cm2, s1) (Cv.Panniyur-1). Transpiration rate has shown, highly significant and positive correlation with depleting soil moisture content (r=0.932, table 15). Statistical analysis showed significant difference with respect to transpiration rate in the last two stages of moisture stress treatment. Cultivar x Treatment x Stages interaction was also significant (at 1% level).

Table 7. Transpiration rate on different sampling dates

S.NO.	CULTIVAR	TRANSPIRATION RATES (Mg, Cm^{-2}, S^{-1})					
		CONTROL			MOISTURE STRESS		
		1	2	3	1	2	3
1.	Aimpiriyan	12.92	13.09	13.08	13.15	6.02	2.16
2.	Arakulam munda	13.02	13.04	12.88	13.19	5.23	0.67
3.	Kalluvally	12.83	13.03	13.02	13.21	3.60	1.38
4.	Karimunda	12.77	12.82	12.90	13.18	3.43	1.32
5.	Narayakodi	12.83	13.06	13.05	13.04	3.42	1.44
6.	Panniyur-1	12.88	13.14	13.03	13.07	5.58	0.26
		NS	NS	NS	NS	*	*
	C D	-	-	-	-	0.57	0.38

* Significant at 5% level

Interactions :

Cultivar x Treatment x Stages : Significant at 1% level

by DMRT L S D : 0.75

Leaf Water Potential:

Leaf water potential recorded on different sampling dates is presented in table 8. In control plants, when moisture supply is not a limiting factor, the leaf water potential ranged from -5.6 to -6.5 bars. However, with the development of stress leaf water potential lowered in all the cultivars. In stressed plants leaf water potential ranged from -5.8 to -20 bars. Cultivars differed significantly with regard to leaf water potential in 2nd and 3rd sampling dates in moisture stress treatment(1% level). Cultivar x treatment x stages interactions were also significant. Leaf water potential showed a significant and positive correlation with depleting soil moisture content ($r = 0.833$, table 15).

Water release curve:

To obtain water release curve for the cultivars studied, relative water content recorded was plotted against leaf water potential. The water release curve for the six cultivars is presented in fig.8. Cultivars having a smaller slope represent the better osmo-regulation than one showing larger slope. Cvs. Kalluvally, Karimunda and Narayakodi showed a smaller slope than Cvs.Aimpiriyan, Arakulam munda and Panniyur -1.

The physiological responses studied viz. stomatal resistance, transpiration rate, leaf water potential, and water release curves present meaningful data that can be utilised for

Table 8. Leaf water potential on different sampling dates

S.NO.	CULTIVAR	LEAF WATER POTENTIAL (- bars)					
		CONTROL			MOISTURE STRESS		
		1	2	3	1	2	3
1.	Aimpiriyan	5.67	5.77	6.50	5.97	10.87	18.77
2.	Arakulam munda	5.60	6.37	6.20	5.83	10.53	19.20
3.	Kalluvally	6.47	6.50	6.23	6.23	9.73	13.43
4.	Karimunda	6.27	6.03	6.43	6.10	9.23	12.27
5.	Narayakodi	6.43	6.30	6.43	6.60	9.23	13.27
6.	Panniyur-1	6.43	5.90	6.53	6.63	11.17	20.00
		NS	NS	NS	NS	*	*
	C D	-	-	-	-	1.19	0.97

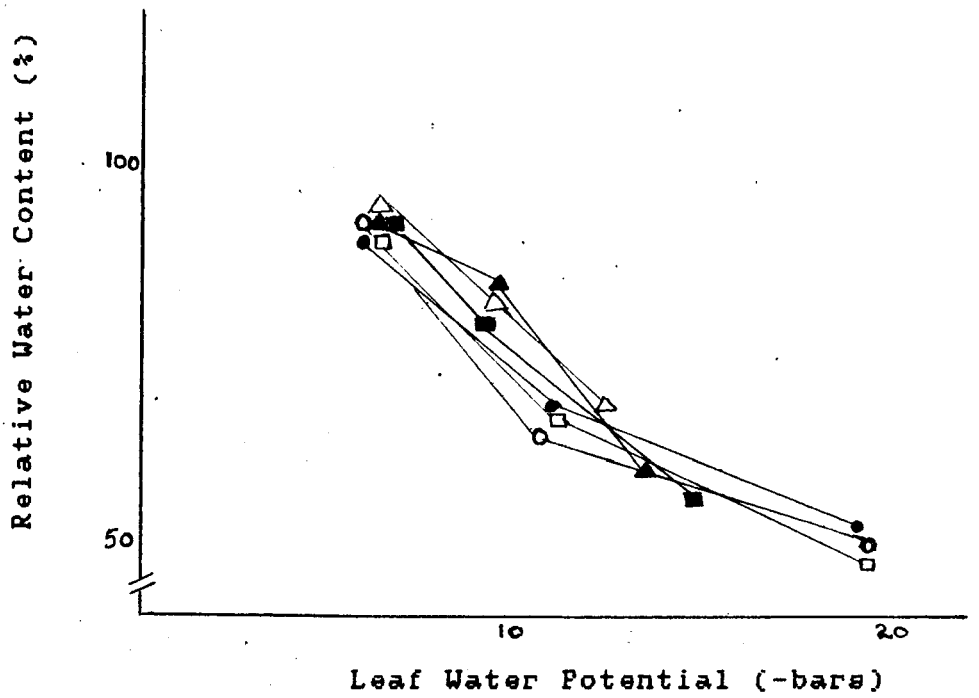
N.S : Not Significant, * Significant at 1% level.

Interaction :

Cultivar x Treatment x Stages : Significant at 1% level

by DMRT : L S D = 1.28

Fig.8 : Water release curve for different cultivars



scoring for drought tolerance, apart from being realistic(these responses are considered a line response to moderate stress, Hanson, 1980).

Photosynthetic pigments, sugars, phenols and free proline changes due to moisture stress:

Responses at biochemical level are expected only when the stress attains critical proportions (Bradford & Hsiap 1982). Total chlorophyll content and pigment level were reported to alter due to moisture stress(Alberte et.al.1977). Sufficient volumes of reports are available on the accumulation of proline(Stewart 1981., Channan Itai et.al.1988. Venkataramana et.al.1988) and sugars (Drossopoulos et.al.1987, Garg et.al.1981, Cortes & Sinclair 1987, Fanjul & Rosher 1984) in different crop species.

Chlorophyll Pigments:

Pigments viz. total chlorophylls, chlorophyll a and b contents for various cultivars in control and moisture stress treatment is presented in table 9. Total chlorophyll ranged from 2.0 to 4.76 (mg/g fr.wt) and 1.1 to 2.6 in control and moisture stressed plants respectively. Chlorophylla ranged from 1.7 to 3.1 (mg/g)in control plants and in moisture stress treatment it ranged from 0.7 to 1.75(mg/g). Chlorophyll b ranged from 0.6 to 1.2 and 0.3 to 0.7(mg/g) in control and moisture stressed plants respectively. Total chlorophylls, chlorophyll a and b declined

Table 9. Chlorophyll pigments as affected by moisture stress
(mg/g)

S.NO.	CULTIVAR	Tot. Chl. *		Chl. a		Chl. b		a/b	
		C	M S	C	M S	C	M S	C	M S
1.	Aimpiriyan	2.95	1.81	2.07	1.22	0.79	0.43	2.72	2.90
2.	Arakulam munda	2.60	1.14	1.72	0.72	0.73	0.34	2.41	2.18
3.	Kalluvally	2.00	1.13	1.28	0.71	0.62	0.32	2.11	2.26
4.	Karimunda	3.09	1.58	2.18	1.11	0.82	0.45	2.72	2.47
5.	Narayakodi	2.55	1.27	1.76	0.86	0.70	0.36	2.56	2.43
6.	Panniyur-1	4.76	2.60	3.10	1.75	1.24	0.73	2.51	2.42

* Cultivar x treatment Interaction significant at 5% Level by DMRT

L.S.D : 0.54

in moisture stressed plants compared to control in all the cultivars. The reduction in total chlorophylls ranged from 38.6 to 56.2%. Cultivar x treatments interaction was significant with respect to total chlorophyll (at 5% level). However, chlorophyll a/b ratio did not difer significantly between treatments or cultivars.

Carotenoid Pigments:

Carotenoid pigments, viz., total carotenoids, carotenes and xanthophylls estimated for various cultivars for both control and moisture stress treatment are presented in table 10. Total carotenoids ranged from 0.57 to 1.19 (mg/g) and 0.32 to 0.57 in control and moisture stress plants respectively. Carotenes ranged from 0.25 to 0.43 (mg/g) in control plants while stress treatment it varied from 0.11 to 0.17 (mg/g). Xanthrophylls ranged from 0.29 to 0.72 and 0.16 to 0.42(mg/g) in control and moisture stressed plants respectively. Cultivar x treatment interaction was significant (at 5% level)with respect to total carotenoid content.

Sugars:

Total soluble sugars estimated for both control and moisture stressed plants is presented in table 11. Sugars ranged from 13.23 to 29.38 (mg/g fr.wt) in control plants while in stressed plants it ranged from 27.82 to 52 (mg/g fr.wt). Accumulation of

Table 10: Carotenoid pigments as affected by moisture stress

(mg/g)

S.NO.	CULTIVAR	Tot. car *		Carotenes		Xanthophylls	
		C	M S	C	M S	C	MS
1.	Aimpiriyan	0.78	0.51	0.34	0.13	0.42	0.38
2.	Arakulam munda	0.74	0.32	0.43	0.14	0.39	0.16
3.	Kalluvally	0.57	0.32	0.25	0.11	0.29	0.23
4.	Karimunda	0.92	0.46	0.34	0.17	0.54	0.28
5.	Narayakodi	0.74	0.32	0.28	0.12	0.43	0.19
6.	Panniyur-1	1.19	0.57	0.34	0.14	0.72	0.42

* Cultivar x Treatment interaction significant at 5% level by DMRT L S D : 0.11

Table 11: Free proline and total sugars in black pepper cultivars

S.NO.	CULTIVAR	Proline (μmoles /g)		Sugars (mg/g)	
		C	M.S	C	M.S
1.	Aimpiriyan	4.14 ±(0.46)	7.52 ±(1.14)	26.43 ±(5.68)	43.13 ±(11.38)
2.	Arakulam munda	4.10 ±(0.38)	6.16 ±(0.81)	13.23 ±(1.97)	31.40 ±(4.89)
3.	Kalluvally	4.29 ±(0.36)	12.11 ±(1.28)	29.38 ±(1.88)	44.68 ±(5.30)
4.	Karimunda	4.25 ±(0.45)	9.58 ±(0.60)	27.41 ±(3.89)	52.00 ±(4.06)
5.	Narayakodi	3.72 ±(0.61)	11.53 ±(1.56)	17.03 ±(2.70)	27.82 ±(2.35)
6.	Panniyur-1	4.14 ±(0.52)	5.61 ±(0.67)	25.42 ±(4.07)	32.32 ±(6.53)

* Values are means of six replications each

± Standard deviation

sugars was highest in Cv.Karimunda followed by Kalluvally. Cv. Narayakodi, recorded lowest sugar content. Total sugars showed significant and negative correlation with depleting soil moisture content (table 15). The higher accumulation of sugars occurred when the soil moisture content reached critical limit (SMC = 11.75%).

Proline content:

Free proline content for control and moisture stress treatment is presented in table 11. In control plants proline content ranged from 3.72 to 4.29 μ moles/g fr.wt of leaf tissue while in treatment plants it ranged from 5.61 to 12.11 (μmoles/g) under stress conditions. Cv. Kalluvally accumulated maximum proline (12.11) followed by Cv.Narayakodi (11.53). The increase in proline content was threefold in the above cultivars while Cv.Panniyur -1 the increase was little. Proline content showed significant and negative correlation with soil moisture content (table 15).

The biochemical parameters studied viz., total sugars, proline content and pigments were found to alter due to moisture stress. These parameters were found to correlate with stress and showed significant difference among genotypes studied. Parameters that showed high significance (total sugars and proline level) with moisture were used in scoring cultivars for drought tolerance.

Nitrate reductase, peroxidase and acid phosphatase activity:

Several enzyme systems were reported to undergo changes due to moisture stress. Hydrolases activity increase while carboxylases/reductases decrease due to moisture stress (Glenn.W.todd 1960). Nitrate reductase activity is reduced due to moisture stress (Paleg & Aspinall 1981) both acid phosphatase and peroxidase activity increased as an influence of moisture stress.

Nitrate reductase activity:

Nitrate reductase activity declined at a rapid pace in moisture stress treatment in all the cultivars studied. However, the degree of reduction varied with cultivars. Fig.13 shows the decline pattern of nitrate reductase activity for different black pepper, cultivars. NR activity was lowest in Cv.Panniyur-1 followed by Cv.Aimpiriyan. Cvs. Kalluvally, Karimunda and Narayakodi showed slightly higher NR activity. Nitrate reductase activity showed positive correlation with depleting soil moisture content. (table 15).

Acid phosphatase activity:

Enzyme acid phosphatase activity increased in all the cultivars studied irrespective of their degree of drought tolerance (table 12). Acid phosphatase activity ranged from 11.4 to18.9 (μmoles/g of p.nitrophenol formed) in control plants and

Fig.13 : NR activity as influenced by M.S. in different cultivars

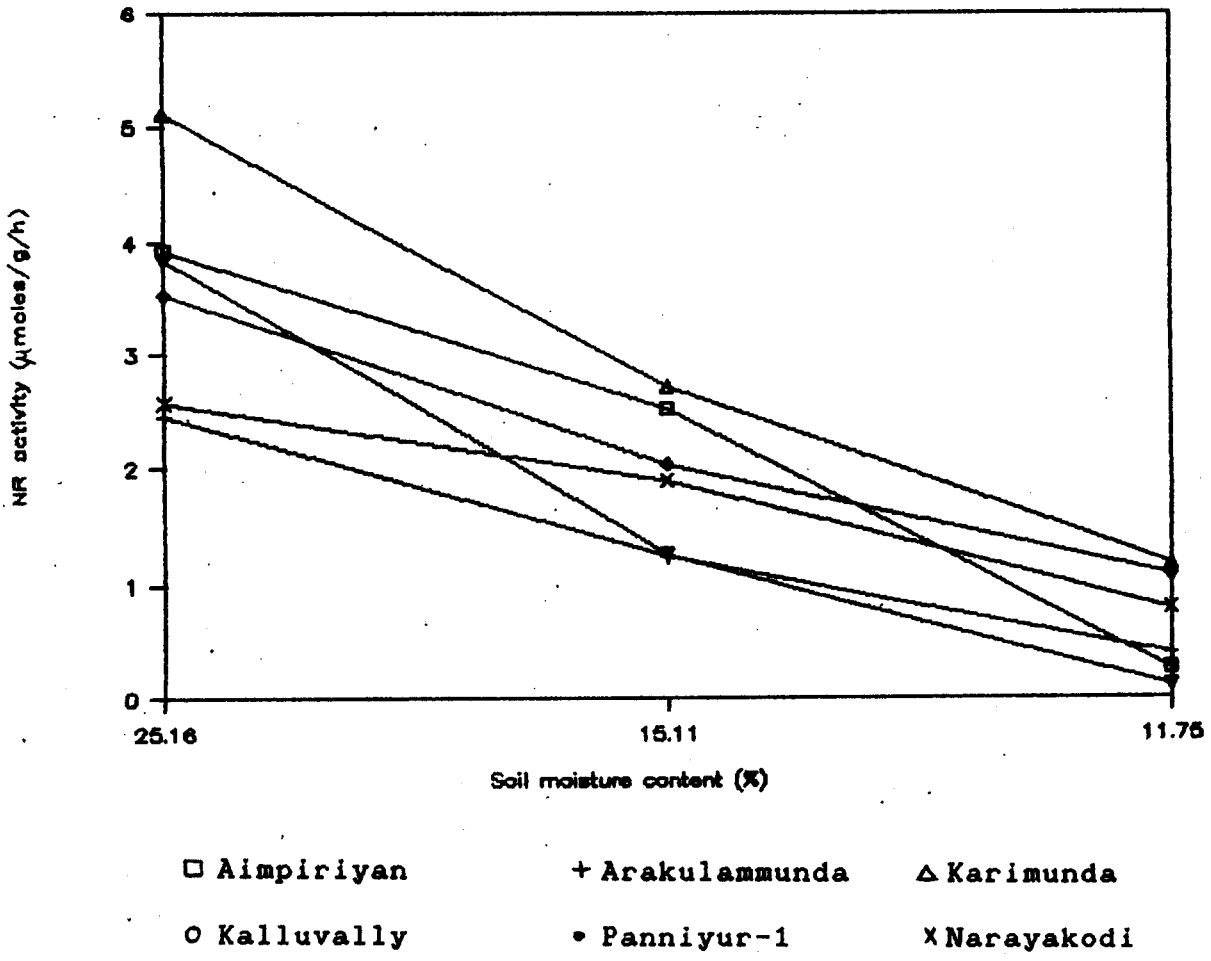


Table 12: Acid phosphatase and Peroxidase activity as influenced by moisture stress

S.NO.	CULTIVAR	PEROXIDASE ACTIVITY * (Unit/g)		ACID PHOSPHATASE ACTIVITY * (μ.mole p.nitrophenol/g/30min)	
		C	M S	C	MS
1.	Aimpiriyan	101 (16.6)	125 (5.0)	13.67 (2.4)	45.17(4.2)
2.	Arakulam munda	114 (2.6)	124 (7.5)	15.07 (1.5)	30.12(1.3)
3.	Kalluvally	102 (3.2)	123 (19.2)	11.40 (2.0)	47.07(2.6)
4.	Karimunda	164 (7.2)	179 (14.1)	15.00 (6.0)	34.87(2.7)
5.	Narayakodi	198 (5.7)	196 (11.0)	18.90 (1.0)	49.66(5.7)
6.	Panniyur-1	83 (5.3)	97 (3.5)	15.00 (1.1)	19.05(1.7)

* Values are means of six replications; Values in parenthesis standard deviation

maximum activity was recorded in Cv.Narayakodi. In moisture stressed plants the acid phosphatase activity ranged from 19.05 to 49.06 (µmoles/g/30 min) the highest again recorded in Cv.Narayakodi. In general most of the cultivars recorded a 2-3 fold increase in the activity of acid phosphatase due to moisture stress.

Peroxidase activity:

Enzyme peroxidase activity recorded in control and moisture stressed plant is presented in table 12. Peroxidase activity increased in almost all the cultivars except Cv. Narayakodi. Peroxidase activity ranged from 83-198 and 97 to 196 units/g/min in control and moisture stressed plants respectively. Although the general trend indicated an increased activity the increase was only marginal.

Alterations in the enzyme activity is an expression of plants subjected to moisture stress. Black pepper cultivars showed decreased activity of Nitrate reductase and higher activity of peroxidase and acid phosphatase similar to earlier reports on other plant species (Paleg & Aspinall, 1981).

Among the three enzymes studied enzyme nitrate reductase has shown differences due to genotypes under moisture stress condition which was significant. Hence, this enzyme activity has been used for scoring for drought tolerance.

Plant response at critical mositure level:

The main objective of fixing critical soil moisture level is to test the repeatability of the trend of responses at developmental, physiological and metabolic level. Generally acceptable method of fixing critical soil moisture content was followed in the present work. For this purpose stomatal resistance (means of all the cultivars studied) was plotted against soil moisture content. Half max of stomatal resistance facilitated fixing of critical moisture content(CMC) Fig.5.Since the critical moisture content determined was between two sampling dates (10th and 16th day after watering), data for different plant response were fitted for the critical moisture content. Fitting of response curves at critical moisture content was done only for the characters that had shown very high correlation with depleting moisture content.

Response of physiological parameters:

The base character used for fixing critical moisture content was stomatal resistance recorded on different dates of sampling. Fig.9a shows the stomatal response for depleting soil moisture content and CMC thus determinded was 14.5%. At CMC the stomatal resistance fitted for various cultivars is presented in table 13. Stomatal resistance ranged from 7.27 to 16.74 S, cm . This range at CMC clearly offers better points for discussuion on stomatal regulation mechanism and the relationship of stomatal resistance with the degree of drought tolerance.

Fig.5 : Stomatal response for depleting soil moisture

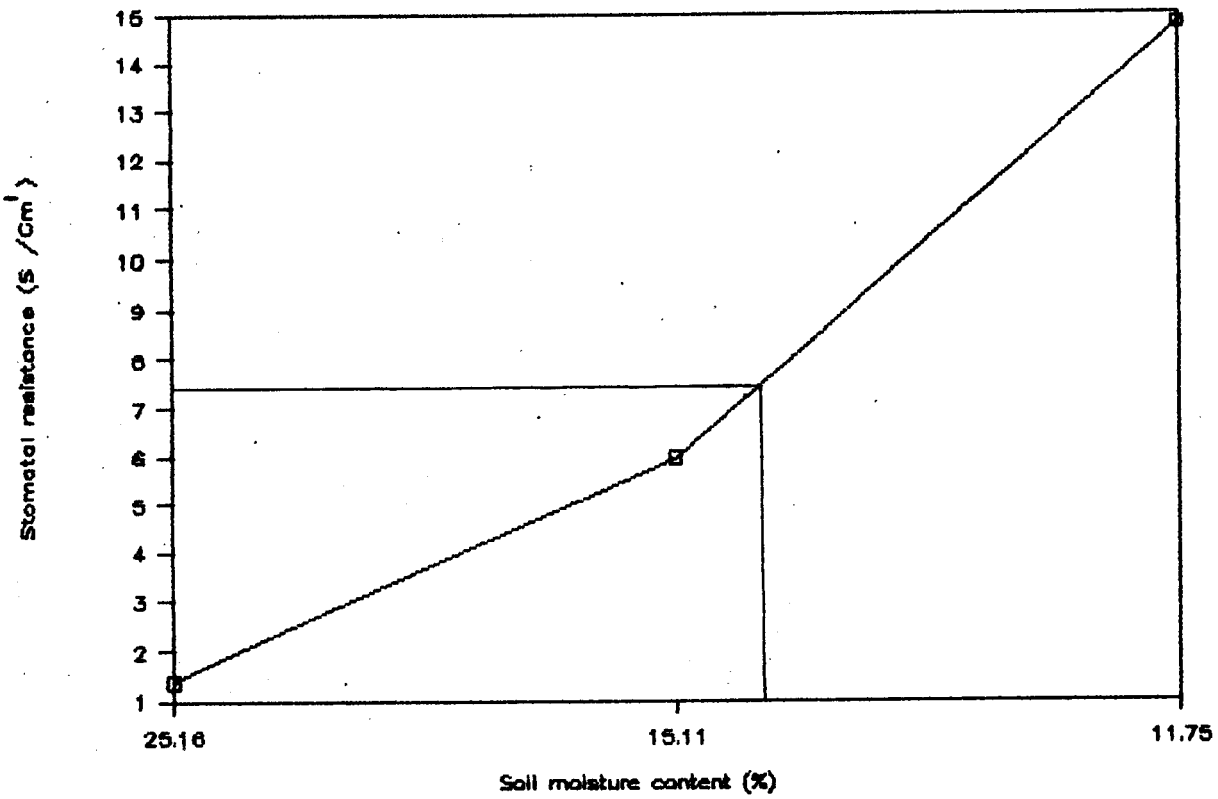


Table 13: Stomatal diffusive resistance, transpiration rate and leaf water potential at critical moisture content

S.NO.	CULTIVAR	SOIL MOISTURE CONTENT (14.5%) *		
		Stomatal Resistance ($S, /Cm^{-1}$)	Transpiration rate ($\mu g /Cm^{-2} /S^{-1}$)	Leaf water Potential (- bars)
1.	Aimpiriyan	7.27	5.77	15.21
2.	Arakulam munda	16.66	5.01	15.55
3.	Kalluvally	10.55	3.45	10.88
4.	Karimunda	10.14	3.29	9.94
5.	Narayakodi	10.59	3.28	10.75
6.	Panniyur-1	16.74	5.35	16.20

* Values obtained by fitting reponse curve for depleting soil moisture content

Response curve for transpiration rate (Fig.9b) shows the optimum transpiration rate desirable for drought tolerant cultivars at CMC. This optimum transpiration rate was used as cut off rate for indexing for drought tolerance (3.7 $\mu g/cm^2/s^1$). Transpiration rate fitted for various cultivars is presented in table 13. Transpiration rate ranged from 3.28 to 5.77 ($\mu g/cm^2/s^1$).

Optimum leaf water potential determined using CMC was -11.2 bars (Fig.10a). Leaf water potential fitted for various cultivars at threshold level of stress ranged from -9.94 to -16.2 bars. (table 13).

Desirable relative water content of leaf tissues obtained at CMC was 70% (Fig.10b) and same fitted for the cultivars ranged from 60-76% (table 14).

Responses of biochemical parameters:

Response of biochemical parameters viz. total sugars and free proline are, presented in figs. 11 a & b. The desired level of sugars and, proline determined was 31 mg/g and 65 μmoles/g fr.wt. respectively. Total sugars ranged from 22.53 to 42.13 mg/g fr.wt. and free proline ranged from 4.5 to 9.8 μmoles/g fr.wt at critical moisture content (table 14).

Nitrate reductase activity in response to depleting soil moisture content is presented in Fig.12a. The optimum level of

Table 14: Relative water content, proline, total sugars and nitrate reductase activity at critical moisture content

S.NO.	CULTIVAR	SOIL MOISTURE CONTENT (14.5%) *			
		RWC(%)	Proline (µmole/g)	Sugars (Mg/g)	NR Activity (µmole/g/hr)
1.	Aimpiriyan	62.17	6.09	34.95	2.40
2.	Arakulam munda	61.89	4.99	25.44	1.19
3.	Kalluvally	76.09	9.81	36.20	1.95
4.	Karimunda	75.21	7.76	42.13	2.59
5.	Narayakodi	75.59	9.34	22.53	1.81
6.	Panniyur-1	59.75	4.54	26.19	1.20

* Values obtained by fitting response curve for depleting soil moisture content

NR activity was 1.7 µmole/g/ht/ NR activity fitted for the cultivars at critical moisture content varied from 1.19 to 2.59 µmoles/g/hr.

The response of the above parameters determined viz. stomatal resistance, transpiration rate, RWC, Leaf water potential, total sugars, proline content and NR activity at critical moisture content were utilised for indexing of drought tolerance. These parameters showed highly significant correlation (positive as well as negative) with depleting soil moisture content.

Plant response correlation with moisture stress:

Plant response to moisture stress vary with the severity as well as the duration of the stress. Only the most sensitive process are altered by mild stress. As the stress increases these changes intensify and additional processes become affected depending on their sensitivity to the stress (Bradford & Hsiao 1982).

Correlation matrix :

Among the twenty four characters studied, eight characters showed highly significant correlation with depleting soil moisture content (table 15).

TABLE 15 : **Plant Response Correlation with moisture stress**

Correlation Matrix

x_1	–	Soil moisture content	x_{13}	–	N R A
x_2	–	Total Chlorophyll	x_{14}	–	Proline
x_3	–	Chlorophyll a	x_{15}	–	Sugar
x_4	–	Chlorophyll b	x_{16}	–	Phenols
x_5	–	Chl. a/b ratio	x_{17}	–	Sugar
x_6	–	Total carotenoids	x_{18}	–	Shoot Wt.
x_7	–	Carotenes	x_{19}	–	Leaf Wt.
x_8	–	Xanthophylls	x_{20}	–	R/S Ratio
x_9	–	Chl /Car	x_{21}	–	Root length
x_{10}	–	R.W.C	x_{22}	–	Shoot length
x_{11}	–	S.L.W.	x_{23}	–	Leaf No.
x_{12}	–	Leaf ψ	x_{24}	–	No. of nodes

Table 15 (contd)

	x1	x2	x3	x4	x5	x6	x7	x8
x1	1.00000							
x2	.69236	1.00000						
x3	.69844	.98004	1.00000					
x4	.69371	.96414	.93860	1.00000				
x5	.07942	.12945	.23994	-.08324	1.00000			
x6	.70110	.90260	.91084	.90525	.09514	1.00000		
x7	.68906	.69009	.72925	.71686	.07690	.85293	1.00000	
x8	.60709	.89791	.88288	.87658	.12967	.89979	.58705	1.00000
x9	-.09180	.08636	.03774	.04414	-.04591	-.27464	-.32039	-.16144
x10	.92707	.57766	.59705	.58333	.07429	.63163	.71542	.47937
x11	.69485	.39087	.40625	.40229	.09592	.50057	.48039	.43273
x12	-.85777	-.52067	.53598	.53341	-.04246	-.57368	.67388	-.42184
x13	.86743	.58152	.61371	.57158	.13408	.63397	.67698	.50210
x14	-.77784	-.59521	-.61525	-.59835	-.11586	-.60822	-.58825	-.52241
x15	.59866	.38791	.38516	.39319	.00569	.35702	.42478	.28123
x16	.16446	.19759	.19166	.18153	.01291	.20852	.13811	-.15387
x17	-.09207	-.36330	-.39838	-.31983	-.27855	-.35918	-.15536	-.45834
x18	-.02621	-.26941	-.31056	-.23305	-.26468	-.23912	-.01334	-.34491
x19	.18971	-.07244	-.11747	-.01810	-.29389	-.07024	.15717	-.20326
x20	-.30253	-.19164	-.16267	-.21109	.11037	-.21978	-.33698	-.13737
x21	-.09582	-.23854	-.25495	-.20835	-.17667	-.23196	-.15936	-.24924
x22	.04402	-.18099	-.16879	-.18179	-.06314	-.10574	.05449	-.20383
x23	.10890	-.25489	-.27745	-.18783	-.28238	-.13152	.05360	-.24071
x24	.09999	-.24511	-.26970	-.16900	-.31266	-.12203	.07286	-.23156
x9	1.00000							
x10	-.17993	1.00000						
x11	-.34215	.72045	1.00000					
x12	.19108	-.95780	-.73712	1.00000				
x13	-.17941	.88382	.68615	-.84972	1.00000			
x14	.08384	-.63425	-.46666	.50919	-.57268	1.00000		
x15	.06494	.60246	.17512	-.47453	.40940	-.60899	1.00000	
x16	-.12812	.18795	.13768	-.12925	.19478	-.23058	-.12787	1.00000
x17	.09507	.02694	-.05397	-.06152	-.09276	.15001	-.06231	-.11044
x18	.06886	.09071	-.05171	-.09854	-.04983	.02932	.17955	-.11866
x19	.09112	.27477	.09009	-.26621	.10884	-.12516	-.30247	.04134
x20	.02555	-.33260	-.15966	.28028	-.22378	.30624	.33506	-.12357
x21	-.05279	-.00758	.04912	.09122	-.00522	.23974	.14068	-.14731
x22	-.21504	.18822	.05446	-.20155	.15669	.05904	-.01631	.23387
x23	-.24100	.20074	.06943	-.19530	.03865	-.07787	-.22373	.09367
x24	-.23660	.20450	.05312	-.19038	.04307	-.04315	-.22605	.08349

Table 15 (contd)

	X17	X18	X19	X20	X21	X22	X23	X24
x17	1.00000							
x18	.80279	1.00000						
x19	.74895	.82149	1.00000					
x20	-.06374	-.55267	-.63541	1.00000				
x21	.38968	.34246	-.29704	-.05936	1.00000			
x22	.45712	.56598	.49552	-.38059	.18582	1.00000		
x23	.60135	.67748	.66308	-.42766	.34473	.65547	1.00000	
x24	.55201	.64039	.62862	-.41564	.32782	.67126	.95273	1.00000

CRITICAL VALUE (1-TAIL, .05) = +Or - .19551
CRITICAL VALUE (2-tail, .05) = +/- .23172

	x	y	x1	x2
x	1.00000			
y	-.83000	1.00000		
x1	-.89407	.81666	1.00000	
x2	.93248	-.84161	-.93955	1.00000

CRITICAL VALUE (1-TAIL, .05) = +Or - .11225
CRITICAL VALUE (2-tail, .05) = +/- .13352

X = Soil Moisture Content
Y = Leaf water potential
x1 = Stomatal resistance
x2 = Transpiration

i) Biomass Characters

Components of biomass viz. leaf weight, stem weight and root weight did not correlate with moisture stress.

* Specific leaf weight showed significant and positive correlation with depleting moisture content (r= 0.694).

* Root/shoot ratio showed negative correlation though not highly significant (r=-0.302).

* Morphological characters viz. root length, shoot length and no.of leaves did not correlate with moisture stress.

ii) Physiological Parameters:

All the physiological parameters studied were found to correlate with depleting moisture content.

* Stomatal resistance showed significant and negative correlation (r= -0.894).

* Transpiration rate, leaf water potential and relative water content showed highly significant and positive correlation with depleting moisture content (r= 0.932,0.830,0.927 respectively).

iii) Biochemical Parameters:

* Free proline and total sugars showed significant and negative correlation with depleting moisture content (r= -0.777 and -0.598 respectively).

* Pigments viz.total chlorophylls, chl. a and chl. b, total carotenoids, carotenes and xanthophylls showed significant and positive correlation with soil moisture content (r= 0.692,0.698,0.693,0.701,0.689 and 0.697 respectively).

* Nitrate reductase activity showed a highly significant and positive correlation with depleting moisture content (r=0.867).

Indexing for drought tolerance:

Characters which had shown very high correlation with moisture stress were selected for indexing for drought tolerance. The indexing is based mainly on stomatal resistance, eg: Stomatal resistance was taken in x axis and each of the other characters selected in y axis (one character at a time.) Half-max line was drawn for both stomatal resistance and respective characters selected.

Half-max for each characters was determined from response curves (Fig 9-12). The half max lines divided the graph into four blocks. The cultivars studied were fitted for the data to whichever block it belongs (Fig.14a-g). Block numbering and scoring was done as follows:

Fig.9a : Response curve : Stomatal Resistance/SMC

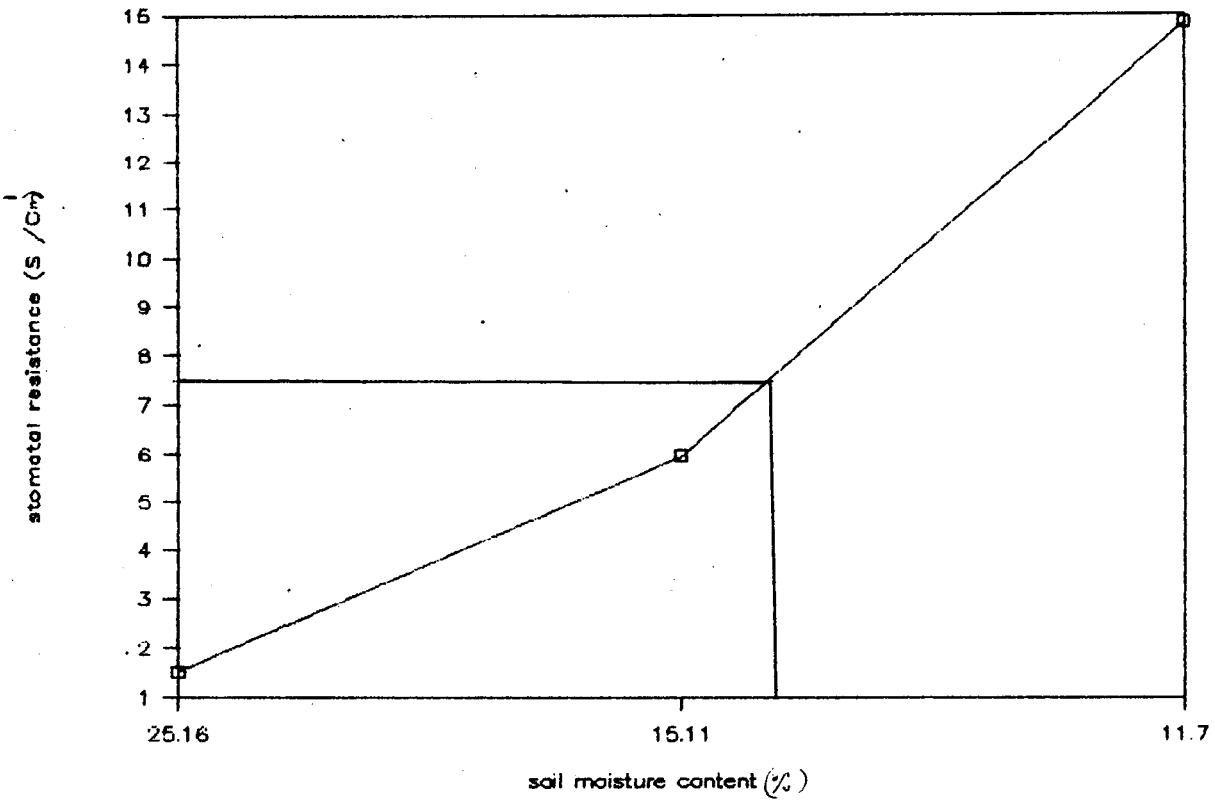


Fig.9b : Response curve : Transpiration Rate /SMC

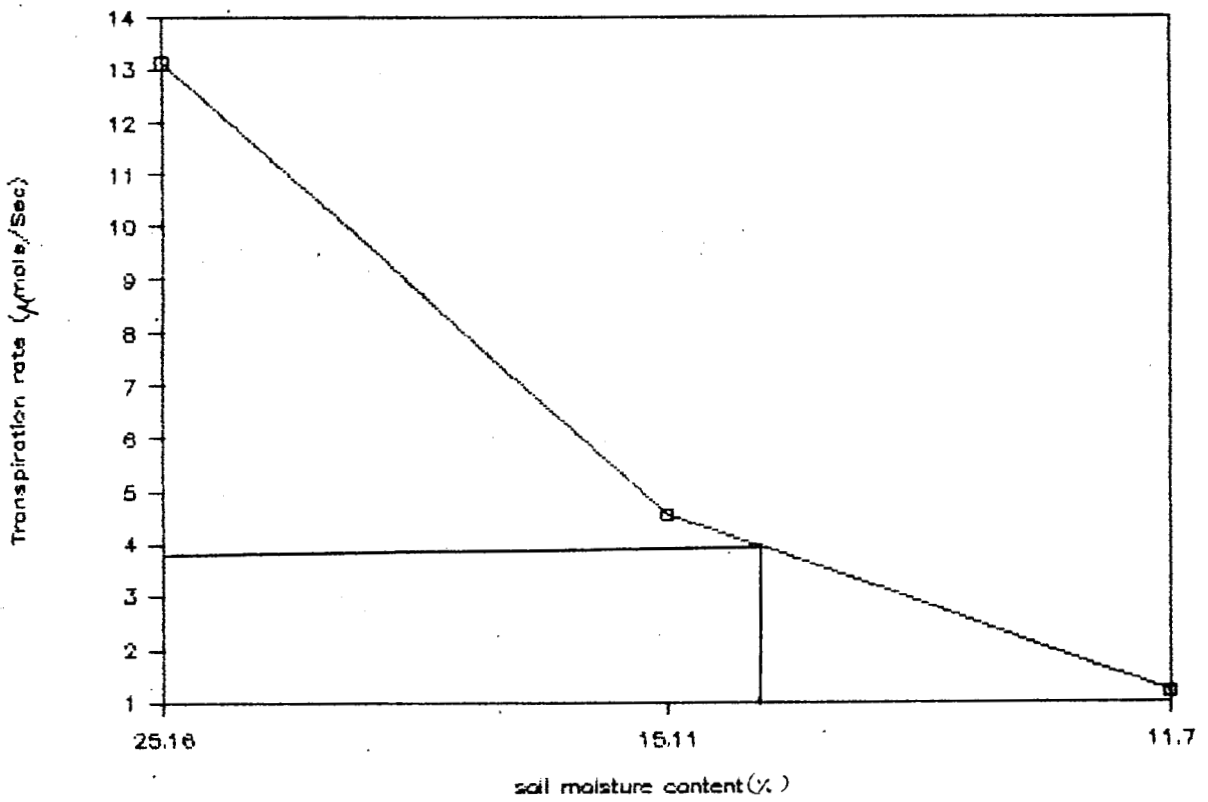


Fig.10a : Response curve : ψ_L / SMC

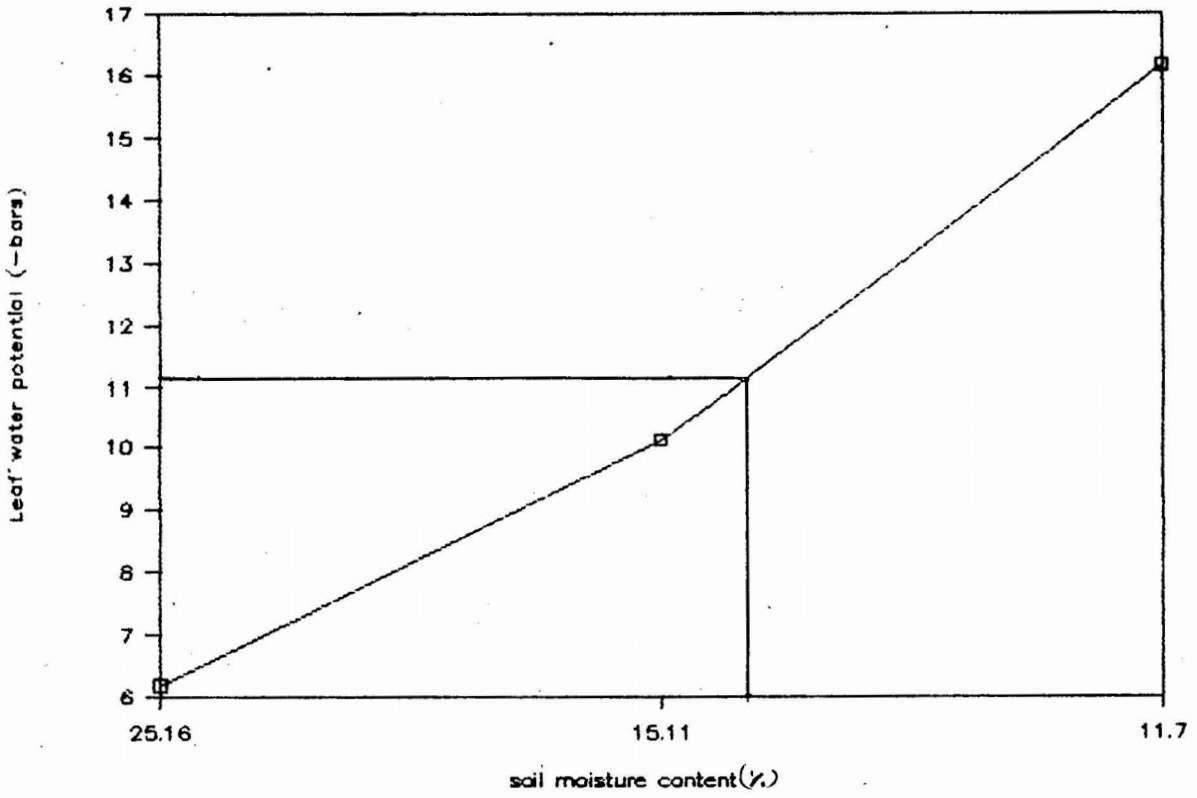


Fig.10b : Response curve : RWC / SMC

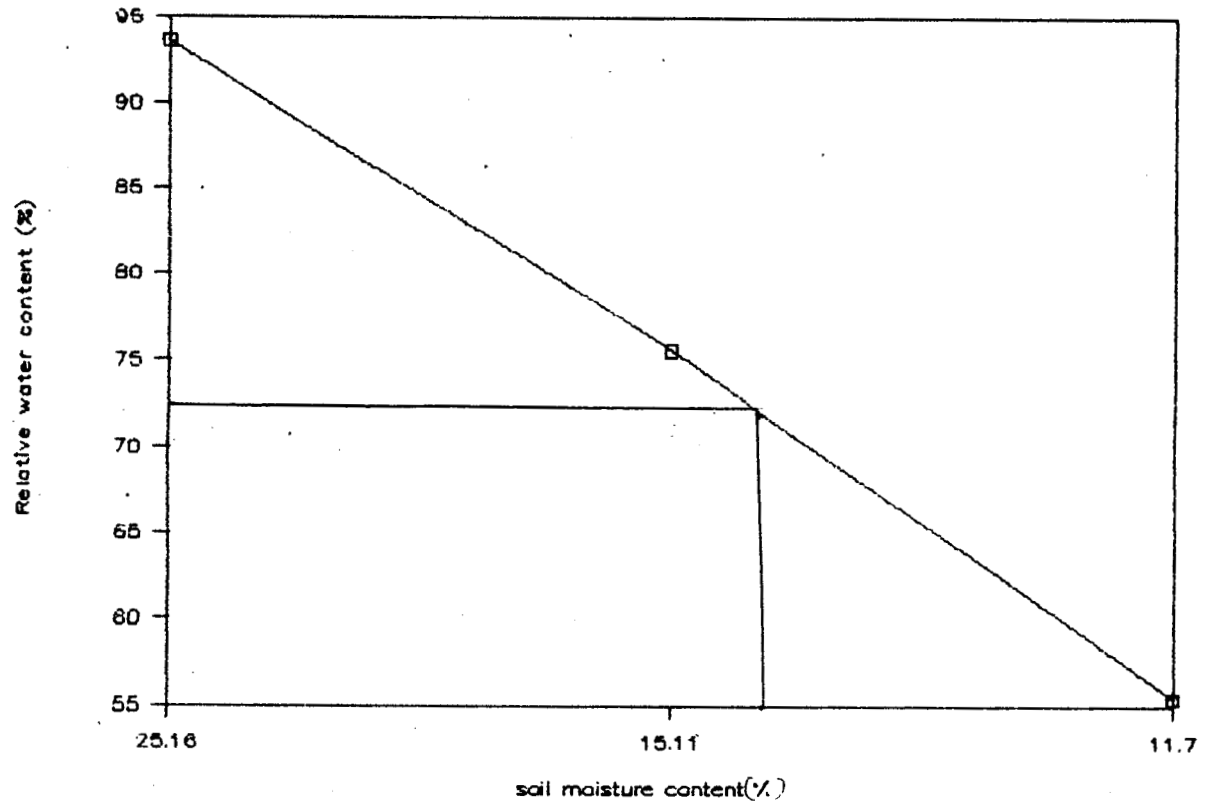


Fig.11a : Response curve : SLW / SMC

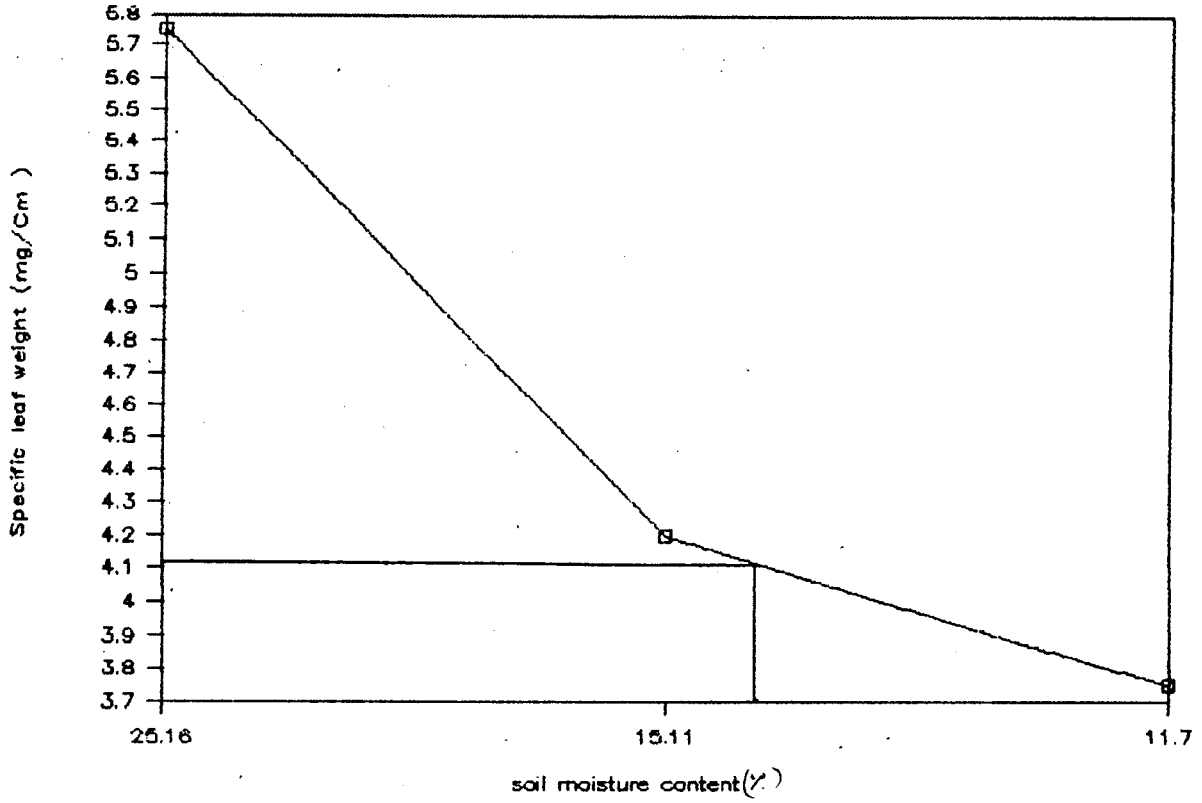


Fig.11b : Response curve : NRA / SMC

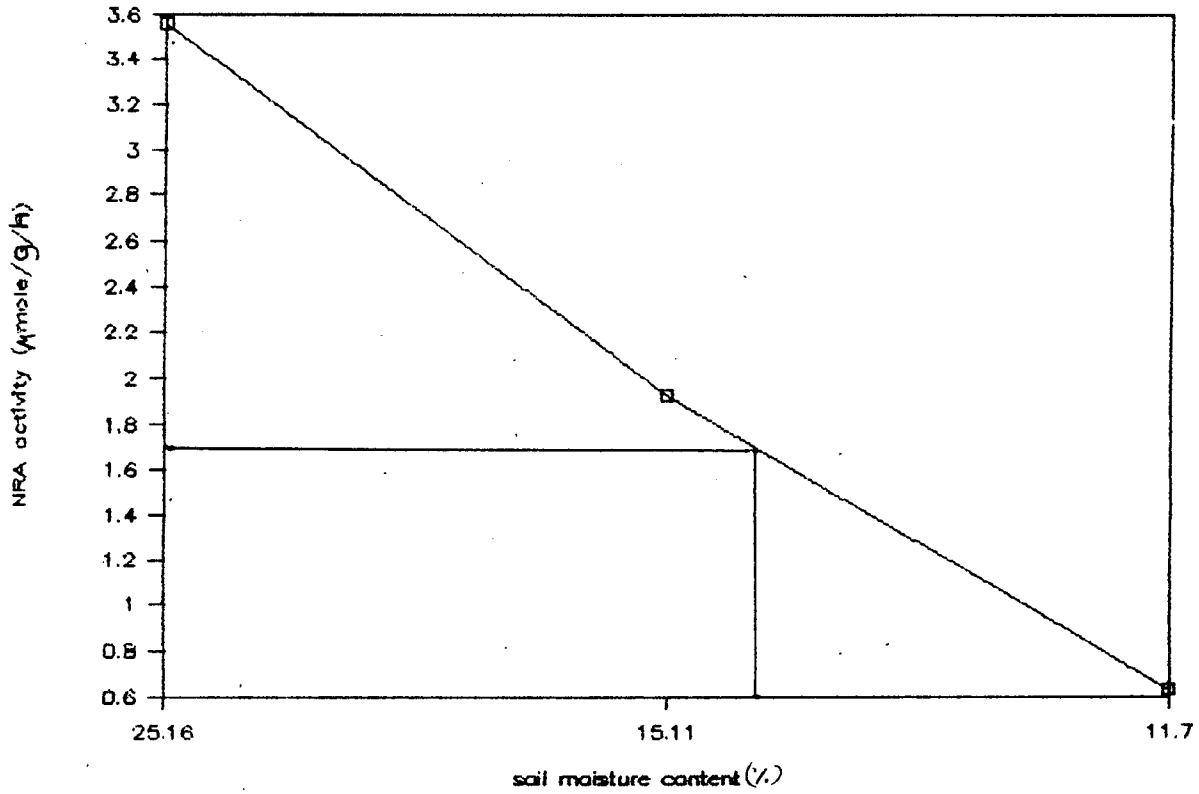


Fig.12a : Response curve : Sugars / SMC

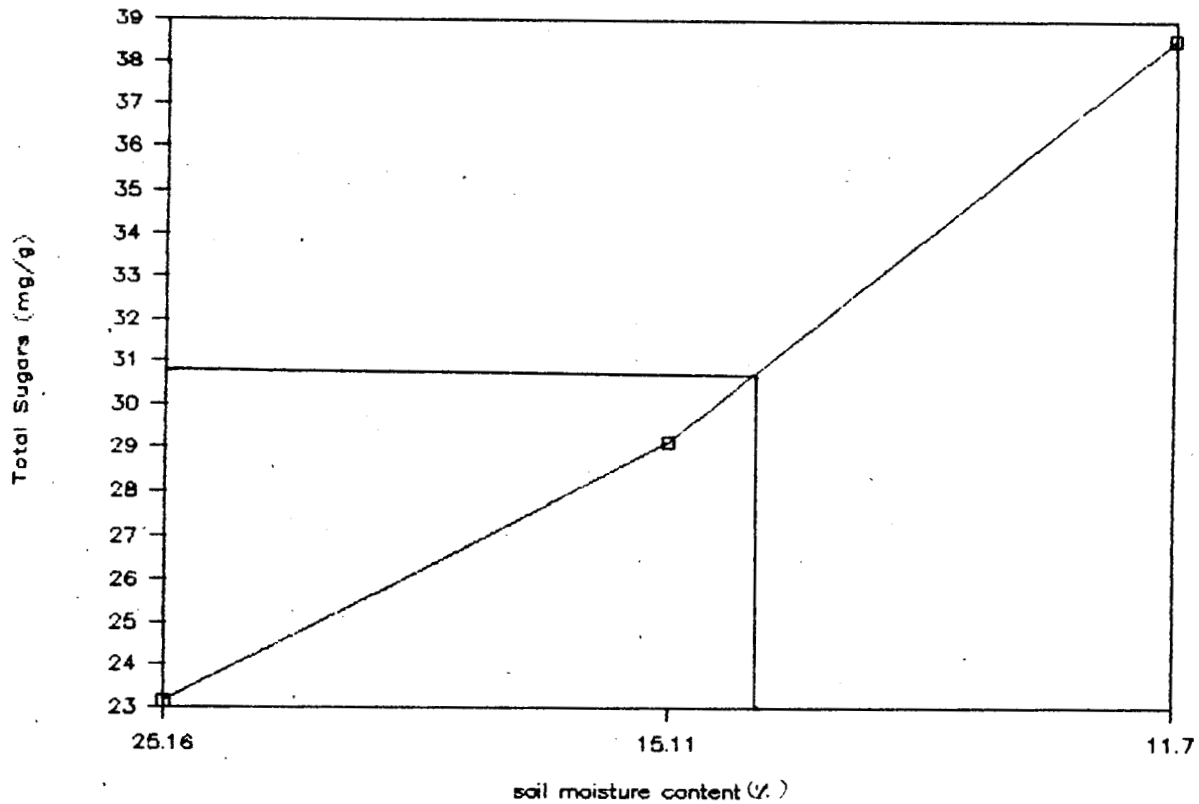


Fig.12b : Response curve : Proline / SMC

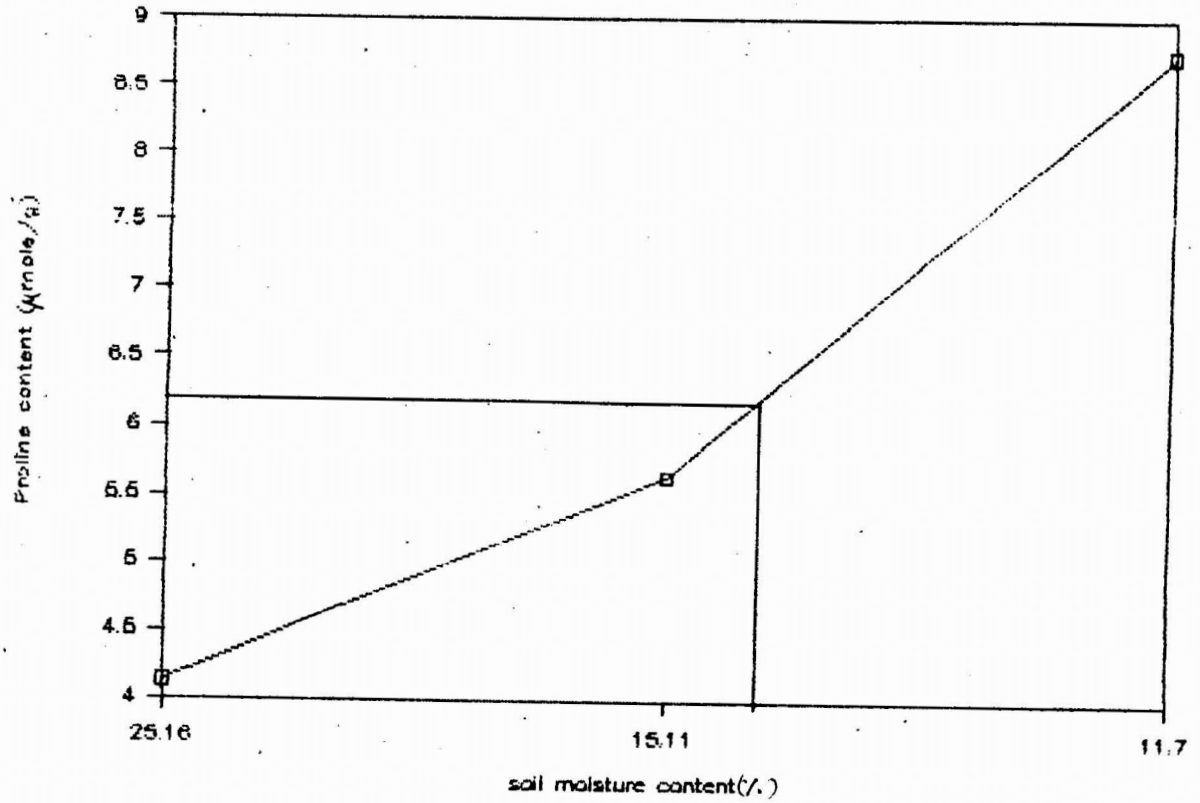
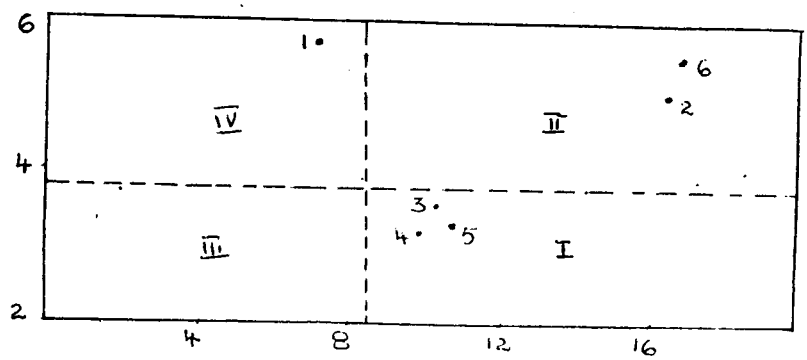


Fig.14 : Indexing for drought tolerance

a) Stomatal resistance Vs. transpiration



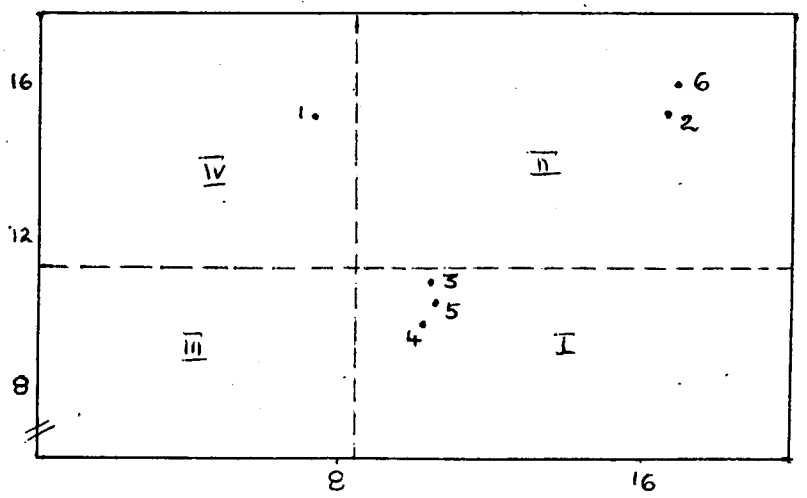
1 Aimpiriyan 2 Arakulammunda 3 Karimunda

4 Kalluvally 6 Panniyur-1 5 Narayakodi

I-IV - See text

Fig.14(Contd.)

b) Stomatal resistance Vs. leaf water potential



c) Stomatal resistance Vs. RWC

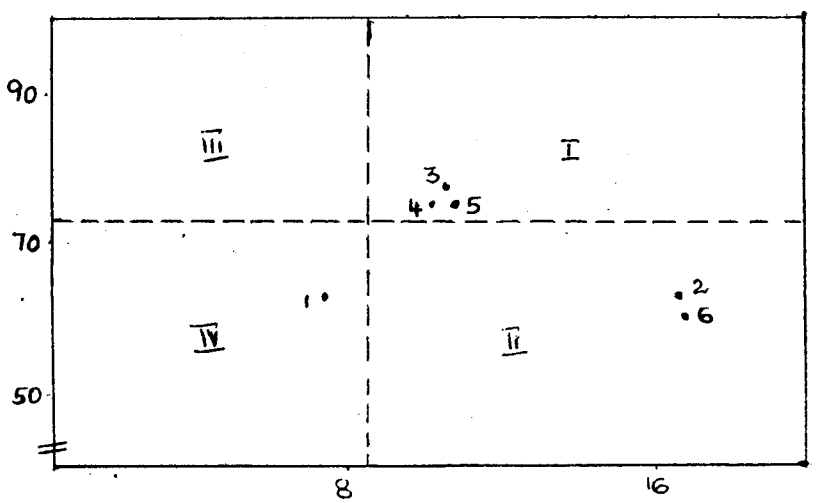
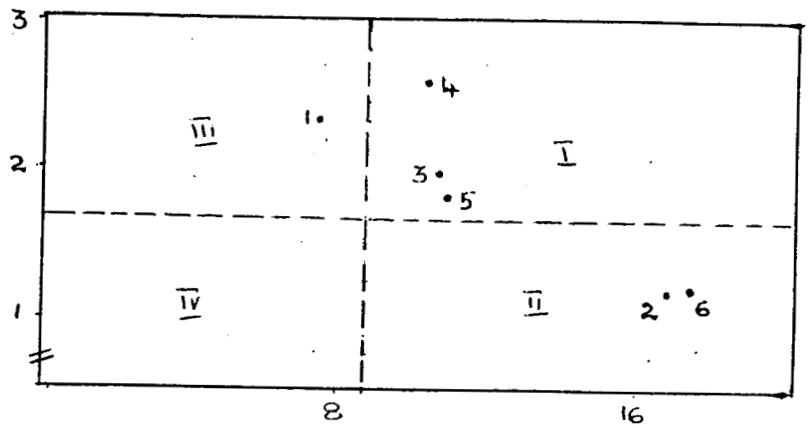


Fig.14 (Contd.)

d) Stomatal resistance Vs. NRA



e) Stomatal resistance Vs. proline content

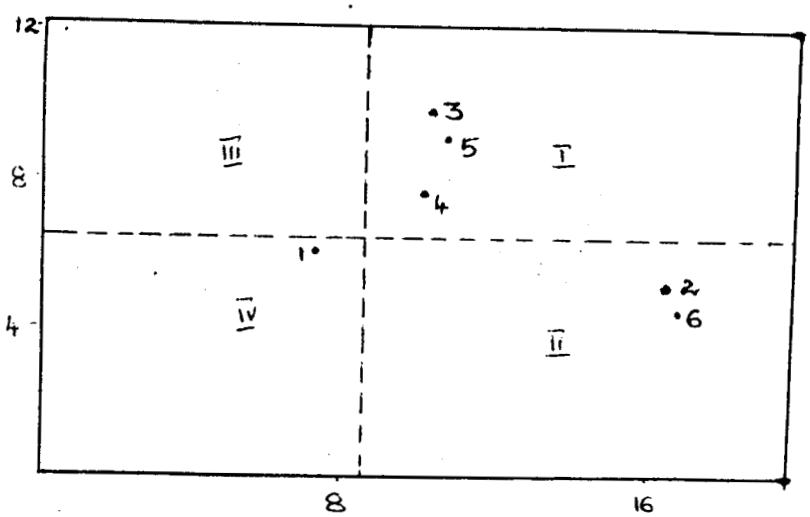
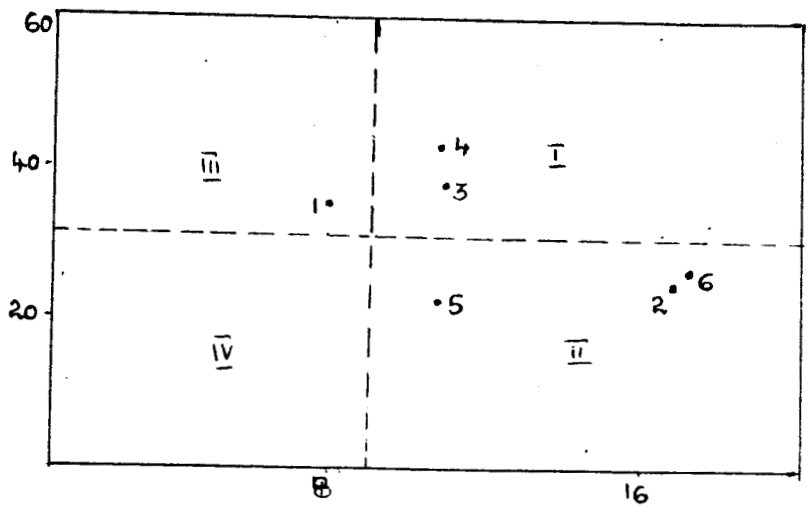
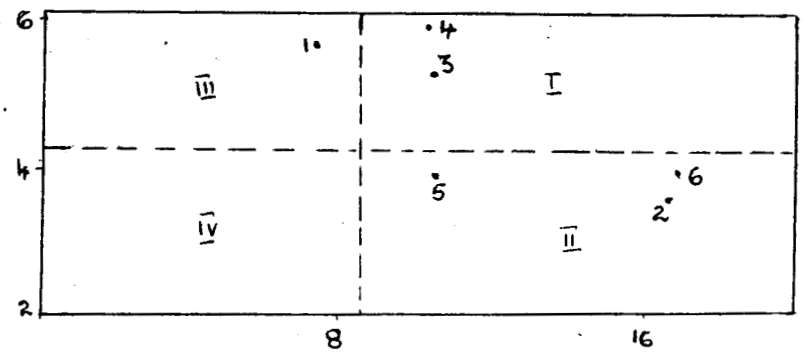


Fig.14 (Contd.)

f) Stomatal resistance Vs. sugar content



g) Stomatal resistance Vs. SLW



Character	Block	Score
High sotmatal resistance and high leaf water potential	I	1
High stomatal resistance and low leaf water potential	II	2
Low stomatal resistance and high leaf water potential	III	3
Low stomatal resistance and low leaf water potential	IV	4

The scoring is in the ascending order from Block I. The lowest score for each character is the most preferred. Table 16 shows the score details for each of the character and aggregate for each of the cultivars studied.

The cultivars which scored least (7) is considered drought tolerant and the relative tolerance is given as follows:

Score Aggregate	Degree of Tolerance
7	Drought Tolerant
8-14	Moderately tolerant
15-21	Moderately sensitive
22-28	Highly sensitive

Cultivars Karimunda and Kalluvally scored 7 and Narayakodi (9) are found to be relatively tolerant ovder the rest. Cultivar Aimpiriyan is found to be very sensitive.

Table 16: Pepper cultivars - scoring for drought tolerance

S.No.	CULTIVAR	SCORING OF CHARACTERS vs STOMATAL RESISTANCE							Drought Index Score
		1	2	3	4	5	6	7	
1.	Aimpiriyan	4	4	4	3	4	3	3	25
2.	Arakulam munda	2	2	2	2	2	2	2	14
3.	Kalluvally	1	1	1	1	1	1	1	7
4.	Karimunda	1	1	1	1	1	1	1	7
5.	Narayakodi	1	1	1	1	1	2	2	9
6.	Panniyur-1	2	2	2	2	2	2	2	14

Characters 1-7 represent transpiration rate, leaf water potential, relative water content, Nitrate reductase acitivity, proline content, sugar content and specific leaf weight respectively.

For relative drought tolerance of cultivars see text page 58*

Screening of promising genotypes:

Screening of germplasm for drought tolerance in most of the agricultural crops is based on their yield performance under rainfed conditions. However, screening based on yield performance takes several years in perennial crops as the actual yield potential is realised only after 4 or 5 years after planting. Hence, methodologies to screen at an early stage would enable breeders to identify tolerant lines and test tolerance in the field in a shorter period. Hence, characters that showed high correlation with depleting soil moisture content were studied in few promising genotypes of pepper viz. Acc.no.1495 (Kottanadan), Ks69,Ks88 (Karimunda), Panchami (Aimpiriyan) Acc.No.931(Kalluvally). Were screened for drought tolerance based on the short listed character i.e., stomatal resistance, transpiration rate, leaf water potential, relative water content, specific leaf weight, total sugars,proline content and nitrate reductase activity.

Screening of promising genotypes : Soil moisture content as influenced by moisture stress is presented in Fig 15. Soil moisture content depleted by about 50% when most of the plants of showed wilting symptoms. Fig. 16 pictures the response curve of stomatal resistance for depleting soil moisture content. This formed the basis for fixing critical moisture content.(CMC=soil moisture content at half-max of stomatal

Fig.15 : Soil moisture content as influenced by moisture stress

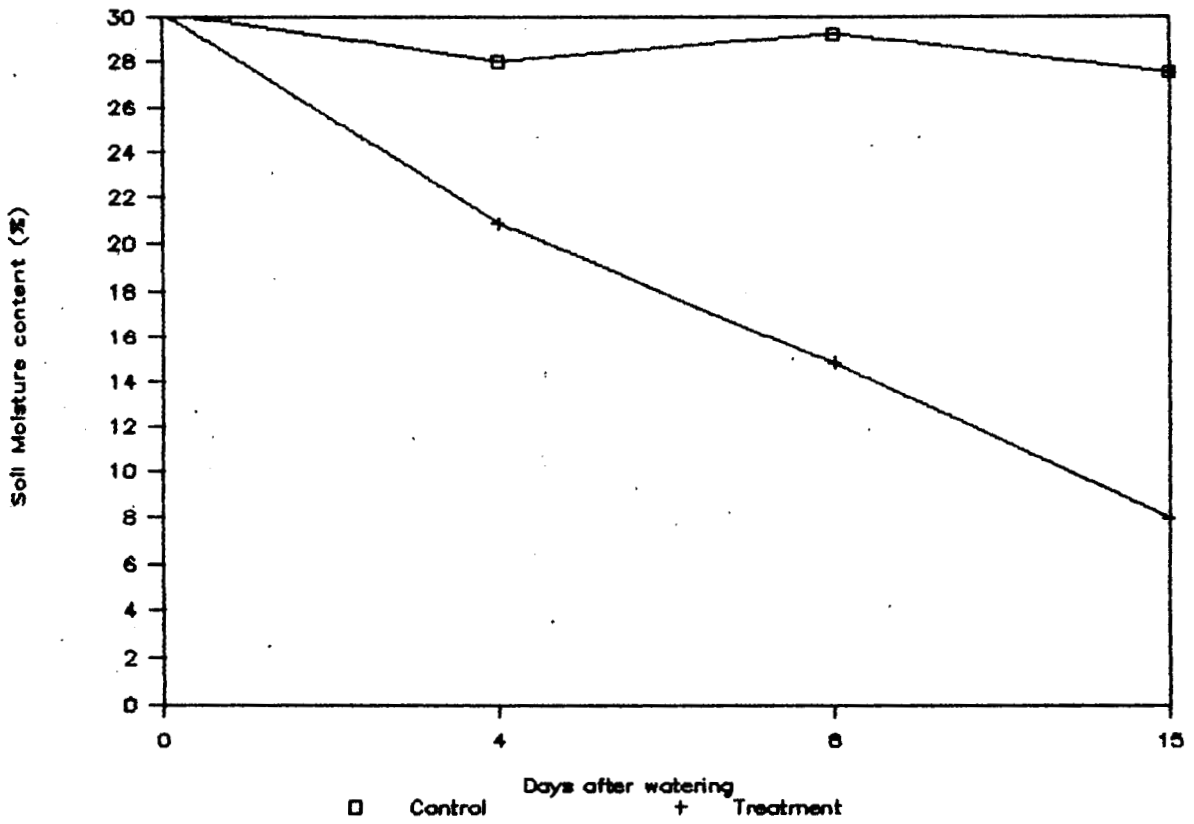
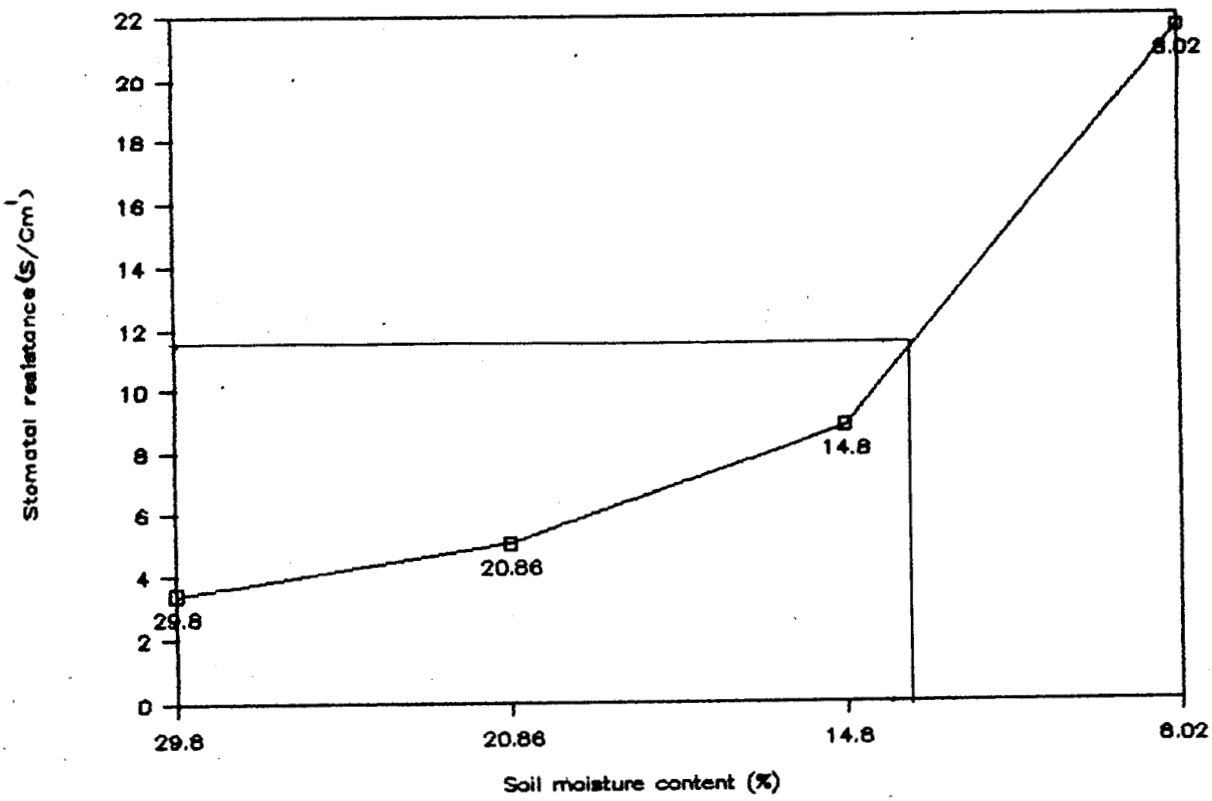


Fig.16 : Stomatal resistance against soil moisture depletion



resistance). The critical moisture content in this experiment was 13.8%.

Stomatal resistance increased in all genotypes when the soil moisture showed depleting trend, however, the response of genotypes varied as realised in the previous experiment. Genotypes 1495, KS69, KS88 responded quickly by showing higher stomatal resistance while genotypes Panchami and Acc.No.931 responded at latter stage only. Stomatal resistance recorded on last sampling stage ranged from 17.3 to 26.75 Ωcm^{-1}. At critical moisture content Accno.1495 & KS69 recorded higher stomatal resistance (table17), this helped the above genotypes to reduce water loss by reducing transpiration rate.

Leaf water potential reduced (reached more negative values) as the soil moisture depleted. Genotypes KS69 and Acc.No.11495 recorded higher leaf water potential compared to KS88, Panchami and Acc.No.931. This indicated the higher turgidity retained by KS69 and 1495 (table 17).

Relative water content worked out for critical moisture content varied from 56-72% and genotypes KS69,1495 and Acc.No.931 showed higher RWC(%) indicating their turgid nature (table 18). Specific leaf weight did not show much variation due to moisture stress. Specific leaf weight ranged from 3.0-4.2 mg/cm2.

Proline content estimated showed the genotypes' differential response to soil moisture stress (table18). Genotypes KS69,1495

Table 17: Stomatal resistance, transpiration rate, leaf water potential at critical moisture content (13.8%)

Variety	Stomatal resistance (S, Cm^{1})	Transpiration rate (μg, Cm^{-2}, S^{1})	Leaf water potential (-bars)
Acc.no. 1495	12.28	1.92	12.60
KS 69	13.65	1.98	10.86
KS 88	10.58	2.14	14.20
Panchami	8.38	2.31	15.60
Acc.no. 931	10.05	2.31	14.80

Table 18: RWC, Sugars, proline, nitrate reductase activity and specific leaf weight at critical moisture content

Variety	RWC(%)	SLW (mg/cm^2)	SUGARS (mg/g)	PROLINE (µmole/g)	NITRATE REDUCTASE ACTIVITY (µmole/g/h)
Acc.no. 1495	68	3.8	26.68	10.26	1.36
KS 69	72	4.2	22.32	11.06	1.24
KS 88	60	3.2	24.14	9.36	1.06
Panchami	56	3.6	22.46	7.44	0.88
Acc.no. 931	62	3.0	23.38	9.16	0.96

and KS88 recorded higher proline content compared to other genotypes. Total sugars varied from 22-26 mg/g and genotypic differences were meager. Nitrate reductase activity showed a declining trend with depleting moisture content. The lowest activity detected was in Genotypes Panchami(0.88 µmole/g/h) and highest in Acc.no.1495 (1.36µmole/g/h).

Scoring for the above genotypes for shortlisted characters is presented in table 19. The genotypes that showed tolerant reactions are Acc.no.1495 (Kottanadan) and KS69 (Karimunda).

Data on screening work has indicated the suitability of the indexing methodology proposed in this work. Genotypes(1495 and KS69) that showed better drought tolerant reaction in pot culture experiment, were planted in the field to test their field tolerance.

Field tolerance : Field tolerance to stress conditions would largely reflect upon the genotypic potential and its response to the environment. The response of a genotype in the field to stress situation would remain unaltered if the trend of stress development as well as agroclimatic conditions remain (unaltered) similar in pot culture experiment.

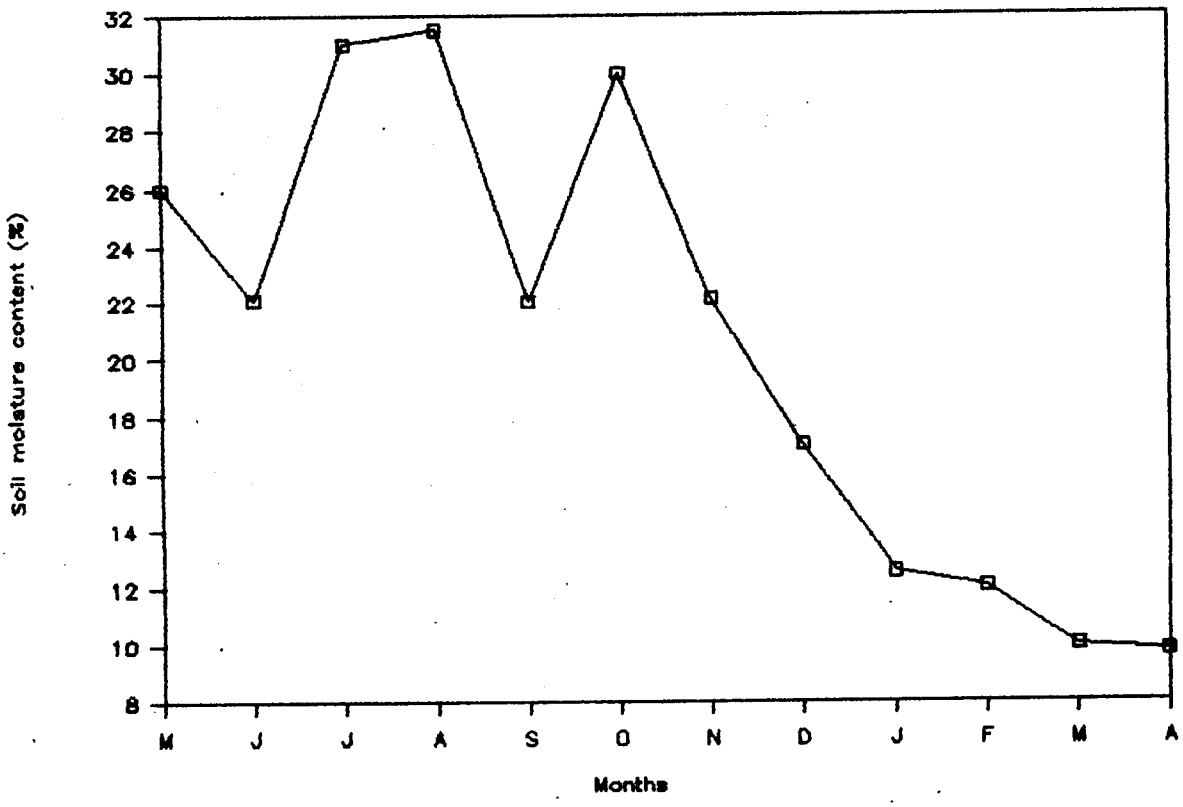
Field observations recorded : Soil moisture depletion pattern, on monthly basis is presented in fig 17(1990-91). Soil moisture content was at field capacity in the months of May to October with a dip in sepetember. This corresponded with rainfall

Table 19: Scoring of promising lines for drought tolerance

S.No.	Genotype	SCORING OF CHARACTERS vs STOMATAL RESISTANCE							Drought Index Score
		1	2	3	4	5	6	7	
1	Acc. no.1495	1	1	1	1	1	1	1	7
2	KS 69	1	1	1	1	2	1	1	8
3	KS 88	4	4	3	4	3	3	3	24
4	Panchami	4	4	4	3	4	3	4	26
5	Acc no.931	4	4	3	4	3	3	4	25

* 1-7 represents transpiration rate, leaf water potential, relative water content, Nitrate reductase acitivity, sugars, proline and specific leaf weight respectively.

Fig.17 : Soil moisture depletion pattern in the field



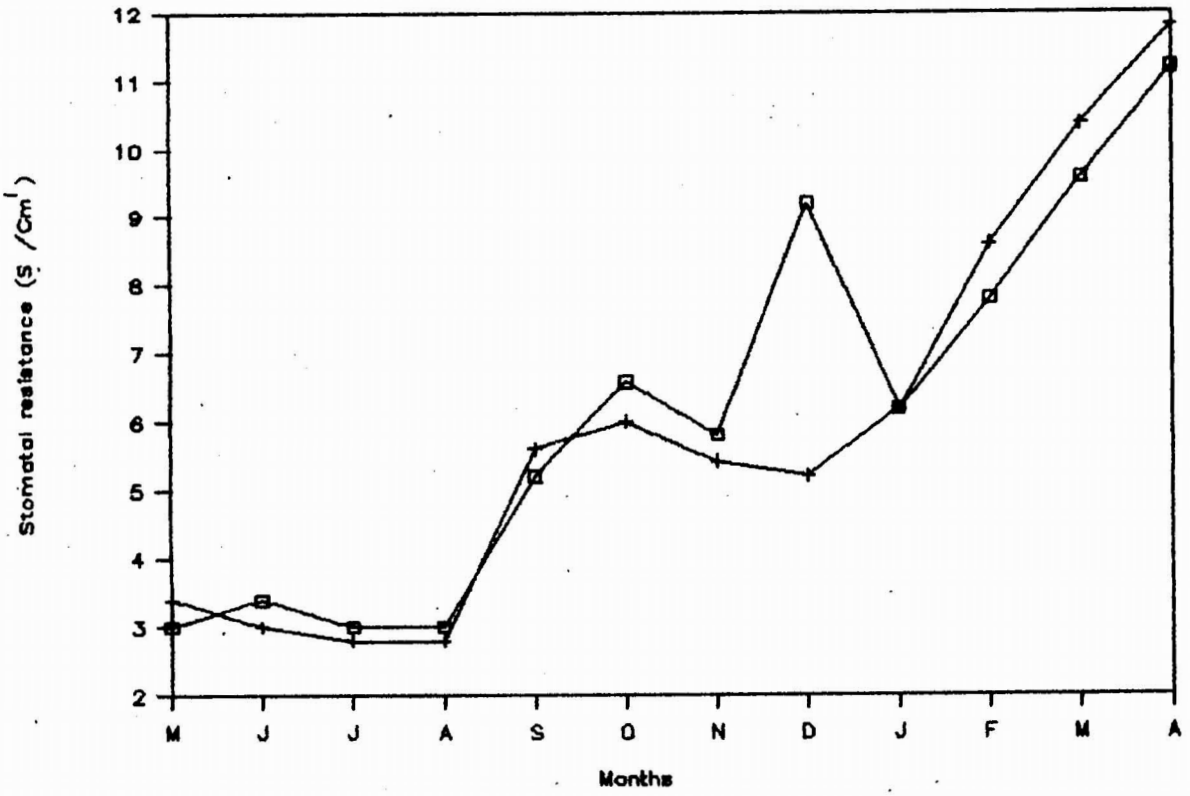
pattern. Soil moisture depleted steadily from the month of November and reached. Critical limits during Feb-April (SMC 14.0-12.0%).

The trend of physiological responses viz., stomatal resistance, transpiration rate and leaf water potential recorded, indicated similarity to that recorded in pot culture experiment. Stomatal resistance recorded in the field plants is presented in Fig 18. The stomatal resistance increased as the soil moisture depleted. Both the genotypes responded in similar way with the development of stress.

Transpiration rate recorded in the field is presented in Fig19. Transpiration was high (7.2 - 9.0 mg/s/cm^2) during the month of May and decreased with the onset of stress to record minimum during the months of Feb- April (2.0-3.0 mg/s/cm^2). The response was similar in both the genotype (KS69& Acc.No.1495). The reduced transpiration during Feb-April helped the plants to tide over the adverse situation of less moisture availability.

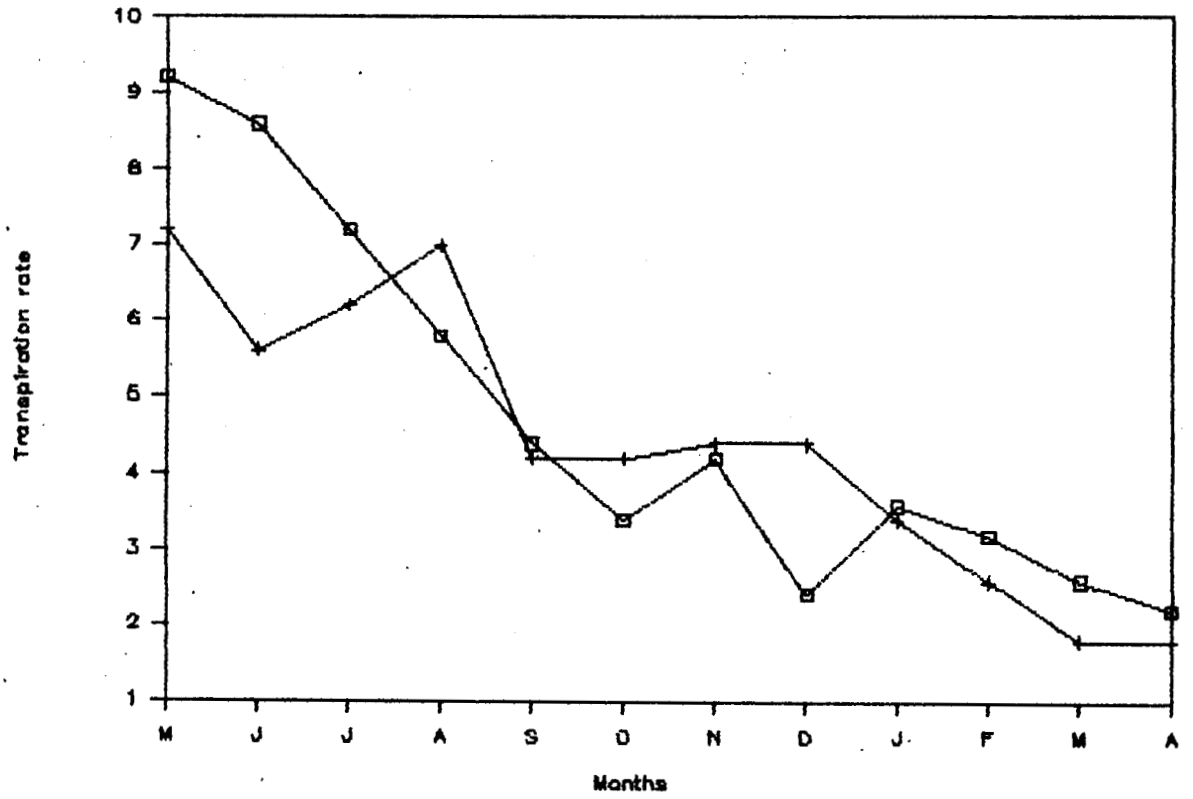
Fig 20 shows the trend of leaf water potential recorded during the year in genotypes KS 69 and 1495, when soil moisture was not a limiting factor (May-August) the leaf water potential was as high as -3 to -5 bars and with the scnsing of soil moisture stress the leaf water potential attained more negative values(-11.8 to -12.0 bars). Response of both the genotypes remained similar with respect of leaf water potential.

Fig.18 : Stomatal resistance response to depleting SMC in the field



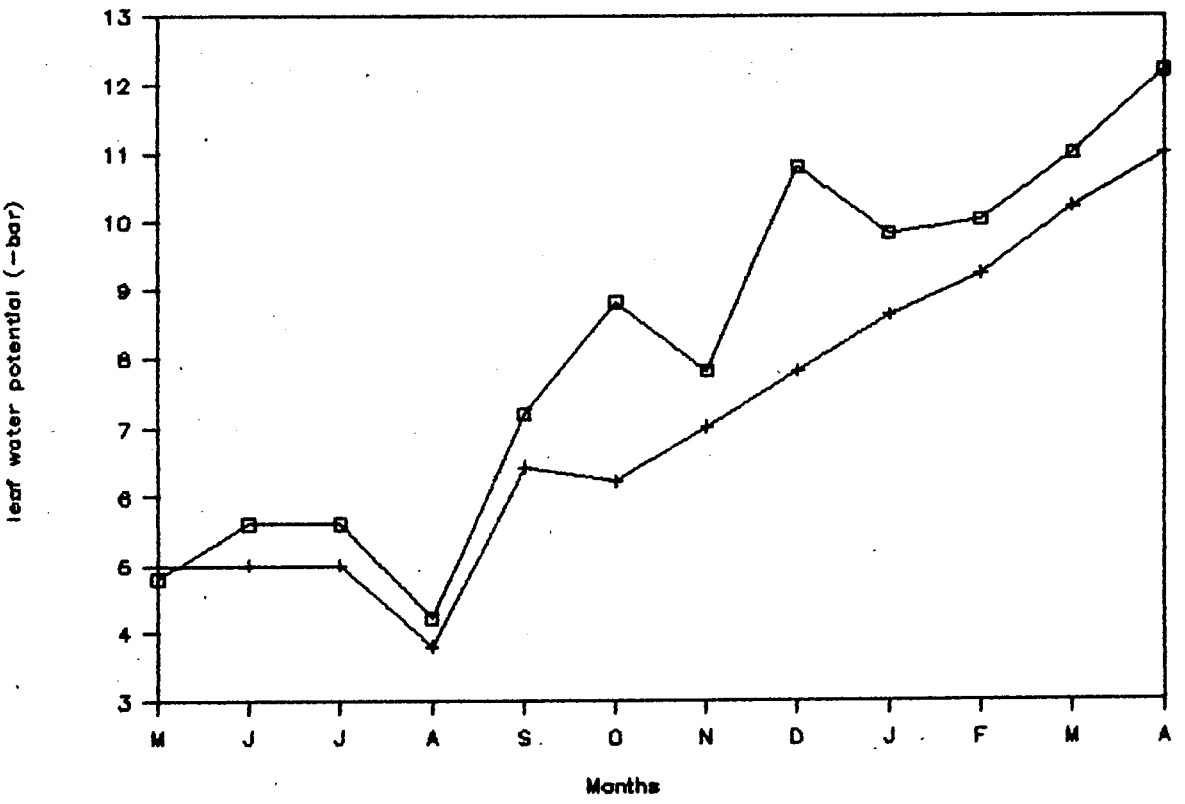
□ Acc. No. 1495 + KS69

Fig.19 : Transpiration rate as influenced by soil moisture content



□ Acc. No. 1495 + KS69

Fig.20 : Leaf water potential as affected by SMC



□ Acc. No. 1495 + KS69

The field data on physiological responses viz, stomatal resistance, transpiration rate and leaf water potential indicate the similarity of trend of the genotypic responses realised in pot culture experiment. Further, the study indicated the suitability of the physiological parameters proposed for indexing for drought tolerance.

Discussions

DISCUSSION

Agroclimatic factors and soil moisture stress development:

The important aspect of (drought) stress study is the agroclimatic conditions prevailed during stress stages. The numerous physiological responses of plants to mositure stress vary with the severity as well as the duration of the stress. Only the most sensitive precess are altered by mild stress. However, at critical stress conditions even the basic metabalism is affected. It is between these two stages that most of the plant responses are indicative of the genic tolerance to less moisture supply. Hence, drought studies involving the plants responses essentially includes all responses viz, growth responses, physiological responses and metabolic responses. The growth responses become positive only when the resources are unlimited (water, nutrients etc). Therefore, invariably in most of the plants the growth responses expected would be nil or negative.

Several of the plant responses are influenced by agroclimatic factors especially under soil moisture stress. Higher temperature and radiation interception coupled with the higher evaporative demand increased the severity of soil moisture stress in coconut (Rajagopal *et.al.* 1989) and in cocoa (Balasimha and Rajagopal 1986).

In the present study a similar situation is realised in black pepper also. Higher temperature and high evaporative demand has intensified the stress and it is reflected by plants responses within fifteen days, in potculture experiment, while in the field the stress development has been slow and steady. The difference in the stress development between potted plants and field plants can be attributed to the resource limitations of respective studies.

Soil moisture depletion pattern in pot experiment and in field experiment when compared indicates the above fact (Fig 4 and 17). The intensity of soil mositure stress is high when atmospheric drought exists along with soil drought. (Rajagopal et. al., 1989). The results agree with the above statement as evidenced is pot experiment where the severity was realised in first two weeks (without watering).

In the present study, relative humidity (40-45%) and temperature (29-33 °C) indicate the higher evaporative demand of the atomosphere. It is obvious from the figures (1-4) that climatic factors played a significant role in increasing the intensity of stress. Statistical analysis of soil moisture content (tabel 1) showed significant differences due to stress, stages of samplings. Difference due to cultivars was significant only in the last sampling date. This indirectly indicates the cultivars capability to extract moisture from the soil.

Critical moisture content fixed is based on a widely accepted method (Rajagopal et.al.1989). Soil moisture content at half max of stomatal diffusive resistance is considered critical beyond which the diffusive resistance would show a steep increase, an indication of plants complete closure of stomata and negligible metabolic activity.

Response of growth characters:

Soil moisture stress reduces the number, rate of expansion and final size of leaves. In species whose entire leaves persist for over an year, water stress of the preceeding year regulates the no., of leaf primardia that form in the succeeding year (Kozlowski 1964 a). Developmental processes viz., expansion growth, leaf production and reproductive development are very sensitive to moisture stress.

Leaf expansion growth:

Biomass production and ultimate productivity of crop species are influenced by expansion growth of the leaves, as it is the means of developing leaf area for light interception, photosynthesis process and canopy stand. In many crop species the process most sensitive to moisture stress appears to be the expansive growth (Hsiao 1973, Bayer 1970 Passioura & Anne Gardner 1990: Kallarackal et.al.1990: Karamonas 1982 and Kemp et.al. 1989).

Reduction in leaf enlargement and the declined rates of leaf expansion with depleting soil moisture is well documented in several works (Karamonas et.al.1982: Tanguiling et. al.,1987: Passioura 1988, Boyer 1988 Hay & Janette 1988, Joly & Hahn 1989, Randall & Sinclair 1989).

Leaf expansion rate both length and widthwise (Fig 6 and 7) declined with the onset of moisture stress in all the cultivar studied. Leaf expansion rate was negligible from 5th day onwards and ceased completely thereafter on 8th day. This indicate the sensitivity of expansion growth to even mild stress. The leaf expansion rate was very low when the soil moisture content was 19.5% and as the soil moisture content declined to 17% the leaf expansion ceased. Leaf area increment shows a similar trend (table 2). The decline in leaf expansion rate is reflected upon the leaf area increment. Cultivars difference in leaf expansion growth under moisture stress is not significant.

Morphological/Biomass characters

Increase in root mass relative to shoot mass has been reported for moisture stressed plants (El.Nadi et.al.1969 and Pearson 1966).There are reports of increased absolute root mass irrespective of reduction in shoot growth (Hsiao and Acevado 1974; Sharp and Devies 1975). Rajagopal et.al.(1989) reported poor partitioning of dry matter in moisture stressed conconut.

Morphological characters viz., root length, shoot length and leaf no., for control and stress treatment is presented in table 5. Differences due to moisture stress is not significant for root length and shoot length. Leaf number showed significant difference due to moisture stress. However, cultivar x treatment interaction was not significant. Partitioning of dry matter to leaf, stem and root shows higher share allocated for leaves followed by stem and root. None of these characters showed significant correlation with moisture stress. Root/shoot ratio showed correlation with moisture stress though not highly significant (table 15). However, difference within cultivars is not significant. The insignificant variation among cultivars and treatment for morphological and biomass partitioning may be due to the fact that the whole experiment's duration was 20-25 days. In such a short duration drastic differences may not be plausible in growth, morphological and biomass characters.

Response of physiological characters:

The first (physiological) adaptive response of any plant species, to soil moisture depletion is checking water loss/reducing transpiration through stomatal closure. In this regard it is desirable to identify, cultivars having moderately high stomatal resistance and not complete closure or irresponsive opening of stomata (to check transpirational loss as well as to continue with basic metabolic activity). Such a cultivar, would have a better water use efficiency under adverse environments.

Stomatal closure provides a mechanism for reducing water loss. A general observation in most of the crop species is the incresed stomatal resistance under moisture stress. In black pepper the increase in stomatal resistance (table 6) in different cultivars has different trends. With the onset of moisture stress a set of cultivars responded quickly to reduce water loss by stomatal regulation (cvs Karimunda, Kalluvally and Narayakodi) The initial increase in stomatal resistance helped the plants to cope up with increasing intensity of stress, while the other group (cvs. Arakulam munda, Panniyur-1 & Aimpiriyan) did not respond till the stress attained critical level, thereafter these cultvars, cut down water loss drastically by stomatal regulation only to wilt. The latter group showed the wilting symptoms first. Higher rates of transpiration is a common occurrence in crop species under adequate moisture supply. However, plants experiencing moisture stress tend to cut down the transpiration to tide over stress period. Higher transpiration rates in control plants indicate the active growth of cultivars under adequate moisture availability (table 7). Transpiration rate declined in most of the cultivars studied. As moisture stress intensified transpiration rate was almost nil in two cultivars where in all probablity the metabolic activity has come to stand still (cvs Arakulam munda & Panniyur-1). Few cultivars have shown controlled stomatal regulation whereby necessary metabolic activities continue, though at a minimum level to sustain drought period.

Similar trend was recorded in conconut. The reduced rates of transpiration in unirrigated palms was attributed to stomatal regulation (Rajagopal et.al.1989). It appears that an approach does occur in plant systems as shown by Hygen(1953). A rapid reduction phase in the rate of transpiration may occur at critical stage of stress beyond which stomatal regulation no more saves the plant.

Leaf water potential and relative water content

Leaf water potential varies greatly depending upon the type of plant and upon environmental conditions. Hsiao et.al.(1976) outlined a number of plant responses to moisture stress which occur well before desiccation becomes lethal. Most responses (eg.cell growth, protein synthesis, enzyme activities etc.) are affected by leaf water potential reduction of less than 1.5 Mpa.

In the present study cultivars difference was significant with regard to leaf water potential. Cultivars which showed the wilting symptoms first recorded a more negative leaf water potential on last sampling date. In stressed plants leaf water potential ranged from -5.8 to -20 bars (table-8); leaf water potential showed a significant and positive correlation with depleting moisture content (table 15 r=0.833).

Water release curve has been shown to have relation with drought tolerance (Jarvis & Jarvis 1963, Connor & Tunslatt 1968).

Cultivars with a smaller slope of the water release curve is considered to have better tolerance. Since a larger potential gradient may result from either due to large osmotic potential at full turgor, a low tissue elasticity or a high ability to accumulate sloutes as tissues water content decline (Aspinall & Paleg 1981).Cultivars Kalluvally, Karimunda and Narayakodi showed a smaller slope than cvs Aimpiriyan, Arakulam munda and Panniyur-1 (fig 8).

Responses at metabolic level:

Osmotic adjustment during moisture stress is considered as a phenomenon of whole plant water relations, it must be based on cellular metabolic changes associated with accumulation of various solutes, amiono acids, ions etc(Raven et.al.1979; Turner & Jones 1980). Apart from accumulation of osmoregulants, pigments appear to be affected by moisture stress. Chlorophyll stability index has been used in screening for heat & drought tolerance (Ravindran et.al.) in cocoa.

In the present study chlorophyll and carotenoid pigments showed significant difference due to moisture stress as evidenced from table 9 & 10. Vasantha et.al.(1989) has reported similar effects due to moisture stress in black pepper. Effects on moisture stress on chlorophyll degradation has been reported by Kurup and Vijayakumar (1989) in pepper.

Moisture stress may have both qualitative and quantitative

effects on plant constituents. Total sugars estimated in different cultivars is presented in table 11. Total sugars has shown significant and negative correlation (table 15) with depleting moisture content. Proline content showed similar trend (table 11) and cultivars differences were also significant. Proline content showed a significant and negative correlation with depleting moisture content (table 15). The results agree with the literature available and reviwed in this work.

Enzyme (s) activity under moisture stress:

Activities of enzymes are very sensitive to moisture stress as water molecules forms the medium for all enzyme activities. Enzyme nitrate reductase has been studied to a greater extent as it is sensitive to even mild stress (Huffakar et.al. (1970). Reduction in nitrate reductase activity to the line of 75-85% has been reported for cotton subjected to moisture stress. (Ganesan et.al.1988). Diurnal course of NR activity was maintained at lower levels in stressed plants compared to unstressed wheat. A similar reduction in NR activity is recorded in (black pepper) the present study. Fig 13 shows the decline pattern of NR activity in different black pepper cultivars. NR activity was lowest in Panniyur-1 and Aimpiriyan. Cvs Kalluvally, Karimunda and Narayakodi showed slightly higher activity. Nitrate reductase activity showed a significant and positive correlation with depleting moisture content (Table 15).

Enzyme acid phosphatase activity is shown to increase under moisture stress condition (Vierra de silva 1968 & 1969, 1974, Nir & Polijakoff- Mayber 1966, Thakkur 1991) In the present study acid phosphatase activity increased in all the cultivars. (table 12).

Peroxidase activity ranged from 83-198 and 97-196 units /g/min in control and moisture stress treatment (table 12). Increase in peroxidase activity due to moisture stress (Zbiec et.al.1986, Li & Liang 1988). However, both these enzymes Viz acid phosphatase and peroxidase activity alterations occured in the last sampling stage when most of the plants showed visible witting symptoms.

The characters studied and discussed so far, has helped to evolve methodology for screening promising genotypes. Out of the twenty four characters studied, seven characters were found to have significant correlation with soil moisture content. Hence, the characters were short listed based on their statistical significance. The characters identified are stomatal resistance, transpiration rate, leaf water potential, relative water content, specific leaf weight, total sugars, proline content and nitrate reductase activity. All these characters showed varietal significance at critical moisture content and enabled to classify them for scoring purpose.

Based on the indexing method evolved comparing characters

with stomatal resistance at critical moisture content, five promising lines were screened. The short listed characters were studied, in these varities. Response curves were fitted for various characters and response at critical moisture content revealed the varietal response to moisture stress. Among the promising lines Acc.No.1495 (Kottanadan) and KS69 (Karimunda) performed better and scored 7 and 8 indicating higher degree of drought tolerance compared to KS88 (Karimunda), Panchami (Aimpiriyan) and Acc.No.931 (Kalluvally). Data on stomatal resistance, transpiration rate, leaf water potential, RWC, Proline content, total sugars and enzyme nitrate reductase activity support the above statement regarding their tolerance to moisture stress (table 17,18 & 19). To elucidate whether, these varieties (1495 and KS69) show similar response in the field, both these varieties in sufficient no,were tested in the field. The field evaluation data (Fig.18,19,20) has indicated the similarity in their response to stress in the field also. These plants were maintained as rainfed crop, and the physiological response viz, stomatal resistance, leaf water potential and transpiration rate recorded on monthly intervals reveal that the plants easily adapt to moisture stress situation.

Further, the stress development itself is very gradual and intensifies only during summer months, so also the physiological responses. The field data suggest that both varieties have better

adaptability to rainfed situtations. The same conclusion was arrived in the pot culture experiment also (See chapter screening of promising lines.). Since the indications are positive the scoring and indexing methodology explained in the first experiment would enable the breeders to indentify drought tolerant lines at early stages of growth itself.

Abstract

ABSTRACT

Plants response to undesirable environments i.e, drought, can be expressed at different levels; developmental, morphological, physiological and biochemical. Among the listed levels of adaptations morphological and phenological adaptations are more sensitive and express itself at an early stage of stress. Whereas, physiological and biochemical responses are less sensitive and show repidity when stress attains critical proportions.

The objective of the present study is to record responses of various physiological, growth and biochemical parameters to moisture stress and to arrive at drought index (DI) for screening large germplasm lines.

The various characters studied include;

Growth parameters: Leaf expansion growth, root length, shoot length, leaf no., biomass partitioning specific leaf weight and Root / Shoot ratio.

Physiological Parameters : Stomatal diffusive resistance transpiration rate, leaf water potential and relative water content.

Biochemical parameters : Pigments viz., chlorophylls and cartenoids, total sugars, free proline, phenols. Enzyme

activities viz., nitrate reductase, peroxidase and acid phosphatase.

Of the twenty four characters studied eight characters showed highly significant correlation with depleting soil moisture content.

* Stomatal resistance showed highly significant and negative correlation with soil moisture depletion (r= -.894)

* Leaf water potential showed significant and positive correlation (r=0.830)

* Transpiration rate and relative water content showed highly significant and positive correlation (r=0.932,0.927 respectively)

* Specific leaf weight showed highly significant and positive correlation (r=.694)

* Components of biomass did not correlate with moisture stress

* Among the biochemical parameters studied total free sugars and proline content showed a significant and negative correlation with depleting moisture content (r= -0.598, -0.777 respectively)

* Nitrate reductase activity showed signigicant and positive correlation (r=.867)

Based on the study a drought index is proposed for screening/identifying drought tolerant pepper cultivars.

The short listed characters were used for indexing purpose with stomatal resistance as basic character.

Based on the above study five promising (high yielders) lines were screened for drought tolerance. (Acc.No.1495 KS 69, KS 88, Panchami and Acc. No.931).The shortlisted characters were studied in the above genotypes, subjecting them to moisture stress in pot culture experiment. The results established the usefulness of these characters (viz., stomatal resistance, leaf water potential,transpiration rate, RWC, SLW proline content, sugars and nitrate reductase activity). Among the genotypes studied Acc.No.1495 and KS 69 performed better (Scoring 7 & 8). Hence, these two lines were planted in the field and their field tolerance was studied.

* Genotypes 1495 (Kottanadan) and KS 69 (Karimunda) responded in the similar fashion in the field also. as in the pot culture experiment. Fig 18-20 shows physiological responses from adequate moisture available situation to drought situations.

* Stomatal resistance increased gradually from the month of september and continues to increase up to April corresponding

with the depleting moisture content. A reverse trend was observed in the case of tranpiration rate while leaf water potential reached more negative values during summer months.

The study has established the importance of the shortlisted characters and methodology (indexing for drought tolerance) that would aid in screening for drought tolerance.

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