PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON WINGED BEAN (*Psophocarpus tetragonolobus* (L.) DC.)

THESIS SUBMITTED TO THE UNIVERSITY OF CALICUT IN PART FULFILMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
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This thesis is Dedicated to

late **Prof. P. S. Krishnan** F.N.A.

and to

My Parents

DECLARATION

I hereby declare that the thesis entitled **Physiological and Biochemical Studies on Winged bean (*Psophocarpus tetragonolobus* (L.) DC)** submitted for the **Ph.D. Degree** of the University of Calicut has not been submitted for the award of any other degree or diploma and that it represents the original work carried out by me.

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CERTIFICATE

This is to certify that the thesis entitled **Physiological and Biochemical Studies on Winged Bean (*Psophocarpus tetragonolobus* (L.)DC)** submitted to the University of Calicut by Sri. K. Harikumar in part fulfilment for the award of the degree of **Doctor of Philosophy** in Botany is a bonafide record of the research work carried out by him under our supervision and guidance. No part of this thesis has previously formed the basis for the award of any other degree or diploma.

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FOREWORD

The author started the investigations on the topic, **Biochemical studies on winged bean (*Psophocarpus tetragonolobus* (L.) DC) seeds** under the supervision and guidance of Prof. P.S. Krishnan, FNA, Emeritus Professor of Biochemistry in the Department of Botany. After the unexpected demise of Prof. Krishnan, the research work was continued under the joint guidance of Dr. S. Nandakumar, Professor of Biochemistry and Dr. Nabeesa Salim, Reader in Physiology, with a slight modification of the topic - **Physiological and Biochemical studies on winged bean (*Psophocarpus tetragonolobus*(L.) DC).**

The winged bean, an under-exploited climbing perennial legume, has considerable potential in the humid tropics to combat protein malnutrition owing to the high protein content of its edible parts (leaves, flowers, green pods, seeds and tubers). It has much importance as a research plant both in the applied and fundamental field. A review of the nutritional values of the winged bean can be found in Claydon (1978). Following the report of the National Academy of Sciences (Anonymous, 1975), two symposia have been held on exploring the potentials of winged bean - one at Manila in 1978 and the other at Colombo in 1982. The "Winged bean Flyer" makes available reports on the latest researches on winged bean in institutions all over the world.

At the Calicut University, the Department of Botany has been actively engaged in fundamental researches on the Physiological, Histochemical and Biochemical aspects of the winged bean plant, the seed in particular. An important aspect of these researches in which the present author has focussed his attention is the Physiology and Biochemistry of pod / seed development and seed germination. The physiological studies include imbibition pattern

by winged bean seeds in the laboratory conditions and seedling emergence pattern in the field conditions.

Seed coat imposed dormancy is found to be a characteristic of winged bean seeds and the distribution of dormancy varies with variety. Similarly, seed polymorphism and dormancy are interrelated. It is well established that seed coat plays a dominant role during imbibition (Mayer and Poljakoff-Mayber, 1989; Mohammed-Yasseen *et al.*, 1994). In an earlier publication on water uptake patterns by 45 varieties of winged bean seeds Nabeesa *et al.* (1988) showed that the seeds of winged bean exhibit physiological heterogeneity in water uptake. On the basis of these studies the seeds of winged bean were grouped into three categories: (i) readily imbibing when in contact with water, (ii) delayed imbibing , (iii) hard seeds which need scarification for imbibition. In physiological studies, the present author has given special emphasis to the inter and intra varietal differences in the pattern of water uptake and the role of seed coat of each seed type. Seed colour and size were taken into consideration during imbibition in Petri dish condition in the laboratory. For these studies, 10 varieties of seeds were selected, namely, V4, V5, V8, V16, EC 28886, Selection 12, PT 15, PT 51*a*, PT 51 *b* and PT 62. The pattern of field emergence of these varieties were also studied to compare them with those of the laboratory germination conditions.

The biochemical studies were confined to only one of the above varieties, namely, V16 due to the greater degree of consistency in terms of seed polymorphism and other characteristics exhibited by this variety.

The biochemical studies during pod / seed development were carried out in axillary source leaf, pod / pod wall, embryo and seed coat. The main sink in winged bean is the pod / seed which is borne in the axill of leaves, the source. During early stages of development, the legume seeds obtain their nutrients from the endosperm surrounding the embryo in the embryo sac. But the assimilates carbon, nitrogen, sugars etc., required later for development of

seed and reserve deposition in the cotyledons are translocated from the mother plant.

The nutritive role of the seed coat is highly significant during embryo development and due attention was given to seed coat in the studies. The storage proteins in legume seeds are built up from a few amino acids transported from the mother plant. Enzymatic proteins in developing seeds may either be utilized as supplementary source material for elaboration of the storage protein or may be retained for the purpose of metabolism in the resting seed. Transaminases play a central role in amino acid metabolism since these result in the synthesis and redistribution of nitrogen from glutamate to a range of other amino acids. Kirk and Leech (1972) showed that all the protein amino acids can be synthesized by aminotransfer reactions from alanine and aspartate. The biochemical aspects studied during pod / seed development included the changes in metabolites like total sugars, proteins, changes in free and total amino acids. The change in total phenolics during pod / seed development was also included since it plays a dominant role in the hardness of the seed coat. Detailed studies were carried out on alanine and aspartate aminotransferases, presumably the most important transamination enzymes in these reactions on pod / seed development. The changes in fresh weight and dry weight were also carried out simultaneously during pod / seed development.

Mobilization of cotyledonary reserves is a major event during the germination of legume seeds. The main cotyledonary reserves in winged bean are protein, lipids and sugars. Therefore, biochemical changes during germination were conducted in the 3 types of seeds upto 3 days of germination. The tissues included dry resting seeds (control) cotyledons, embryonic axis, plumule and radicle. The amino acids generated by proteolysis of reserve proteins are translocated to, and utilized by the growing embryonic axis or used for the synthesis of enzyme proteins in the cotyledons.

Amino acid formation and transformation, therefore, constitute an important aspect of the metabolism in germinating seeds. Transaminases are important in germinating seeds as well. So, detailed studies were conducted on changes in alanine and aspartate aminotransferases, free and total amino acids, proteins and total sugars during germination. Here also the changes in fresh and dry weight were carried out simultaneously.

PRESENTATION OF THE THESIS

The thesis is divided into 7 chapters. The thesis commences with **Introduction** of the plant in detail. The Introductory chapter is followed by six chapters. Chapter II deals with a detailed survey of **Review of Literature** relevant in the field. The third chapter covers **Material and Methods** in detail. It starts with the cultivation of the plant in the Botanical Garden of Calicut University since the success of researches depended on the availability of pure lines of seeds with stable characteristics. The pattern of imbibition and field emergence of seedlings are also described in detail. Sampling for physiological and biochemical studies are described with the help of tables. The procedures for estimations are described with authentic references. Standardization of the optimal conditions for the assay of the aminotransferases are also given in detail.

The fourth chapter deals with **Seed Polymorphism and Pattern of Water Uptake and Varietal Variations in Winged Bean (*Psophocarpus tetragonolobus* (L.) DC)**. The results are followed by detailed discussion regarding with uptake and seed coat imposed dormancy in 3 types of seeds.

The Pattern of Field Emergence in 10 Varieties of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC) are presented in chapter V. The results are discussed with imbibition pattern in the laboratory condition.

The sixth chapter deals with **Biochemical Studies in Winged Bean (*Psophocarpus tetragonolobus* (L.) DC) During Pod / Seed Development**

commencing from the day of anthesis upto 60 days. The studies include changes in fresh and dry weight, total sugars, free and total amino acids, total phenolics, proteins as well as activities of alanine and aspartate aminotransferases. The results are presented on the basis of per gram fresh and dry tissue and on a whole tissue basis. The chapter ends with detailed discussion giving emphasis to source-sink relationship.

The Biochemical Studies in 3 Types of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC) Seeds During Germination represents chapter VII. Studies were conducted at various stages of germination upto 3 days in cotyledons, embryonic axis, plumule and radicle. During the course of germination study in the 3 types of seeds of V16 variety, changes in fresh and dry weight, total sugars, free and total amino acids and proteins were carried out. Also, activities of alanine and aspartate aminotransferases were studied. The results are presented on the basis of per gram fresh and dry tissue and on a whole tissue basis and discussed in relation to source-sink and reserve mobilization.

Chapter VII is followed by the **General Conclusions** in which the author has attempted to high-light the major findings the present investigations.

The dissertation ends with the **References** section.

CONTENTS

INTRODUCTION

INTRODUCTION

The winged bean (*Psophocarpus tetragonolobus* (L.) DC), a tropical legume, is an important source of high protein human food in southeast Asia, Papua New Guinea, and Indonesia (Burkill, 1906; Masefield, 1973; Claydon, 1978; Pickersgill, 1980; Harder *et al.*, 1990). Existence of winged bean in India has been reported from 1799 onwards (Chandel *et al.*, 1978; Srinivasan *et al.*, 1978). World-wide interest in the winged bean as a food legume has increased in the past few decades (Masefield, 1973; Anonymous, 1975). It is an exceptional legume and almost all parts of the plant can be eaten. The tubers, young pods, seeds, leaves, flowers and shoots, are rich in protein, amino acids, oils, vitamins and minerals (Claydon, 1978). It is hailed as the soybean of the future as a valuable supplement in human nutrition by virtue of its high proteins and satisfactory quantity of oil (Anonymous, 1975; Gillespie and Blagrove, 1978). Compared with other edible legumes, the winged bean plant appears to have more and larger nodules, which may account for the remarkable nitrification and the plant's exceptionally high protein content (Masefield, 1973; Karikari, 1978).

The origin of winged bean is probably Papua New Guinea and southeast Asia. Eight of the nine species of *Psophocarpus* are native to tropical Africa with a single extension of the cultivated *Psophocarpus tetragonolobus* to south east Asia and Papua New Guinea (Harder *et al.*, 1990).

The winged bean, the only cultivar among the genus *Psophocarpus* belongs to the tribe *Phaseolae*, in the sub family Fabaceae of Leguminosae. It is a perennial climbing short-day plant, cultivated as an annual with indeterminate growth. In India the best time for planting is from the last week of June to the end of July, after the severity of the south west monsoon. Seeds can be dibbled at a depth of 3 to 5 cm. Seedling will emerge from the ground in 5 to 10 days. Staking the plant with poles gives rise to high yields of pods and seeds. Usually flowering will commence after 50 to 60 days of sowing. Like many legumes cultivated in the tropics, the winged bean can be grown as an intercrop with tapioca, bananas, sugarcane, sweet potatoes, or other green vegetables.

Earlier reports suggest that the plant is free of serious pests and diseases (Anonymous, 1975). However, a number of insect pests and diseases which cause serious crop loses have been identified. Some diseases such as pod rot, leaf scorch, bacterial wilt and pests like aphids are the major ones (Srinivasan *et al.*, 1978; Khan, 1982; Shanthi-Chandra *et al.*, 1990).

The basic pigmentation of the stem is green and purple, green being the most common. The leaves are green, trifoliate similar to the genus *Phaseolus*. The majority of leaves are deltoid (94.6%), the rest being lanceolate (Chomchalov *et al.*, 1978). The leaves contain 5 to 15% protein and high amount of vitamins (Anonymous, 1975). The extensive root system, with unusual amount of nodulation, may help the plant to grow in nitrogen poor soils, reflecting its ability to obtain fixed nitrogen via its root nodules. The fibrous roots of certain varieties form tubers. The tubers

contain 10 to 12% crude protein on fresh weight basis, an amount exceeding that of any other tuber crop. According to Herath and Fernandez (1978), reproductive pruning increased tuber yield, whereas vegetative pruning reduced tuber production.

The inflorescence is an axillary, erect raceme which bears 3-12 bluish, purple or white flowers. The calyx tube is five - lobed with green or purple colour. The standard petal is large and broad, two irregularly obovate wing petals, and an obtuse, incurved keel. They enclose the ten stamens, all but one of which are united by their filaments at the base; the remaining one is free. The multi-ovulate ovary has a long incurved style and terminates in a globose stigma with dense hairs around and below it. The winged bean is cleistogamous and largely self pollinating since anthesis occurs prior to flower opening (Anonymous, 1975; Erskine and Bala, 1976; Pospisil, *et al.*, 1978). However, a low but significant level of heterozygosity can be seen in several areas. Experiments conducted in Papua New Guinea showed that the level of cross pollination varied from 0 to 7.6% (Khan, 1976; Erskine, 1978; Summerfield and Roberts, 1982). Carpenter bees (*Xylocopa aruana*) plays a major role for the considerable rate of out-crossing (Erskine, 1978). Usually the flowers open between 8 and 10 am and close in the late afternoon.

Pod formation is visible 5 to 6 days after anthesis. Some varieties rarely produce more than one pod per inflorescence, whereas in others the occurrence of more than one pod per cluster is common. About 60 days duration is necessary for the pods to mature and dry out (Anonymous, 1975; Pospisil, *et al.*, 1978; Data and Pratt, 1980). The length of the four-winged,

slightly bent pod varies from 6-38 cm and the seed number 5 to 20 per pod (Anonymous, 1975). Wide variation can be seen in pod colour, shape and the wing characteristics (Khan, 1978). The basic colours of the pod are green, pink or purple. Pod shape as seen in cross section is rectangular, semiflat or flat. The wings may be undulate, serrate or lobed.

The green tender pods themselves have high protein content. Anonymous (1975) reported 1. 9 to 2.9% protein and 3.1 to 3.8% carbohydrate in immature pods, on fresh weight basis (water content 76 to 92%). The green pod is also rich in calcium, iron, phosphorus, vitamins and other minerals.

Seed colour shows wide variation, with brown as the most dominant. The seeds might also be white, yellow, black, purple or mottled. The winged bean also shows variation in their testa hardness, like other legumes. Nabeesa *et al.* (1988) reported three types of seeds on the basis of the pattern of water uptake : (i) readily imbibing when in contact with water (ii) delayed imbibing and (iii) impermeable or hard seeds, which need scarification for germination. The dry weight of seeds varied between 62 to 417 mg with average of 224 mg per seed (Anonymous, 1975). The highest seed weight reported was 558 mg for a Sri Lankan selection (Herath *et al.*, 1978).

The mature seeds contain 29.8 to 37.4% protein, 15 to 20% fat and 28 to 31.6% carbohydrates on fresh weight basis (water content 6.7 to 24.6%). The seeds are rich in calcium, magnesium, phosphorus, iron, vitamins and minerals (Anonymous, 1975). The amino acid composition is similar to that of soybean which is deficient in sulphur-containing amino acids. It is rich in lysine and can supplement cereal diets that are lysine

deficient. The seeds contain a high content of unsaturated fatty acids. The seed oil is rich in tocopherol, an antioxidant, that improves the utilization of vitamin A in the human body. This is important since vitamin A deficiency is common in many tropical countries. The people in Indonesia are said to use the plant extracts traditionally to treat eye and ear infection, cure dyspepsia and venereal diseases, and in Sri Lanka it is used to treat diabetes (Burkill, 1966).

The occurrence of trypsin inhibitor in mature winged bean seeds is well known (Sohonie and Bhandarkar, 1954). But its activity can be destroyed almost completely by simple heat treatment.

The winged bean plant and its various parts without exception, are sufficiently rich in important nutrients that make them comparable to other protein rich legumes such as soybean. This makes the plant a crop of economic and nutritional importance especially among the poor in tropical countries to whom it forms part of their staple diet.

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

Imbibition and Germination

The kinetics of water uptake by the seed integrate the uptake by its individual parts. The different organs of the embryo as well as the different tissues of the seed differ in chemical composition, physical organization, physiological activities and water retention characteristics (Stiles, 1948).

Hyde (1954) described the hilum of legume species as a hygroscopic valve that opens at low relative humidities, permitting the loss of water and causing internal seed drying; under wet conditions it closes when expanding cells close the fissure. According to Bewley and Black (1983) greater water uptake occurs through the micropyle than the rest of the testa. Although the hilar fissure seemed to provide the primary path of water entry, the entire system of the seed coat, hilum and micropyle might constitute an integrated water absorption process in legume seeds (Sefa-Dedeh and Stanley, 1979; Deshpande and Cheryan, 1986).

Germination commences with the uptake of water by the dry seed - imbibition - and is completed when a part of the embryo, usually the radicle , extends to penetrate the structures that surrounded it. The uptake of water by dry seeds is a triphasic process: (i) an initial period of rapid uptake (ii) a lag period or transition phase in which little water is absorbed; and (iii) A further increase in water uptake occurs only after germination is completed, as the embryonic axis elongates. Because dormant seeds do not complete germination, they can't enter phase iii (Koller and Hadas, 1982; Bradford,

1986 ; Bewley, 1997).

Although the initial uptake of water is of a physical nature, a number of biological processes occur during this period. The respiratory rate increases rapidly early in the initial phase (Bewley and Black, 1983; Mayer and Poljakoff-Mayber, 1989). Studies with embryos of Lima bean (Klein and Pollock, 1968) and soybean (Webster and Leopold, 1977; Bramlage *et al.*, 1978) showed that proliferation and reorganization of the membrane system (plasmalemma, endoplasmic reticulum and mitochondria) start as early as 20 minutes after the onset of rehydration. ATP seems to be found during the first hours of imbibition in cucumber and mung bean seeds, due to the activities of pre-existing mitochondria in cotyledons which phosphorylate ADP to ATP during early hours of imbibition (Morohashi and Sugimoto, 1988).

Seed germination culminates in embryo growth and radicle protrusion through the tissues surrounding the embryo, the seed coat. According to Bewley and Black, (1983) the first visible and early measurable signs of germination are commonly the increase in length and fresh weight of the radicle -visible germination. Germination is characterized by the hydrolysis of reserve, including lipids, proteins and carbohydrates from the storage tissues. The products such as sugars and amino acids are subsequently translocated to the embryonic axis for synthesizing cellular constituents required for growth and differentiation (Bewley and Black, 1983; Mayer and Poljakoff-Mayber, 1989).

Hard seeds in leguminous plants have been attributed to both genetic and environmental factors (Rolston, 1978). Among the environmental factors are: (a) soil fertility (b) light (photoperiod) (c) relative humidity and (d)

temperature. Gutterman and Heydecker (1973) reported that reducing day length during the last 8 days of ripening increased the water permeability of *Ononis sicula*. Water impermeability in wild pea, *Pisum elatius*, *Cercis siliguastram* and *Robinia pseudoacacia* can be manipulated by oxygen (Marbach and Mayer, 1974). Quinlivan (1971) reviewed the effect of relative humidity on impermeability and its relationship to seed moisture content. Rowland and Gusta (1976), studied the seed moisture and imbibition temperature of faba beans and peas and their results agreed with that of Hobbs and Obendorf (1972) and Roos and Manalo (1976). According to them germination and seedling vigour are adversely affected by low seed moisture and low imbibition temperatures. Storage for extended period of time at high temperature and humidity in creased the occurrence of hardness in most legumes. Jones and Boulter (1983 *a, b*) found that two factors were responsible for increased hardness in beans stored at high humidity: (i) reduction in the rate of cotyledonary cell separation due to reduced middle lamella (pectin) solubility and (ii) reduction in the ability of the seeds to imbibe water.

The importance of the seed coat in seed longevity has long been noted (Becquerel, 1906). Seeds with hard seed coats are generally long lived (Bass, 1980; Priestley, 1986).Lang (1965) concluded that in most seeds the seed coat is inert and does not contribute directly to the metabolic activity of the system during germination, Nevertheless, it may prevent germination because it:

a. interferes with water uptake

b. interferes with gaseous exchange

c. contains chemical inhibitors

d. act as a barrier against the escape of inhibitors from the embryo.

e. modifies the light reaching the embryo.

f. exert a mechanical restraint (Bewley and Black, 1983).

The family leguminosae is one of the most commonly known to possess seeds with impermeable coats (Tran and Cavanagh, 1984). Chomchalow (1978), working with 167 lines of winged bean in Thailand, concluded that the seed coat was rather impermeable to water and the seed coat got harder during storage. Contradictory to this, Nabeesa Salim and Lalitha (1997), found that seed coat imposed dormancy breaks during storage of winged bean seeds.

Saio (1976) observed that the small hard soybeans contained higher amounts of crude fibre and calcium than the normal beans, resulting in their resistance to water absorption. Bhalla and Slattery (1984) studied the seed coat impermeability of clover seed and found intense deposition of callose in the inner parenchymatous cells, or the nutrient layers of impermeable seeds, whereas, no such deposits was noticed in permeable seeds. The testa structure of hard and soft seeded varieties of *Lupinus angustifolius* was investigated by Valenti *et al.* (1989) who found that the lack of grooves and the unbroken and more prominent light line are thought to be involved in the impermeability of hard seeds.

Several workers have emphasized the unusually tough and massive nature of the seed coat of winged bean, and special treatments required to promote germination (Cerny, 1978; Kordylas *et al.*, 1978; Martin, 1978;

Vietmeyer, 1978; Lam-Sanchez and Tondato, 1983). Sastrapradja (1978) found that winged bean seeds imbibe water much more slowly than the rest of the food legumes tested in Indonesia. Scarifying with sulphuric acid (Woomer, *et al.*, 1978; Csizinsky, 1980; Ruter and Ingram, 1991) and overnight soaking in water (Khan, 1978) have been recommended for hastening germination. Deshpande and Cheryan (1986) studied the micro structure and water uptake of *Phaseolus* and winged bean seeds and suggested that the very thick and compact arrangement of seed coat layer, the small hilar fissure and the very fine filtration system provided by the highly developed tracheid bar seemed to have contributed to the extremely slow hydration of winged bean seeds, especially, the low initial water uptake.

Legume seed lots, in general, contain varying proportion of 'hard' seeds. The phenomena of 'physiological heterogeneity' ('polymorphism', in its broad sense; Roberts, 1972), have been well presented in winged bean (Nabeesa *et al.*, 1988). By studying the imbibition pattern of 45 lines of winged bean, these authors sorted out 3 types of seeds: (i) 'readily imbibing', (ii) 'delayed imbibing', and (iii) 'hard seeds' (scarified types) which need scarification for water uptake.

A hard seed coat is considered an undesirable characteristic, as this high level of resistance to deterioration is accompanied by resistance to germination and cooking and consumption (Mohamed-Yaseen, *et al* 1994).

Phenolic compounds in the seed coat

The seed coat chemically (Van Sumere *et al.*, 1972) and physically (Esau, 1977) protects an embryo from harsh environmental conditions. Kannenberg and Allard (1964) reported that coloured snap beans had greater

seed coat dry weight and thickness and less permeability to water than the white seeds. Lignin composed about 15% of the total weight of the coloured lima bean seeds but 1% of white seeds (Wyatt, 1977). Marbach and Mayer (1974; 1975) suggested that during dehydration of the seeds, phenolic compounds in the seed coat are oxidized in the presence of catechol oxidase and this might render the seed coats impermeable to water. According to Marbach and Mayer (1979) and Werker *et al.* (1979) the water impermeability of the testa of peas is due to the continuous, very hard layer of pectinaceous caps of the palisade cells, and the presence of quinones in a continuous layer of cells around the seed both in the lumen and cell wall. Drying seeds in the absence of oxygen resulted in seed coats without pigmentation and high permeability to water, while drying in the air or in oxygen resulted in coloured seed coats and less permeability (Mayer and Poljakoff-Mayber, 1989). Soybeans with black seed coats imbibe water more slowly than unpigmented soybeans (Tully *et al.*, 1981). Kadam *et al.* (1982) studied the phenolic compounds of winged bean and found that phenolics decrease in concentration as winged bean seeds mature in the pods. In a study of the inheritance of water permeability in soybean seed, Shahi and Pandey (1982) found a linkage between seed coat colour and seed impermeability. Yellow seeds are more permeable than black seeds. Prasad and Weigle (1976) reported that seed coat cracking and leakage was greater in white seeded snap bean than black seeded making the former more susceptible to attack by soil pathogens. Black seeded soybeans were more resistant to deterioration in high humidity conditions than pale varieties (Starzinger *et al.*, 1982). Recently it has been suggested that the resistance of coloured seed coats to deterioration was as a result of impermeably thick seed coats formed by the oxidation of phenolic

compounds by polyphenol oxidase or peroxidase, the activity of which in soybean seed was mainly localized in the seed coats (Gillikin and Graham, 1991).

Phenolic compounds in seed coats play another role in seed longevity and act as a chemical defense against microorganisms (Haslam, 1979; Harborne, 1988). Monomeric phenols, which are more soluble than polymerized forms, may act as inhibitors to fungal growth and seed germination under humid storage conditions (Halloin, 1986). According to Iwanowska *et al.* (1994) secondary metabolites may play a primary role both in seed protection and in the regulation of seed germination.

Pod development

Physiological and biochemical changes accompanying maturation of the seeds and pods in legumes have been described by several authors (Bisson and Jones, 1932; Bain and Mercer, 1966; Flinn and Pate, 1968; Abu-Shakra, *et al.*, 1970). The ultrastructure of developing and mature cells of the cotyledons in garden pea (*Pisum sativum* L.) has been studied by Varner and Schidlovsky (1963) and Bain and Mercer (1966). Studies of the seed protein have been made in garden pea by Osborne (1924), Danielsson (1952), Varner and Schidlovsky (1963), Fox *et al.* (1964), Zarkados *et al.* (1965) and in chick pea seeds by Abu-shakra *et al.* (1970).

In a number of species of plants it has been shown that after anthesis seed moisture content changed in distinct phases. (Bedford and Mathews 1976; Barlow *et al.*, 1980; Onckelen - Van *et al.*, 1980). According to them initiation of each phase appeared to be associated with some fundamental changes in the seeds.

According to Mayer and Poljakoff-Mayber (1989), four stages may be distinguished in seed development:

1. histodifferentiation - which is actually the seed formation;

2. maturation, during which embryo reaches its maximal size and most of the storage materials are accumulated;

3. desiccation, which is closely connected with maturation.

4. quiescence, which is the resting stage after desiccation.

Desiccation period seems to be an essential stage of development in which a switch-over occurs from a pattern associated with development to characteristic of germination (Kermode and Bewley, 1986; Kermode *et al.*, 1986).

According to the reports of Revelli *et al.* (1978), pod formation was visible after 5 to 6 days of flower opening in winged bean. Pospisil *et al.* (1978) suggested that the development of pods in winged bean is very fast and occurs in two stages. The longitudinal growth of pods lasts for 16 to 20 days during the course of which the pod attain their full length; an additional 45 days or so is needed for the pods to mature and the seeds to ripen and dry.

Data and Pratt (1980) studied the patterns of pod growth, development and respiration at various stages after anthesis in winged bean and suggested that pod growth in length, width and fresh weight and seed growth in length and width showed sigmoid growth patterns, with significantly linear period of rapid growth extending over many days. Seed fresh weight followed a diauxic growth curve. The respiration rates of intact pods varied with pod age at harvest, reflecting the different growth stages.

Little attention has been given to the ultrastructural aspects of various stages of ovule development. However, in *Vicia faba* some studies have been done (Neumann and Weber, 1978; Nieden *et al.*, 1982; Nieden *et al.*,1984; Johansson and Walles, 1994).

Studies on pod development in chick pea, garden pea and cow pea seeds showed that moisture content, total sugars and protein decreased with maturity (Bisson and Jones, 1932; Culpepper, 1936; Hoover and Dennison, 1953; Worthington and Burns, 1971).

Hocking and Pate (1977) have published a detailed study on mobilization of minerals to developing seeds of legumes. Nitrogen metabolism of developing and germinating seeds of pigeon pea (Rao and Rao, 1978) showed that the total nitrogen of the embryo increased throughout the development, whereas, that of the seed coat showed a gradual decrease.

Murray and Kennedy (1980) studied the activities of enzymes of nitrogen metabolism in seed coats and cotyledons during embryo development in pea seeds and found that on a fresh weight basis the highest activities of asparagine and alanine and aspartate aminotransferases were exhibited in the seed coats in early stages, whereas, the highest activities of glutamine synthetase, glutamate synthase and glutamate dehydrogenase were shown in the cotyledons in early stages of development.

According to Peoples *et al.* (1985), extracts of pod, seed coats and embryo during development of cow pea all exhibited measurable activities of the ureide catabolizing enzymes allantoinase and urease, the asparagine utilizing enzyme asparaginase, the ammonia assimilating GS:GOGAT system, and the transaminases aspartate aminotransferase and alanine

aminotransferase.

Rauf (1980) in his studies on the free amino acids in the developing seed parts and pod of *Cicer arietinum*, *Pisum sativum* and *Vicia faba*, upto 45 days after anthesis, found that in pod wall and seed coat, generally higher levels of free amino acids were present initially and their levels declined continuously with development.

According to Egli *et al.* (1981) and Saroop *et al.* (1998) the accumulation of dry matter by the seed is associated primarily with expansion of the cotyledonary cells and this requires uptake of water. Kadam *et al.* (1982) reported that phenolics decreased in concentration as seeds mature in the pods of winged bean. Phenolic acids occur commonly in the seed hulls of legume species (Sosulski and Dabrowski, 1984).

Data and Bautista (1983) while studying the chemical changes in the developing pod of winged bean found rapid synthesis of total and non reducing sugars, fat and fibre upto 10 days after anthesis. At 25 to 60 days after anthesis, starch, total sugar and fat content in the seeds increased briefly while protein and moisture decreased. Protein slightly increased after 35 days. Chemical constituents of pericarp decreased after 20 days except fibre content.

Garcia and Palmer (1980) analysed starch content of five cultivars of winged bean and found no starch in mature seeds. Contradictory to this view, Kute *et al.*, (1984) reported the presence of starch; their content ranged from 5.66 to 6.22% in mature seeds. These authors also analysed the sugar content and trypsin inhibitor activity of 3 varieties of winged bean during seed development and found that the starch and total sugar content increased during

the early stages of development followed by a decrease during subsequent maturation of seeds, and they concluded that winged bean does not receive adequate supplies of carbohydrate from the parent plant, and sugars and starch are utilized for the synthesis of proteins and lipids.

Sauvaire *et al.* (1984) studied the changes in growth, proteins and free amino acids of developing seed and pod of fenugreek and reported that the production of soluble nitrogenous compounds and amino acids precedes the synthesis of storage proteins, which takes place prior to dehydration of seeds.

Source-sink relationship and pod growth pattern in soybean have been reported by Egli *et al.* (1985) and they stated that reduction in the supply of assimilates during the linear phase of seed development lowered the seed growth rate, but did not affect final seed size because of a longer duration of seed growth. Also, nitrogen stress during seed development did not affect seed growth rate but shortened the duration of seed growth and reduced final seed size.

The accumulation of lipids and changes in lipid and fatty acid composition in developing winged bean seeds from three to seven weeks after anthesis have been studied by Khor and Chan (1988). They found progressive accumulation of lipids in developing seeds from 3 to 6 weeks after flowering. Neutral lipids, mainly triacylglycerols accumulated progressively in the developing seeds, whereas polar lipids, glyco- and phospholipids decreased rapidly as the seed developed and matured. Analysis of the fatty acid composition of seed total lipids and major lipid classes indicated substantial changes in the proportion of certain fatty acids during seed development.

The role of phytohormones in developing seeds was studied by many

authors. According to Wheeler (1972), in wheat, the highest levels of auxin in the developing kernel are associated with the time of intensive accumulation of dry matter. Berry and Bewley (1992), Patrick (1990) and Thomas (1993) reported that changes in abscisic acid (ABA) concentration and osmotic potential of the locular tissues regulate seed desiccation and induce dormancy of the embryo to prevent precocious germination in the pod. The gibberellins synthesized in developing seeds are much concerned with both seed and fruit growth by stimulating cell division and to maintain cell expansion (Gillaspy *et al.*, 1993). Wang *et al.* (1993) reported that during early phases of tomato fruit development sucrose synthase rather than acid invertase plays a dominant role in regulating the import of carbon into the fruit by metabolizing the imported sucrose.

Source-Sink relationship

Growth and development of plants are dependent upon the energy gained by fixing carbon dioxide into carbohydrates during photosynthesis and the translocation of newly fixed photoassimilates from the site of synthesis, "the source" to regions of utilization "the sink" (Sonnewald and Wilwitzer, 1992).

Sink strength has been considered as a product of sink size and sink activity i.e., the potential capacity of sink tissues to accumulate assimilates (Ho, 1988; Ho *et al.*, 1989).

The important reviews devoted to aspects of nitrogen metabolism are by Raven and Smith (1976) and Pate (1980); carbon assimilation and transport (Giaquinta, 1983); partitioning of assimilates (Gifford and Evans, 1981;Pate *et al.*, 1988; Wolswinkel, 1992) and assimilate unloading in sink organs (Thorne, 1985; Wolswinkel, 1985; Murray, 1987).

According to Bewley and Black (1994) during the early stages of development, the legume (non-endospermic) seed obtains its nutrients from the endosperm surrounding the embryo in the embryo sac. But when embryo growth accelerates, the endosperm may be absorbed and the embryo comes to occupy virtually the whole seed. The assimilates required later for reserve deposition in the cotyledons are translocated from the mother plant preferentially to the sink closest to the assimilatory leaf. This is facilitated by the vascular strand running through the pod and then passes through the funiculus, into the integuments.

Passage of assimilates from seed coat to developing embryo certainly involves apoplastic transport, since embryo and endosperm do not have symplastic continuity with parent plant (Bewley and Black, 1994; Peoples and Gifford, 1990).

Studying the structural aspects of the pathways of nutrient flow to the developing embryo and cotyledons of garden pea, Hardham (1976) has shown that the amount of vascular tissue in the funicle and ovule increases markedly between the time of cotyledon initiation and the commencement of storage protein synthesis and deposition.

Peoples and Gifford (1990) suggested that sink cells maintain the sucrose concentration gradient needed to sustain continued unloading by conversion to other soluble products by hydrolysis of sucrose, or conversion to insoluble products like starch.

According to Thorne (1982), translocation of assimilates into importing regions is dependent on the metabolic status of the sink. Treatments of sink with inhibitors of metabolism such as low temperature,

chemical inhibitors etc. decrease assimilated transport to these regions. Wolswinkel and Ammerlaan (1983) found in developing seeds of *Vicia faba* that 25 mM K^+ does not inhibit the release of sucrose and amino acids. They have also found that after the release of assimilates into the seed coat apoplast sucrose is more intensively taken by the seed coat tissues than amino acids.

Studies conducted by Huffaker (1982) suggested that root nodules may induce alteration among seeds either directly or indirectly. Nitrogen fixed by the nodules is taken up by the maturing and filling seed because of its powerful sink effect. When supply from the nodules is not adequate, the reserves in the leaves are drawn upon by the developing seed.

Experiments were conducted in Papua New Guinea by Bala and Stephenson (1978) in winged bean, to evaluate tuber production under conditions of vegetative pruning and reproductive pruning. The results indicated that pods and seeds constituted more powerful sinks than tubers.

Studying the influence of sink on photosynthesis in garden pea, Nath and Bhardwaj (1987) showed that photosynthetic rate of the leaf is controlled by the sink activity. Removal of sink (pod) reduced the photosynthetic activity of the source (subtending leaf) whereas, removal of source enhanced the photosynthetic activity of the sink.

Singh and Singh (1991) studied the source-sink relationship in Okra *(Abelmoschus esculentus* (L.) Moench) and found that complete removal of sink diverted the photosynthate for vegetative promotion. Removal of source caused significant reduction in vegetative as well as reproductive growth of the plants. They concluded that growth and development of different plant parts were compensatory and the relationship between source and sink seems

to be interdependent.

According to Bhatt and Rao (1993), the stem of Okra could be acting as a storage for assimilate, and the pod was strongly but not exclusively dependent on the subtending leaf for photoassimilates.

Mobilization of nitrogen from leaves usually contributes most of the seed's nitrogen requirement (from 16-40% of seed N), but fruit parts like pod wall are also capable of nitrogen redistribution to seeds (Hocking *et al.*, 1984; Peoples and Dalling, 1988).

The studies of Pate and Flinn (1973; 1977), Queberdeaux and Chollet (1975), Oliker *et al.* (1978 *a*) and Pate and Herridge (1978) indicated that photosynthetic capacity of the legume pod does not contribute much to the reserve materials of the seed. The main contribution is apparently through the refixation of the respiratory carbon dioxide of the developing seeds and pod tissue itself.

According to the studies of Flinn and Pate (1970), Turgeon and Webb (1975) and Wardlaw and Moncur (1976), the main sources of carbon for the developing seed is the leaflets, stipules and pods which will provide two thirds of the total carbon required for the ripening process.

Rawson and Evans(1971) and Kipps and Boulter (1974) have reported that post-anthesis photosynthate usually supply 70% or more of the carbon required during fruit growth.

Sheoran *et al.* (1987) in their CO_2 exchange studies using $^{14}CO_2$ in chick pea during seed development found that pod wall fixed net CO_2 in light during the early stages upto 21 days after anthesis, which was found to

contribute about 20% to seed dry matter.

The normal growth of a developing and expanding leaf initially depends on phloem-imported nutrients. With further expansion of the leaf, its capacity for assimilating atmospheric CO_2 gradually increases and the direction of transport in the phloem is eventually reversed as carbohydrates accumulate, import stops and the export of soluble sugar begins (Turgeon and Webb, 1973; Fellows and Geiger, 1974; Turgeon *et al.*, 1975).

Recently, a new technique "the empty seed coat technique" has been developed to measure unloading of assimilates from the seed coat of developing legume seeds. After surgical removal of the embryo from a developing ovule, leaving most of the maternal assimilate-delivering tissue intact and functioning, the empty ovule can be filled with a suitable medium to measure nutrient release from the seed coat (Murray, 1979; 1987; Patrick, 1983; Thorne, 1985; Thorne and Rainbird, 1983; Wolswinkel and Ammerlaan, 1983; 1984; Wolswinkel and De Ruiter, 1985).

Murray (1979) while studying the nutritive role of the seed coats during embryo development in *Pisum sativum* found that the liquid contents of the embryo sac are considered to arise as a secretion from the tegmen. High concentration of amino acids, NH_4^+, and orthophosphate were measured in this liquid.

During the passage of translocated substances across the seed coat, they can be transformed. Murray (1986) found that in developing seeds of garden pea, most of the alanine secreted into the embryo sac is synthesized in the seed coats. According to Peoples and Gifford (1990), a range of amino acids are synthesized in seed coats by enzymes capable of metabolizing

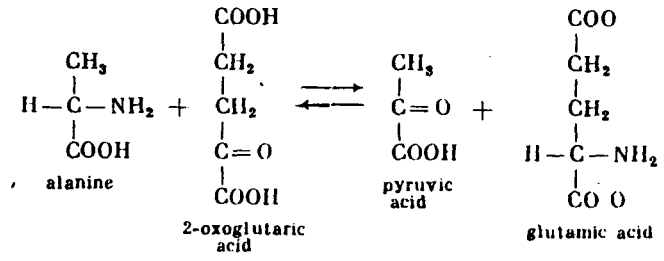
asparagine or by break down of proteins. Sucrose may be partially hydrolyzed.

It is known that the seed coats of *Pisum sativum* acquire reserves of starch and protein, which are mobilized as the seed coats senesce and as the embryos mature (Flinn and Pate, 1968; Murray and Collier, 1977). Mineral reserves are also mobilized from seed coat to embryo with varying degrees of retention.

According to Fisher (1967) and Fransceschi and Giaquinta (1983 *a;* 1983 *b*; 1983 *c*), in the leaves of certain C-3 dicot legumes such as soybean, mung bean and winged bean, a unique cell layer called the paraveinal mesophyll exists in the center of the leaves at the level of the phloem and spans the interveinal space. This is a highly specialized cellular network, presumably associated with assimilate transport and compartmentation (Giaquinta, 1983).

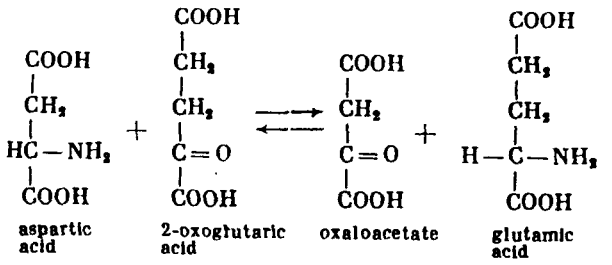
Transaminases

Since the first demonstration of enzymatic transamination made by Braunstein and Kritzmann (1937), using pigeon muscle preparation, considerable information has been accumulated regarding the occurrence and properties of aminotransferases or transaminases in bacteria, animal and plant tissues. These are enzymes catalysing the transfer of an amino group, plus a proton and an electron pair, from one amino donor compound to the carbonyl position of an amino acceptor compound (Fowden, 1965; Miflin and Lea, 1982; Ireland, 1990; Anderson and Beardall, 1991). Usually an amino acid acts as the amino donor and a 2-keto acid as the amino acceptor (Braunstein, 1973). The result is the formation of a new amino acid plus the keto acid analogue of the amino acid which originally served as the amino donor.



Alanine aminotransferase
(GPT, E.C 2.6.1.2)

Aspartate aminotransferase
(GOT, E.C.2.6.1.1)



Nitrogen, following its initial assimilation into glutamine and glutamate (Miflin and Lea, 1982) can be distributed to many other compounds by the action of aminotransferases. The final step in the synthesis of several amino acids is a transamination of the 2-keto analogue of the amino acid (Forest, and Wightman, 1972 *a*).

Earlier works of Virtanen and Laine (1938), Kritzmann (1939), Leonard and Burris (1947) and Smith and Williams (1951) have established the wide spread occurrence of aspartate aminotransferase (glutamic oxaloacetic - transaminase, GOT, E.C.2.6.1.1) and alanine aminotransferase (glutamic pyruvic - transaminase, GPT, E.C 2.6.1.2).

In higher plants, aspartate and alanine aminotransferases have been demonstrated in cell free extracts of corn radicle, pea, white and blue lupine, barley, oat and mung bean seedlings by Wilson *et al.* (1954). In wheat germ, semipurified aspartate and alanine aminotransferases have been isolated and studied by Cruickshank and Isherwood (1958).

A characteristic of aminotransferases is the requirement of pyridoxal phosphate as a coenzyme (Lu and Mazelis, 1975). The most extensively studied aminotransferase in plants is the enzyme responsible for catalyzing the transamination of aspartic acid. Smith and Williams (1951) were the first workers to present clear evidence for the occurrence of this aminotransferase in germinating embryos of several monocot and dicot plants. High activity of this enzyme was later demonstrated in wheat germ by Cruickshank and Isherwood (1958). Since these early studies, preparations of this enzyme have been purified to various levels from a range of plants such as cauliflower buds (Davis and Ellis, 1961; Ellis and Davis, 1961), germinating pea seeds (Wong and Cossins, 1969), bush bean roots (Forest and Wightman, 1971; 1972 *a;* 1973), oat leaves (Reed and Hess, 1975), spinach leaves (Huang *et al.*, 1976) and in crude homogenate of germinating cow pea seeds (Madhusudanan *et al.*, 1982) and in pea root nodules (Appels and Haaker, 1991).

Alanine aminotransferase (E.C.2.6.1.2) has been isolated and partially purified from leaves of *Artiplex spongiosa* (Hatch, 1973), tomato fruit (Gazeau-Reyjal and Crouzet, 1976) and pumpkin cotyledons (Splittstoesser *et al.*, 1976).

The early studies on the transamination reactions in plants have been reviewed by Sanwal *et al.* (1964), Fowden, (1965, 1967), Kretovich, (1965) and Wightman and Forest, (1978). According to Cameron and Cossins (1967) and Thomas (1972), transaminase is one of the enzymes persisting and probably participating in the metabolic activity of the dry seed. Ghildiyal and Sinha (1971) studied the activities of aspartate and alanine

aminotransferases during germination, growth and seed development in bengal gram and concluded that: (i) glutamic acid can transfer amino group to pyruvate, oxaloacetate, glyoxylate etc. at all stages of growth and development (ii) the aminotransferase activity of the same system differs at the varietal level (iii) there appears a possibility of fairly high rate of non-enzymatic transamination and also of exchange reactions.

Collins and Wilson (1972; 1975) studied the amino acid metabolism during early germination of embryonic axis and cotyledon of *Phaseolus vulgaris* and embryo and endosperm of *Hordeum vulgare,* using tritiated water. According to these authors, alanine, aspartate, glutamate and γ-aminobutyrate were rapidly labelled (3 minutes), whereas, later (15 minutes) acids of the Krebs cycle became radioactive. Because keto acids are chemically unstable, they are probably stored as amino acids and produced by deamination and transamination soon after the start of imbibition.

Studies on the subcellular localization and developmental changes of aspartate aminotransferase isoenzymes in the cotyledons of cucumber seedlings by Liu and Huang (1977) demonstrated that the enzyme in the cotyledon extracts from seedlings at various ages was resolved into six distinct isoenzymes by starch gel electrophoresis. These isoenzymes were localized in the glyoxysomes, cytosol and chloroplast. Also, no enzyme activity was detected in isolated mitochondria. According to Murray and Kennedy (1980), the highest activities of both aspartate and alanine aminotransferases were in the developing seed coat of pea seed during embryo development. Lillo (1984) studied the diurnal variations of nitrogen metabolizing enzymes in barley leaves and found that alanine aminotransferase activity increased 37 to 82%

during the photoperiod, whereas, aspartate aminotransferase activity showed only minor diurnal variations. In developing wheat grain endosperm Garg *et al.*, (1985) reported that the activity of alanine aminotransferase was higher than that of testa and pericarp. Sudhakar and Veeranjaneyulu (1988) studied the effect of salt stress on some enzymes of nitrogen metabolism in the shoots and roots of *Dolichos biflorus* and found that the activity of GOT was higher than GPT under salt stress. The activity of alanine aminotransferase in barley root tissue exhibited four-fold increased activity during several days of anaerobic induction (Good and Crossby, 1989). Nabeesa (unpublished) found that by decoating and forcing of cotyledonary axillary bud growth during seed germination in winged bean, the GOT activity in cotyledon, plumule and radicle was prominently higher than GPT, upto 10 days of germination.

Kinetic studies on peanut cotyledons (Mazelis and Fowden, 1969) in bush bean (Forest and Wightman, 1972 *b*), in tomato shoot (Gibson *et al.*, 1972), in oat leaves (Reed and Hess, 1975) have shown that no exogenous pyridoxal phosphate was required for the expression of enzyme activity in plants due to the much tighter binding encountered between protein and coenzyme moiety.

Investigations of the pH optima of several plant aminotransferases have shown that these enzymes show optimum activity in the pH range 7.5 to 8.9, and the range for maximum activity is usually quite broad, though some times sharp. Rarely a pH optimum has been reported to be in the acidic region (Hasse *et al.*, 1967), but more often near-maximum activity has been seen at pH values that are neutral or slightly higher. Wong and Cossins (1969) extracted soluble aspartate aminotransferases from pea cotyledons with the

pH optimum of 8.0. According to Forest and Wightman (1971, 1972 *a*), the pH optimum was found to be 8.5 for aspartate and alanine aminotransferases in cotyledons and growing tissues of bush bean seedlings.

The soluble aspartate aminotransferase purified from bush bean roots was found to have a M.W. of 1,28,00 daltons, as determined by gel filtration of Sephadex G-200 (Forest and Wightman, 1972 *b*) and that from wheat germ had a M.W. of 75,000 daltons when estimated by gel filtration on Sephadex G-100 (Verjee and Evered, 1969). Alanine aminotransferase isolated from tomato fruit was shown to have a M.W. of 100,000 as estimated by gel chromatography (Rech and Crouzat, 1974).

Patwardhan (1960) showed the participation of Fe^{++} ion as a cofactor in promoting the activity of glutamate oxaloacetate transaminase isolated from *Dolichos lablab* but Wightman and Forest (1978) found that Fe^{++} had no effect on the activity of multispecific aspartate aminotransferase from bush bean seedlings. According to Wong and Cossins (1969) the addition of Mg^{++} and Mn^{++} stimulated aspartate aminotransferase activity in cell free extracts of pea cotyledons.

The intracellular distribution of aspartate and alanine aminotransferases has been extensively studied in both animal and plant tissues and these enzymes are found to be present in chloroplast, cytosol and mitochondria (Kirk and Leech, 1972; Ireland and Joy, 1985). Considerable interest has been expressed by the presence of aminotransferases in the chloroplast and their possible role in amino acid formation, and hence protein synthesis in this organelle (Hedley and Stoddart, 1971; Hatch and Mau, 1973; Thomas and Stoddart, 1974).

The most comprehensive study of the aminotransferases of the chloroplast is that of Kirk and Leech (1972) using aqueously isolated chloroplasts from *Vicia faba*. These authors concluded that glutamate which forms 33% of the free amino acid pool of the chloroplasts is the primary product of photosynthetic amino acid synthesis. Secondary transfer from glutamate results in the formation of aspartate and alanine, and from these two compounds all the other protein amino acids can be synthesized. In contrast to the well known view of the central role of glutamate in ammonia assimilation and amino acid synthesis in chloroplasts, O'Neal and Joy (1973) and Miflin and Lea (1976; 1977) have shown that glutamine rather than glutamate must be regarded as the primary product of ammonia assimilation in leaf chloroplasts.

Cooper and Beevers (1969) showed that the glyoxysome of castor bean endosperm contains aspartate- a-ketoglutarate aminotransferase. Studies of Rehfeld and Tolbert (1972) in spinach leaf peroxisomes have shown to contain two specific aminotransferases which utilize glyoxylate as the amino acceptor in the synthesis of glycine.

The aminotransferases of the peroxisome play an important role in the process of photorespiration, a gluconeogenic pathway requiring the co-operation between peroxisomes, mitochondria, chloroplasts and cytosol (Tolbert, 1971; Rehfeld and Tolbert, 1972). This process provides an essential link between carbohydrate and amino acid metabolism in plant cells.

The involvement of aminotransferases in the biosynthesis of secondary plant products has been studied in several alkaloid containing plants (Hasse *et al.*, 1967). The work of Roberts (1977) has demonstrated the presence of

an L-alanine aminotransferase in *Conium maculatum*. Secondary metabolites arising from amino acids play a part in the 'hardness' of the legume seeds. (Gibson *et al.*, 1972; Roberts, 1977; Werker, 1980/81).

MATERIAL AND METHODS

CONTENTS

MATERIALS AND METHODS

Cultivation of winged bean

About 100 varieties of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) procured originally from National Botanical Research Institute (NBRI), Lucknow, Kerala Agricultural University, Thrissur, and National Bureau of Plant Genetic Resources (NBPGR), Regional Station, Thrissur, are being raised and maintained in the Botanical gardens of the Calicut University.

The seed sowing was carried out every year during June/July, after the severity of the southwest monsoon. The seeds were sown in 1 x 1.5 m beds, each bed separated by a distance of about 0.5 m. Before sowing the seeds, the soil was ploughed well manually and powdered dry cow dung, 5 kg per bed, was added into the soil. Thirty seeds were sown in a bed in 5 rows of 6 seeds each, at a depth of 2.5 cm. The beds were watered daily in the evening by means of tap water.

The plumular hook started emerging in 5-7 days and was complete in 10 to 20 days. The initial growth of seedling was slow, but once established, it grew vigorously. After the plants attained a height of 10 to 15 cm (after 3 to 4 leaves emerged), 5% urea solution was added to the soil. After one month, when the plants started throwing whips, they were staked with 5 to 6 ft. long poles fixed vertically in each bed, to function as support for 4-5 plants per pole. When the plants started flowering, that is, after 2 months, mulching was done with dried *Calicopteris* foliage.

Harvest pattern

The pods were harvested only after drying on the plant. In general, winged

bean pods take 45 to 60 days to dry up after anthesis. The harvesting was carried out once a week. The pods were then sun-dried for a day and shelled.

Storage

The aborted seeds and other waste parts of pod wall were removed. The seeds were transferred to clean dry plastic bottles and labelled, and stored at room temperature. Every month or so, the seeds were exposed to the sun for about 3 hour.

I. Physiological Studies

Varieties selected

The winged bean varieties selected for the present study were V 4, V 5, V 8, V 16, EC 28886, Selection 12, PT 15, PT 51*a*, PT 51*b* and PT 62 These varieties were proved to be ideal test material because of testa colour, larger size of seeds and tetragonal green pods.

Seed collection

The flowers of winged bean are cleistogamous and largely self pollinating, since anthesis occurs prior to flower opening (Erskine and Bala, 1976). However, it was suggested that some cross pollination has been occurring and it varied from 0 to 7.6% (Khan, 1976; Erskine, 1978; Summerfield and Roberts, 1982). Heterogeneity in seed/plant characters was observed in this campus which might have been due also to phenotypic plasticity in response to environmental variations (Gutterman, 1982). A number of vectors are likely to be involved in cross pollination; among the more prominent in the campus are: *Xylocopa aruana*, the carpenter bee. In order to minimize, if not prevent cross pollination, the

inflorescence was enclosed in perforated white paper bag the day prior to the lowest flower opening. The bags were removed 3 days after anthesis and the pods were tagged.

The tagged pods were collected when completely dry on the mother plant. The pods were dried again spreading in the sun for a day. This ensured reasonable constancy in moisture content of the seeds in a batch, since water uptake pattern of air-dried seeds can be influenced by moisture status of the seed (Roberts, 1972; Bewley and Black, 1983). The pods were weighed and gently opened along the ventral suture. The seeds were picked by hand. Aborted seeds, if any, were discarded. The seed was surface - cleaned with cloth and weighed. Each seed was wrapped in butter paper, numbered serially as in pod and stored dry in the dark in plastic bottles at room temperature until use.

An interesting and special feature of winged bean seeds was the heterogeneity in water uptake pattern during imbibition and this character persisted from generation to generation. The seeds of winged bean were composed of a mixture of 3 types, namely, readily imbibing, delayed imbibing and hard seeds in all varieties mentioned above except in PT 51, where all seeds were readily imbibing.

Imbibition studies

Unless otherwise stated, the seeds were used within 3 weeks of collection. The volume of each weighed seed was determined by water displacement method. The seeds were then spread in serially numbered positions in Petri dish (9.0 cm diameter), aligning the seed position with the number marked on the filter paper lined on the bottom of the lower plate. Distilled water was added to cover about

3/4 of majority of the seeds, taking care that the hilum was in contact with water. The absorption of water was allowed to take place at room temperature, the covered Petri dishes being spread on top of work tables in the laboratory.

Weights of individual seeds were recorded every 2 h until 12 h and then at 18 h, 24 h and 48 h. The volumes of imbibing seeds were measured at 6, 12, 18, 24 and 48 h. Before noting weight and volume, the seeds were wiped dry with cloth. After the weighing, the seeds were returned to the original position in Petri dish.

During imbibition, the leachate coming out of the seeds contribute to the proliferation of fungi immediately around seeds (Mayne *et al.*, 1969; Halloin, 1986; Mohammed-Yasseen, 1991; Splittstoesser *et al.*, 1994). In order to check the proliferation of fungi, which possibly may influence the water uptake pattern, the water in the Petri dish was sucked away after every 12 h without disturbing the seed position and fresh water was added.

The seeds which did not imbibe in 24 h (no change in weight and volume) were scarified by slicing about 1 mm^2 area of testa on the dorsal side. These seeds, designated as scarified type or hard seeds, were transferred to separate dish, bearing the appropriate numbers. Distilled water was added and weight and volume were noted at specified intervals as described above.

Seeds which absorbed water from the commencement, that is, showing increase in weight at the end of 2 h, were designated as 'imbibing'. Seeds which started taking up water only after 2 h were designated as 'delayed' and seeds which did not imbibe after 24 h as 'hard' seeds.

At the end of 48 h of imbibition, the testa was peeled off from the seeds and used for dry weight determination. The testa dry weight was calculated on the basis of percentage of oven dried seed as well as per seed. The moisture content of seed was expressed as percentage of the fresh weight of seed. All experiments were carried out six times, permitting statistical evaluation of the significance of the data.

Dry weight determination

For dry weight determination, tissue was taken in a pre-weighed bottle and weighed in a chemical balance, followed by 1 hour heating in a hot air oven at 100°C, and then drying at 65-70°C. The dry weight was recorded every day, after cooling to room temperature in a desiccator. This was repeated until constant weights were attained.

Germination studies under field conditions

All the 10 varieties namely V 4, V 5, V 8, V 16, EC 28886, Selection 12, PT 15, PT 51*a*, PT 51*b* and PT 62 were used to study the pattern of germination under field conditions in order to compare the germination pattern under Petri dish conditions. Thirty seeds were sown in 5 rows of 6 seeds each in a 1 x 1.5 m bed. The plumular hook started emerging from the 5th day onwards. The germination count was done every morning by noting the plumular hook until the numbers stabilized. The germinability percentage was calculated and plotted against days after sowing. The germination capacity, or germinability, was calculated as the maximum percentage of seeds germinating. Under the field conditions germination was completed in 10 to 20 days after sowing.

II. Biochemical Studies

Variety selected

For biochemical studies the variety used was V16. This variety has proved to be an ideal material because of its high yield, seed colour (brown), larger sized seeds etc. Heterogeneity in water uptake pattern of these varieties was found to be maximum, i.e. the seed lot was composed of imbibing, delayed and hard seeds in the proportion 32.00, 20.00 and 48.00 percent respectively.

Sampling of Materials for Pod/Seed Development Studies

For studying the various biochemical aspects in the flower and its axillant leaf during different stages of development, the inflorescence was tagged on the day of anthesis.

The schedule for sample collection for enzyme assays, proteins, dry weight determination, total sugars, phenolics, total and free amino acids are given in table 1.

TABLE 1

The plant parts (tissues) were collected in the morning between 8 and 9 am and washed with distilled water. The washed tissues were blotted with filter paper and weight noted. The weight of pod wall, embryo and testa were separately noted wherever necessary before pooling.

The 60th day leaf and pod wall were not analysed, since the leaf abscised after 48th day of anthesis and the pod wall was completely dry on the mother plant.

TABLE 1. Types of various tissues used for analysis during pod/seed development in *P. tetragonolobus*

Age of pod/seed (days after anthesis)	Tissue analysed
0	Whole pod Source leaf
3	" "
6	" "
12	Pod wall Embryo Source leaf
18	Pod wall Embryo Testa Source leaf
24	" " " "
36	" " " "
48	" " " "
60	Embryo Testa

Sampling

After noting the whole weight, the collected tissues were pooled separately into leaf, whole pod, pod wall, whole seed, embryo and testa, depending upon the type of tissue analysed. Usually 3-4 pods and leaves were used at a time, but more numbers were used in cases where tissue weight is very low. From the pooled samples, tissues were weighed randomly for enzyme assay, protein estimations and other biochemical analyses.

Sampling for Germination Studies

Seventy five seeds one month after harvest were taken and weighed. They were then sterilized with 0.1% mercuric chloride for 2-3 minutes. After sterilizing, the seeds were washed several times with sterile distilled water. The seeds were

then spread over a large Petri dish lined with filter paper. Sterile distilled water was added to cover about 3/4 of majority of the seeds, taking care that the hilum was in contact with water and kept at room temperature. The water in the Petri dish was pipetted away after every 12 h without disturbing the seed position and fresh distilled water was added.

The dry resting seed is called 0 hour seed or control. After 2 h, the imbibing seeds were sorted out (by visual observation) to another Petri dish and marked as imbibing. The original Petri dish contained the non-imbibed seeds. After another 2 h, the seeds which started imbibing were designated as 'delayed' type and were retained in the Petri dish. The next day, i.e., after 24 h, the seeds which were still non-imbibed were marked as 'hard' types. They were then transferred to another Petri dish after scarification and distilled water added and kept for imbibition.

For all the three types of seeds controls (zero hour seeds) were common since the heterogeneity could be recorded only after imbibition. The seeds which failed to imbibe at the end of 24 h were transferred to separate Petri dishes after scarification (zero time) and samples were taken at 2, 4, 6, 12, 24, 48 and 72 h.

Sampling

Sampling of imbibing seeds for biochemical studies commenced at the end of 2 h of imbibition. Subsequent samples were collected at 4, 6, 12, 24, 48 and 72 h. The dry seeds were considered as 0 h sample or control.

At the end of 24 and 48 h of water uptake, the imbibed seeds were cut into cotyledons and embryonic axis and after 72 h, the embryonic axis was cut into plumule and radicle. After random sampling, the seed/seeding parts were washed, blotted and whole fresh weight noted. After noting the weight, the samples were

pooled separately into cotyledon, embryonic axis, plumule and radicle. The different types of tissue samples collected for dry weight determination, estimation of sugars, total and free amino acids, alanine and aspartate aminotransferases assay and estimation of proteins in imbibing, delayed and scarified seeds are listed in table 2.

TABLE 2. Seed/Seedling parts employed for biochemical analysis of imbibing/germinating seeds of *P. tetragonolobus*

Period of contact with water, h	Tissue analysed
0	Embryo
2	,,
4	,,
6	,,
12	,,
24	Cotyledon Embryonic axis.
48	,, ,,
72	Cotyledon Plumule Radicle.

1. Estimation of sugars, free amino acids and phenolics

The principle consisted of extracting these components with aqueous (80% v/v) ethanol and estimating them in the extract after removal of ethanol.

Preparation of the extract

The tissue (1.0 g) was ground with 80% (v/v) ethanol to give an approximately 5% (w/v) homogenate. The homogenate was refluxed for 4 hours on a steam bath. The suspension was centrifuged and the supernatant collected. The residue was again ground and refluxed for 2 hours. Following centrifugation the combined supernatant was dried at 60°C and the residue was taken up in 2

ml distilled water. This was centrifuged to get a clear extract.

Ion exchange chromatography

The separation of sugars and amino acids was done according to Kliewer (1964) with the aid of ion exchanger Dowex 50 x 8 (U.S. Mesh (NA^+) BDH Chemicals, England). The ion exchanger was washed first with distilled water and then treated with 0.5 N HCl for 1-2 hours. The resin was washed thoroughly with distilled water to remove the last traces of acid. A column of 15 x 1.0 cm was prepared by pouring the resin suspension into a burette. Care was taken to fill the column uniformly without trapping air bubbles.

Sample loading

Using a 2 ml pipette, the sample was spread uniformly over the top of the column without disturbing the resin packing. The sample was allowed to sink slowly into the resin and washed down with a small quantity of water.

Elution

Distilled water was added slowly with a 5 ml pipette to expel the sugar fraction, the amino acid staying back by ion exchange reaction. Flow rate was controlled with the stopcock at 3 drops/minute. About 40-50 ml of the (sugar fraction) was collected in this way. After this 50 ml of 2N ammonia solution was added to the column and the amino acids fraction was collected as above. The two eluates were separately oven dried at 60°C and dissolved separately in 2 ml of distilled water (for sugars) and 10% n-propanol (for amino acids). After centrifugal clarification, the supernatants were used for sugar and amino acid estimations, described below.

a. Estimation of total sugars

Total sugars were estimated according to Montgomery (1957). To 0.1 ml of sample, 0.1 ml of 80% (v/v) phenol was added followed by 5.0 ml of concentrated sulphuric acid, added in a quick discharge from a burette. After cooling the tubes, the optical density was measured at 540 nm using a Spectronic-21 spectrophotometer. Analytical grade glucose was used as standard.

b. Total free amino acids

The total amino acids were estimated with ninhydrin by the method of Lee and Takahashi (1966). To 0.2 ml of sample, 0.4 ml citrate buffer, 0.5 M (pH 5.5) was added followed by 2.4 ml of glycerol (98%). 1.0 ml of 1% ninhydrin (in 0.5 M citrate buffer pH 5.5) was added and the tubes well shake and kept for 12 minutes in a boiling water bath. After cooling in tap water, the optical densities were measured at 570 nm in a Spectronic 21 spectrophotometer.

c. Individual amino acids by thin layer chromatography

The concentrated amino acid fraction following ion exchange chromatography (described earlier) was used for separation on TLC. Amino acids were separated according to Brenner *et al.* (1969). Silica gel-G (E.Merck) was used as the adsorbent. The plates, after coating the gel, were air dried for 10-20 minutes and then placed vertically in a hot air oven and activated by heating to 110-120°C for 1 hour.

The solvent systems used were : n-Butanol-acetic acid-water in the ratio 4:1:1(v/v) for the first dimension and phenol-water in the ratio 75:25 (w/v) for second dimension. The spots were detected by spraying ninhydrin according to Krebs *et al.* (1969). The spots were eluted with 80% ethanol, centrifuged and the

optical densities measured at 540 nm in Spectronic-21 spectrophotometer.

d. Total phenolics

Total phenolics were estimated according to the method of Folin and Denis (1915), as modified by Swain and Hillis (1959) and Goldstein and Swain (1963) by the use of phosphotungstic-phosphomolybdic reagent (Folin-Denis reagent). To 2.0 ml of sample (supernatant after refluxing the tissue in 80% (v/v) ethanol as described earlier was evaporated to near dryness to remove ethanol and the sample appropriately diluted with distilled water), 2.0 ml of 1N sodium carbonate solution was added, followed by 2 ml. 0.25 N Folin-Denis reagent. The optical density was measured at 725 nm. Tannic acid was used as the standard.

2. Assay of alanine and aspartate aminotransferases (GPT and GOT) activities

Preparation of homogenate

Medium

Only pod wall of 12th day were used for preliminary standardisation experiment. Homogenates prepared in buffer without supplements tended to give brown homogenous due to the oxidation of phenolics. Kadam et al. (1982) reported that winged bean is rich in phenolics. It is well established that phenolics inhibit enzyme during homogenization unless precautionary measures are adopted.

Therefore, a basal medium consisting of 10 mM Tris -HCl, pH 7.0 and supplemented with different final concentrations of 2-mercaptoethanol (2.5, 5 and 7.5 mM) were employed for the preparation of 10% (w/v) homogenates of 12 day pods. Following grinding of the tissues in ice-cold medium in a chilled glass mortar with pestle for 10-15 min. in the presence of acid-washed sand as

abrasive, the homogenates were made to volume and stored in ice until used for enzyme activity determinations. The optimum 2-mercaptoethanol was found out and the same composition of the medium was maintained subsequently for the preparation of homogenates of other tissues as well.

Standardisation of optimum conditions for the assay of alanine and aspartate aminotransferases in winged bean

The progress of transaminases can be detected by the formation of any one or more of the components. Alanine and aspartate aminotransferase activities were measured by estimating the pyruvic acid. (For GOT oxaloacetic acid formed is quantitatively converted to pyruvic acid by treatment with aniline-citrate).

The initial velocity of an enzyme reaction depends upon a number of factors such as pH, substrate concentration, incubation temperature, enzyme concentration etc. These factors are to be standardised in order to get the optimum conditions for the measurement of maximum activities. The following experiments were conducted for standardization of the assay system.

Assay

For standardization experiments, a basal assay system patterned after Tonhazy *et al.* (1950) was followed. The pyruvic acid formed was estimated according to Friedeman and Haugen (1943) with slight modification. The assay system contained 0.4 ml of 200 mM Tris-HCl buffer (pH 7.5), 0.2 ml of 5% homogenate, 0.2ml of 200 mM alanine/aspartate (respectively for GPT and GOT) and 0.1 ml of 10 µg pyridoxal phosphate. The reaction was started with the addition of 0.1 ml of 100 mM α-ketoglutaric acid (pH 7.5) in the experimental tubes and kept for incubation at 37°C for 15 minutes. The tubes were shaken

gently every 5 minutes. After 15 minutes of incubation, the reaction was terminated by adding 0.1 ml 100% (w/v) cold trichloroacetic acid (TCA) in all the tubes followed by the addition of α-ketoglutaric acid to the control tubes. Aniline citrate, 0.1 ml, was added to all the tubes containing aspartate as substrate and the tubes incubated at 37°C for 10 minutes. After incubation, 1.0 ml, dinitrophenylhydrazine reagent was added to all the tubes and kept for incubation at 37°C for another 5 minutes. Then 2.0 ml water saturated toluene was added to all tubes followed by vigorous shaking. The tubes were centrifuged for 10 minutes at 2100 g. After centrifugation, 1.0 ml of toluene layer was pipetted out and transferred to 5 ml of alcoholic KOH. After 5 minutes, 1.0 ml of distilled water was added. The optical densities were measured in a Spectronic-21 spectrophotometer at 540 nm. Pyruvate was used as standard.

pH-activity relationship

For the study of optimum pH, 12th day old pod wall, seeds, axillant leaves, 60 day old dry seeds and harvested mature seeds after storage for 1 month, were used. Tris-HCl buffer in the pH range 7.0 to 8.6 with 0.2 unit intervals were employed for the assay. Other experimental conditions were as described in the basal assay system.

Enzyme concentration

Crude homogenates (5% w/v) prepared from seeds of 12 day old after anthesis were used for this study. The influence of enzyme concentration was determined at the previously determined pH optimum (7.6 for GPT and 8.0 for GOT). The concentration of the enzyme in the assay system, expressed as ml of the homogenates varied in the range 0.05 to 0.3 ml. The final volume of the assay system was adjusted to 1.0 ml by reducing the volume of buffer whereever

necessary. The other conditions of the assay were as described earlier.

Substrate concentration

(a) alanine/aspartate

L-Alanine and L-aspartate at final concentrations of 10, 20, 40, 60 and 80 mM in the assay system were used for determining the optimum concentration of substrates in GPT and GOT assays respectively. The pH of alanine and aspartate were adjusted to 7.6 and 8.0 respectively. The assay was carried out at the optimum pH and optimum enzyme concentration as determined in the previous experiments. The rest of the procedure was as described earlier.

(b) α -ketoglutaric acid

The effect of α-ketoglutaric acid, the second substrate (the other substrate being alanine for GPT and aspartate for GOT) on the velocity of reaction was tested using the conditions already optimized with respect to pH, enzyme concentration and amino acid concentration in the previous experiments. The final concentration of α-ketoglutaric acid in both GPT and GOT assay systems varied in the range 5mM to 20mM. The other details of the procedure were as described earlier.

Incubation time

For determining the optimum incubation time, the assay was carried out at the pre-determined optimal conditions of pH, enzyme concentration and substrate concentrations. The assay system was incubated at 37°C and the reaction was terminated by adding 0.1 ml of 100% cold TCA at different time intervals of 10,20,30,40 and 50 minutes. The other experimental conditions were as described earlier.

Optimum temperature

The effect of varying the incubation temperature on the enzyme activities was studied at the pre-determined optimal conditions with respect to pH, enzyme and substrate concentrations and incubation period. The assay systems were incubated at varying temperatures in a thermostatic serological water bath at 20,25,30,35,40 and 45°C. At the end of 30 minutes of incubation, the reaction was terminated and the activities determined at each temperature as described earlier.

Effect of exogenous pyridoxal phosphate

A characteristic of aminotransferases is the requirement of pyridoxal phosphate as a coenzyme (Wightman and Forest, 1978). To check the effect of added pyridoxal phosphate, the assay system was modified by incorporating pyridoxal phosphate at final concentrations of 5,10,15 and 20 μg per ml in the assay system for GOT and GPT. The assay system employed was one in which the various conditions were already optimized (as detailed earlier).

Standard procedure for enzyme assay

From the foregoing standardization experiments an optimized assay system with respect to the various condition and factors for the activity determination of alanine and aspartate aminotransferases was arrived at. This assay system was subsequently employed for the measurements of aminotransferases in the tissues of winged bean.

The standard assay system consisted of 0.4 ml of 200 mM Tris-HCl buffer (pH 7.6 for alanine aminotransferase and 8.0 for aspartate aminotransferase), enzyme in the proportionality range, 0.2 ml of 200 mM L-alanine / L-aspartate

and distilled water to 0.9 ml. The reaction was initiated by the addition 0.10 ml of 100 mM α-ketoglutaric acid (pH 7.5) in the experimental tubes and incubated at 30°C for 30 minutes. The tubes were shaken gently every 5 minutes. The reaction was terminated by adding 0.1 ml of 100% (w/v) cold TCA in all the tubes followed by adding 0.1ml of α-ketoglutaric acid in the control tubes. Aniline-citrate, 0.1ml, was added to all the tubes containing aspartate as substrate and the tubes incubated at 37°C for 10 minutes. One ml. each of DNPH reagent (0.1% w/v) was added to all tubes and the tubes kept for incubation at 37°C for 5 minutes. Two ml water-saturated toluene was added to all tubes followed by vigorous shaking. Following centrifugation for 10 minutes at 2100 g, 1.0 ml of toluene layer was pipetted out and transferred to 5 ml of alcoholic KOH and finally 1.0 ml of distilled water was added and mixed. The optical density was measured in a Spectronic-21 spectrophotometer at 540 nm. Pyruvate was used as standard.

3. Estimation of protein

Protein estimation was done according to the method of Lowry *et al.* (1951), as modified by Khanna *et al.* (1969). For estimation of protein 0.5 ml of homogenate already prepared for enzyme assay (10% w/v) was mixed with 0.5 ml 10% (w/v) TCA, so that the final concentration of TCA was 5%. It was kept in an ice bath for a minimum of 30 minutes to facilitate flocculation of precipitate and centrifuged at room temperature for 10 minutes. The supernatant was decanted off and the residue was washed thrice with 2% TCA.

The acid-washed residue was then washed with perchloric acid twice (15% w/v) to remove starches, if any, followed by washing with 80% acetone twice, and then with anhydrous acetone twice to remove pigments and phenolics. The

residue was dissolved in 5 ml of 0.1 N sodium hydroxide by heating the tubes in a bath of boiling water for 10 minutes. After centrifugal clarification, aliquots were drawn from the supernatant for colour development using the Folin-Ciocalteu reagent. Bovine serum albumin was used as standard.

Expression of data

Unless otherwise mentioned, all analytical values are represented on the basis of per g fresh/dry tissue (concentration) or the whole tissue basis (content).

Unit of enzyme activity

One unit of activity corresponds to the formation of one micro mole of keto acid formed per 30 minutes at 30°C under the standard experimental conditions.

Specific activity

The specific activity was defined as units of activity per mg protein.

Statistics

All analytical experiments were replicated 4-6 times and values reported are the mean S.D.

Equipments, Chemicals and Reagents

Equipments

Colorimetric measurements were carried out using Spectronic-21 spectrometer (USA). For pH determinations, Systronics (India) and Toshniwal (India) digital pH meters were used.

For centrifugations, Remi T8 and Remi R8C (India) table top centrifuges

were used. For weighing purposes, Metler (Switzerland) electronic balance and K-Roy (India) single pan balances were used. For freezing and cold storage purposes. Blue star (India) and Remi (India) freezers were used.

Thin layer Chromatography was carried out using Toshniwal (India) thin layer chromatography unit. Double distilled water was used for all experimental purposes and was distilled using the all-glass distillation unit of Scientronic India.

Hot air oven (Scientronic, India), Serological water bath (NSW, India) Heating mantles (Concord, India) were also used for the experiments. All glassware were of Borosil (India) make.

Chemicals and reagents

Tris (hydroxymethyl)-aminomethane was from Fluka (Switzerland) and Merck (India). Bovine serum albumin was purchased from Sigma (U.S.A). α - ketoglutaric acid and sodium pyruvate were from E-Merck (Germany). Aspartic acid and alanine as substrates (for aminotransferases assay) standard amino acid kit for thin layer chromatography and ion exchange resin (Dowex 50 x 8) were purchased from BDH (England).

Other chemicals and reagents used were of analytical grade wherever necessary and were purchased from BDH (India), Merck (India) Glaxo Laboratories (India), Sarabhai M.Chemicals (India), Qualigens Fine Chemicals (India), Sisco Research Laboratories (India), and HiMedia Laboratories (India).

CHAPTER 4

Seed Polymorphism and Pattern of water uptake and Varietal Variations in Winged bean (*Psophocarpus tetragonolobus* (L.) DC.)

Contents

Seed Polymorphism and Pattern of Water Uptake and Varietal Variations in Winged bean *(Psophocarpus tetragonolobus (L.) DC)*

Introduction

In an earlier publication on water uptake pattern by 45 varieties of winged bean (Nabeesa *et al.*, 1988), it was clear that winged bean seeds differing in weight and colour exhibited differences in imbibition pattern. These authors made use of seed lots comprising the total harvest spread over about 2 months. Some of the seed lots were procured from National Botanical Research Institute, Lucknow, where the environmental conditions differ greatly from those at Calicut. In further pursuance of the studies on the water absorption by winged bean, the author raised the plants under the condition of Calicut and examined the intervarietal and intravarietal variations. The author has also looked for any interpod differences in a given variety. By experimenting with a number of representative varieties of winged bean, it has been possible to draw conclusion about intrapod and interpod and varietal differences (if any) in the pattern of water uptake.

Since the seed coat plays a dominant role in imbibition (Bewley and Black, 1983; Mayer and Poljakoff-Mayber, 1989; Mohammed - Yasseen, *et al.*, 1994), the water absorption pattern is found to be related to the testa weight and colour. So, in the present study the testa weight and colour of individual seeds of various varieties were also included.

Materials and Methods

(These were as described in Material and Methods)

Results

The seed colour, seed (dry) weight, percentage distribution of seed types and number of seeds per pod are shown in table 3. The average weight of individual seeds, dry weight per seed, dry weight percentage, of seeds and testa weight percentage for the three seed types are shown in table 4. The percentage increase of weight and volume of seeds during imbibition and time of testa breaking are shown in table 5 and figures 1 to 10. The time of testa breaking was between 24 to 30 h in all the varieties except PT 51 *a* and 51 *b*, where it was 18 to 24 h period.

Table 3 -5

Figures 1-10

V 4

The seed colour of this variety was brown. This variety consisted of two types of seeds, imbibing (37.88%) and scarified (62.2%). The average weight of a single seed was 266.11 mg for imbibing type and 283.10 mg for scarified type. The percentage of dry testa was more for scarified type of seeds than the imbibing type (Table 4).

During imbibition the imbibing types showed linear increase in weight and volume (Figure 1). In this type, the volume quotient exceeded weight quotient until 48 h. In the case of scarified seeds also the volume was quotient was higher throughout. Maximum rate of imbibition occurred in both cases between 6-12 h intervals. In the case of imbibing type the percentage increase of both weight and volume was higher than scarified types at 48 h.

Table 3. Colour, weight, percentage distribution of dormancy and number of seeds in 10 varieties of winged bean

Variety	Seed colour	Weight of Seed (Dry), mg		Percentage distribution of seed types **			Average No. of seeds / pod
		Maximum*	Minimum*	Imbibing	Delayed	Scarified	
V4	Brown	357	115	37.88	-------	62.20	11.00
V5	Purple	418	50	35.30	41.20	23.52	8.50
V8	Brown	427	220	6.55	26.23	67.21	10.16
V16	Brown	425	168	32.00	20.00	48.00	12.50
EC 28886	Gray	553	178	66.20	------	33.80	10.33
Selection 12	Brown	330	159	57.90	28.90	13.08	10.00
PT 15	Brown	385	226	38.88	------	61.10	9.50
PT 51*a*	White with white hilum	352	98	100.00	------	------	10.50
PT 51*b*	White with dark hilum	361	175	100.00	------	------	11.00
PT 62	Dark Brown	510	250	12.40	------	87.70	9.50

* Out of total seeds from 6 pods, maximum and minimum seed weight

** Classified on the basis of imbibition behaviour.

Table 4. Dry weight distribution pattern of winged bean seeds

Variety	Weight / seed (air dried), mg			Dry weight / seed, mg			Percentage of dry testa			Dry weight % of seeds
	Imbibing	Delayed	Scarified	Imbibing	Delayed	Scarified	Imbibing	Delayed	Scarified	
V 4	266.11±24.00	-----	283.10±24.57	236.47±21.34 (29.67±0.40)	-----	251.58± 21.85 (31.07±2.88)	12.85±0.56	-----	13.44± 0.72	89.90±0.16
V 5	160.67 ±23.76	243.06±42.98	229.62±23.48	143.25±21.18 (17.42±0.02)	216.71±38.32 (26.35±4.65)	204.90±20.93 (24.83±20.16)	11.96±0.76	12.67±0.43	14.80±0.58	89.16±0.27
V 8	276.66±38.04	313.54±26.19	311.84±18.42	248.05±24.13 (28.61±3.93)	255.37±49.30 (32.42±2.67)	219.59±16.51 (32.25±0.44)	12.18±0.77	12.61±0.56	15.27±0.93	89.66±0.14
V 16	271.33±44.55	298.38±19.71	271.42±39.40	239.71±39.36 (31.61±3.93)	263.61±17.41 (34.76±2.29)	239.78±34.81 (31.61±4.58)	11.10±0.27	11.64±0.54	13.86±0.61	88.35±0.12
EC 28886	336.30±45.37	-----	277.28±38.25	302.13±40.76 (34.17±5.32)	-----	249.10±34.36 (28.18±5.49)	10.25±0.49	-----	14.22±1.54	89.84±0.16
Selection 12	246.90±21.92	260.37±19.68	237.16±26.50	224.20±19.90 (22.69±2.01)	236.44±17.87 (23.93±1.80)	215.36±24.10 (21.8±2.44)	12.32±1.53	13.43±1.89	15.83±2.95	90.81±0.40
PT 15	317.22±20.90	-----	315.75±33.16	285.58±18.87 (32.29±2.66)	-----	284.34±22.20 (31.45±3.27)	11.16±0.67	-----	12.40±1.95	90.03±0.40
PT 51 *a*	233.36±10.02	-----	-----	210.23±9.03 (23.18±0.97)	-----	-----	9.31±0.62	-----	-----	90.08±0.19
PT 51 *b*	286.36±15.62	-----	-----	257.11±14.03 (29.24±1.60)	-----	-----	9.32±0.44	-----	-----	89.85±0.18
PT 62	368.90±56.45	-----	367.98±73.99	332.15±50.82 (36.75±7.93)	-----	331.32±66.63 (36.65±7.36)	11.74±0.12	-----	13.59±1.61	90.04±0.04

(Values in parenthesis are moisture content, mg)

Table 5. Percentage increase in weight and volume of seeds during imbibition in 10 varieties of winged bean

Variety	Seed type	Period of contact with water, h.										Time of testa breaking, h.
		0	2	4	6	8	10	12	18	24	48	
V4	Imbibing	0 (0)	3.4	11	20 (24)	39	47	66 (70)	91 (98)	108 (117)	155 (160)	24-30
	Delayed	-	-	-	-	-	-	-	-	-	-	-
	Scarified	0 (0)	7	21	33 (38)	47	64	84 (91)	102 (112)	109 (119)	131 (138)	-
V5	Imbibing	0 (0)	14	49	73 (64)	85	109	115 (103)	135 (123)	140 (128)	151 (136)	-
	Delayed	0 (0)	-	-	9 (14)	16	22	37 (49)	71 (88)	87 (105)	117 (135)	-
	Scarified	0 (0)	13	27	50 (43)	61	81	88 (78)	102 (94)	110 (100)	123	-
V8	Imbibing	0 (0)	9	26	42 (44)	49	61	75 (79)	92 (101)	100 (113)	122 (126)	-
	Delayed	0 (0)	-	-	6 (8)	9	14	21 (27)	56 (64)	85 (84)	109 (111)	-
	Scarified	0 (0)	5	12	27 (32)	33	53	66 (73)	96 (99)	97 (104)	115 (118)	-
V16	Imbibing	0 (0)	3	5	12 (11)	26	45	61 (52)	95 (76)	104 (77)	111 (96)	-
	Delayed	0 (0)	-	-	-	14	18	27 (25)	53 (34)	77 (55)	123 (92)	-
	Scarified	0 (0)	5	19	35 (22)	48	61	79 (70)	99 (80)	106 (86)	120 (100)	-
EC 28886	Imbibing	0 (0)	10	18	28 (37)	39	51	72 (83)	84 (101)	98 (119)	117 (141)	-
	Delayed	0 (0)	-	-	-	-	-	-	-	-	-	-
	Scarified	0 (0)	8	14	21 (29)	35	52	65 (77)	87 (100)	116 (118)	122 (129)	-
Selection -12	Imbibing	0 (0)	15	35	51 (52)	76	87	97 (98)	110 (110)	119 (121)	137 (139)	-
	Delayed	0 (0)	0	14	24 (27)	25	35	53 (55)	84 (83)	101 (102)	125 (128)	-
	Scarified	0 (0)	25	45	73 (64)	83	91	96 (87)	106 (103)	114 (109)	148 (144)	-
PT 15	Imbibing	0 (0)	3	10	23 (26)	37	55	72 (75)	92 (97)	117 (115)	127 (131)	-
	Delayed	0 (0)	-	-	-	-	-	-	-	-	-	-
	Scarified	0 (0)	3	13	29 (33)	48	66	85 (88)	103 (108)	119 (123)	134 (139)	-
PT 51 *a*	Imbibing	0 (0)	22	48	71 (74)	85	95	104 (107)	117 (118)	123 (127)	160 (163)	18-24
	Delayed	0 (0)	-	-	-	-	-	-	-	-	-	
	Scarified	0 (0)	-	-	-	-	-	-	-	-	-	
PT 51 *b*	Imbibing	0 (0)	20	38	53 (61)	77	87	98 (100)	113 (115)	122 (123)	172 (180)	18-24
	Delayed	0 (0)	-	-	-	-	-	-	-	-	-	
	Scarified	0 (0)	-	-	-	-	-	-	-	-	-	
PT 62	Imbibing	0 (0)	2	11	24 (25)	42	62	82 (79)	99 (101)	117 (119)	140 (138)	24-30
	Delayed	0 (0)	-	-	-	-	-	-	-	-	-	
	Scarified	0 (0)	3	16	31 (33)	46	63	80 (83)	95 (100)	112 (118)	131 (136)	

(Values in parenthesis are percentage increase of volume)

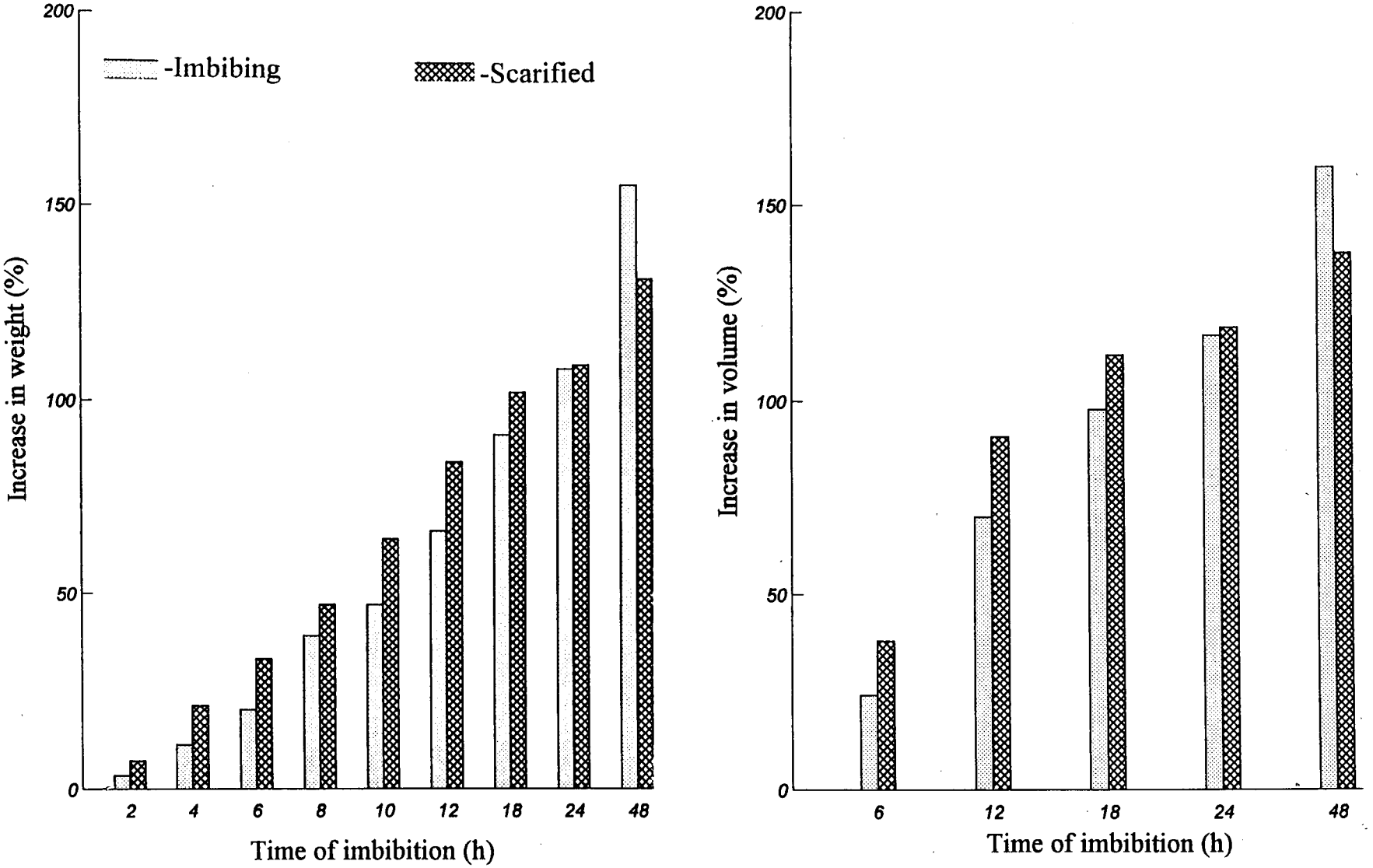


Figure 1. Percentage increase in weight and volume in V-4 variety.

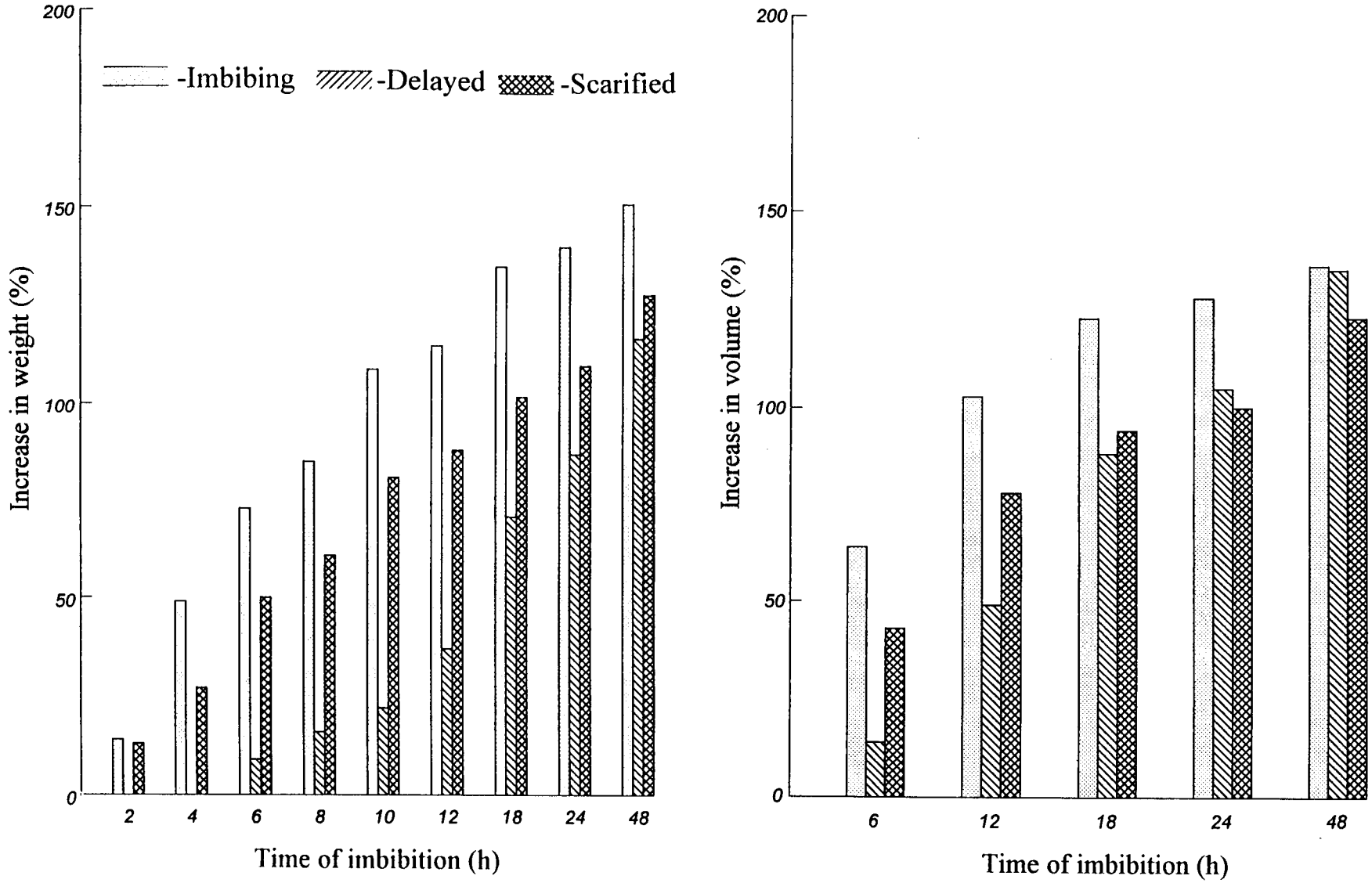


Figure 2. Percentage increase in weight and volume in V-5 variety.

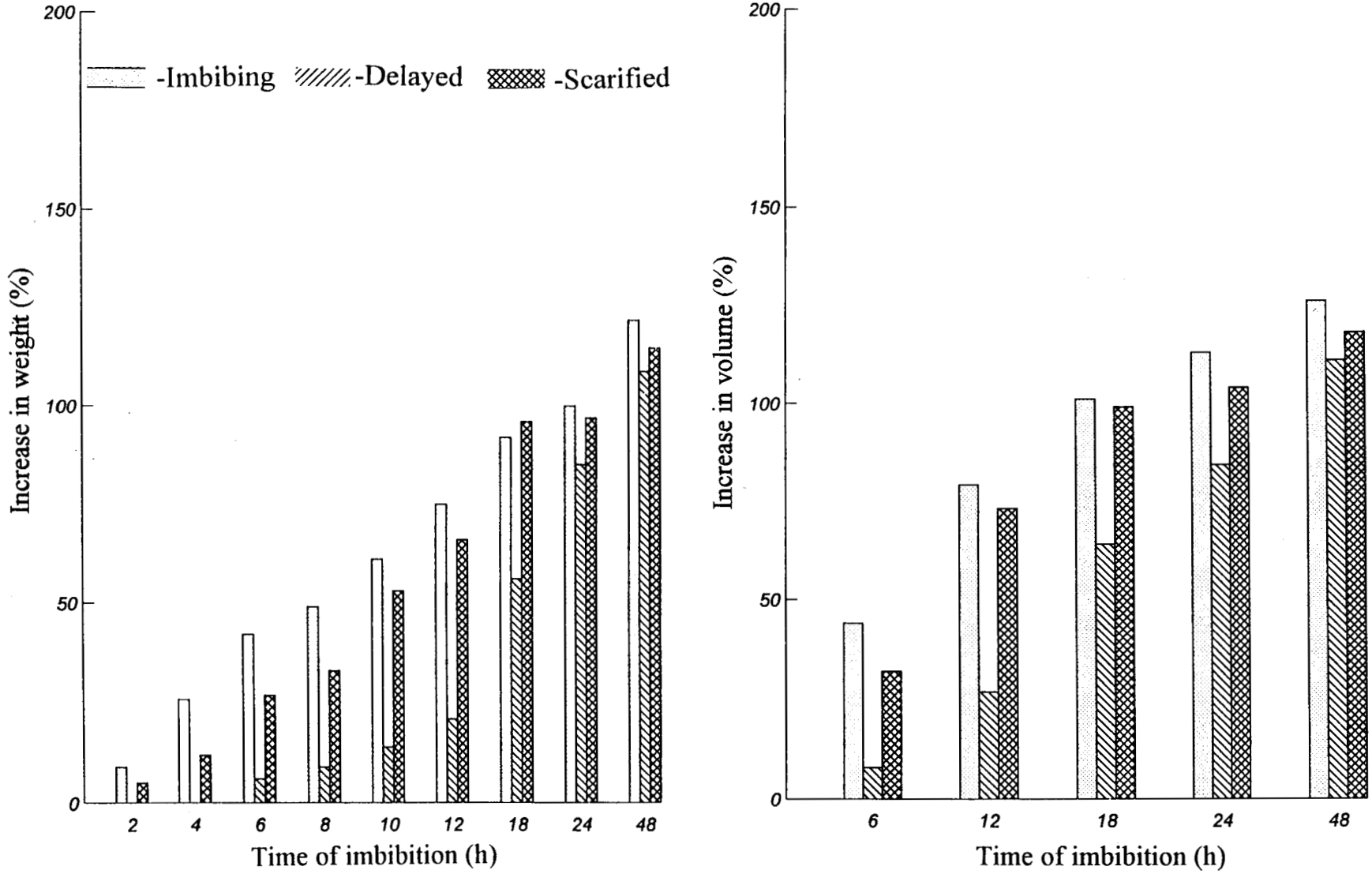


Figure 3. Percentage increase in weight and volume in V-8 variety.

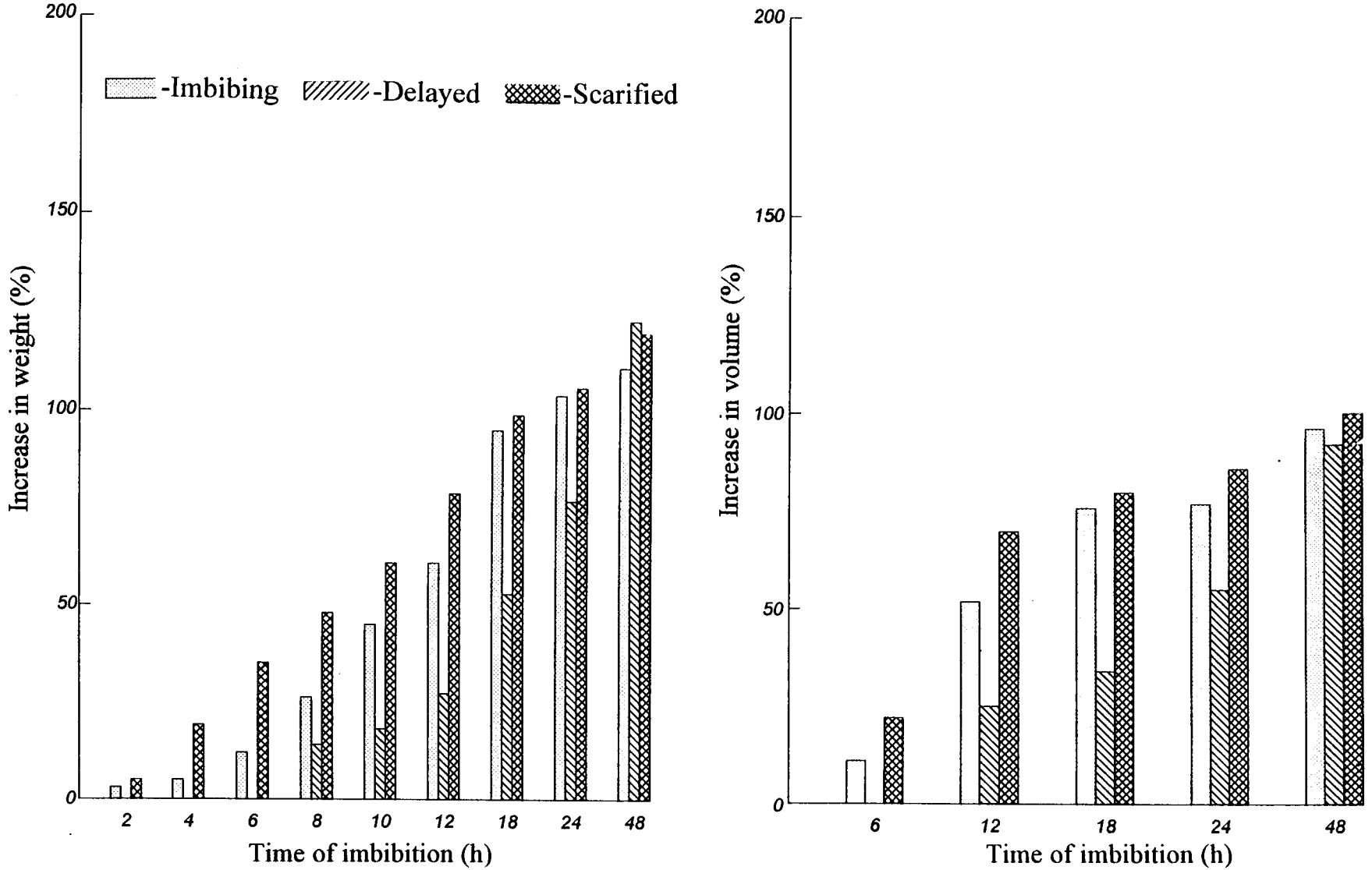


Figure 4. Percentage increase in weight and volume in V-16 variety.

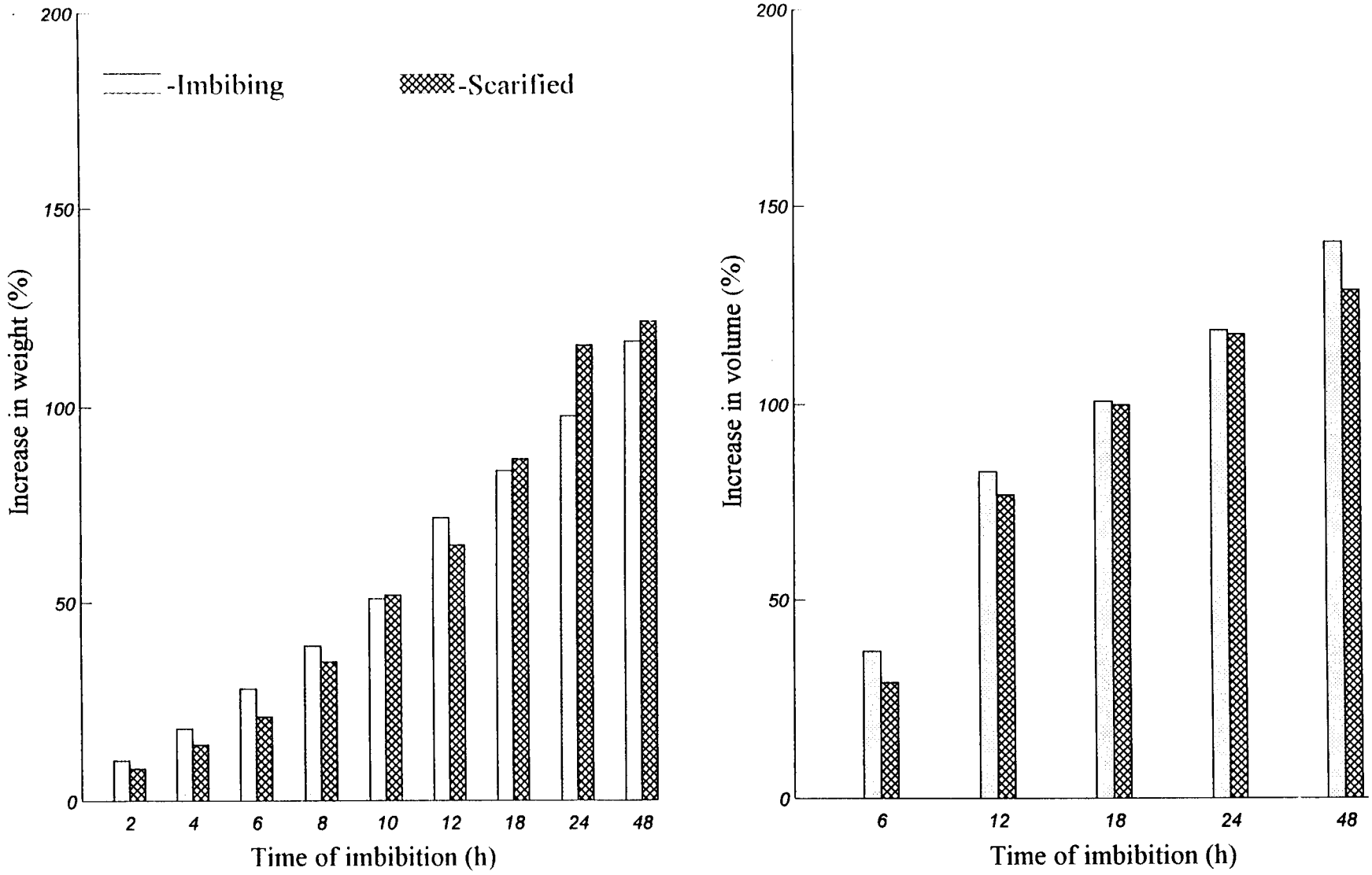


Figure 5. Percentage increase in weight and volume in EC-28886 variety.

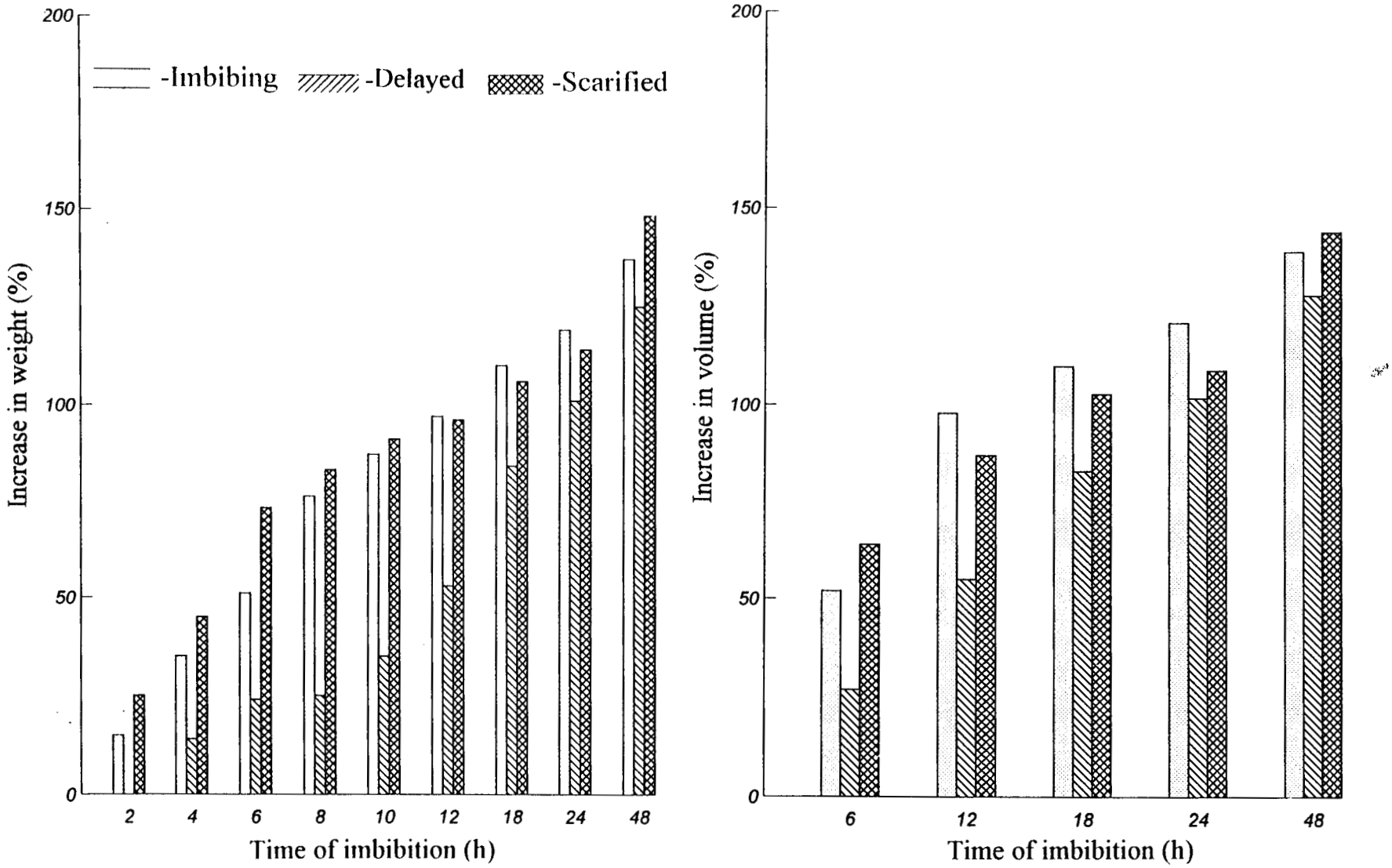


Figure 6. Percentage increase in weight and volume in Selection-12 variety.

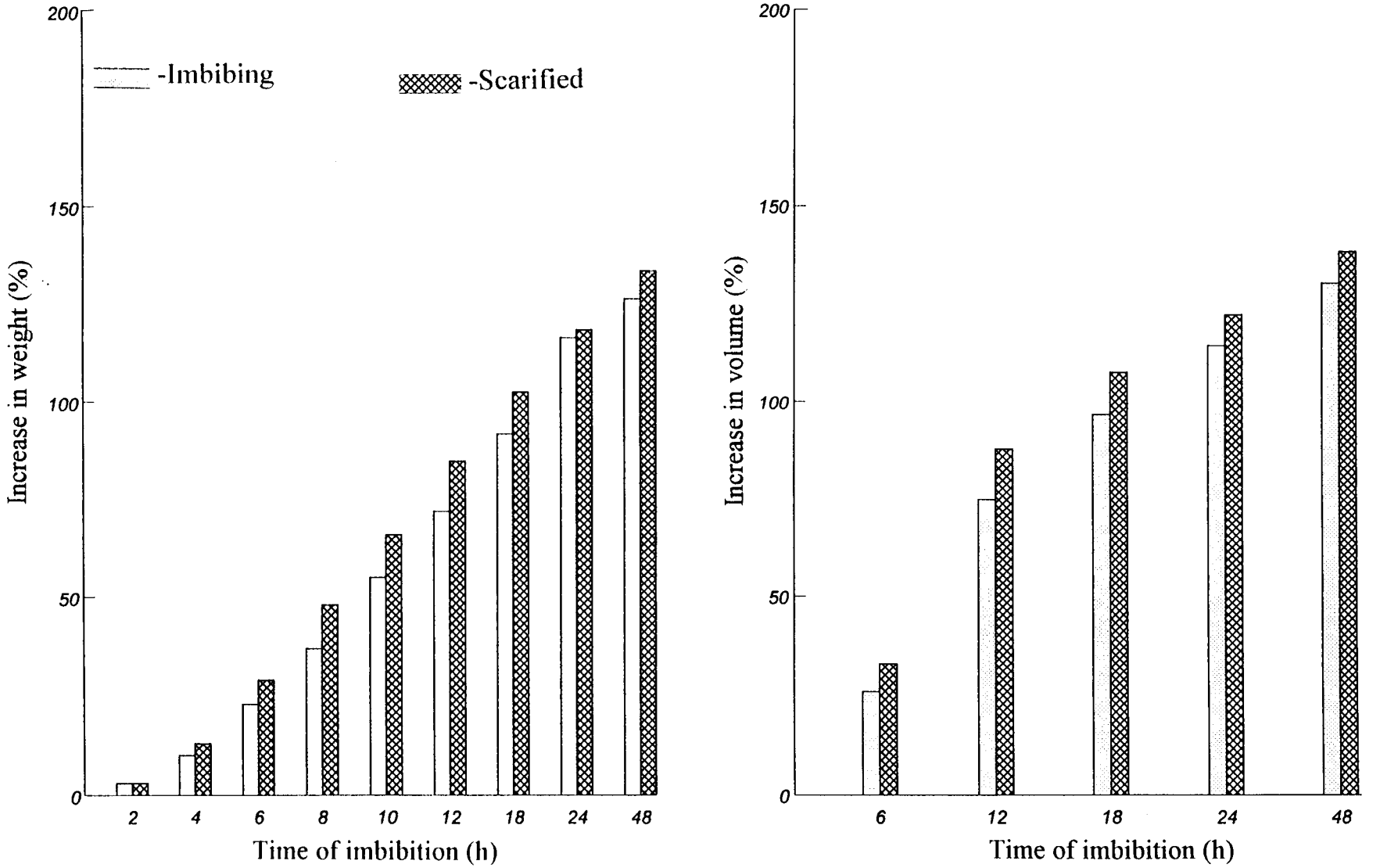


Figure 7. Percentage increase in weight and volume in PT-15 variety.

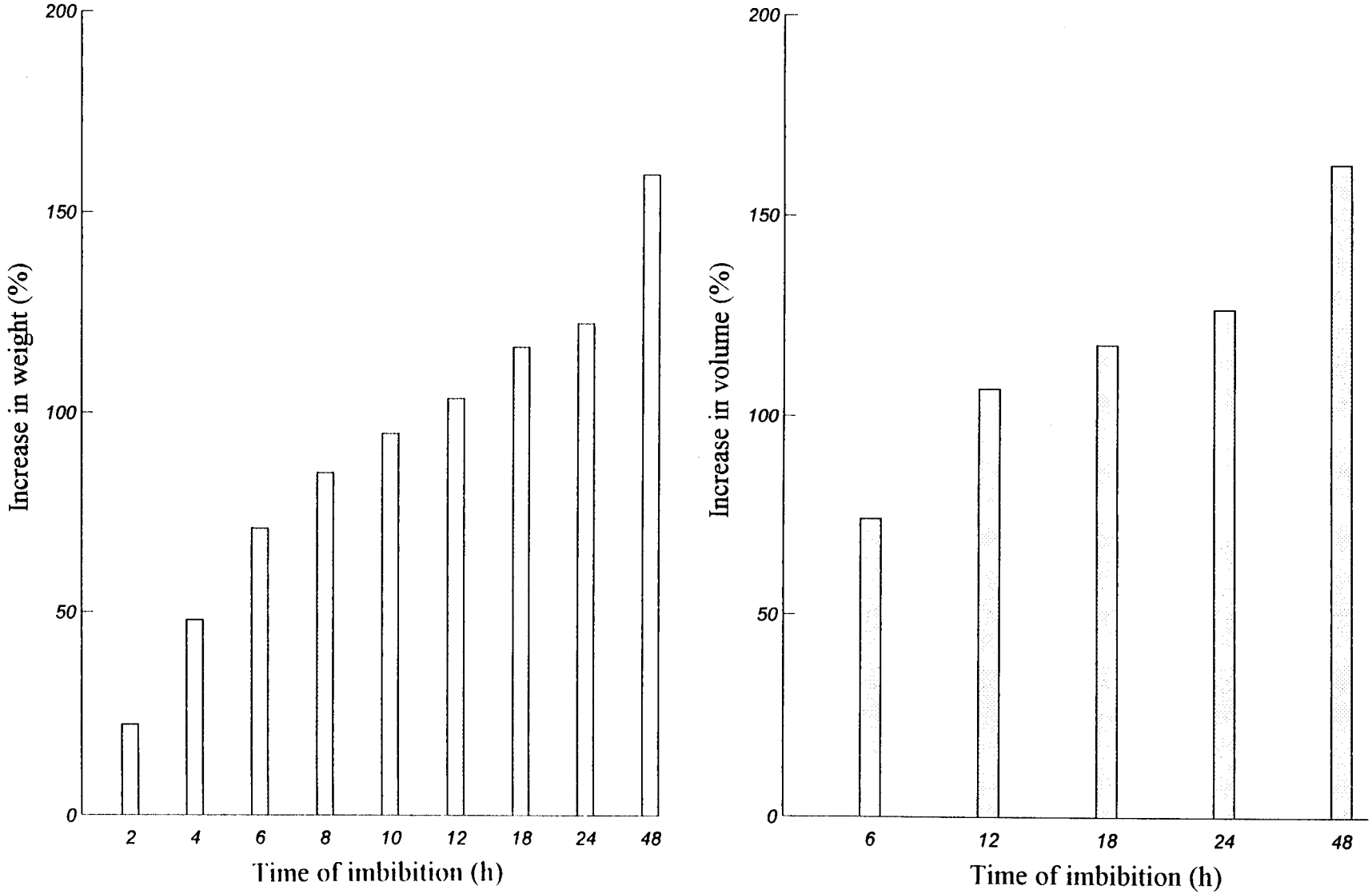


Figure 8. Percentage increase in weight and volume in PT-51*a* variety (Imbibing).

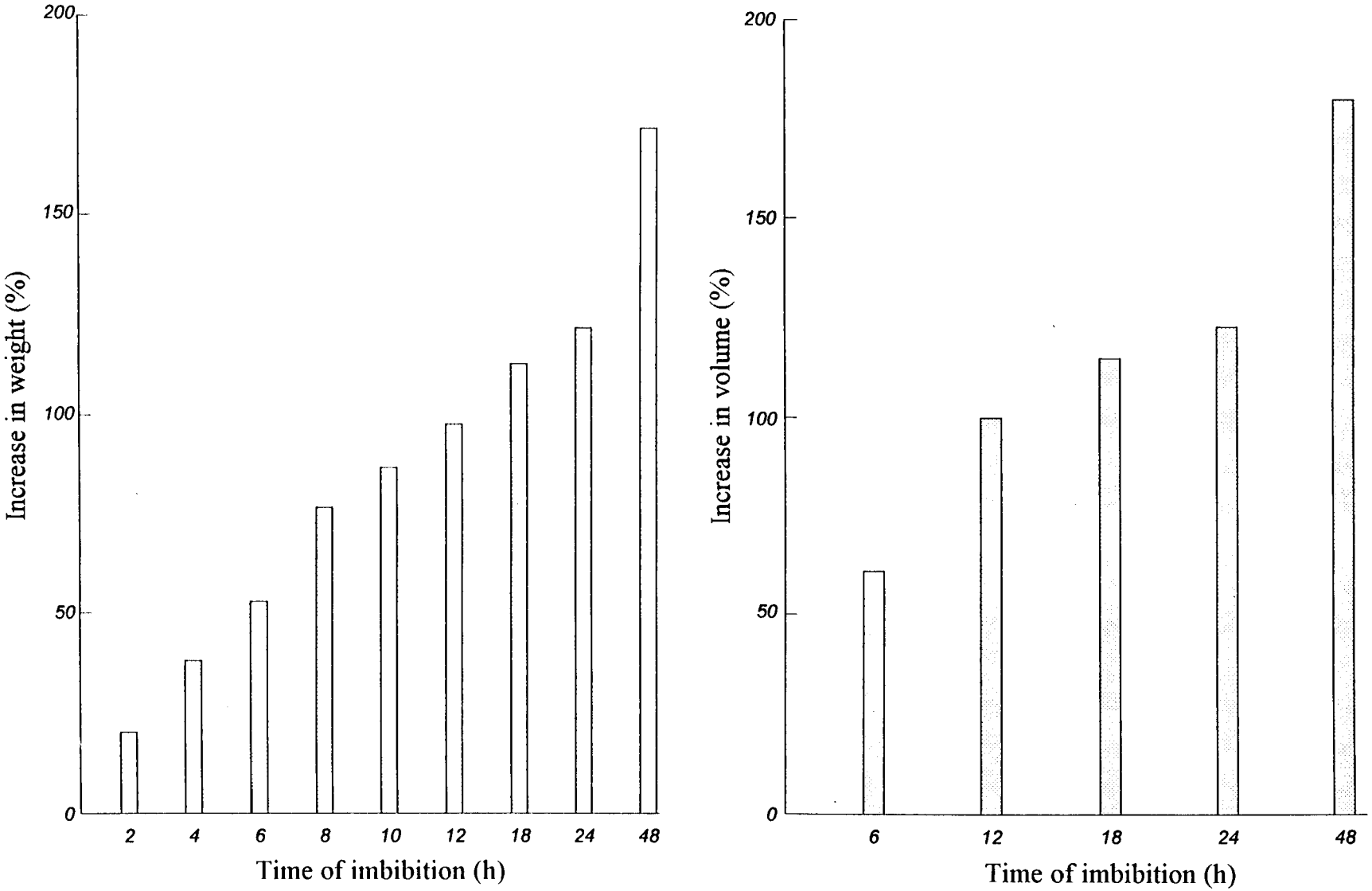


Figure 9. Percentage increase in weight and volume in PT-51*b* variety (Imbibing).

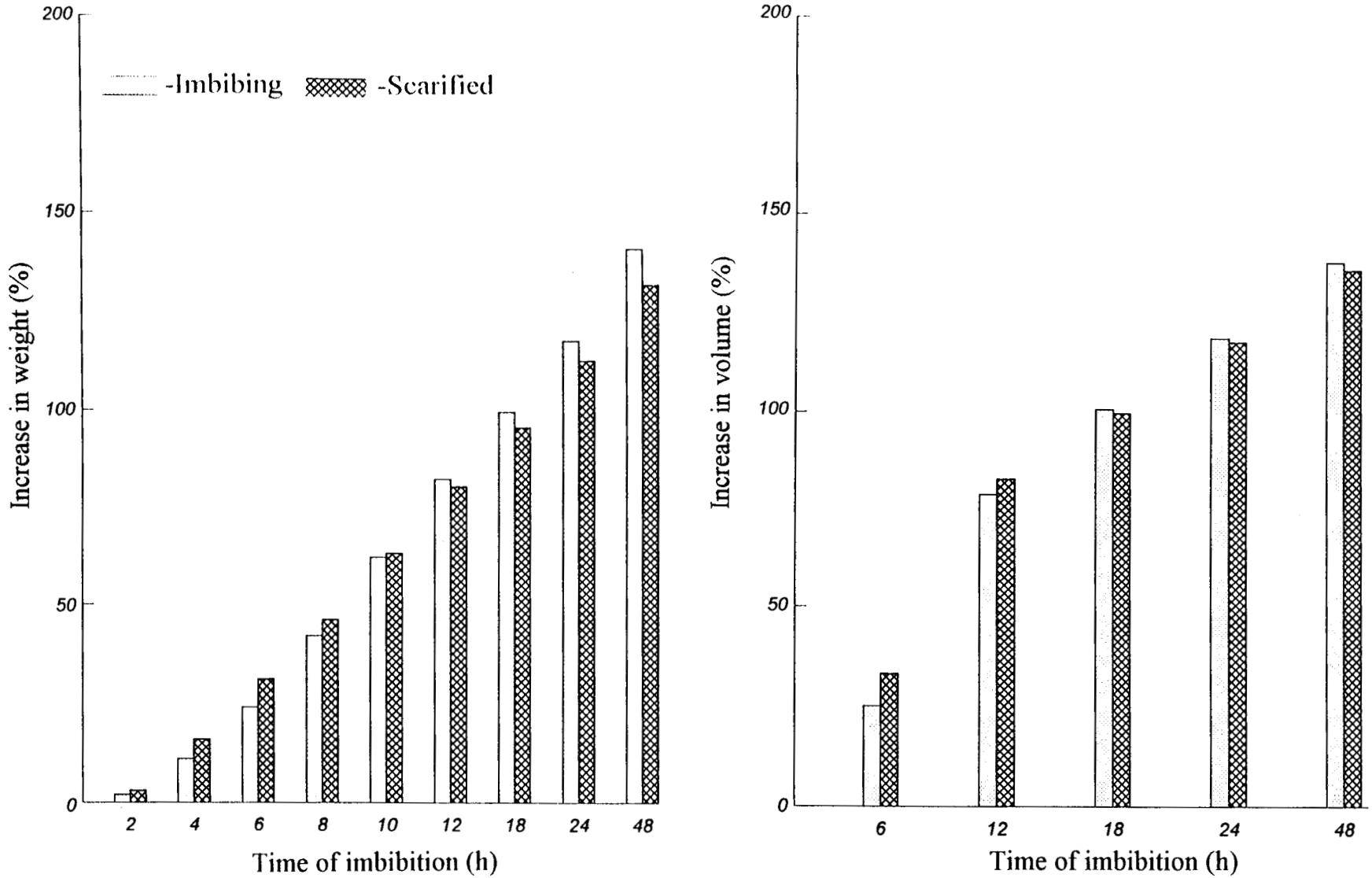


Figure 10. Percentage increase in weight and volume in PT-62 variety.

V 5

The seed colour of this variety was purple. This variety consisted of all the 3 types of seeds; imbibing (35.30%), delayed (41.20%) and scarified (23.50%). The average weight of seeds was 160.67 mg for imbibing, 243.06 mg for delayed and 229.62 mg for scarified type of seeds (Table 4). The percentage of dry testa was minimum for imbibing type and maximum for scarified type of seeds and the delayed type showed an intermediate value (Table 4).

The imbibing type showed almost linear increase in weight and volume upto 12 h (Figure 2). The maximum rate of water uptake occurred between 0-6 h interval. The weight quotient exceeded volume quotient upto 48 h of imbibition.

In the case of delayed type the volume quotient was higher than weight quotient throughout (Figure 2). Here, imbibition started only after 4 h. Then the increase in both weight and volume quotients was sharp and steady up to 18 h. The percentage of weight and volume of delayed seeds at 18 h was only about half than the imbibing types at 18 h. Here maximum rate of imbibition occurred between 6-12 h interval (Table 5).

Like the imbibing seeds, scarified seeds also showed higher weight quotient throughout. Here also up to 12 h the increase of both quotients were sharp and steady. Between 12 h and 48 h, the rate decreased. Here the maximum rate of water absorption was between 0-6 h interval.

V 8

Seed of this variety was brown in colour. This variety consisted of

imbibing (6.55%), delayed (26.23%) and scarified (67.21%). The average weight of seeds was 276.66 mg for imbibing, 313.54 mg for delayed and 311.84 mg for scarified types. The percentage of dry testa is almost same for imbibing and delayed seeds, while the scarified seeds showed higher testa weight than the other 2 types (Table 4).

The imbibing type showed linear increase in weight and volume quotient. The volume quotient exceeded weight quotient throughout (Figure 3. and Table 4). The maximum rate of weight and volume quotient was between 0-6 h interval.

The delayed type started imbibing only after 4 h. The highest rate of absorption was between 12 and 18 h. The increase in weight and volume quotient in this type was almost steady throughout and the volume quotient exceeded weight quotient throughout (Figure 3).

The scarified seeds showed the highest rate of absorption between 6 and 12 h. Upto 18 h the uptake of water was steady and linear. Between 18-24 h, very little uptake of water took place. Then upto 48 h the uptake of water increase sharply. Like the imbibing and delayed seeds, here also the volume quotient exceeded weight quotient throughout (Figure 3 and Table 5).

V 16

The seed colour of this variety was brown. This variety consisted of all the 3 types of seeds; imbibing (32%) delayed (20%) and scarified (48%). The average weight of seeds of imbibing and scarified types are equal but for delayed, the weight was more (Table 4). The percentage of dry testa is maximum in scarified types and the other types exhibited almost the same testa percentage

weight.

Water uptake upto 6 h was very slow in the imbibing seeds. From 6-18 h there was a sharp increase in percentage of weight and volume (Figure 4). The weight quotient exceeded volume quotient upto 48 h. In this type, the highest rate of water uptake was between 6-12 h (Table 5).

The delayed seeds started imbibing only after 6 h (Figure 4, Table 5). From 6-12 h, the maximum rate of imbibition occurred. From 12 h to 48 h, the increase in weight quotient was sharp and steady. The weight quotient exceeded volume quotient throughout.

The scarified seeds showed maximum rate of water uptake during the 6 to 12 h interval (Figure 4). The weight quotient exceeded volume quotient throughout.

EC 28886

The seeds were gray in colour. This variety was made up of imbibing (66.2%) and scarified (33.8%) types. The average seed weight was more for imbibing than for scarified types (Table 4). The percentage of dry testa was 10.25 for imbibing and 14.22 for scarified type (Table 4).

The imbibing type showed steady uptake of water (Figure 5). The rate of increase of both weight and volume quotient were maximum during the first 12 h interval. The volume quotient exceeded weight quotient throughout.

Water uptake was linear in scarified seeds. Here also volume quotient exceeded weight quotient throughout. The maximum rate of imbibition took place between 6-12 h interval (Figure 5).

Selection 12

The seed colour of this variety was brown and consisted of all the 3 types of seeds; imbibing (57.9%), delayed (28.9%) and scarified (13.08%). The average weight of seeds was 246.90 mg for imbibing, 260.37 for delayed and 237.16 for scarified seeds. The percentage dry weight of testa was minimum (12.32%) for imbibing, intermediate (13.43%) for delayed and maximum (15.83%) for scarified type of seeds (Table 4).

In the case of imbibing types, imbibition upto 12 h was sharp and steady. Here the weight and volume quotients were almost equal throughout the stages of imbibition (Table 5, Figure 6).

The delayed seeds started imbibing after 2 h and was steady upto 6 h. Sixth to 8 h was a lag period. Both weight and volume quotients were almost equal in all stages upto 48 h. From 12 to 24 h the increase in weight and volume quotient was sharp and linear (Fig. 6).

In the case of scarified seeds, the imbibition rate was higher during 0 to 6 h period. From 6-24 h, the rate was slow. The weight quotient exceeded volume quotient throughout.

PT 15

The seed colour of this variety was brown and was made up of only imbibing (39.61) and scarified (60.30%) seeds (Table 3). The percentage of dry testa was 11.16% for imbibing and 12.40% for scarified seeds(Table 4).

In the case of imbibing seeds, imbibition upto 6 h was slow. From 6 h to 12 h, a sharp increase in weight and volume quotient occurred. The volume quotient exceeded weight quotient throughout (Figure 7).

In the case of scarified seeds also, the increase in weight quotient was slow upto 6 h. From 6 to 12 h a sharp and steady increase occurred. In both imbibing and scarified seeds, maximum rate of water absorption happened between 6-12 h interval. In this type the volume quotient exceeded weight quotient throughout.

PT 51*a*

The seeds of this variety were white with white hilum and were 100% imbibers (Table 3). The average weight of seeds was 233.36 mg. The percentage dry weight of testa was 9.31% (Table 4).

The rate of increase in weight and volume was maximum during the first 6 h (Table. 5, Figure 8). There was a sharp increase from 24 to 48 h. The volume and weight quotients were nearly equal over the entire period.

PT 51*b*

The seeds of this variety were white coloured with black hilum. The seeds were made up of 100% imbibers (Table 3). The average weight of seeds was 286 .36mg. The percentage dry weight of testa was 9.32 (Table 4).

The pattern of weight and volume increase was nearly the same as PT 51*a* (Table 5). The rate of increase was the highest during the first 8 h interval. There was marked uptake of water also during the 24 to 48 h interval.

PT 62

The seed colour of this variety was brown. This variety was made up of imbibing (12.40%) and scarified (87.70%) seeds (Table. 3). The percentage of dry testa was minimum (11.74%) for imbibing and maximum (13.59%) for

scarified seeds (Table. 4).

Imbibition was slow up to 2 h and then a sharp and steady increase occurred up to 12 h. Both weight and volume quotients were almost equal upto 48 h of imbibition (Table 5).

In the case of scarified seeds also, imbibition was slow upto 2 h, and then a sharp and steady increase occurred upto 12 h. The volume quotient was higher throughout.

Discussion

The majority of winged bean varieties (8 out of 10) used in the present study of water uptake pattern, contained normally imbibing seeds, in addition to varying proportions of seeds with different degrees of impermeability to water. Three types of seeds were recognized *ie.* (i) 'imbibing' which showed water uptake soon after coming in contact with water (ii) 'delayed', which started imbibition after a lag period of 2-6 hours and (iii) 'hard' seeds which proved to be impermeable at the end of 24 hours but commenced imbibition on scarification. PT 51*a* and PT 51*b* were 100% imbibers; V 4, EC 28886, PT 15 and PT 62 consisted of imbibing and scarified seeds, whereas, in V 5, V 8, V 16 and selection 12, all the 3 types occurred (Table 5).

Seed coat-imposed dormancy, wide spread in the plant kingdom (Harrington, 1972; Ballard, 1973; Rolston, 1978; Simon, 1984) is particularly prominent in the leguminous genera (Tran and Cavanagh, 1984). Nabeesa *et al.* (1988) suggested that hard or scarified seeds could be considered as manifesting 'deep' dormancy and the delayed seeds as manifesting different degrees of shallow dormancy. According to these authors the occurrence of seeds with

different levels of hardness along with normal seeds appears to be an ecological adaptation for spreading the emergence over several days, so that some plants emerging in hospitable environments survive. So, in the present study four varieties, namely, V 5, V 8, V 16 and selection 12 are found to be more adapted for survival.

According to Barton (1965 *b*), Heydecker (1972), Bewley and Black (1983), Simon (1984), Kamaladevi (1985) and Nabeesa *et al.* (1988), imbibition and germination in winged bean have been related to seed size and weight. In *Lespedeza stipulacea*, 48-72% of small seeds were hard as compared with 1-3% in large seeds and 26-50% in those of intermediate size (Middleton, 1983). By analogy with this, the heaviest seeds may be expected to be imbibers, the lightest to be of the scarified type and seeds with intermediate size and weight to be of the delayed type. Contradictory to this statement, in the present study no such relationship existed except in EC 28886, where the average seed weight of imbibing type was 336.30 mg and that of scarified type 277.28 mg (Table 4). In this variety the percentage distribution of imbibing type was 66.20 and scarified was 33.80% (Table 3). In the four varieties which showed all the 3 types of seeds, the delayed type had higher seed weight than imbibing and scarified types, whereas, in V 16, PT 15 and PT 62, the imbibing and scarified seed weight was almost the same (Table 4).

The initial weight of seeds varied among varieties (Table 3) but there was no consistent pattern among varieties between weight and permeability. The heaviest seeds occurred in EC 28886 (553 mg) and the lightest in V 5 (50 mg).

A number of studies has proved the linkage between colour, thickness

and weight of testa with permeability characteristics. Kannenberg and Allard (1964) reported that coloured snap beans had greater seed coat dry weight and thickness and less permeability to water than the white seeds. The studies by Moore (1972) and Werker *et al.* (1979) in legume seeds, and by Egley *et al.* (1983) with malvaceous seed support the association of seed coat colour with development of impermeability. During dehydration of seeds, phenolic compounds in the seed coats are oxidized in the presence of catechol oxidase and this might render the seed coats impermeable to water (Marbach and Mayer, 1974, 1975). According to Wyatt (1977), lignin composed of about 15% of the total weight of coloured lima bean seeds but only 1% in white seeds. Soybeans with black seed coats imbibe water more slowly than unpigmented soybeans (Tully *et al.*, 1981). In a study of inheritance of water permeability in soybean seed, Shahi and Pandey (1982) found a linkage between seed coat colour and seed impermeability. Yellow seeds were more permeable than black seeds.

In winged bean seeds also correlation can be drawn between testa colour and impermeability. As testa colour intensity increases, impermeability is imposed. Among the winged bean varieties studied, two (PT51*a* and PT1*b*) were wholly imbibers, where the testa was white in colour (Table 3). In two varieties (EC 28886 and selection 12), the seed coat colour was gray and light brown respectively and these varieties constituted maximum imbibers comparatively. Five varieties (V 4, V 8, V 16, PT 15 and PT 62) were brown in colour and the percentage of scarified seeds was higher in these varieties. The highest percent of scarified seeds was in PT62 where the testa colour was dark brown (Table 3). In one variety (V 5) the coat colour was purple, where most

of the seed lot were made up of delayed and scarified types.

Drying seeds in the absence of oxygened in seed coats without pigmentation and high permeability to water, while drying in the air or oxygen resulted in coloured seed coats and less permeability (Mayer and Poljakoff-Mayber, 1989). Recently it was suggested that the resistance of coloured seed coats to water uptake was as result of impermeably thick seed coats created by the oxidation of phenolic compounds by polyphenol oxidase or peroxidase and majority of peroxidase activity in soybean seed was localized in the seed coat, suggesting that it may play a role in the hardening of the seed coat (Gillikin and Graham, 1991).

In the present study, correlation can be drawn between the testa thickness and permeability characteristics as suggested by Kannenberg and Allard (1964), Marbach and Mayer (1974; 1975) and Wyatt (1977). The seeds with more testa weight and thickness may be expected to be 'hard' and with less testa weight and thickness to be imbibers and unless and otherwise stated the testa weight of imbibers may be low and hard seeds to be high and that of the delayed type to be intermediate. In variety PT 51*a* and 51*b* which consisted of only imbibers, the percentage of dry testa, was very low when compared with other varieties (Table 4). The time of testa breaking in these varieties was between 18-24 h which reflects the comparatively thin nature of the testa. In all other varieties the testa breaking happened between 24-30 h (Table 5).

The percentage of dry testa of imbibing type was minimum and scarified type was maximum and delayed type (in V 5, V 8, V6 and Selection 12) showed intermediate value. Also, from the studies it can be seen that the testa weight of imbibing, delayed and scarified seeds was different in each variety and this

may be due to the qualitative and quantitative differences in the phenolic content of the polymorphic seeds as reported by Khan and Unger (1986) in *Artiplex* seeds.

Initial water uptake by dry seeds proceeds at a rapid rate so that in legumes very little further absorption occurs after 12 h at room temperature (Swanson *et al.*, 1985). In every variety of winged bean studied in all the 3 types, water absorption did not cease in 12 h (Table 5). According to Waggoner and Parlange (1976), winged bean being large seeded, the water front may take a longer time to advance to the innermost regions of the seeds, so that the exterior region may pass the lag phase of imbibition, while the innermost tissues commenced imbibition. In imbibing and hard types of all the 10 varieties tested, water uptake upto 2 h was very slow, after that upto 12 h the rate was rapid (Table 5). Deshpande and Cheryan (1986) reported that in terms of the relative surface area, the hilum and micropyle were the most important structures influencing the initial water uptake of beans. Apparently water uptake was influenced by the seed coat only after 30-60 min. of soaking. When the primary path of water entry was established, water absorption seemed to proceed rapidly. In *Onopordium nervosum* seeds, Perez-Garcia and Pita (1989) found that coat resistance decreased significantly ($P < 0.01$) as the imbibition period increased, and the decrease in resistance was greater in seeds incubated at 25°C than in those at 15°C and they concluded that the hydrolytic activity increases at higher temperature as proposed also by Halmer *et al.* (1976).

The delayed types (V 5, V 8, V 16 and Selection 12) showed low level of hydration at the end of 12 h when compared with imbibing and scarified types and same was the case at 18 and 24 h and an anticipated equalization of hydration

at the end of 48 h was not always fulfilled (Table 5).

The time course of water uptake during seed germination is conventionally expressed as a smooth linear curve. The uptake of water by dry seeds is characterized by an initial phase of rapid uptake with saturation kinetics, followed by a lag phase (transition phase) with very gradual to negligible uptake, and this in turn is followed by a third phase of rapid and exponentially increasing uptake (Koller and Hadas, 1982). In PT 51*a* and 51*b* (both 100% imbibers) and the imbibing seeds of V 5, water uptake did not proceed smoothly (Table 5, Figures 8 and 9); the water uptake was sharp and abruptly accelerated upto 12 h interval in these varieties. Due to this process water damage (Moore, 1972) may occur to the cotyledons (Dickson *et al.*, 1973) and particularly to the axis tissue (Dunn *et al.*, 1980). In the case of scarified seeds water absorption may be expected to be hastened but without change in the final level of hydration. In V 16, EC 28886, PT 15 and selection 12, the percentage increase in weight quotient was higher in the scarified seeds than the imbibing seeds at all intervals upto 48 h (Table 5). On the other hand, in some varieties (V 4, V 5, V 8 and PT 62), the percentage increase of weight quotient in the scarified seeds at all the intervals upto 48 h was lower than the imbibing seeds. The highest weight quotient attained at the end of 48 h was in PT 51*b* followed by PT 51*a* and lowest in the imbibing types of V 16 (Table 5). The difference in hydration level among seed types of intra and intervarieties may be due to the size, shape, structure and composition of the testa as well as the embryo.

The weight and volume increase during imbibition may have markedly different dynamics. Imbibing seeds do not swell simultaneously and uniformly throughout their tissues, the testa and axis are completely wetted at a relatively

early stage of imbibition (Bewley and Black, 1983). The relation between water absorbed and seed volume was reported by Shaykewich (1973) to be linear for rape seed, but for wheat and maize the increase in volume was greater than the amount of water absorbed. In the studies by Leopold (1983), with 15 species of seeds, inclusive of soybean, the volume quotient outstripped weight quotient at the end of 24 h. In the present study in V 4, delayed seeds of V 5, V 8, EC 28886, PT 51*a*, 51*b*, PT 15 and imbibing and delayed seeds of selection 12, the volume quotient was higher than the weight quotient (Table 5); the reverse was the condition in V 5 (imbibing and scarified types), V 16, PT 62 and scarified seeds of selection 12. In winged bean seeds the chief component which imbibes water is the protein. The swelling of seeds, therefore, reflects the storage materials present in the seeds (Mayer and Poljakoff-Mayber, 1989). According to Stiles (1948) the kinetics of water uptake by the seed integrate the uptake by its individual parts.

CHAPTER 5

Pattern of Field Emergence in 10 Varieties of Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) Seeds.

Contents

Pattern of Field Emergence in 10 Varieties of Winged bean (*Psophocarpus tetragonolobus* (L.) DC) Seeds

Introduction

The winged bean (*Psophocarpus tetragonolobus* (L.) DC.) holds out considerable promise as the source of good protein for the population in the tropics (Anonymous, 1975). The establishment of winged bean plant is a problem due to poor seed germination, which is mainly attributed to the hard seed coat. Several workers have emphasized the unusually tough and massive nature of the seed coat of the winged bean, difficulty in water penetration and special treatments to promote germination (Cerny, 1978; Khan, 1978; Martin, 1978; Vietmeyer, 1978; Lam-sanchez and Tondato, 1983). Using 45 varieties of winged bean seeds, Nabeesa *et al.* (1988) studied the imbibition pattern under laboratory condition and found three types of seeds; readily imbibing, delayed imbibing and hard seeds depending on their testa hardness.

The present author in an earlier study with fresh seeds of 10 varieties of winged bean seeds, under Petri dish conditions and found the unevenness in imbibition pattern due to varying hardness of the seed coat. The following is a study on the pattern of field germination of seeds in 10 varieties of winged bean in order to compare with the germination pattern under Petri dish conditions.

Material and Methods

(These were as described in Material and Methods)

Results

The emergence of the majority of the varieties was spread over a period of 5 to 8 days, depending on the variety. In two varieties (PT 51*a* and PT 51*b*) the emergence was completed in 3 days (day 5 to day 8). In PT 62 the emergence was observed upto 16th day. The greater part of the emergence in every case took place within 3 days (between 5th and 8th). Four varieties (V 16, EC 28886, PT 51*b*, selection 12) showed above 90% emergence during a period of 5 to 15 days and one variety (V 4) showed only 50% germination spread over a period of 5 to 10 days. The percentage of emerged seedlings are shown in table 6 and figures 11-15.

Table 6

Figures 11-15

V 4

Only 50% germination occurred in this variety at the end of 10 days with no further increase thereafter. The seedlings started emerging from 5th day after sowing and 43% seedlings emerged between 5-7 days (Figure 11).

V 5

Total germinability percentage was 67. The seedlings started emerging from 5th day after sowing and 60% of the seedlings emerged between 5 to 8th day (Figure 11).

V 8

Total germinability percentage was 80. The seedlings started emerging from 5th day after sowing and 60% emergence occurred between 5 and 8th day

Table 6. Percentage of emerged seedlings in 10 varieties of winged bean.

Variety	Days after sowing											
	5	6	7	8	9	10	11	12	13	14	15	16
V 4	20	23	43	43	47	50	50	50	50	50	50	50
V 5	3	10	50	60	60	60	60	63	63	63	67	67
V 8	3	10	40	60	63	67	67	77	77	77	80	80
V 16	33	50	93	93	93	93	93	97	97	97	97	97
EC 28886	0	0	43	83	87	87	90	90	93	93	93	93
Selection 12	0	20	60	70	73	77	80	80	87	90	93	93
PT 15	0	7	50	57	63	67	73	73	73	73	73	73
PT 51 *a*	36	77	83	90	90	90	90	90	90	90	90	90
PT 51 *b*	73	87	90	93	93	93	93	93	93	93	93	93
PT 62	0	10	17	23	30	47	53	63	80	80	83	87

(Percentage out of 30 seeds sown/variety)

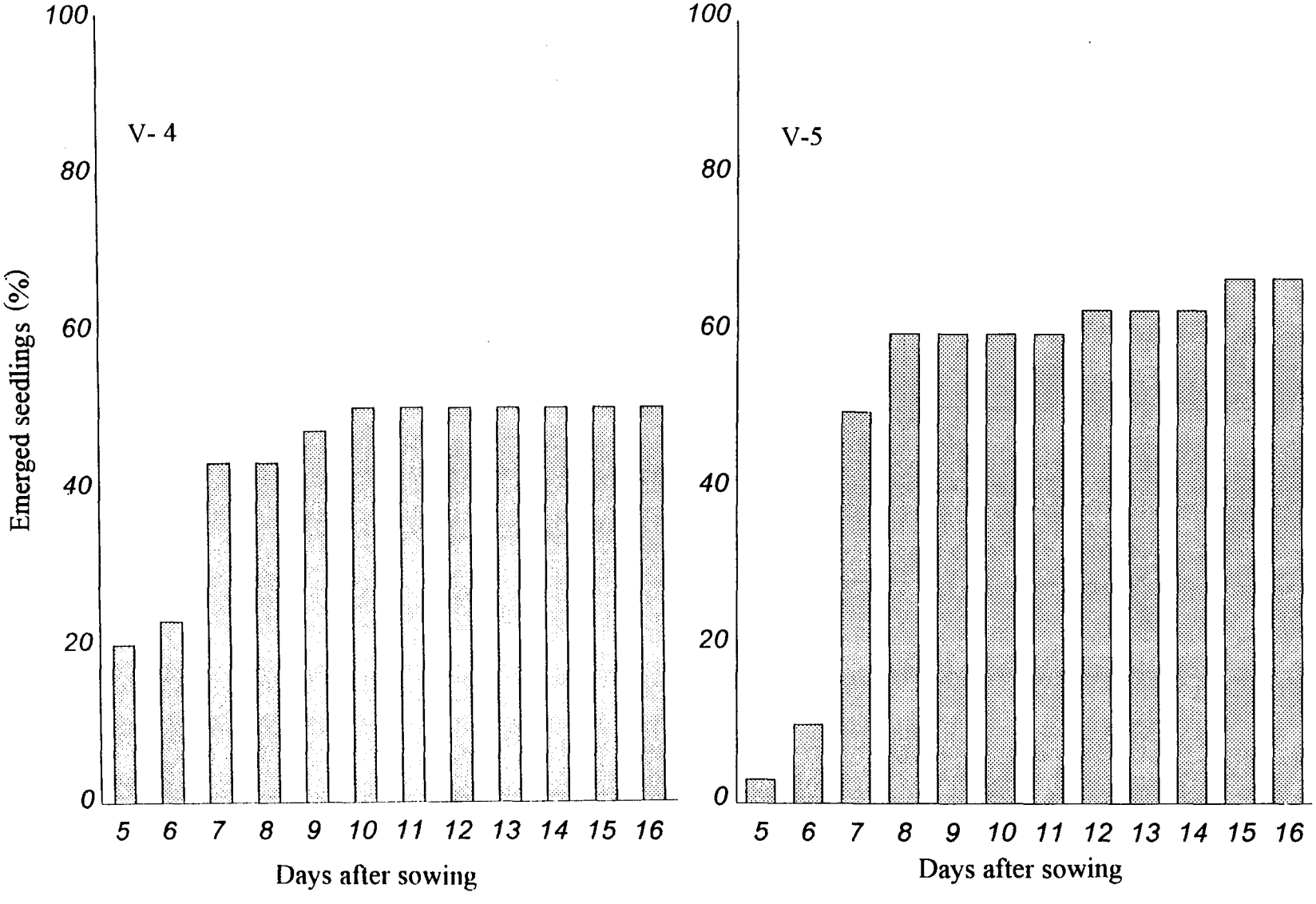


Figure 11. Percentage of emerged seedlings in V-4 and V-5 varieties.

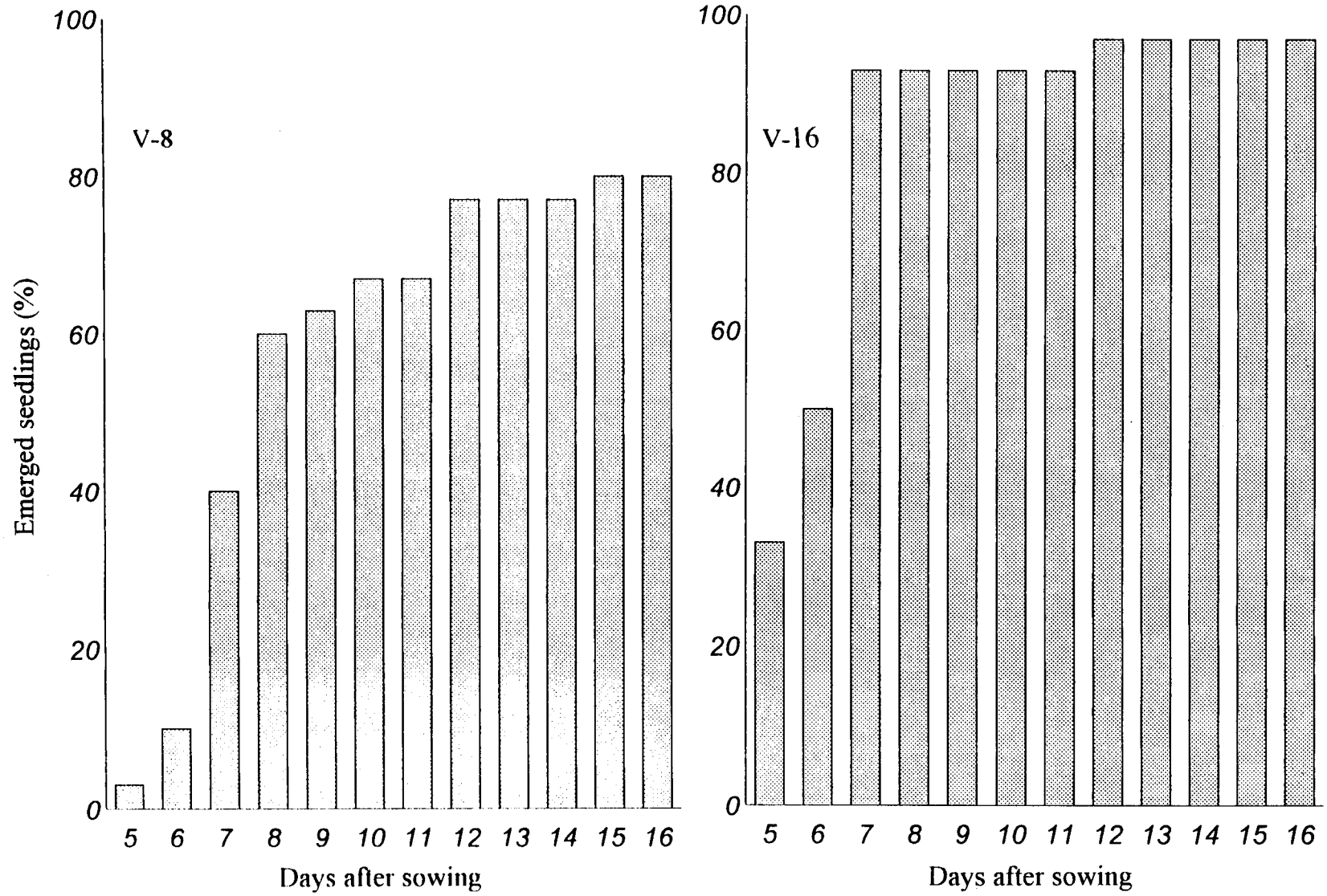


Figure 12. Percentage of emerged seedlings in V-8 and V-16 varieties.

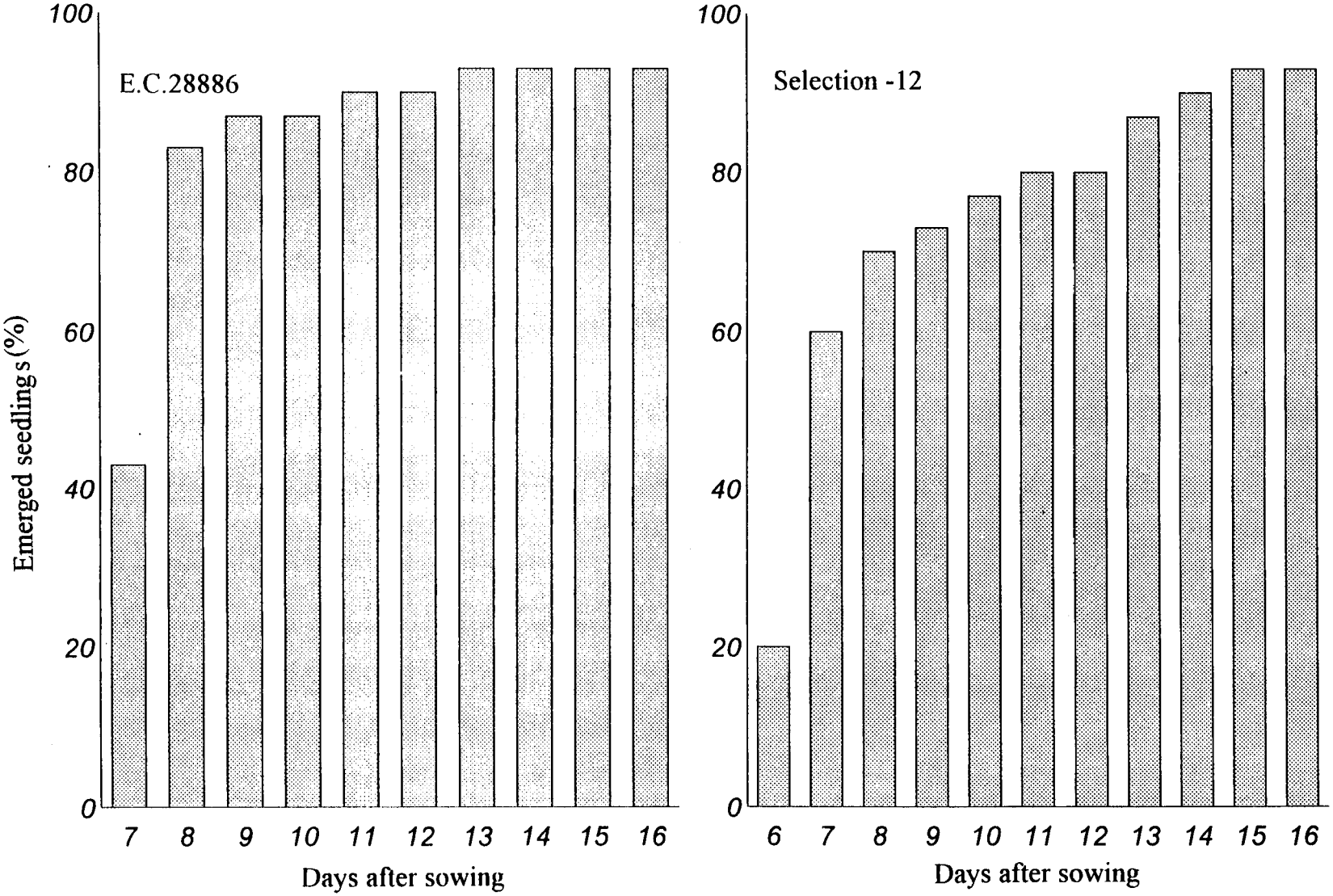


Figure 13. Percentage of emerged seedlings in EC-28886 and Selection-12 varieties.

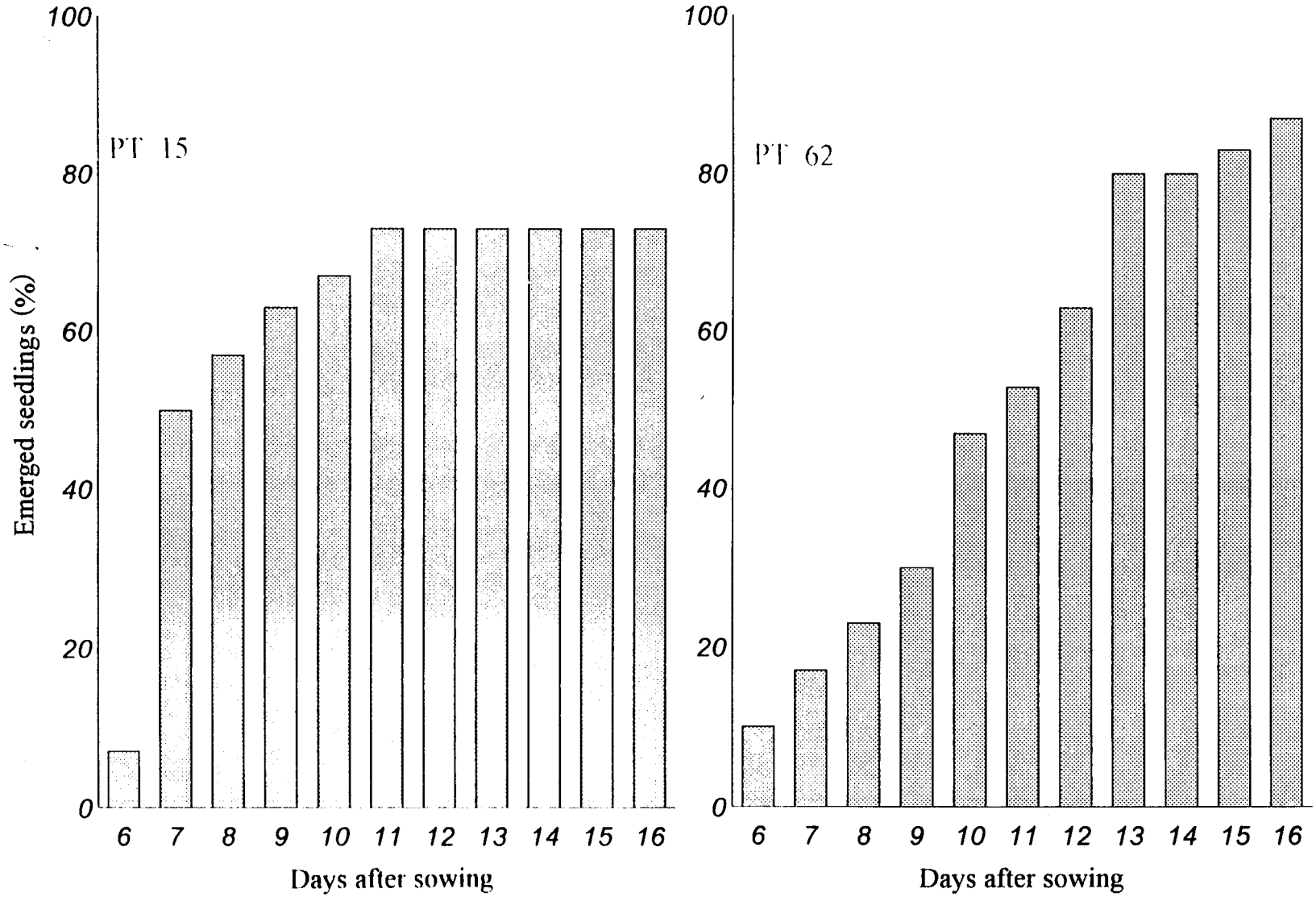


Figure 14. Percentage of emerged seedlings in PT-15 and PT-62 varieties.

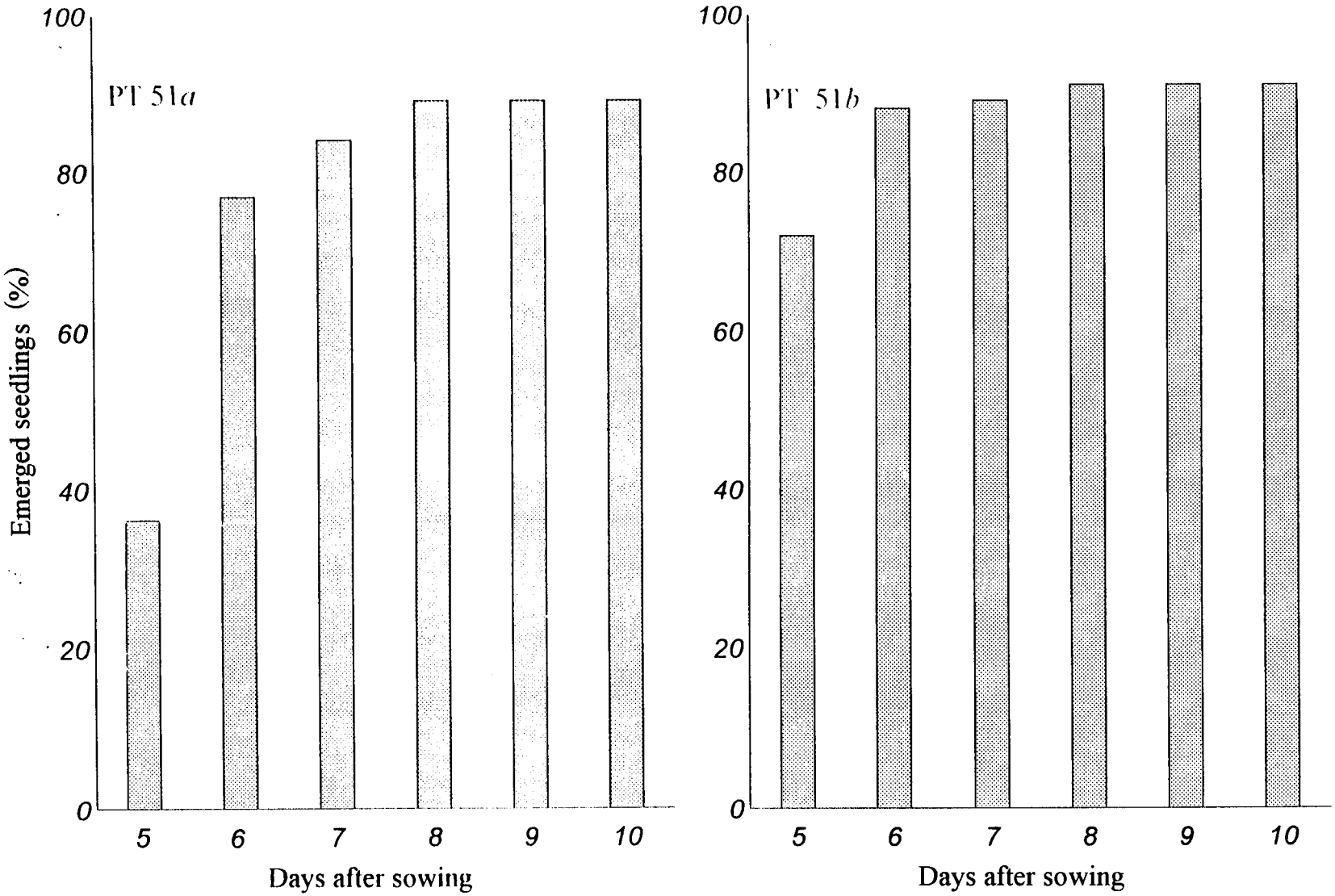


Figure 15. Percentage of emerged seedlings in PT-51*a* and PT-51*b* varieties.

after sowing and completed at the 15th day (Figure 12).

V 16

The seedlings started emerging on the 5th day after sowing. In this variety the total germinability percentage was 97. Here 93% of the seedlings emerged between 5 and 7th day after sowing and completed on the 12th day (Figure 12).

EC 28886

The seedlings started emerging on the 7th day after sowing and 87% of the seedlings emerged between 7th and 9th day. The total germinability percentage in this variety was 93%. The seedling emergence completed between 7-11 days (Figure 13).

Selection 12

The total germinability percentage was 93%. The seedlings started emerging on the 6th day and 20% of emerged on the same day. Seventy percentage of the seedlings emerged between 6-8 days and completed between 6-15 days (Figure 13).

PT 15

The total germinability percentage was 73%. The seedlings started emerging on the 6th day and only 7% emerged on the same day. Fifty seven percent seedlings emerged between 6-8 days and completed on the 11th day (Figure 14).

PT 51*a*

In this variety the seedlings started emerging from the 5th day. The germinability percentage was 89. Seventy seven percentage of the seedlings

emerged between 5 - 6 days and completed between 8th day after sowing (Figure 15).

PT 51*b*

Here the total germinability percentage was 91. The seedlings started emerging from the 5th day and 72% of the seedlings emerged on the same day. The germination completed in the 8th day after sowing (Figure 15).

PT 62

The total germinability percentage was 87%. The seedling started emerging on the 6th day and 10% emerged on that day. Only 23% seedlings emerged between 6-8 days. Germination completed between 6-16 days after sowing (Figure 14).

Discussion

The spread of seed germination over a period of time has been observed in many species (Koller, 1972). The size, shape, structure and composition of seeds can determine their germination behavior in different environments (Mayer and Poljakoff-Mayber, 1989). Johnson and Madhusudanan (1989) found that the unevenness in field emergence of fresh seeds is a characteristic feature of winged bean seeds.

Physiological heterogeneity have been reported by Koller (1969) in winged bean seeds and according to the author the seed lot is made up of two broad types, (a) quiescent seeds, germinating readily when provided with moisture, appropriate temperature, oxygen and light and (b) dormant seeds, which though fully viable, do not germinate when supplied with factors normally considered adequate for germination. The seeds which do not imbibe, are

considered to be 'hard' (Brown, 1972; Mac Kay, 1972) and the dormancy is associated, at least in part, with the testa which is acting as a barrier to water and oxygen (Rolston, 1978).

Hard seeds occur most frequently in the small seeded legumes such as red clover and lucerne (Mac Kay, 1972), but winged bean, though large seeded, also exhibits this phenomenon. According to Harper (1957) the dormancy exhibited by the winged bean is of the "innate" type, known also as primary, or natural, or inherent, or endogenous type.

A scatter in the time taken for germination of individual seeds is generally taken as an expression of deterioration (Heydecker, 1972). In winged bean, however, the scatter is not due to deterioration but due to the presence of certain amount of hard seeds. In a separate study (unpublished) of this laboratory using scarified seeds, imbibed seeds (24 h imbibed in Petri dishes) and decoated seeds, improved uniformity and nearly hundred percent emergence from fresh and 3 month stored seeds were observed and it is concluded that intact adherent testa is a barrier to germination in winged bean.

Seed coat imposed dormancy is widespread in the plant kingdom and has been investigated and excellently reviewed by Barton, (1965 *a,b*), Harrington, (1972), Ballard, (1973), Rolston, (1978) and Simon (1984). This seed characteristic is particularly prominent in the leguminous genera (Tran and Cavanagh, 1984). According to Nabeesa *et al.*, (1988) the presence of seeds with different levels of hardness along with normal seeds in winged bean appears to be an ecological adaptation for spreading the emergence over several days, so that some plants emerging in hospitable environment survive.

As per the observation (Table 6) in the field emergence studies, out of

10 varieties, no variety showed 100% germination. The maximum emergence occurred in V 16, which was 97% and minimum in V4, where only 50% seeds emerged (Table 6). When a comparison was made between the pattern of germination under Petri dish condition with that of the field as reported earlier, in the former, hundred percent germination occurred in all the 10 varieties whereas, in the latter no variety showed cent percent germination.

Even though varietal variation in germinability exists under laboratory and field conditions, the variation in germination between the two environmental conditions is more significant since the field (soil atmosphere) may inhibit germination to some extent. This decrease in germination cannot be attributed to the seed lot since cent percent germination were obtained in Petri dish condition. Almost similar results were obtained by the studies of Hegarty, (1974). He found no correlation between level of laboratory germination and field emergence for spring wheat. In contrast, Mac Kay *et al.* (1970) reported that relative field emergence of onion seedlings was closely correlated with laboratory germination.

Hegarty (1974) compared emergence from 20 seed lots of calabrese (*Brassica oleracea* var. *italica*) on four sowing dates and found that the percentage of field emergence and laboratory germination varied between the two extremes of 0.2 - 0.9 in the first sowings and 0.4 - 0.9 in the last two sowings showing that laboratory germination was not a reliable predictor of field emergence under the conditions into which the seeds were sown. According to Perry (1976) the field environment limits the expression of seeds potential performance and, moreover, interacts with seed vigour. The physical soil environment is dominated by the controlling factors of temperature and

water availability, both of which have optima above and below which emergence declines. A number of causes prevent seeds in the field reaching the high germination percentages compared to observations in the laboratory conditions. It has long been appreciated that spatial variation in the micro environment is an important cause of this discrepancy and that for example, the microtopography of the soil surface may determine which seeds and which species germinate and which remain dormant in the soil (Harper *et al.*, 1965).

According to Mayer and Poljakoff-Mayber (1989), a seed can germinate, if it must be placed in an environmental condition favorable for the process-adequate supply of water, a suitable temperature and composition of the gases in the atmosphere, light, soil conditions. The ecological conditions prevailing in a given habitat will affect germination, the determining factor being probably the micro-climatic condition prevailing in the immediate vicinity of the seed. There is some correlation between the environmental requirement for germination and the ecological condition recurring in the habitat of the plant and the seeds. Excessive water levels can be deleterious to germination in soil; emergence from sugar beet seed was reduced by irrigating after sowing to simulate high rainfall (Perry, 1973). However, Aura (1975) believed that inhibition of sugar beet germination was due to restriction of oxygen diffusion by excess water.

Fehr (1973) suggested that depth of sowing is often critical for successful seedling emergence; for example, deep sowing reduced emergence from soybean seed by 40%. Lack of ability to survive deep sowing lead Popay and Sanders (1975) to recommend deep ploughing to control the barley grass weed.

High soil salinity presents problems similar to those of drought on germination. Ghorashy and Kheradman (1972) showed that germination of four different varieties of sunflower was inhibited by 2% sodium chloride.

Soils are quite heterogenous and germination and seedling establishment may be determined by microheterogeneity in the soil. In germination studies there is often a discrepancy between the germination percentage achieved under laboratory conditions and that achieved, for the same stock of seeds, in the field. It is very difficult to reproduce in the laboratory the constantly changing conditions which occur in the field. For this reason extrapolation of the results of laboratory experiments to field conditions should be carried out with great caution (Mayer and Poljakoff-Mayber, 1989).

CHAPTER 6

Biochemical Studies in Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) During Pod/Seed Development.

Contents

Biochemical Studies in Winged bean(*Psophocarpus tetragonolobus* (L.)DC) During Pod/Seed Development

Introduction

Winged bean (*Psophocarpus tetragonolobus* (L) DC), a tropical legume is a promising source of oil and protein and is listed as one of the under exploited legumes (Anonymous, 1975). The winged bean plant has exceptional nutritional value and is different from most legumes in that every parts of it is consumed. The seed proteins have an amino acid composition similar to that of soybean protein with methionine and cysteine being limiting amino acids (Ekpenyong and Borchers, 1982). Several workers have studied the changes in growth pattern and chemical composition of the seeds during various stages of development in winged bean (Data and Pratt, 1980; Data and Bautista, 1983; Kadam *et al.*, 1982). But no report exist on the metabolic changes in relation to sink-source activity during winged bean seed development with reference to leaf tissues.

The biosynthesis of storage proteins in seeds occurs during the last two-thirds of their development, commencing after cell division and formation of the embryo body is completed and ceasing during the latter stages of maturation drying (Higgins 1984). The storage substances are synthesized from assimilates transported into the seed from other parts of the plant (source) while the developing seed (and the fruit) serves as a "sink". According to Flinn and Pate (1970), highest rates of export from leaf and pod take place when the seeds show their greatest demand for carbon and at this time a relatively large proportion of the exported assimilates are diverted to the seeds. The seed coat has a special role in the transport of assimilates from the parent plant to the embryo. It apparently constitutes the

chief interface through which sucrose and other nitrogenous compounds are unloaded into the seed. During the passage of translocated substances across the seed coat they can be transferred. For example, a range of amino acids are synthesized in seed coats (Murray and Kennedy, 1980; Peoples and Gifford, 1990).

Transaminases play a central role in amino acid metabolism since these results in the synthesis and redistribution of nitrogen from glutamate to a range of other amino acids. Kirk and Leech (1972) studied the aminotransferases activity of whole chloroplasts in *Vicia faba* using 21 amino acids and three ketoacid acceptors- a- ketoglutarate, pyruvate and oxaloacetate. They showed that all the protein-amino acids could be synthesized in the intact chloroplast by aminotransfer reactions from alanine or aspartate. They concluded that glutamate which forms 33% of the free amino acid pool of the chloroplasts, is the primary product of photosynthetic amino acid synthesis. Secondary transfer from glutamate results in the formation of aspartate and alanine and from these two compounds all the other protein-amino acids can be synthesized. They contribute to the maintenance of relatively stable amino acid pools.

Apart from being the structural units of proteins, amino acids are involved in transporting N between roots, leaves and fruits and also seen as N and C source for the production of most of the secondary products like alkaloids and phenolics. Winged bean plants are abundant in phenolics. (Kadam *et al.*, 1982).

The main objectives of this study by the present author are to determine the time course of various aspects of growth and maturation of pod / seeds, the changes in free amino acids, proteins, soluble sugars and phenolics as well as changes in aspartate and alanine aminotransferases in the axillant source leaf, axillary pod / pod wall, embryo and testa during various stages of development of

the seed.

Materials and Methods

(These were as described in Materials and Methods)

Results

Changes in fresh weights (whole tissue)

The changes in fresh weight were recorded in leaf, pod/pod wall, embryo and testa from 0 to 60th day of anthesis. In general, the fresh weight increased with maturity and attained maximum on 36th day of anthesis and subsequently decreased as water was lost from the senescing plant parts. The values are represented in Table 7 and figure 16.

Table 7

Figure 16

(a) Leaf

Total fresh weight of whole leaf did not show marked changes initially. In the first 6 following anthesis the increase was about 20%. Thereafter the weight remained nearly the same. By the 36th day the fresh weight was over 73% higher than that on 0 day. A decrease of 33% was noticed from 36th to 48th day. The 60th day fresh weight of the leaf was not recorded since it got abscised from the mother plant by then.

(b) Pod/pod wall

The pod/pod wall showed higher total fresh weight among all the tissues analysed. The increase in fresh weight of the pods was comparatively very slow during the early stages of development than later stages. From 12th day onwards,

Table 7. Changes in fresh weight of different plant parts (whole) following anthesis in the winged bean

Days after anthesis	Weight of plant parts, g			
	Leaf	Pod / Pod wall	Embryo	Testa
0	0.575±0.01	0.046±0.01	---	---
3	0.640±0.01	0.125±0.01	----	---
6	0.688±0.08	0.312±0.01	----	---
12	0.696±0.03	7.68±0.18	0.088±0.01	---
18	0.715±0.06	18.87±1.90	0.278±0.01	0.108±0.01
24	0.884±0.02	29.24±2.03	0.317±0.02	0.135±0.01
36	0.997±0.09	32.53±2.89	0.672±0.05	0.164±0.01
48	0.666±0.03	18.71±1.32	0.382±0.04	0.056±0.00
60	---	---	0.362±0.02	0.053±0.01

(The values are the mean of a minimum of 4 individual determinations)

Table 8. Dry weight percentage of different plant parts following anthesis in the winged bean

Days after anthesis	Dry weight (%)			
	Leaf	Pod / Pod wall	Embryo	Testa
0	19.85±2.05	8.09±0.25	---	---
3	20.03±1.86	9.36±1.01	---	---
6	20.16±1.89	11.32±0.33	---	---
12	20.90±1.86	12.23±1.68	22.90±0.78	---
18	21.73±2.07	14.12±0.86	31.99±0.68	27.25±1.36
24	24.01±1.25	15.59±1.00	36.49±1.17	32.62±3.17
36	25.35±4.05	25.51±1.53	47.83±2.18	33.47±0.51
48	46.53±1.38	55.36±1.21	84.59±1.35	84.38±1.25
60	---	90.51±2.61	89.38±0.82	88.72±2.00

(The values are the mean of a minimum of 6 individual determinations)

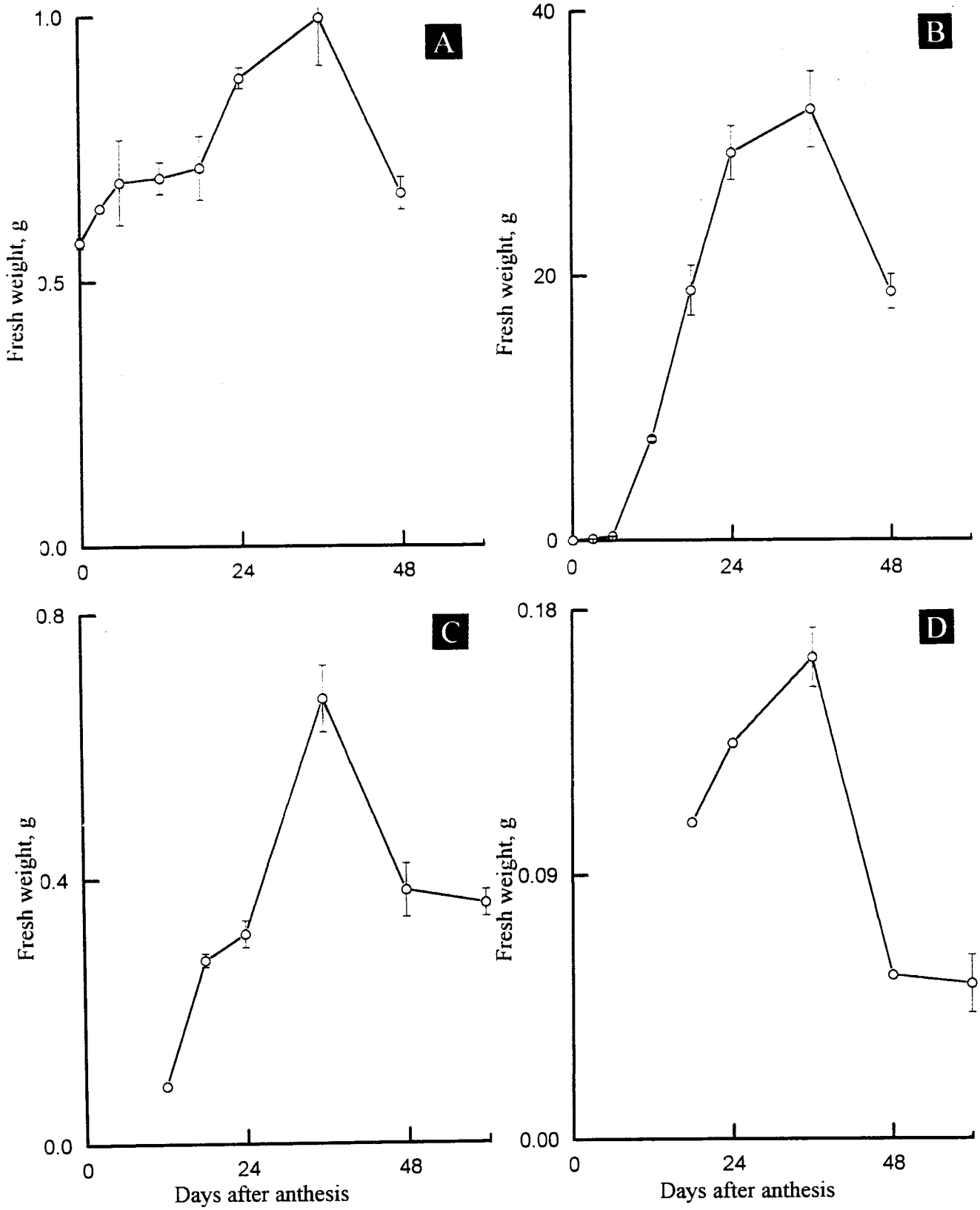


Figure 16. Changes in fresh weight of different plant parts (whole) following anthesis in the winged bean. A-Leaf, B-Pod wall, C-Embryo and D-Testa.

the fresh weight of pod started its rapid increase and attained 90% of its maximum value by 24th day. The increase in fresh weight was slow after 24th day reaching its maximum fresh weight on 36th day. After 36th day of anthesis the fresh weight of pod wall decreased with maturity accompanied by rapid water loss.

(c) Embryo

The fresh weight of embryo on 12th day was very low; afterwards the increase was rapid and attained maximum fresh weight on 36th day of anthesis. Compared to the fresh weight of embryo on 36th day, the 48th and 60th day embryos weighted only about half.

(d) Testa

The increase in fresh weight of the testa was also very slow during the earlier stages which attained maximum weight on 36th day. Like the embryo, the decrease in fresh weight of the testa was also very fast after 36th day. The 60th day testa weight was only one third when compared with 36th day testa.

Changes in percentage dry weight

In all the four plant parts analysed, 36th day was characterised by the onset of a sharp increase in percent dry weight. There was a steep increase in dry weight after 36th day and the percentage dry weight remained nearly the same for embryo and testa by the 48th day. The dry weight of leaves was determined only upto 48th day. The values are represented in table 8 and figure 17.

Table 8

Figure 17

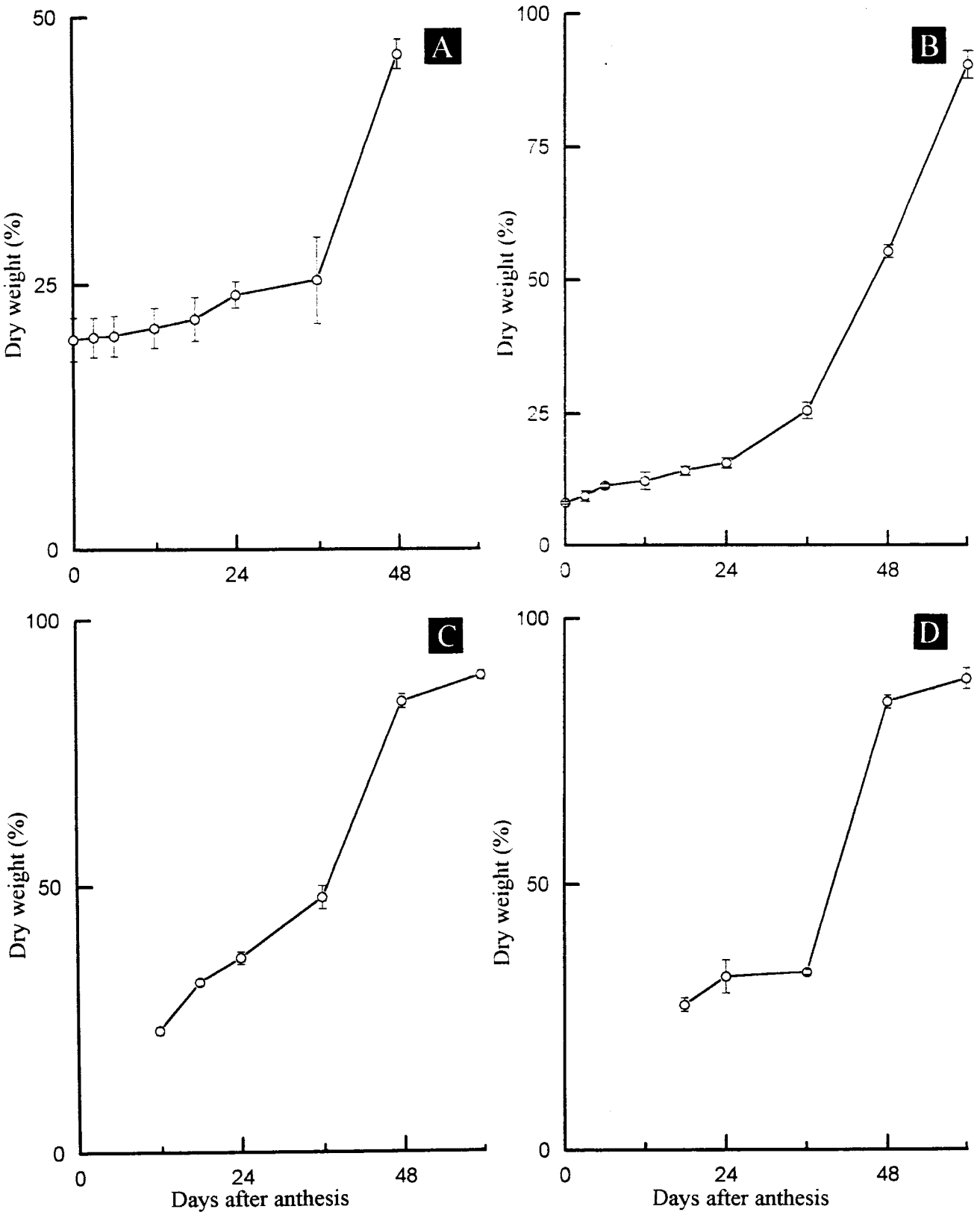


Figure 17. Changes in dry weight (%) of different plant parts following anthesis in the winged bean. A-Leaf, B-Pod wall, C-Embryo and D-Testa.

A6

(a) Leaf

The increase in dry weight percentage was very slow in leaves 0 day upto 18th day (19.85 to 21.73). Thereafter it increased more prominently. The maximum increases was registered between 36th and 48th day (25.35 to 46.53).

(b) Pod/pod wall

There was only a gradual increase in percentage dry weight in the earlier stages of pod development upto 24th day. The increase were very prominent from 24th day, to reach the maximum of 90% by the 60th day.

(c) Embryo

In the embryos, the dry weight percentage gradually increased from the 12th day upto 36th day. From 36th day onwards, the increase in percentage dry weight was faster and reached maximum on 60th day, which was almost double than the 36th day embryo.

(d) Testa

The increase in dry weight percentage of the testa was gradual upto 36th day of anthesis. The increase was very prominent after 36th day and registered about 85% by the end of 48th day, with a further increase to about 90% by the 60th day of anthesis.

Changes in total sugars

Of the various tissues analysed, the 24th day samples showed maximum amount of sugars. The values are expressed as per gram fresh/dry tissue (concentration) and per whole tissue basis (content) and are represented in table 9 and figures 18 and 19.

Table 9

Figures 18 and 19

Concentration

(a) Leaf

Both on fresh tissue and dry tissue basis, the total sugar concentration in leaf showed gradual increase upto 24th day of anthesis and decreased subsequently. The maximum sugar concentrations was registered on 24th day and minimum by the 48 th day of anthesis.

(b) Pod/pod wall

The concentrations of total sugars of the pods were high on the day of anthesis which decreased upto 6th day. From 12th day onwards, the concentration of total sugars gradually increased (in the pod wall) attaining a peak on 24th day after anthesis and again decreased. The decrease was more prominent after 36th day and registered the minimum concentration by the end of 48th day.

(c) Embryo

On a per gram fresh tissue basis, the total sugar concentration of the 12th day embryo steeply increased upto 24th day followed by a drop on 36th day and then increased gradually forming the maximum on 60th day. But on a per gram dry tissue basis, the maximum concentration of total sugars was on 18th day and decreased gradually with maturity of the tissue.

(d) Testa

In testa, the concentration of total sugars was maximum on 24th day of anthesis and decreased gradually with development and maturity of the testa. The

Table 9. Changes in total sugars in different plant parts following anthesis in winged bean

Days after anthesis	Tissue analysed	Per g, tissue, mg		Whole tissue, mg
		Fresh	Dry	
0	Leaf	5.58±0.63	28.16±3.19	3.20±0.35
	Pod	10.58±1.13	128.56±14.54	0.49±0.03
3	Leaf	13.31±1.31	66.45±6.54	8.51±0.83
	Pod	9.82±2.35	104.91±3.63	1.22±0.04
6	Leaf	17.92±1.05	88.94±5.22	12.34±0.73
	Pod	9.84±0.60	86.96±5.38	3.07±0.18
12	Leaf	19.12±0.95	91.51±4.54	13.30±0.66
	Pod wall	13.78±0.11	112.72±0.90	105.83±0.84
	Embryo	45.74±4.63	199.75±20.18	4.02±0.40
18	Leaf	22.85±2.27	105.17±10.47	16.33±1.62
	Pod wall	19.96±1.70	141.43±12.07	376.64±32.07
	Embryo	76.99±1.39	240.68±4.36	21.40±0.38
	Testa	22.32±1.69	81.93±6.22	2.41±0.18
24	Leaf	32.41±0.44	134.98±1.83	28.65±0.38
	Pod wall	22.55±0.54	144.34±3.40	659.36±15.78
	Embryo	80.48±3.69	220.56±10.13	25.51±1.16
	Testa	31.11±1.05	95.39±3.22	4.19±0.14
36	Leaf	14.25±0.38	56.25±1.52	14.20±0.37
	Pod wall	18.64±0.32	72.98±1.29	605.38±10.30
	Embryo	63.15±9.15	132.02±19.12	42.43±6.14
	Testa	23.43±0.88	70.02±2.65	3.84±0.14
48	Leaf	5.43±0.38	11.69±0.82	3.61±0.25
	Pod wall	3.84±0.23	6.93±0.41	71.84±4.30
	Embryo	77.56±2.71	91.69±3.21	29.62±1.03
	Testa	10.28±1.03	12.17±1.23	0.57±0.05
60	Embryo	84.08±8.58	94.05±9.60	30.43±3.10
	Testa	11.26±0.51	12.69±0.58	0.59±0.02

(The values are the mean of minimum of 6 individual determinations)

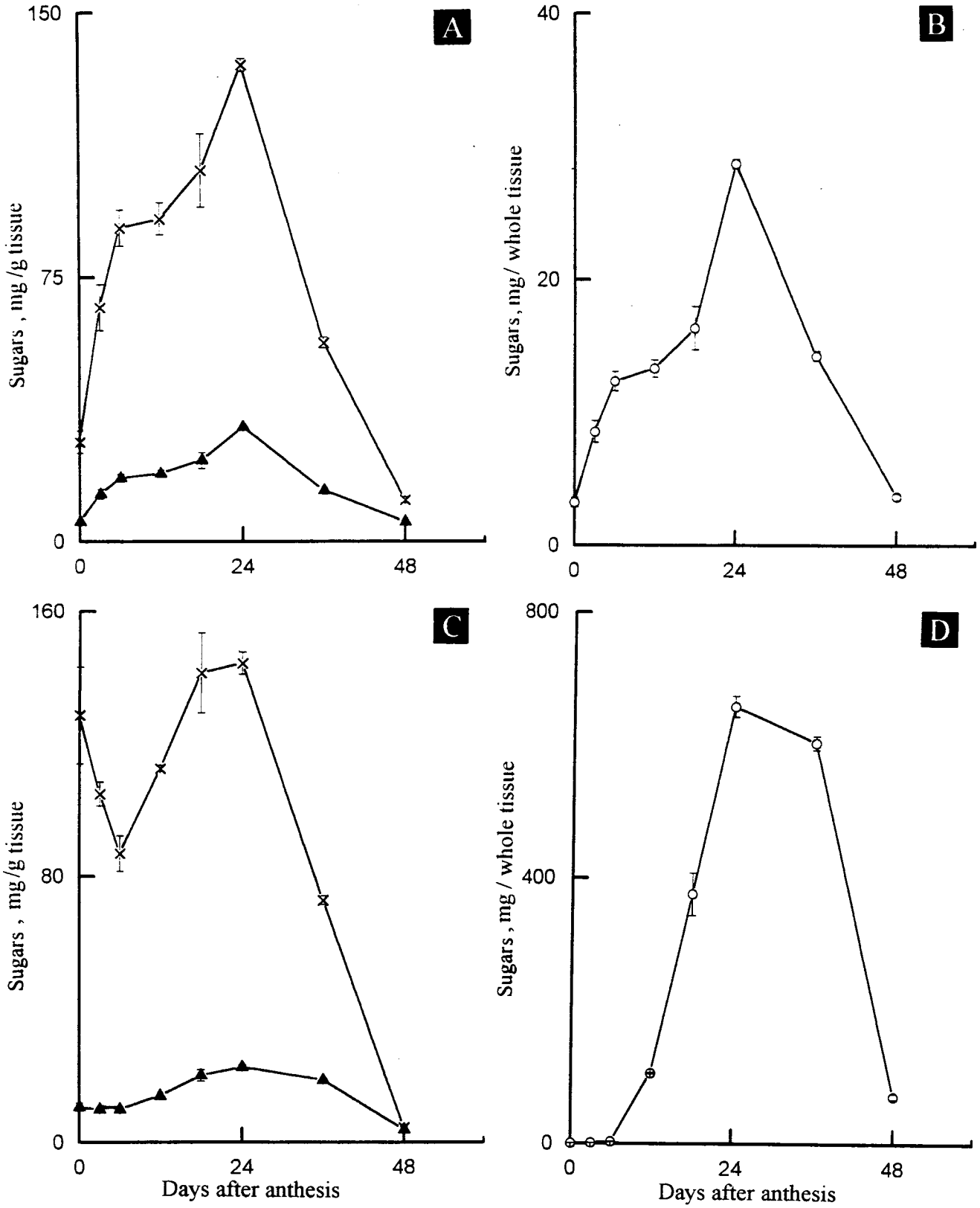


Figure 18. Changes in total sugars in leaf and pod/pod wall following anthesis in the winged bean. -▲- Fresh tissue -×- Dry tissue -○- Whole tissue

A&B-Leaf C&D-Pod/Pod wall

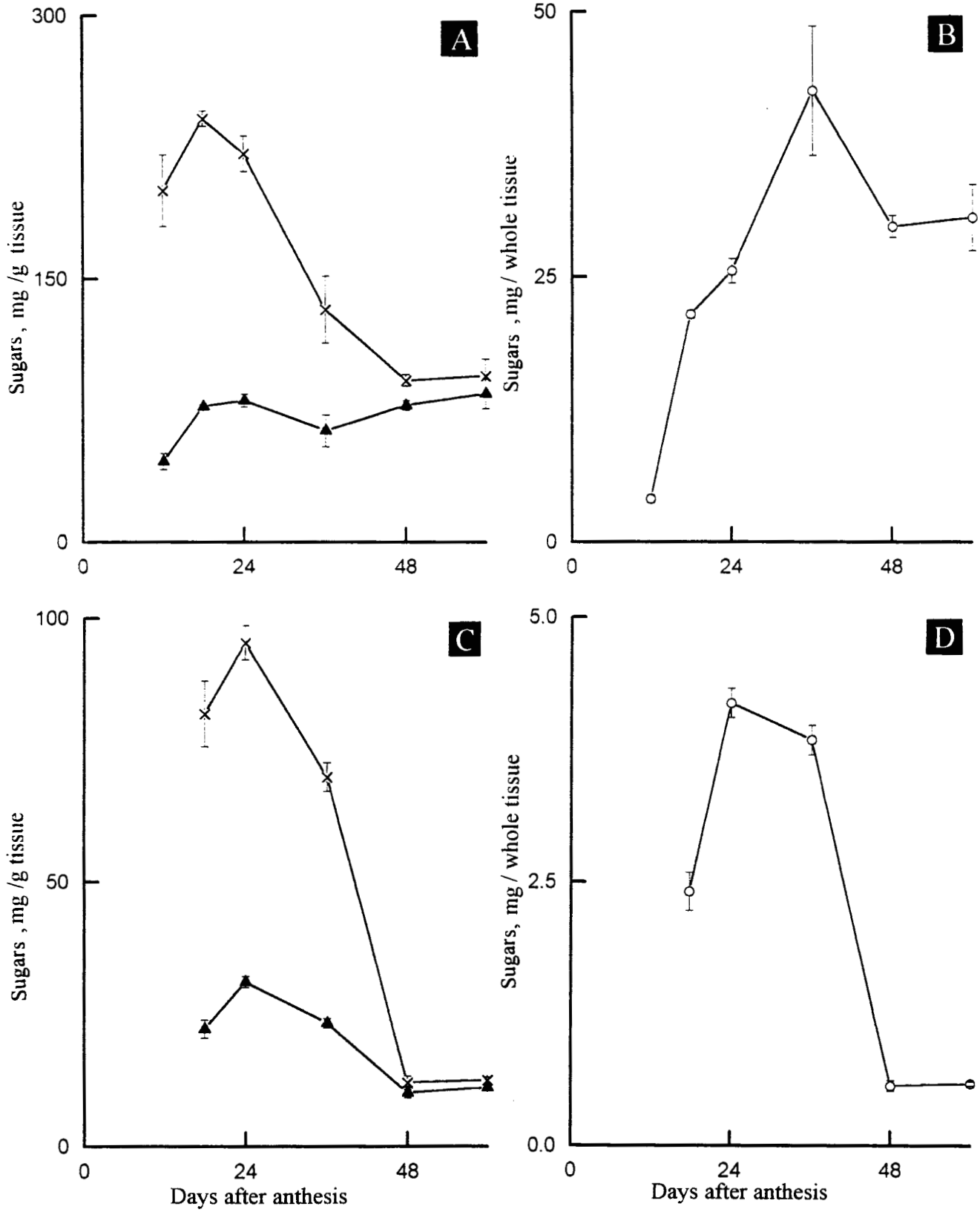


Figure 19. Changes in total sugars in embryo and testa following anthesis in the winged bean. –▲– Fresh tissue –×- Dry tissue –○– Whole tissue

A&B-Embryo C&D-Testa

pattern of changes was same on fresh and dry tissue basis.

Content

(a) Leaf

The total sugar content of the leaf gradually increased from zero day of anthesis and reached maximum content on 24th day and then declined gradually. By the 48th day the content was nearly the same as on 0 day.

(b) Pod/pod wall

The total sugar content was very low during the earlier stages. The content increased sharply after 6th day of anthesis and attained a peak on 24th day. The total sugar content on 48th day pod wall was only about 10% of that of the 36th day pod wall.

(c) Embryo

The total sugar content of the embryo was very low on the 12th day. A prominent increase was noticed on 18th day and the maximum sugar content was registered by the end of 36th day of anthesis. The content of total sugars gradually decreased with development and maturity of the embryo.

(d) Testa

The total sugar content of the testa was maximum on 24th day which slightly decreased on 36th day of anthesis. Further prominent decrease was noted after 36th day reaching a minimum by the end of 48th day of anthesis. The value remained almost the same at the end of 60th day.

Changes in total free amino acids (Lee and Takahashi method)

In general, the concentration of total amino acids was highest during the

earlier stages of development in all tissues analysed and decreased gradually with development and maturity. The values are represented in table 10 and figures 20 and 21.

Table 10

Figures 20 and 21

Concentration

(a) Leaf

In the leaves, the concentration of total amino acids, on a fresh tissue basis, was maximum on zero day of anthesis and declined gradually upto 12th day and again showed an increase on 18th day and gradually decreased thereafter. The decrease was more prominent after 36th day of anthesis. On a dry tissue basis, nearly the same pattern persisted.

(b) Pod/pod wall

The concentration of total amino acids in the pods were highest on the day of anthesis on a fresh tissue basis. There was hardly any fluctuation in the concentration upto 6th day. On the 12th day, the concentration of total amino acids in the pod wall showed a steep decrease. Thereafter, the changes, upto 48th day were marginal. However, on a dry tissue basis, the decrease from 0 day to 12th day was very prominent (more than 80%). From day 12th to day 24 th the changes were less prominent. After 24th day the concentration gradually decreased to reach the minimum value at the end of 48th day.

(c) Embryo

On a fresh weight basis, the concentration of total amino acids was high in

Table 10 : Changes in total free amino acids in different plant parts following anthesis in the winged bean

Days after anthesis	Tissue analysed	Per g, tissue,μ moles		Whole tissue, μ moles
		Fresh	Dry	
0	Leaf	40.19±0.87	202.47± 4.40	23.13±0.50
	Pod	28.60±0.56	353.62± 8.70	1.32±0.02
3	Leaf	35.53±1.72	173.81± 2.64	22.76±1.10
	Pod	26.48±0.96	282.90±10.29	3.31±0.12
6	Leaf	28.21±0.59	140.91± 1.62	19.40±0.40
	Pod	28.84±0.96	254.79± 8.48	8.99±0.29
12	Leaf	23.70±0.54	113.09± 2.60	16.49±0.37
	Pod wall	8.28±0.37	67.77± 3.06	63.59±2.84
	Embryo	43.04±1.30	187.96± 5.69	3.78±0.11
18	Leaf	32.79±0.62	150.92± 2.88	23.44±0.44
	Pod wall	9.90±0.73	70.14± 5.18	186.81±13.77
	Embryo	31.86±0.49	99.62± 1.55	8.86±0.13
	Testa	27.65±1.55	101.49± 5.72	2.98±0.16
24	Leaf	22.30±0.76	92.91± 3.18	19.71±0.67
	Pod wall	12.62±0.33	80.95± 2.16	369.00±9.64
	Embryo	35.32±1.12	96.80± 3.17	11.19±0.35
	Testa	17.37±1.00	53.25± 3.09	2.34±0.13
36	Leaf	18.51±1.54	73.04± 6.08	18.45±1.52
	Pod wall	6.62±0.33	25.95± 1.31	215.34±10.73
	Embryo	48.41±2.04	101.22± 4.26	32.53±1.37
	Testa	14.27±0.51	42.66± 1.53	2.34±0.08
48	Leaf	2.93±0.11	6.29± 0.25	1.95±0.07
	Pod wall	4.59±0.23	8.29± 0.41	85.87±4.30
	Embryo	32.65±1.12	38.59± 1.33	12.47±0.42
	Testa	5.45±0.32	6.46± 0.38	0.30±0.01
60	Embryo	35.15±0.14	39.28± 0.15	12.72±0.05
	Testa	4.18±0.16	4.72± 0.18	0.22±0.00

(The values are the mean of minimum of 4 individual determinations by the Lee and Takahashi method)

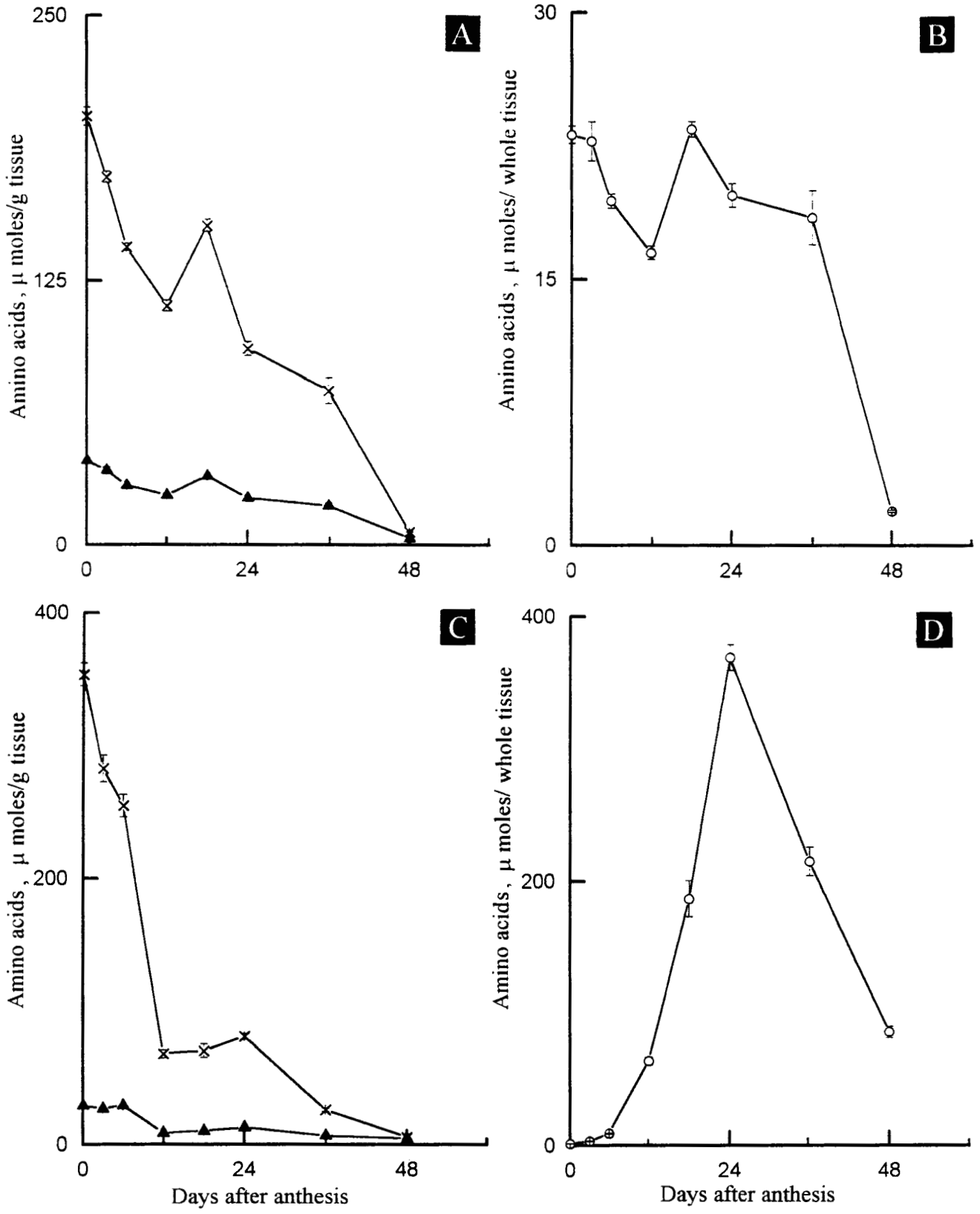


Figure 20. Changes in total free amino acids in leaf and pod/pod wall following anthesis in the winged bean. -▲–Fresh tissue -×- Dry tissue -○– Whole tissue

A&B-Leaf C&D-Pod/Pod wall

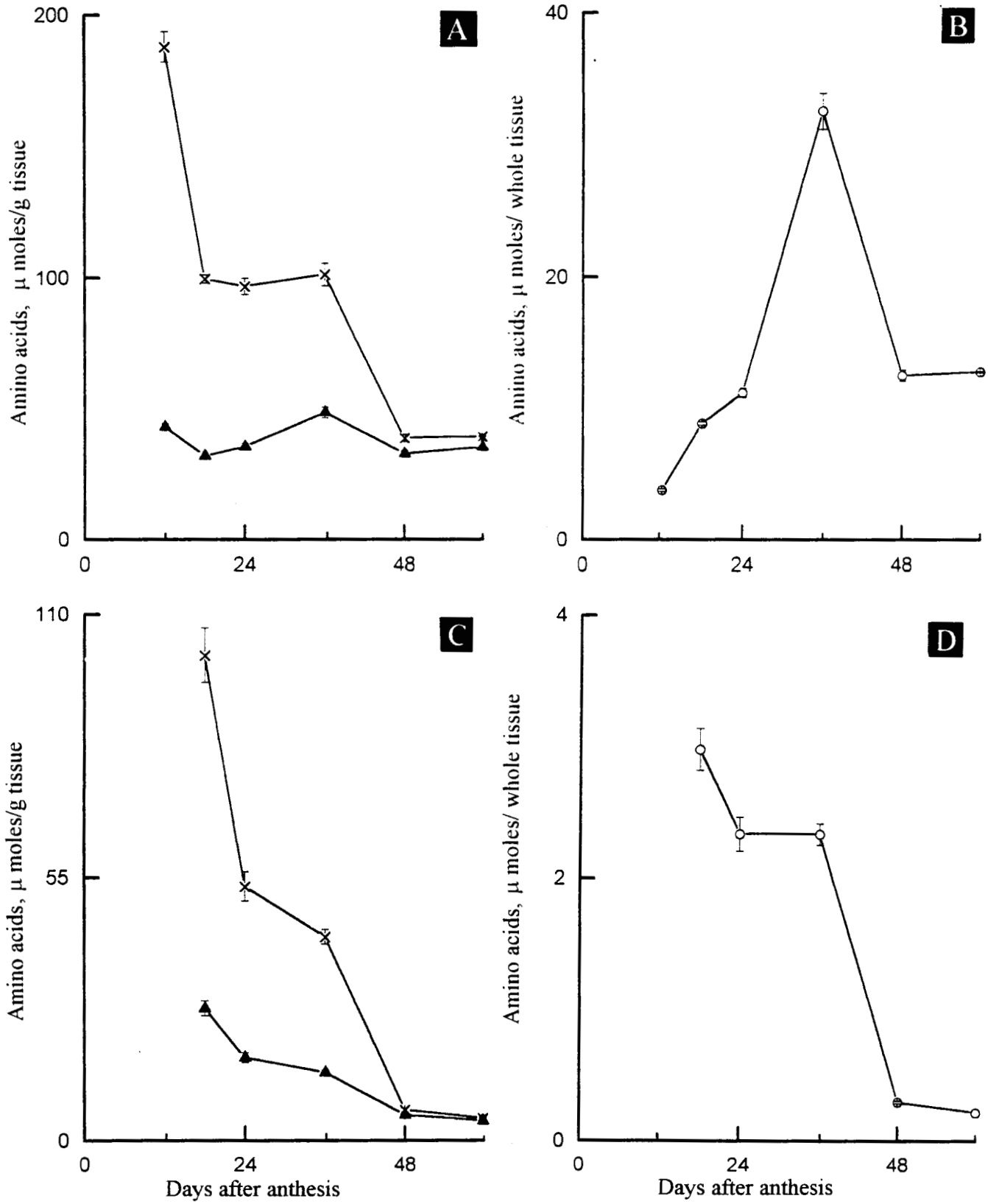


Figure 21. Changes in total free amino acids in embryo and testa following anthesis in the winged bean.–▲–Fresh tissue -×- Dry tissue –○–Whole tissue

A&B-Embryo C&D-Testa

the 12th day embryos which slightly decreased on 18th day and again increased gradually. The maximum concentration of total amino acids was recorded on 36th day of anthesis. On a dry weight basis, the concentrations of total amino acids decreased by about 50% from day 12th to day 18th. The changes were minimal from day 18th to day 36th. By the 48th day the value reached the lowest level.

(d) Testa

The total amino acids in the testa was highest in the initial stage (18th day) both on fresh and dry tissue basis. The values decreased gradually in subsequent stages to attain a low value by the 48th day. At the end of 60th day the decrease was marginal.

Content

(a) Leaf

The content of total amino acids gradually decreased from zero day of anthesis upto 12th day and again increased on 18th day forming a peak which was almost same as that of zero day. It gradually decreased in subsequent stages of development and the decrease was most prominent after 36th day of anthesis to reach the lowest value by 48th day.

(b) Pod/pod wall

The total amino acid content of the pod gradually increased from 0 day of anthesis upto 6th day and very prominently increased afterwards forming a peak on 24th day of anthesis. The total amino acid content decreased after 24th day and the decrease was more prominent after 36th day.

(c) Embryo

The total amino acid content of the embryo gradually increased from 12th day of anthesis and attained a peak on 36th day which was about 10 times higher than the 12th day embryo and steeply decreased afterwards.

(d) Testa

In the testa, the total amino acid content was high on 18th day which on 24th day showed a slight decrease and was unaltered on 36th day. After 36th day of anthesis, the content of total amino acids steeply decreased reaching a minimum on 60th day.

Individual free amino acids

The values for individual amino acids and total amino acids (sum of individual amino acids) are represented in table 11 A, B and C.

Table 11 A, B and C.

The most abundant amino acid was glutamic acid in most of the tissues analysed at almost all stages. Detectable amounts of methionine and arginine were present only in some stages. Leucine and isoleucine were not clearly separated from each other on the chromatogram and were determined as a pair, for the same reason phenylalanine and tryptophan as well as asparagine and glutamine were determined in pairs. The total free amino acids were also expressed by adding up the individual amino acids of each tissue of a particular stage. The data are presented in Table 11 A to C.

Table 11 A. Changes in individual free amino acids in different plant parts following anthesis in the winged bean

Days after anthesis	Tissue analysed	Amino acids, μ moles per g. fresh tissue																	Total
		Asp	Thr	Ser	Glt	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Leaf	3.24	2.14	0.81	6.33	ND	4.02	3.74	1.30	3.36	ND	1.63	3.55	1.75	2.38	0.94	1.74	0.53	37.46
	Pod	2.24	1.62	0.50	2.67	0.79	2.78	2.45	1.50	1.43	0.76	ND	2.26	0.70	2.45	1.64	0.68	ND	24.47
3	Leaf	4.50	1.89	0.58	5.72	T	3.51	3.44	T	2.71	T	1.38	3.04	1.21	2.78	0.72	1.18	T	32.67
	Pod	4.04	0.59	1.05	4.92	0.22	1.79	1.85	T	1.08	0.65	T	2.22	0.66	4.23	1.18	0.84	ND	25.32
6	Leaf	4.14	0.82	T	4.51	ND	1.99	3.99	1.30	1.72	ND	1.19	3.08	0.90	2.69	0.80	0.93	T	28.06
	Pod	4.36	0.70	0.46	5.20	0.27	2.95	2.25	0.30	1.29	T	0.58	2.49	0.67	2.05	1.22	T	ND	24.79
12	Leaf	3.72	0.80	T	4.63	ND	0.79	1.29	0.50	T	T	0.64	1.46	1.49	1.42	0.85	0.74	0.61	19.12
	Pod wall	0.79	0.35	0.44	1.25	ND	1.04	0.54	ND	T	ND	ND	0.88	0.77	0.48	0.45	T	ND	6.99
	Embryo	4.53	1.16	2.28	7.98	T	3.53	2.90	T	3.16	0.78	2.79	2.78	1.58	2.75	2.17	1.90	T	40.29
18	Leaf	2.78	0.66	0.95	4.41	1.27	4.27	2.57	1.10	1.80	0.39	1.02	2.77	1.44	1.93	1.50	2.36	0.61	31.88
	Pod wall	1.02	0.38	T	1.59	T	1.48	0.74	ND	0.47	ND	ND	0.93	T	0.44	0.64	T	ND	7.69
	Embryo	4.59	1.07	0.52	4.22	0.34	3.27	2.35	T	0.58	T	1.05	3.04	1.14	2.77	1.17	0.96	T	27.07
	Testa	3.18	0.58	T	6.48	0.35	2.77	3.01	T	1.15	ND	0.65	2.24	0.73	2.08	0.47	0.81	ND	24.50
24	Leaf	2.98	0.85	0.81	2.03	T	1.62	1.24	1.20	1.19	0.24	1.10	1.39	0.67	2.20	1.43	0.81	ND	19.76
	Pod wall	1.06	0.63	0.40	1.30	T	1.24	0.92	ND	T	ND	ND	1.24	1.10	0.79	0.83	0.65	ND	10.16
	Embryo	4.53	1.12	0.61	5.16	0.38	3.27	2.14	1.41	0.89	T	1.22	2.96	1.17	2.74	1.01	0.96	ND	29.57
	Testa	1.83	T	0.71	3.11	T	1.20	0.93	T	0.60	ND	ND	1.64	0.46	2.24	0.64	0.72	ND	14.08
36	Leaf	1.50	0.27	0.62	3.81	T	1.18	1.63	0.80	1.11	1.12	ND	1.28	1.14	1.30	0.79	0.81	ND	17.36
	Pod wall	0.59	0.27	T	0.84	ND	0.66	0.74	ND	T	ND	ND	0.89	0.62	0.85	0.43	T	ND	5.89
	Embryo	4.72	2.23	1.82	6.43	0.74	4.53	4.14	1.10	2.43	T	1.16	2.94	2.47	2.43	2.80	2.01	T	41.95
	Testa	1.50	ND	0.41	2.32	T	1.25	1.05	ND	0.50	ND	ND	1.12	1.52	1.58	0.85	T	ND	12.09
48	Leaf	0.54	ND	ND	0.40	ND	0.30	T	ND	T	ND	ND	T	0.33	ND	0.13	ND	T	1.70
	Pod wall	0.30	0.23	T	0.51	T	0.42	0.31	ND	ND	ND	ND	0.48	0.50	0.64	0.33	0.35	ND	4.07
	Embryo	3.03	0.71	0.54	6.95	0.71	3.11	3.47	0.64	1.51	ND	ND	1.43	1.30	1.21	1.05	0.71	ND	25.64
	Testa	0.42	ND	T	0.49	T	0.39	T	ND	ND	ND	ND	0.93	0.63	0.96	0.50	ND	T	4.76
60	Embryo	3.50	1.30	0.89	7.02	1.02	2.69	3.59	T	1.57	ND	1.10	1.98	1.88	1.92	3.15	1.89	0.89	32.88
	Testa	0.25	ND	T	0.44	ND	0.22	T	ND	T	ND	ND	0.92	0.36	0.42	0.70	ND	0.36	3.67

(The values are the mean of two individual determinations. T – Trace, ND – Not detected. The data are based on TLC separation and estimation of amino acids)

Table 11 B. Changes in individual free amino acids in different plant parts following anthesis in the winged bean

Days after anthesis	Tissue analysed	Amino acids, μ moles per g. dry tissue																	Total
		Asp	Thr	Ser	Glt	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Leaf	16.32	10.78	4.08	31.88	ND	20.25	18.84	6.54	16.92	ND	8.21	17.88	8.81	11.98	4.73	8.76	2.67	188.66
	Pod	27.68	20.02	6.18	33.00	9.76	34.35	30.28	18.54	17.67	9.39	ND	27.93	8.65	30.27	20.27	8.40	ND	302.40
3	Leaf	22.46	9.43	2.89	28.55	T	17.52	17.17	T	13.52	T	6.88	15.17	6.04	13.87	3.59	5.89	T	162.95
	Pod	43.16	6.30	11.21	52.56	2.35	19.12	19.76	T	11.53	6.94	T	23.17	7.05	45.18	12.60	8.97	ND	270.44
6	Leaf	20.53	4.06	T	22.37	ND	9.87	19.79	6.44	8.53	ND	5.90	15.27	4.46	13.34	3.96	4.61	T	139.13
	Pod	38.53	6.18	4.06	45.93	2.38	26.26	19.87	2.65	11.39	T	5.12	29.99	5.91	18.10	10.77	T	ND	219.14
12	Leaf	17.79	1.43	T	22.15	ND	4.64	6.17	2.39	T	T	3.06	6.98	7.12	8.84	4.06	3.54	2.91	91.08
	Pod wall	6.45	2.86	3.59	10.22	ND	8.50	4.41	ND	T	ND	ND	7.19	6.29	3.92	3.67	T	ND	57.08
	Embryo	19.78	5.06	9.95	34.84	T	15.41	12.66	T	13.79	3.40	12.18	12.13	6.89	12.01	9.47	8.29	T	175.86
18	Leaf	12.79	3.03	4.37	20.29	5.84	19.65	11.82	5.06	8.28	1.79	4.69	12.74	6.62	0.88	6.90	10.86	2.80	146.41
	Pod wall	7.22	2.69	T	11.26	T	10.48	5.24	ND	3.32	ND	ND	6.58	T	3.11	4.53	T	ND	84.42
	Embryo	14.34	3.34	1.62	13.19	1.06	10.22	7.34	T	1.81	T	3.28	9.50	3.56	8.65	3.65	3.00	T	84.55
	Testa	11.66	2.12	T	23.77	1.28	10.16	11.04	T	4.22	ND	2.38	2.22	2.67	7.63	1.72	2.97	ND	83.85
24	Leaf	12.41	3.54	3.37	8.45	T	6.74	5.16	4.99	4.95	0.99	4.58	5.75	2.79	9.16	5.64	3.37	ND	91.08
	Pod wall	6.79	4.04	2.56	8.33	T	7.95	5.90	ND	T	ND	ND	7.95	7.05	5.06	5.32	4.16	ND	70.17
	Embryo	12.42	3.06	1.67	14.14	1.04	8.96	5.86	3.86	2.43	T	3.34	0.11	3.20	7.50	2.76	2.63	ND	80.48
	Testa	5.61	T	2.17	9.53	T	3.67	2.85	T	1.83	ND	ND	5.03	1.41	6.86	1.96	2.20	ND	53.04
36	Leaf	5.91	1.06	2.44	15.02	T	4.65	6.42	3.15	4.37	4.41	ND	5.04	4.49	5.12	3.11	3.79	ND	68.38
	Pod wall	2.31	1.05	T	3.29	ND	2.58	2.90	ND	T	ND	ND	3.48	2.43	3.33	1.68	T	ND	23.05
	Embryo	7.77	4.66	3.80	8.45	1.54	9.48	8.65	2.29	5.08	T	2.42	6.13	5.16	5.08	5.85	4.20	T	85.55
	Testa	4.48	ND	1.22	0.94	T	3.73	3.10	ND	1.49	ND	ND	3.34	4.54	4.72	2.53	T	ND	36.08
48	Leaf	1.16	ND	ND	0.85	ND	0.64	T	ND	T	ND	ND	T	0.70	ND	0.27	ND	T	3.63
	Pod wall	0.54	0.41	T	0.92	T	0.72	0.55	ND	ND	ND	ND	0.86	0.90	1.14	0.59	0.63	ND	7.26
	Embryo	3.59	0.83	0.63	8.21	1.50	3.67	0.10	0.73	1.78	ND	ND	1.69	1.53	1.43	1.24	0.83	ND	30.26
	Testa	0.49	ND	T	0.58	T	0.46	T	ND	ND	ND	ND	1.10	0.74	1.58	0.59	ND	T	5.54
60	Embryo	3.91	1.45	0.99	7.85	1.14	2.33	4.01	T	1.75	ND	1.23	2.21	2.10	2.14	3.52	2.11	0.98	37.72
	Testa	0.28	ND	T	0.49	ND	0.24	T	ND	T	ND	ND	1.03	0.40	0.47	0.78	ND	0.40	4.09

(The values are the mean of two individual determinations. T – Trace, ND – Not detected)

Table 11 C. Changes in individual free amino acids in different plant parts following anthesis in the winged bean

Days after anthesis	Tissue analysed	Amino acids, μ moles per whole tissue																	Total
		Asp	Thr	Ser	Glt	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Leaf	1.86	1.23	0.46	3.63	ND	2.31	2.14	0.74	1.93	ND	0.93	2.04	1.01	1.30	0.54	1.00	0.03	21.50
	Pod	0.10	0.07	0.02	0.12	0.04	0.12	0.11	0.07	0.06	0.03	ND	0.10	0.03	0.11	0.07	0.03	ND	1.09
3	Leaf	2.88	1.21	0.37	3.66	T	2.24	2.20	T	1.73	T	0.88	1.94	0.77	1.77	0.46	0.75	T	20.90
	Pod	0.50	0.07	0.13	0.61	0.02	1.79	1.85	T	1.08	0.65	T	2.22	0.66	4.23	1.18	0.84	ND	25.32
6	Leaf	2.84	0.56	T	3.10	ND	1.37	2.74	0.89	1.18	ND	0.82	2.12	0.62	1.84	0.55	0.64	T	19.28
	Pod	1.36	0.22	0.14	1.62	0.08	0.92	0.70	0.09	0.40	T	0.18	0.77	0.21	0.63	0.38	T	ND	7.69
12	Leaf	2.58	0.21	T	3.22	ND	0.67	0.89	0.34	T	T	0.44	1.01	1.04	1.28	0.59	0.52	0.42	13.57
	Pod wall	6.06	2.68	3.37	9.60	ND	7.98	4.14	ND	T	ND	ND	6.75	5.91	3.68	3.45	T	ND	53.62
	Embryo	0.39	0.10	0.20	0.70	T	0.31	0.25	T	0.27	0.06	0.24	0.24	0.14	0.24	0.19	0.16	T	3.49
18	Leaf	1.98	0.47	0.67	3.15	0.91	3.05	1.83	0.78	1.28	0.27	0.72	1.90	1.02	1.34	1.07	1.68	0.43	22.71
	Pod wall	19.24	7.17	T	30.00	T	27.92	13.96	ND	8.26	ND	ND	17.54	T	8.30	12.07	T	ND	145.06
	Embryo	1.27	0.32	0.14	1.17	0.09	0.90	0.65	T	0.16	T	0.29	0.84	0.31	0.76	0.32	0.26	T	7.49
	Testa	0.34	0.06	T	0.69	0.04	0.29	0.32	T	0.12	ND	0.07	0.24	0.07	0.22	0.05	0.08	ND	2.61
24	Leaf	2.63	0.31	0.71	1.73	T	1.16	1.09	1.20	1.05	0.21	0.97	1.22	0.59	1.94	1.26	0.71	ND	17.40
	Pod wall	30.99	18.42	11.69	38.01	T	36.25	26.90	ND	T	ND	ND	36.25	32.16	23.09	24.26	19.00	ND	297.02
	Embryo	1.43	0.35	0.19	1.63	0.12	1.03	0.67	0.44	0.28	T	0.38	0.93	0.37	0.86	0.32	0.80	ND	9.31
	Testa	0.25	T	0.09	0.41	T	0.16	0.12	T	0.08	ND	ND	0.22	0.06	0.30	0.08	0.09	ND	1.87
36	Leaf	0.26	0.61	3.79	T	1.17	1.62	0.79	1.10	1.11	ND	ND	1.27	1.13	1.29	0.78	0.81	ND	17.21
	Pod wall	19.19	8.78	T	27.32	ND	21.46	24.07	ND	T	ND	ND	28.95	20.16	27.64	13.98	T	ND	191.55
	Embryo	3.17	1.49	1.22	4.32	0.49	3.04	2.78	0.73	1.63	T	0.77	1.97	1.66	1.64	1.88	1.35	T	28.13
	Testa	0.24	ND	0.06	0.38	T	0.20	0.17	ND	0.08	ND	ND	0.18	0.24	0.26	0.14	T	ND	1.94
48	Leaf	0.35	ND	ND	0.26	ND	0.19	T	ND	T	ND	ND	T	0.21	ND	0.08	ND	T	1.10
	Pod wall	5.61	4.30	T	9.54	T	7.85	5.80	ND	ND	ND	ND	8.98	9.35	11.96	6.17	8.79	ND	76.12
	Embryo	1.15	0.27	0.20	2.65	0.71	1.18	1.32	0.23	0.57	ND	ND	0.54	0.49	0.46	0.40	0.27	ND	9.75
	Testa	0.02	ND	T	0.03	T	0.02	T	ND	ND	ND	ND	0.05	0.03	0.07	0.02	ND	T	0.24
60	Embryo	1.26	0.47	0.32	2.54	0.36	0.75	1.29	T	0.56	ND	0.39	0.71	0.68	0.69	0.14	0.68	0.31	12.07
	Testa	0.01	ND	T	0.02	ND	0.01	T	ND	T	ND	ND	0.05	0.02	0.03	0.04	ND	0.02	0.20

(The values are the mean of two individual determinations. T – Trace, ND – Not detected)

Concentration (fresh tissue basis)

(a) Leaf

On the first day of anthesis-the zero day leaf-glutamic acid was the most abundant amino acid followed by glycine. Aspartic acid, threonine, alanine, valine, leucine(s), tyrosine, phenylalanine+tryptophan and asparagine+glutamine were also present in appreciable amounts. Other amino acids were present in assayable amounts. Proline and methionine could not be detected at this stage.

In the third day sample, glutamic acid, though its amount showed a slight decrease, was the most abundant amino acid. Aspartic acid, which showed an increase, was the second highest. The concentrations of glycine, alanine, threonine, serine, leucine(s), phenylalanine+tryptophan, asparagine+glutamine also decreased. Arginine and cysteine were reduced to traces, while proline and methionine, not detected in the zero day, increased to trace amounts.

Glutamic acid and aspartic acid followed the same pattern in the 6th day sample also. A common feature observed in the 6th day leaf sample was the decrease in concentration of almost all amino acids except alanine, which showed a slight increase when compared with zero and third day leaf. Leucine(s) and lysine maintained the level. Cysteine, traces only in the 3rd day sample, was in high concentration and reached the zero day value. The concentration of threonine and glycine were reduced to half. Serine was reduced to trace while proline and methionine could not be detected. Arginine maintained the trace level.

In the 12th day sample, glutamic acid was the most abundant amino acid followed by aspartic acid. The concentration of almost all amino acids were reduced in the 12th day sample. Glycine, asparagine+glutamine, leucine(s) and

phenylalanine + tryptophan were reduced to half while greater decreases were observed in the case of alanine and cysteine. Also, arginine, which was present only in traces showed a fairly good increase. Serine maintained the trace level. Proline could not be detected. Valine present in fair amounts in earlier stages was reduced to traces. The level of methionine increased to traces. Assayable amounts of other amino acids were present.

Glutamic acid maintained its highest amount in the 18th day sample, immediately followed by glycine, which showed a four-fold increase from the previous stage. The amounts of lysine and histidine, the basic amino acids, increased 2-fold for lysine and 3-fold for histidine. Alanine, asparagine + glutamine leucine(s) and phenylalanine + tryptophan also increased in concentration. Arginine, however, remained constant in value. The amounts of serine valine and methionine increased fairly from the trace level. Assayable amounts of other amino acids were also present.

Among all amino acids, aspartic acid was the highest on the 24th day. The other dicarboxylic acid, glutamic acid was the second highest. At this stage the concentration of the amino acids showed a decrease from the previous stage in their amounts except aspartic acid, threonine, cysteine, asparagine + glutamine and phenylalanine+tryptophan. Alanine and tyrosine became half while histidine reduced to one third when compared to the previous stage. Arginine could not be detected in this sample, though assayable amount was detected in the previous stage. The level of proline was reduced to traces.

The concentration of glutamic acid was almost doubled in the 36th day and was the highest followed by alanine. Fairly good amount of aspartic acid, glycine, valine, methionine leucine(s), phenylalanine+tryptophan and tyrosine were present.

Methionine showed more than four fold increase. Asparagine + glutamine and arginine could not be detected in this stage. Proline was only in traces.

In general, amounts of amino acids in the 48th day sample were comparatively very low. Most of the amino acids could not be detected or, if present, were only in traces. Among the five amino acids detected, aspartic acid was the most abundant followed by glutamic acid. Assayable amounts of glycine, tyrosine and lysine were also present. Alanine, valine, leucine(s) and arginine were present only in traces.

(b) Pod/pod wall

Glycine was the most abundant amino acid in the zero day sample, immediately followed by glutamic acid. Alanine, aspartic acid, lysine, threonine, valine, cysteine, leucine(s) and phenylalanine+tryptophan also showed appreciable amounts. Other amino acids were also presented in fairly good amounts but asparagine+glutamine and arginine could not be detected.

In the 3rd day sample, glutamic acid became the most abundant amino acid followed by aspartic acid. The concentration of both were almost doubled. The concentration of most of the amino acids like serine, alanine, glycine, leucine(s) etc decreased while that of aspartic acid, glutamic acid, tyrosine, phenylalanine+tryptophan, glutamine increased to trace level. The concentration of proline was very low, but in assayable amount. Here also arginine could not be detected.

On the 6th day, the concentration of the amino acids like glutamic acid, aspartic acid, glycine alanine and leucine(s) further increased. However, the amount of phenylalanine + tryptophan was reduced to half. Here also the dicarboxylic

acids were the dominant amino acids. Methionine and histidine were reduced to trace levels while arginine could not be detected. The concentration of cysteine and asparagine+glutamine increased from trace level. Assayable amounts of lysine, valine etc were present.

Pod wall and embryo were separately analysed from the 12th day onwards. Glutamic acid was the most abundant amino acid of the 12th day pod wall followed by glycine. Appreciable amounts of aspartic acid, phenylalanine+tryptophan, tyrosine and leucine (s) were also present. Proline, cysteine, methionine, asparagine + glutamine and arginine could not be detected. Histidine and valine were in traces.

In the 18th day sample also glutamic acid was the most abundant amino acid closely followed by glycine. The amount of aspartic acid, alanine, valine and lysine increased while threonine, leucine(s), phenylalanine+tryptophan maintained the level. Serine, proline, tyrosine and histidine were in traces. Here also cysteine, methionine, asparagine+glutamine and arginine could not be detected.

In the 24th day sample, the amount of aspartic acid, alanine, leucine(s), phenylalanine+tryptophan and lysine increased. Glutamic acid was the most abundant, followed by glycine; both showed a decrease in their concentration from the previous stage. Proline was only in traces whereas valine got reduced to trace level. Serine, tyrosine and histidine increased in assayable amounts from trace level. Cysteine, methionine, asparagine+glutamine and arginine could not be detected.

Glutamic acid, leucine(s) and phenylalanine + tryptophan showed the highest values in the 36th day sample followed by alanine and glycine. Assayable amounts of aspartic acid, lysine, tyrosine etc were also present. Proline, cysteine,

methionine, asparagine + glutamine and arginine could not be detected. Serine and histidine were reduced to traces while valine maintained the trace level.

In the 48th day sample, most of the amino acids could not be detected. Glutamic acid and tyrosine were the most abundant. Glycine was only in third position. Proline increased to trace level while serine maintained the trace level. Appreciable amounts of alanine, leucine(s), tyrosine, phenylalanine + tryptophan, lysine and histidine were also present.

(c) Embryo

The embryos were analysed from 12th day onwards. Glutamic acid was the most prominent amino acid on 12th day followed by aspartic acid. Glycine, valine, asparagine+ glutamine, leucine(s), phenylalanine+tryptophan, lysine and histidine were also present in appreciable amounts. Proline, cysteine and arginine were present only in traces.

On 18th day aspartic acid became the most prominent amino acid followed by the other dicarboxylic acid, glutamic acid. Glycine was the third highest. The amount of alanine, asparagine + glutamine, leucine(s), tyrosine, phenylalanine+tryptophan, lysine and threonine were also in measurable amounts. Proline concentration increased from trace level while methionine concentration was reduced to trace level. Cysteine and arginine maintained the trace level.

On 24th day, glutamic acid again maintained its highest position leaving aspartic acid in the second position. At this stage, almost all amino acids maintained their previous levels. The amount of cysteine increased from trace level while methionine maintained its trace level. Arginine was not detected in this stage.

Both dicarboxylic acids maintained their positions on 36th day embryo.

Glycine became the third highest amino acid followed by alanine. The amount of threonie, proline, alanine and tyrosine were doubled. Fairly good amount of serine, valine, lysine, histidine, asparagine + glutamine, phenylalanine + tryptophan were present. Here arginine and methionine were present only in traces.

In the 48th day sample, the amounts of amino acids decreased significantly. In this sample, glutamic acid was the most prominent leaving alanine in the second position. Glycine was the third highest followed by aspartic acid. Assayable amounts of threonine, serine, cysteine, leucine(s), phenylalanine + tryptophan and histidine were also present. The amount of valine, tyrosine, lysine etc was fairly good. Arginine and methionine were not detected in this samples. Proline and asparagine + glutamine decreased to trace level.

Except methionine, most of the amino acids were detected in the 60th day embryo. The level of cysteine was reduced to trace. In this stage, glutamic acid was the most abundant amino acid followed by alanine and aspartic acid. Fairly good amounts of asparagine + glutamine, leucine(s), glycine, valine, tyrosine, phenylalanine + tryptophan, lysine and histidine were present. Assayable amounts of other amino acids were also present.

(d) Testa

This was analysed from 18th day onwards. Methionine and arginine were not detected in this stage. Cysteine and serine were present only in traces. The most prominent amino acid in the 18th day testa was glutamic acid followed by aspartic acid. Alanine was the third highest. The amounts of valine, leucine(s), phenylalanine + tryptophan were also fairly good. Assayable amounts of other amino acids were also present. Serine and cysteine were only in traces whereas methionine and arginine could not be detected.

On 24th day the amount of threonine and proline were reduced to trace, while serine increased from trace level. Cysteine maintained the trace level. Here also the most prominent amino acid was glutamic acid followed by phenylalanine + tryptophan, the third highest being aspartic acid. Appreciable amounts of glycine, alanine, leucine(s) were also noted. Assayable amounts of other amino acids were present. Methionine, asparagine + glutamine and arginine could not be detected.

Glutamic acid maintained the highest level in the 36th day sample followed by phenylalanine + tryptophan. The amounts of tyrosine and aspartic acid were almost equal and was in the third position. Threonine, cysteine, methionine, asparagine + glutamine and arginine were not detected in this stage. Histidine was reduced to trace level, while proline maintained its trace amount. Assayable amounts of other amino acids were present in this stage.

On 48th day, most of the amino acids were reduced in quantity from the previous stage. Serine and alanine were reduced to traces. Proline maintained the trace level while arginine increased to trace level. At this stage, the most prominent amino acids were phenylalanine + tryptophan followed by leucine(s), tyrosine, lysine, glutamic acid, aspartic acid and glycine. Threonine, cysteine, valine, methionine, asparagine + glutamine and histidine were not detected. Assayable amounts of other amino acids were also present.

On the 60th day testa leucine(s) was the most prominent amino acid component followed by lysine, glutamic acid, phenylalanine + tryptophan, tyrosine arginine, aspartic acid and glycine. Threonine, proline, cysteine, methionine, asparagine + glutamine and histidine were not detected. Serine and alanine maintained their trace levels while valine increased to trace level.

Total free amino acids

The total free amino acids was arrived at by summing up the individually determined values of the amino acids following thin layer chromatographic separation. The values are presented in tables 11 A, B and C.

Concentration

The analytical data are represented in Table 11 A and 11 B.

(a) Leaf

The leaf tissue on zero day of anthesis had the highest concentration of total free amino acids. The concentration steadily decreased with development of the leaf upto 12th day and attained a minor increase on 18th day, following which there was a gradual decrease. In the 48th day sample, the total amino acids concentration was very low, being only about 10% that of 36th day sample.

(b) Pod/Pod wall

The concentration of total free amino acids in the pod was almost constant from day 0 to day 6. Expressed on a per gram pod wall basis, the total free amino acids registered a steep decrease by the 12th day. A tendency for increase was seen by day 18th with a further increase by day 24th. Thereafter the concentration gradually decreased to the lowest value by day 48th. On a dry weight basis too, nearly the same pattern as that of fresh tissue persisted.

(c) Embryo

The concentration of total free amino acids in the embryo was high on 12th day which decreased by 66% on 18th day and showed a pronounced increase on 36th day forming a peak. At 48th day, it again decreased sharply and then again

slightly increased on 60th day. But on a per gram dry tissue basis, the highest value was on 12th day. Thereafter the value was nearly constant upto 36th day. By 48th day there was almost a 65% reduction from the previous stage which was nearly maintained thereafter (up to 60th day).

(d) Testa

In testa, maximum total amino acid concentration was on 18th day both on fresh and dry tissue basis. There was gradual decrease thereafter and by the 48th day the value reached a minimum both on fresh and dry tissue basis.

Content

The analytical data are represented in Table 11 C

The general pattern of changes in amino acids concentration, both on per g fresh tissue basis and on whole tissue basis is expected to remain the same. However, quantitative changes in total free amino acids would occur as the total weight of the individual tissues varied with development.

(a) Leaf

The content per leaf remained nearly the same from 0 day to 6th day. A decrease on 12th day (about 30%) was more than compensated for by the 18th day. Uniform, reduced contents were seen thereafter upto 36th day. The nearly senescing leaf by the 48th day had only negligible total amino acid content when compared to all other stages.

(b) Pod/pod wall

Amino acid content per pod increased gradually from a low value to reach a seven-fold increase in the 6th day pod. Similarly pod wall of day 12th had shown a

seven-fold higher value than the 6th day pod. The total free amino acid content steadily increased with the development of the pod, resulting in a peak on day 24th, following which there was a gradual decrease. A marked decrease in amino acid content occurred at 48th day sample which was only about one third of the 36th day sample.

(c) Embryo

The total amino acid content per embryo was minimum on 12th day sample, which increased steadily attaining a peak on 36th day. The amino acid content on 48th day showed a marked decrease. On day 60th, the content of total free amino acids slightly increased.

(d) Testa

Among the different plant parts, minimum changes in total free amino acid content was noticed in the whole testa irrespective of the stage of development. This is in contrast to the marked changes in other tissues. The content of total amino acids in testa was maximum on 18th day of anthesis, which decreased gradually to reach the minimum value at the end of the 60th day.

Changes in total phenolics

The values are represented in table 12 and figures 22 and 23.

Table 12

Figures 22 and 23

Concentration

(a) Leaf

On a fresh tissue basis, the concentration of total phenolics was the

Table 12 Changes in phenolics in different plant parts following anthesis in the winged bean

Days after anthesis	Tissue analysed	Per g, tissue, mg		Whole tissue, mg
		Fresh	Dry	
0	Leaf	3.56±0.20	17.97±1.02	2.04±0.11
	Pod	1.50±0.11	18.66±1.49	0.07±0.00
3	Leaf	6.71±0.12	33.51±0.59	4.29±0.07
	Pod	1.95±0.08	20.95±0.96	0.24±0.01
6	Leaf	5.20±0.15	25.83±0.76	3.57±0.10
	Pod	3.93±0.10	34.78±0.95	1.22±0.03
12	Leaf	3.17±0.05	15.21±0.24	2.20±0.03
	Pod wall	2.63±0.02	21.50±0.16	20.15±0.15
	Embryo	5.54±0.10	24.20±0.44	0.48±0.01
18	Leaf	3.44±0.04	15.49±0.84	2.45±0.03
	Pod wall	1.62±0.08	11.53±0.66	30.62±1.75
	Embryo	0.91±0.06	2.91±0.33	0.02±0.00
	Testa	2.47±0.09	8.95±0.36	0.26±0.00
24	Leaf	4.36±0.15	18.17±0.64	3.85±0.13
	Pod wall	1.76±0.02	11.58±0.18	52.72±0.84
	Embryo	1.73±0.24	4.76±0.68	0.55±0.07
	Testa	9.96±0.77	30.54±2.38	1.33±0.10
36	Leaf	4.41±0.07	17.42±0.26	4.39±0.07
	Pod wall	3.08±0.11	12.09±0.44	100.29±3.67
	Embryo	1.92±0.17	4.02±0.21	1.28±0.06
	Testa	18.85±0.70	56.33±2.11	3.08±0.11
48	Leaf	3.94±0.07	8.48±0.15	2.62±0.05
	Pod wall	28.04±0.49	50.04±0.88	524.64±9.17
	Embryo	3.18±0.09	3.76±0.11	1.21±0.03
	Testa	31.17±1.15	36.95±1.37	1.74±0.06
60	Embryo	2.74±0.15	3.07±0.16	0.99±0.05
	Testa	37.67±0.86	42.38±0.96	1.98±0.04

(The values are the mean of minimum of 6 individual determinations)

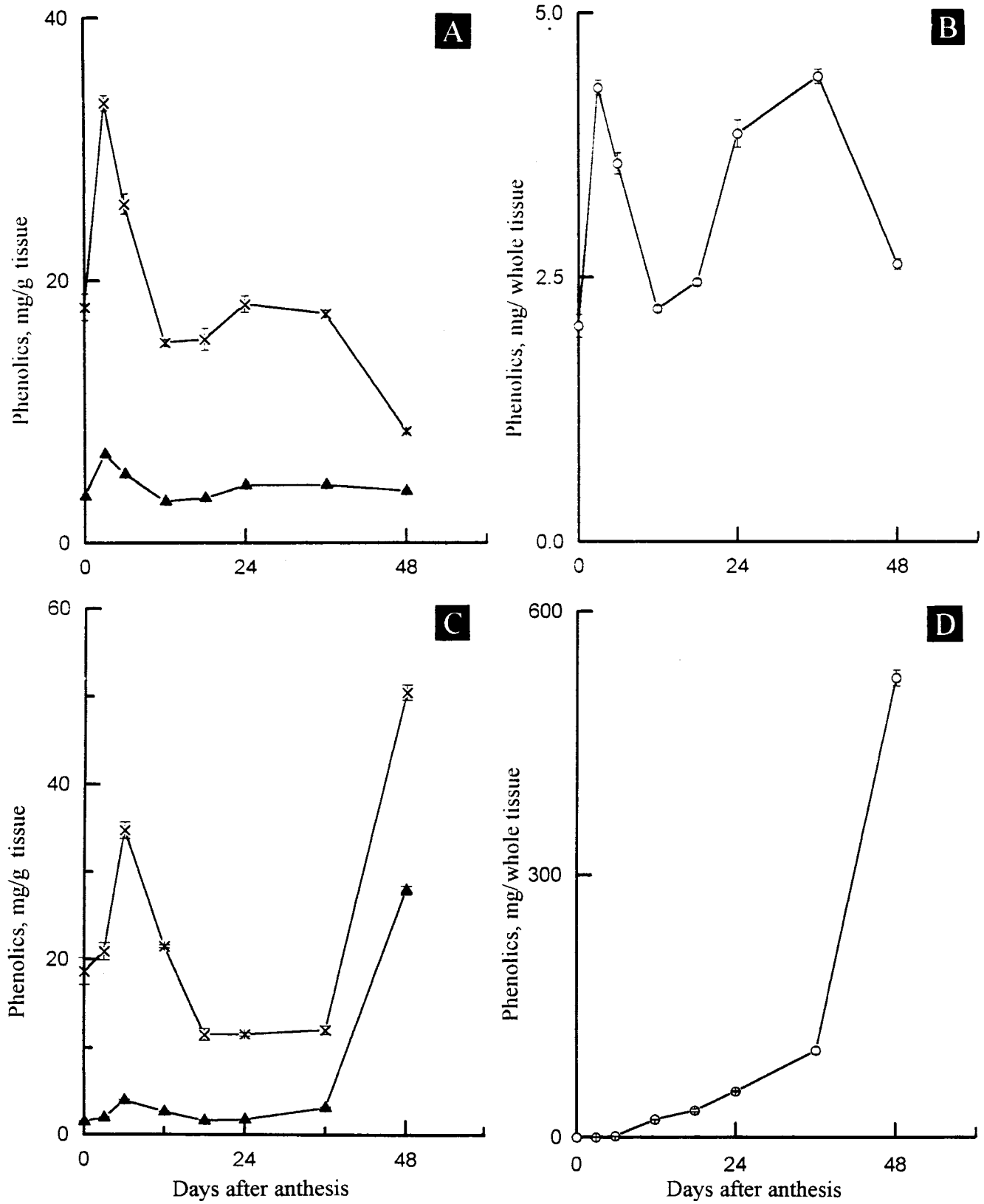


Figure 22. Changes in phenolics in leaf and pod/pod wall following anthesis in the winged bean. –▲– Fresh tissue –×– Dry tissue –○– Whole tissue

A&B-Leaf C&D-Pod/Pod wall

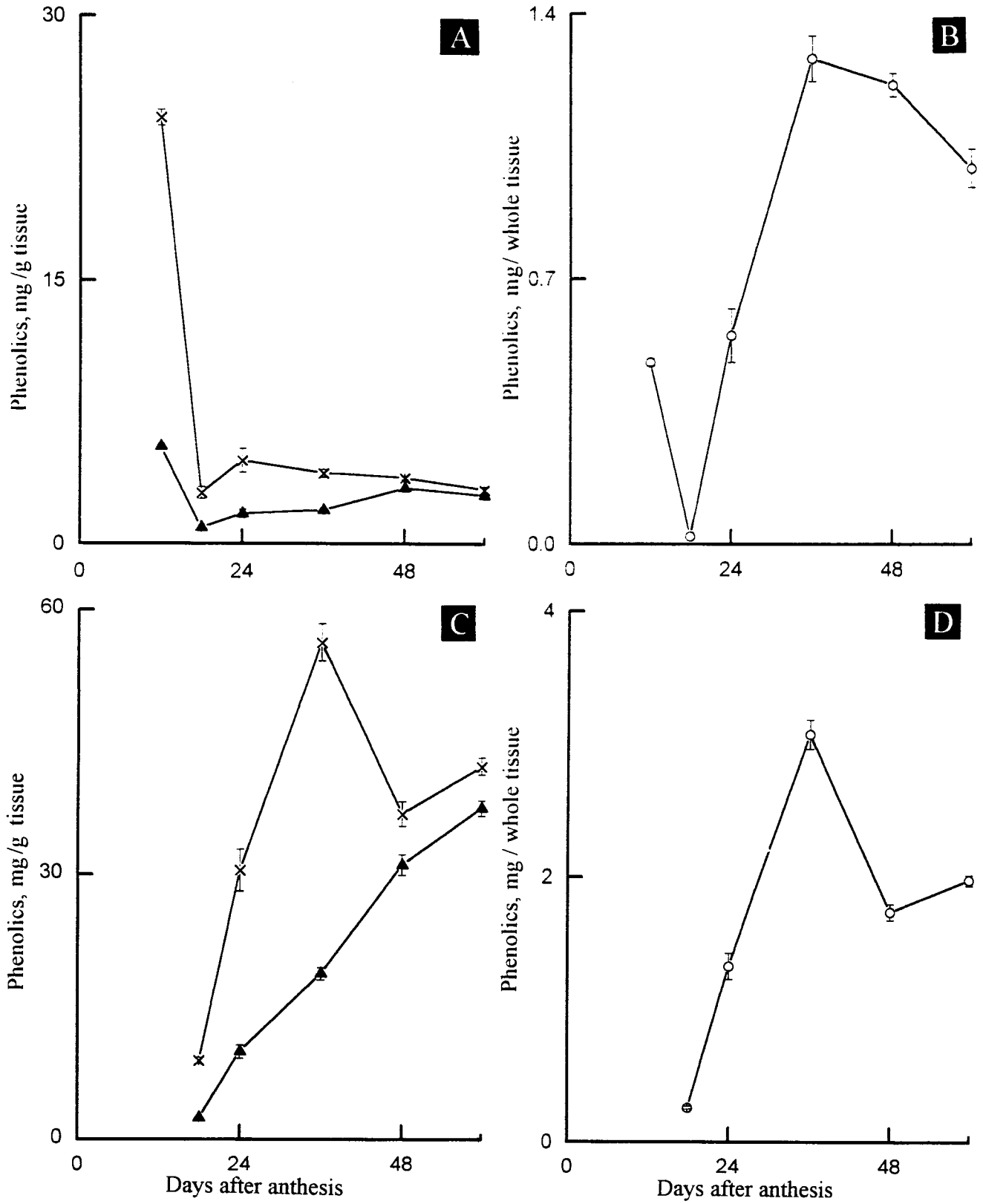


Figure 23. Changes in phenolics in embryo and testa following anthesis in the winged bean. -▲– Fresh tissue -×- Dry tissue -○- Whole tissue

A&B-Embryo C&D-Testa

maximum in day 3 leaf sample, which was almost double that of zero day leaf. It gradually decreased upto 12 day and then again increased gradually till the 36th day. The value tended to decrease thereafter. Quantitatively, a near identical pattern was seen on a dry tissue basis, the highest concentration of phenolics being in the 3rd day leaf. However, the lowest value was on 48th day which was only half that of the previous stage.

(b) Pod/pod wall

In pods, on a fresh tissue basis, total phenolics gradually increased from zero day sample attaining a peak on 6th day and then decreased. The level was maintained upto 24th day. A near doubling of the value was noticed by 36th day. The remarkable increase occurred by 48th day to reach the maximum value which was 9 times that of the previous stage. The pattern remained the same on a dry tissue basis also.

(c) Embryo

The 12th day embryo, the earliest embryo sample, had the highest phenolics concentration on a fresh weight basis. A prominent decrease to one sixth of this value was noticed by the 18th day. The 24th and 36day samples showed a near doubling of this value, with a further increase towards maturity (48th day). The pattern remained nearly the same on a dry tissue basis, the maximum value being in the young 12th day embryo.

(d) Testa

The testa showed a different pattern of phenolic changes. Here the total phenolics on a fresh tissue basis showed consistent increase from 18th day till the 60th day. The maximum concentration of total phenolics was on 60th day. On a

per gram dry tissue basis, the peak value was reached by the 36th day with a decrease noticed thereafter.

content

(a) Leaf

The content of total phenolics on day 3 leaf was more than double when compared with zero day leaf, which gradually decreased from 6th day onwards. From 18th day onwards, the total phenolics content started increasing and attained a peak on 36th day, which was almost same as on day 3 leaf and decreased by half on 48th day.

(b) Pod/pod wall

The amount of total phenolics in the pod on zero day of anthesis was very low. The increase in the content was very slow upto 6 days, but was steep on 12th day and further increased in subsequent stages. The 48th day pod wall showed the highest amount of total phenolics which was 5-fold as that in the 36th day sample.

(c) Embryo

The amount of total phenolics in the embryo on day 18th was very negligible when compared with 12th day embryo, which gradually increased with maturity and attained a maximum on 36th day and decreased gradually in subsequent stages.

(d) Testa

The total phenolics in the testa showed a gradual increase from 18th day attaining a peak on 36th day which decreased to about 50% on 48th day. The 60th day total phenolics in the testa was only marginally higher than that in the previous stage.

Standardisation of enzyme assay systems

(a) Homogenization medium

The homogenates prepared using 12th day old pod walls showed 'browning' during grinding due to the presence of phenolics. Different final concentrations of 2-mercaptoethanol (2.5, 5.0 and 7.5 mM) were incorporated into the basal medium consisting of 10 mM Tris-HCl buffer, pH 7.0 for the preparation of 10 % (w/v) homogenate. The results are shown in figure 24.

Figure 24

Incorporation of 2-mercaptoethanol into the basal medium showed linear increase in GPT activity with 2.5 mM. Thereafter, the activity showed only slight increase with 5 mM and 7.5 mM 2-mercaptoethanol. But in the case of GOT, incorporation of 2-mercaptoethanol showed only slight increase with 2.5 mM which decreased to all most the same as the control with 5.mM 2-mercaptoethanol which again slightly increased with 7.5 mM . In subsequent experiments, 5.mM 2-mercaptoethanol was supplemented with 10 mM Tris-HCl buffer, pH 7.0 were employed for homogenates using other tissues also.

(b) pH activity relationship

Using Tris-HCl buffer in the pH range 7.0 to 8.6, alanine amino transferases showed a pH optimum of 7.6 and aspartate aminotransferase showed a pH optimum of 8.0 for the tissue studied (figures 25 and 26).

Figures 25 and 26

(c) Enzyme concentration

Tested in the range 0.10 ml 0.30 ml using a 10%(w/v) homogenate of 12 th

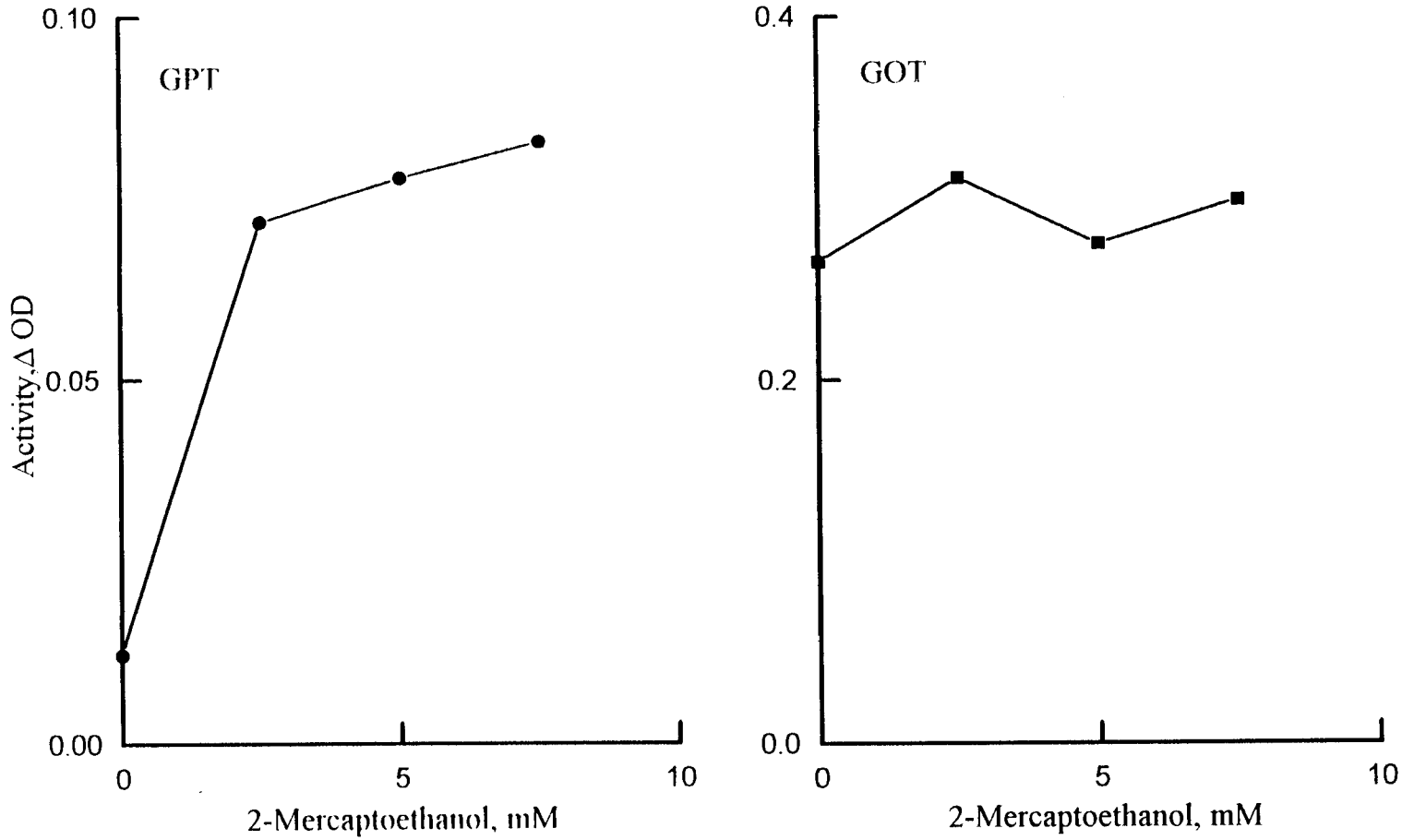


Figure 24. Incorporation of 2-mercaptoethanol during homogenization of pod wall tissue and its effect on GPT and GOT

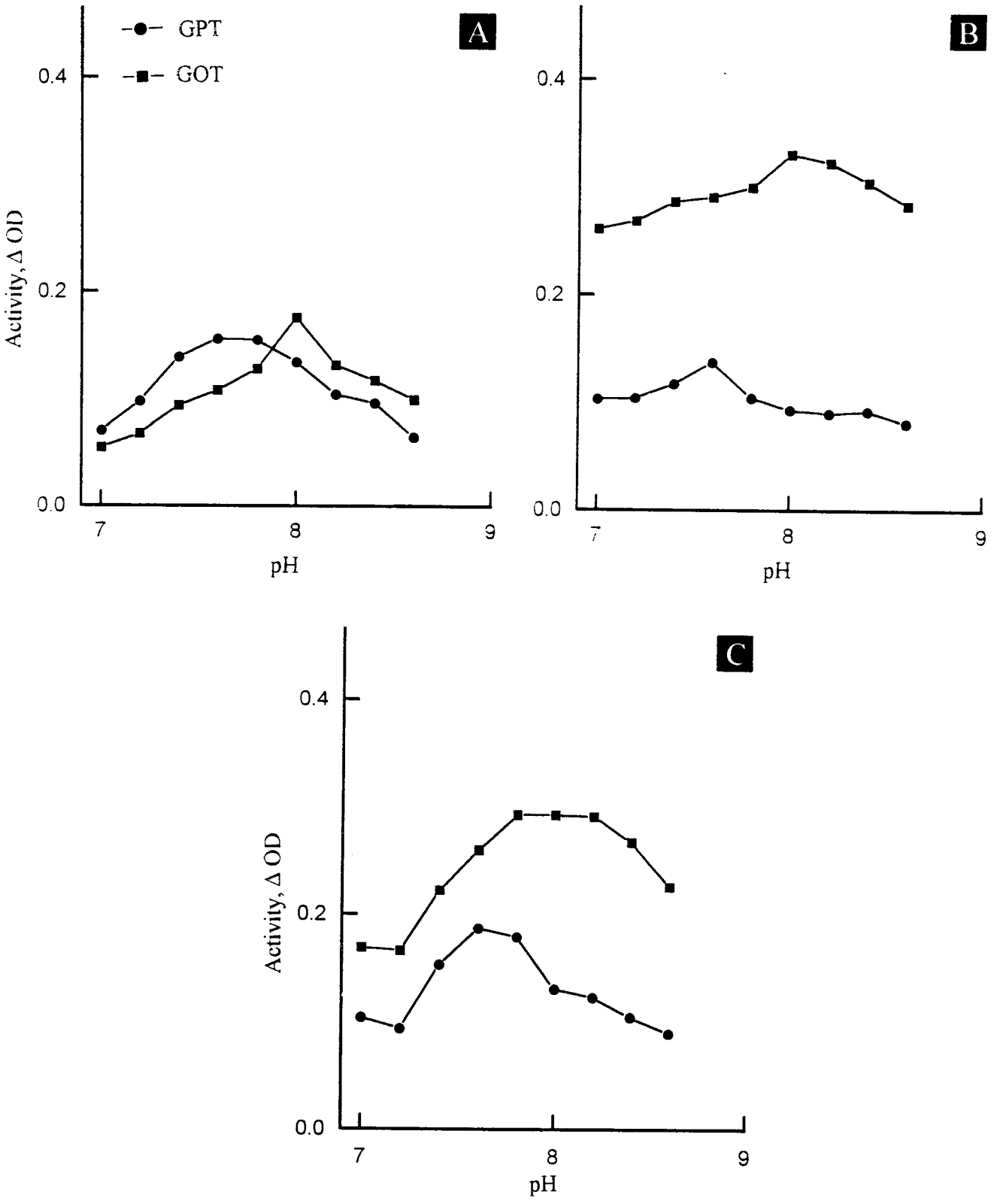


Figure 25. pH activity relationship for GPT and GOT in different tissues of winged bean. A- Leaf, B-Pod wall and C-Young embryo.

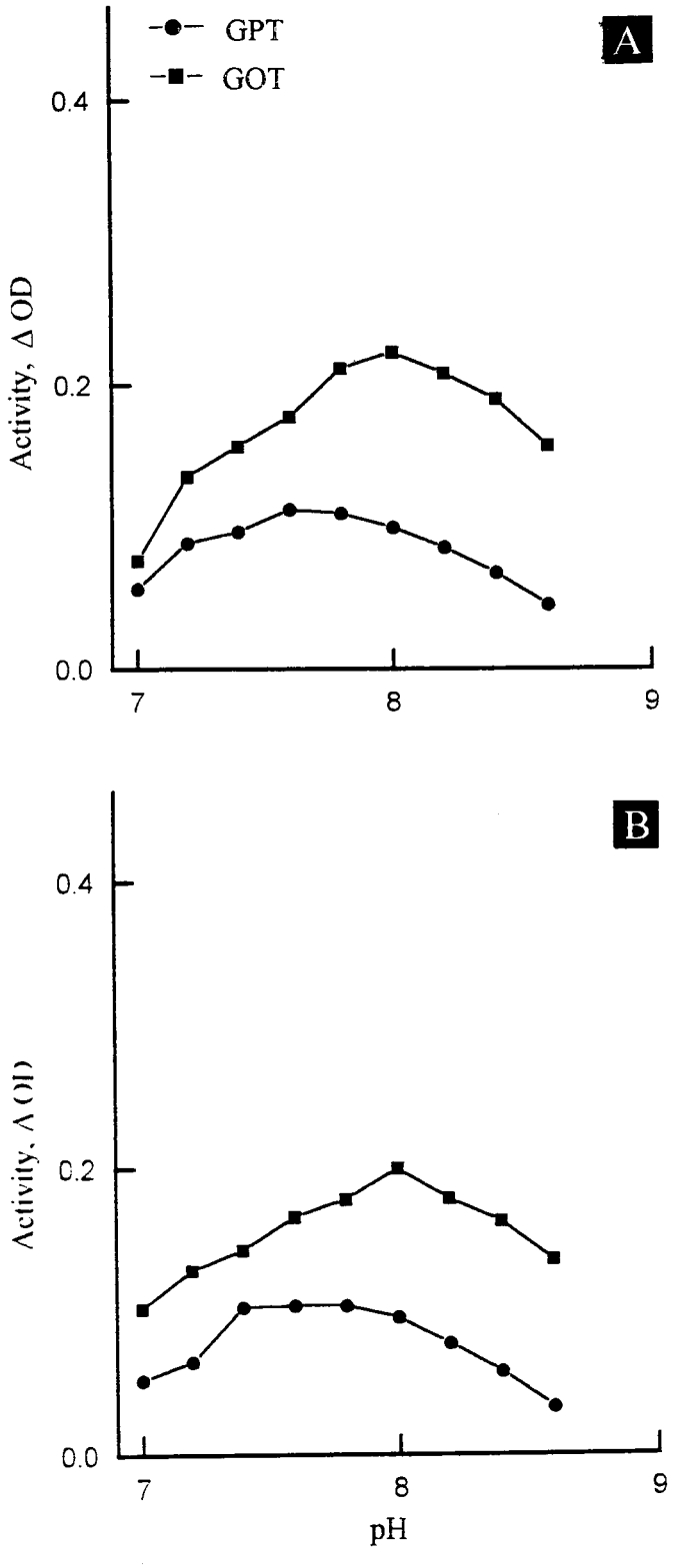


Figure 26. pH activity relationship for GPT and GOT in different tissues of winged bean. A- Cotyledon-freshly harvested seed and B-Cotyledon-one month storage after harvest.

day old seeds (see material and methods page). The activities of alanine aminotransferase and aspartate aminotransferase were nearly linear upto 0.20 ml. In all further experiments, the amount of enzyme used for the assays in the different tissues never exceeded 0.20 ml equivalent of a 10% (w/v) homogenate (figure 27 A).

(d) Substrate concentration

(i) Alanine/aspartate

The substrate saturation curves are represented in figure 27 B.

Figure 27 B.

The initial velocity of reaction increased progressively with increase in the substrate concentration in both GPT and GOT. A rectangular hyperbolic curve was obtained. The enzyme was nearly saturated at a final concentration of 40 mM for both alanine and aspartate. In all subsequent experiments therefore, 40 mM was fixed as the saturating amino acid concentration for assay of each enzyme.

(ii) α-ketoglutaric acid

The optimum concentration of a-ketoglutaric acid, the second substrate was tested with the predetermine pH and other optimal conditions. The V max was reached at a concentration of 10 mM of a- ketoglutarate for both GPT and GOT. Thereafter, increasing the concentration in fact registered a progressive decrease in the velocity of reaction. For routine assays, the final concentration of the ketoacid was fixed at 10 mM (Figure 27 C).

(e) Incubation time

Tested in the range 10-50 minutes, there was a near linear relationship

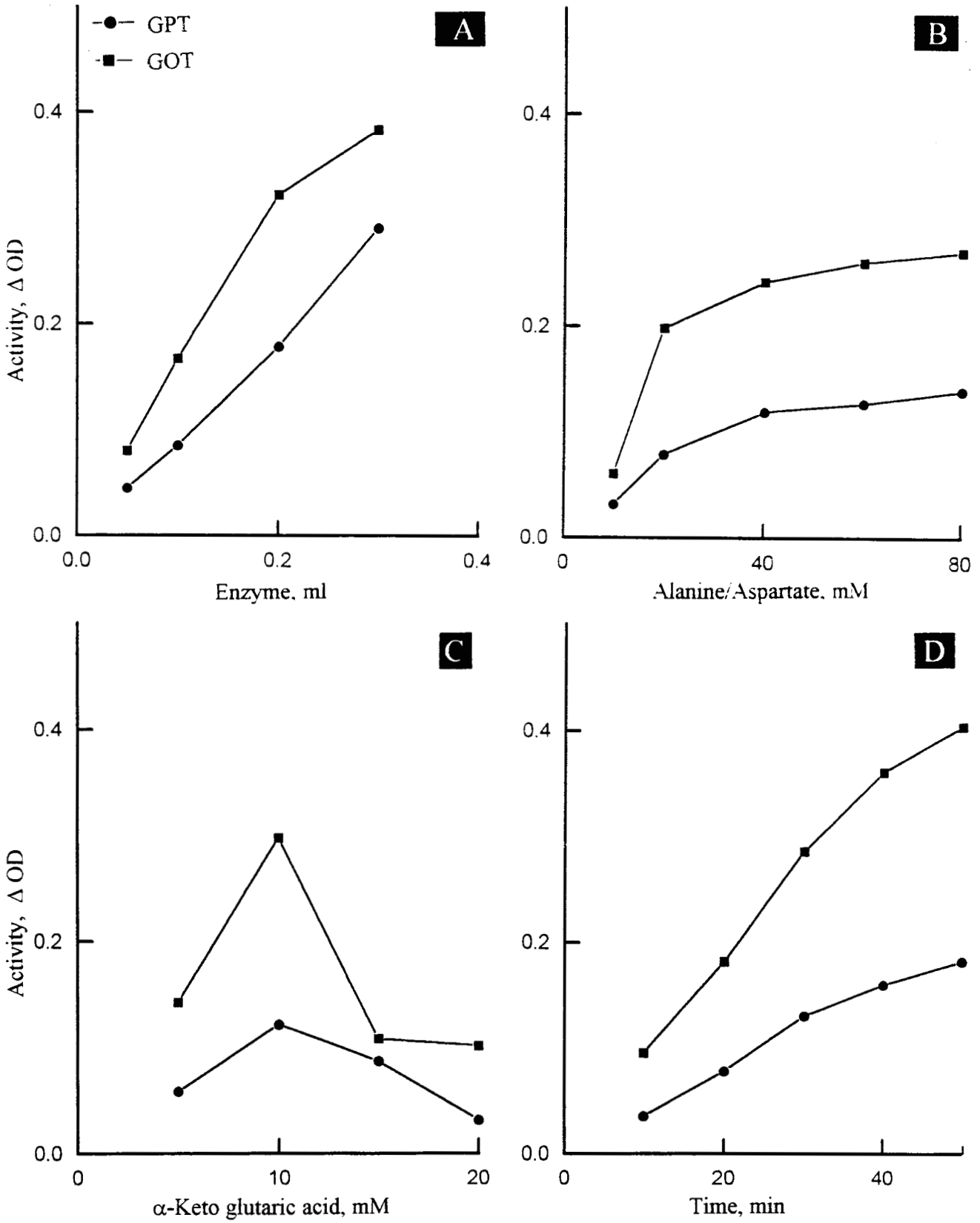


Figure 27. Effect of enzyme concentration, substrate concentrations and time on aminotransferases activities. A-Enzyme concentration, B-Substrates-Alanine/ Aspartate, C-α-ketoglutarate and D-Incubation time.

between incubation time and velocity of reaction for both GPT and GOT. The activity obtained in 30 minutes was sufficiently high for photometric measurements and hence in all subsequent experiments the incubation period was fixed at 30 minutes. (Figure 27 D).

(f) Optimum incubation temperature

This was tested at the predetermined optimum pH, substrate concentrations and incubation period both for GPT and GOT. The activities were measured in the temperature range of 20-45°C. The activities showed progressive increases upto 35°C for an incubation period of 30 minutes. Thereafter, marked decreases occurred in both GPT and GOT activities indicating that the stability of the enzyme was greater at temperature below 35 C. More than 50% reduction in activity occurred when the temperature increased from 35° to 45° C. Since the two enzymes showed greater temperature stability at values below 35°C, the optimum incubation temperature was fixed at 30°C (figure 28 A).

(g) Effect of exogenous pyridoxal phosphate

For reasons already mentioned in the Material and Methods (page 45), it was necessary to verify whether exogenously added pyridoxal phosphate had any effect on GPT and GOT activities in the tissues homogenates of winged bean. Tested in the range 10-20 ug, pyridoxal phosphate had virtually no effect on the enzyme activities, pointing to the non-essentiality of exogenous pyridoxal phosphate during assay. Thus, in routine assays pyridoxal phosphate was not incorporated as an additive (Figure 28 B).

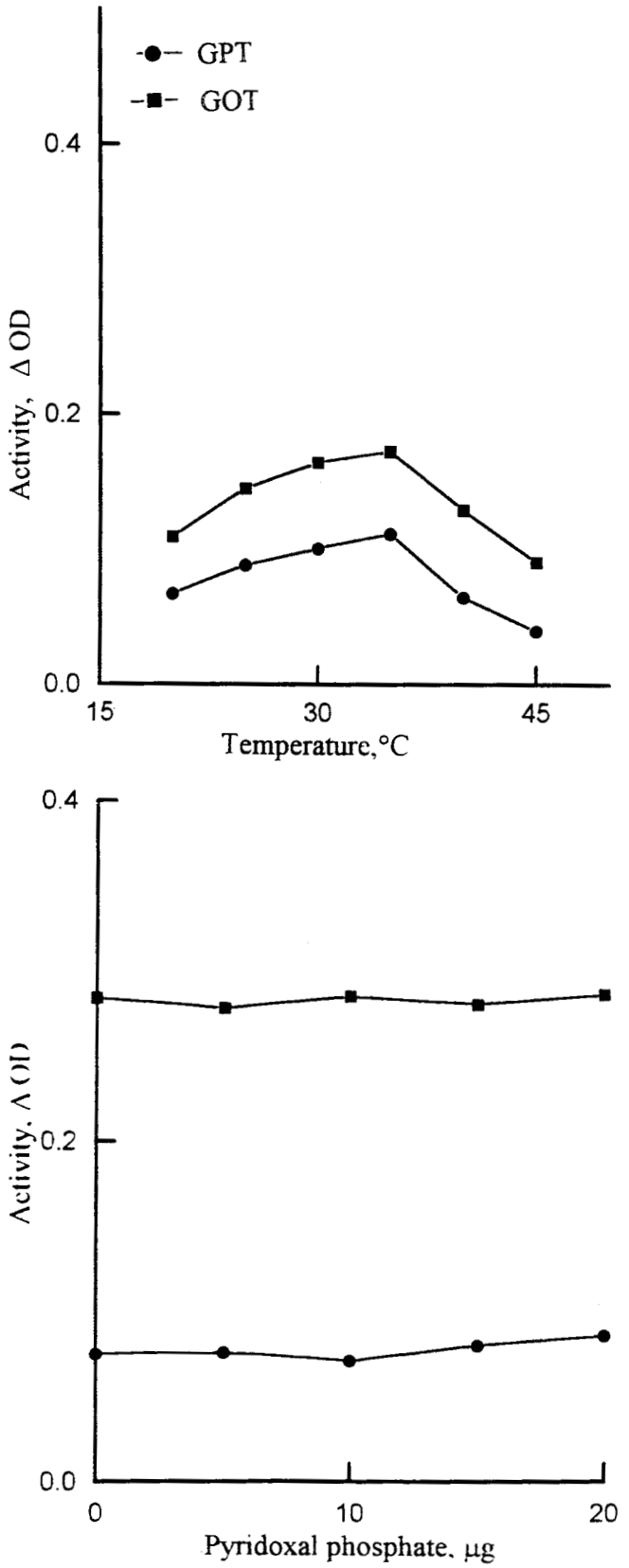


Figure 28. Effect of exogenous pyridoxal phosphate, the co-enzyme and temperature on aminotransferases activities.

Alanine and aspartate aminotransferases activity in different plant parts following anthesis.

The values are expressed as per gram fresh, dry and whole tissue basis. The analytical data are reported in table 13 and figures 29, 30, 31 and 32.

Table 13

Figures 29, 30, 31 and 32.

(a) Leaf

On a per gram fresh tissue basis the activity of GPT increased more than 2-fold by day 3, with a further increase on day 6 to register a peak. By day 18th , the value decreased to almost one-third. A doubling was noticed by the 24th day to reach a second peak of activity. Within the next six day period, there was a steep fall in GPT activity to almost one-seventh of the previous level and a further drop by the 48th day. GOT, on the other hand increased only by about 50% from day 0 to day 3. A nearly constant level of activity was maintained over a period of several days, from day 6 to day 36th. A decrease to less than half was noticed by the 48th day. A comparison of GPT and GOT at a particular stage of development showed that except at anthesis and later towards the maturity of seeds, the axillary leaf had lower GPT levels than GOT. Whereas the 0 day was characterised by nearly equal activities, the 36th day and 48th day leaf had higher GPT activities. (Table 13).

On a whole tissue basis also the pattern of changes, as expected, was the same as on a per gram fresh tissue basis (Table 13).

Quantitatively the activities registered higher values on a per gram dry tissue basis which was to be expected. The general pattern of changes remained the same (Figure 29, A, B and C).

Table 13 : Activities of alanine and aspartate aminotransferases in different plant parts following anthesis in the winged bean

Days after anthesis	Tissue analysed	Transaminase activity, units.					
		Per g. fresh tissue		Per g. dry tissue		Per whole tissue	
		GPT	GOT	GPT	GOT	GPT	GOT
0	Leaf	91.13±3.28	82.53±6.22	459.10±16.56	415.76±31.35	52.40±1.89	47.45±3.57
	Pod	52.13±3.93	171.93±7.03	644.39±48.60	2125.27±86.98	2.42±0.18	7.98±0.32
3	Leaf	214.81±11.25	115.83±9.73	1072.46±56.18	578.30±48.59	137.47±7.20	74.13±6.22
	Pod	21.29±3.51	124.92±4.07	270.22±37.52	1334.96±44.01	3.15±0.43	15.61±0.51
6	Leaf	300.55±4.37	141.11±14.84	1490.85±21.70	699.98±7.64	206.77±3.01	97.08±10.21
	Pod	19.33±1.68	152.91±3.50	170.81±14.84	1350.82±30.97	6.03±0.55	47.70±1.09
12	Leaf	292.42±27.95	133.38±6.79	1399.16±133.74	642.46±34.04	203.52±19.44	92.83±4.73
	Pod wall	22.28±3.00	69.98±5.12	182.23±27.49	572.22±41.89	171.16±25.82	537.46±39.35
	Embryo	56.80±4.84	109.85±2.37	248.46±20.85	479.69±10.38	4.99±0.44	9.66±0.21
18	Leaf	103.57±10.54	142.73±11.48	476.63±48.53	656.84±52.84	74.05±7.54	102.04±8.20
	Pod wall	62.99±8.65	78.38±10.40	446.09±61.32	555.22±72.83	1188.61±163.39	1479.37±194.06
	Embryo	87.03±4.56	185.36±5.59	272.04±14.26	579.43±17.49	24.19±1.26	51.52±1.55
	Testa	37.88±6.12	71.67±8.55	138.93±22.46	263.01±31.40	4.08±0.66	7.73±0.92
24	Leaf	214.32±20.37	140.91±15.33	892.62±84.87	586.90±63.85	173.50±23.56	124.56±13.55
	Pod wall	34.86±4.29	95.18±9.01	223.60±27.55	610.54±57.79	1019.30±125.61	2783.20±263.45
	Embryo	315.52±37.67	231.73±19.68	864.45±103.33	635.04±53.93	100.01±11.94	73.45±6.23
	Testa	31.51±5.67	164.08±19.70	96.60±17.40	502.98±60.33	4.24±0.76	22.14±2.65
36	Leaf	32.48±3.39	132.68±7.30	128.14±13.39	523.41±28.80	32.38±3.37	132.28±7.27
	Pod wall	21.04±3.82	64.00±3.83	82.47±14.98	250.92±15.05	684.42±124.35	2082.35±124.93
	Embryo	142.59±8.22	247.21±10.10	298.12±17.19	516.86±21.12	95.87±5.49	166.12±6.78
	Testa	11.85±1.90	37.32±5.82	35.40±5.69	111.52±17.40	1.93±0.31	6.11±0.95
48	Leaf	23.49±3.48	50.14±6.94	50.48±7.48	107.75±14.93	15.64±2.31	33.38±4.62
	Pod wall	21.01±2.94	44.24±7.17	37.95±5.31	71.91±12.95	393.00±55.16	827.33±134.29
	Embryo	200.76±24.83	206.24±27.09	237.32±29.36	243.81±32.03	76.69±9.48	78.76±10.34
	Testa	12.67±1.27	26.13±4.18	14.57±1.63	30.97±04.95	0.67±0.07	1.46±0.02
60	Embryo	93.65±6.88	132.51±11.49	104.78±7.70	148.25±12.85	33.89±2.49	47.96±4.15
	Testa	7.09±0.98	10.32±1.77	7.99±1.10	11.62±01.99	0.37±0.04	0.54±0.09

(The values are the mean of a minimum of 6 individual determinations)

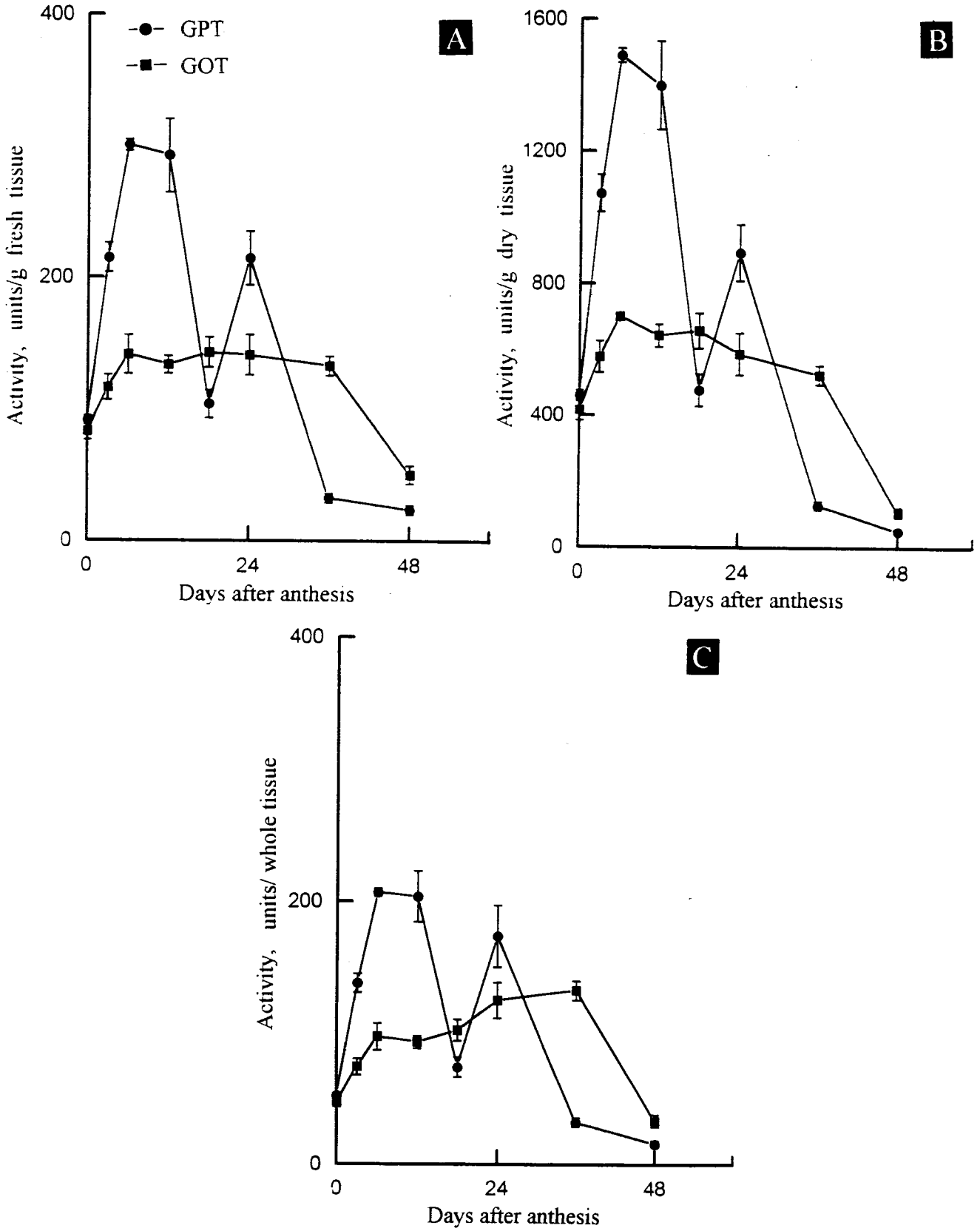


Figure 29. Activities of alanine and aspartate aminotransferases (GPT and GOT) in leaf following anthesis in the winged bean.

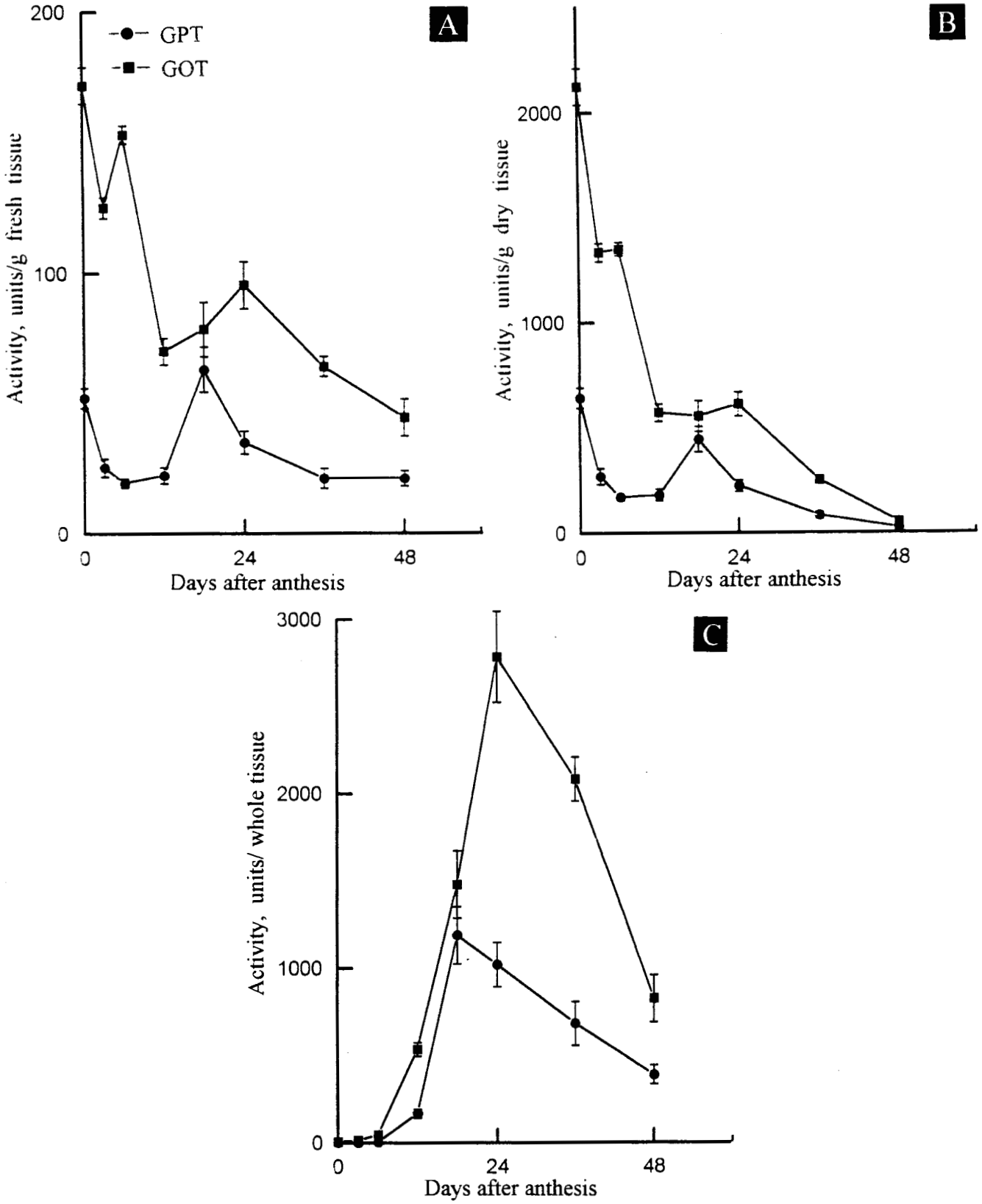


Figure 30. Activities of alanine and aspartate aminotransferases (GPT and GOT) in pod/pod wall following anthesis in the winged bean.

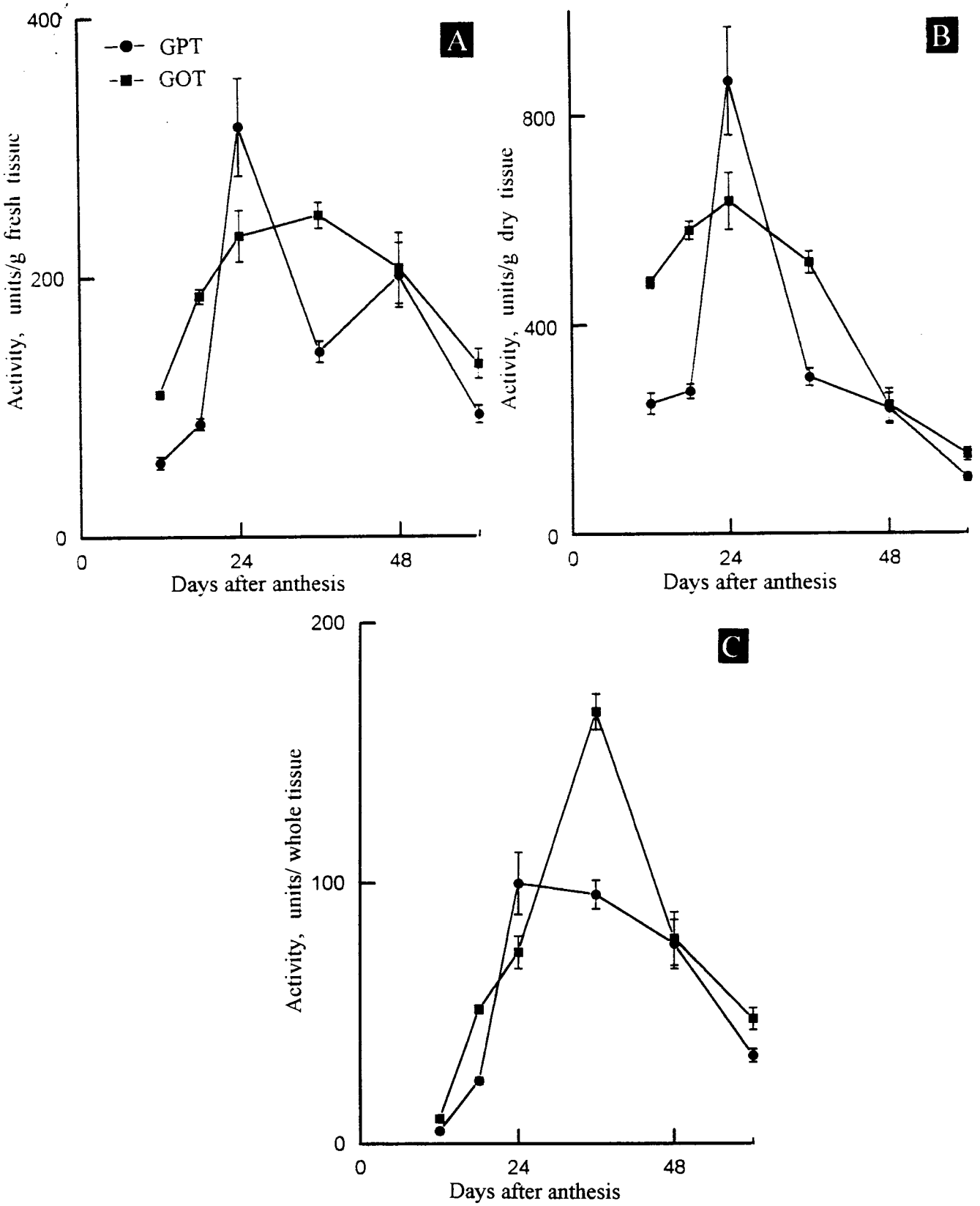


Figure 31. Activities of alanine and aspartate aminotransferases (GPT and GOT) in embryo following anthesis in the winged bean.

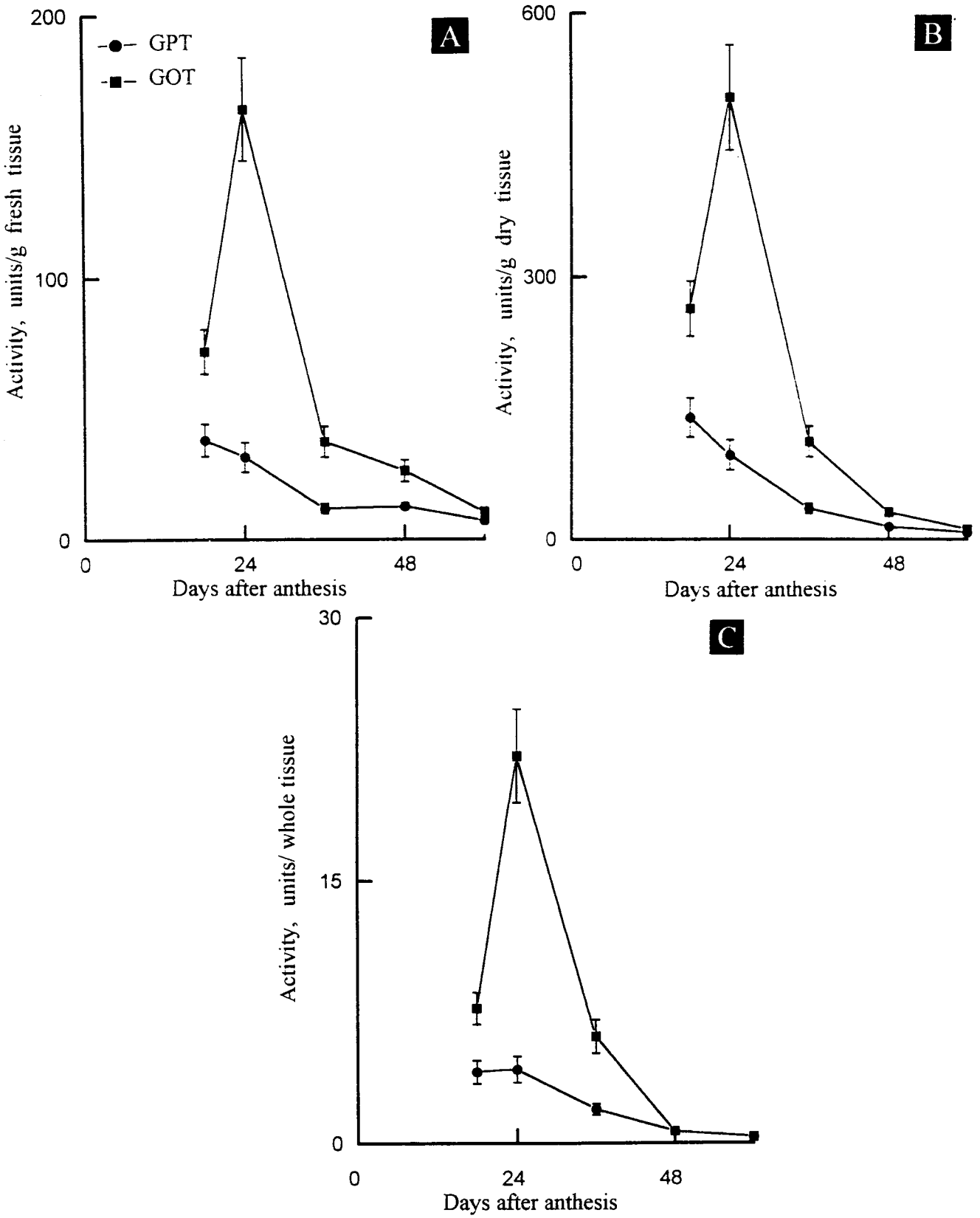


Figure 32. Activities of alanine and aspartate aminotransferases (GPT and GOT) in testa following anthesis in the winged bean.

(b) Pod/pod wall

The activity of alanine and aspartate aminotransferase were determined in whole pods on 0,3rd and 6th day after anthesis. On subsequent stages, pod wall and embryo were separately analysed.

On a per gram fresh tissue basis, the activity of GPT gradually decreased upto 6th day. The activity increased slightly on 12th day with a further three-fold increase on 18th day forming a peak and decreased gradually in subsequent stages. But on a dry tissue basis, the highest activity of GPT was on zero day of anthesis. In the case of GOT, the highest activity was on zero day on a fresh and dry tissue basis. The activity, on fresh tissue basis, decreased on 3rd day and increased on 6th day. On 12th day, the GOT activity again decreased which was less than half that of 6th day. On 18th day onwards the GOT activity started increasing upto 24th day and afterwards decreased gradually.

In the case of whole tissues, both the activity of GPT and GOT was very low at the day of anthesis and increased gradually upto 6th day. The increase was steep afterwards reaching a peak on 18th day for GPT and 24th day for GOT and decreased gradually in subsequent stages.

A comparison of GPT and GOT at a particular stage or development showed that the activity of GOT was much higher in all stages of development on a per gram fresh, dry and on a whole tissue basis (Table 13).

(c) Embryo

On 12th day the embryos were analysed without separating into testa and embryo, since the seeds were too small. On a fresh tissue basis, the activity of GPT by day 18th showed more than 50% increase than the 12th day embryo.

On 24th day the activity of GPT showed nearly four-fold increase than the 18th day embryo and registered a peak. After a period of 12th day in the 36th day embryo, the activity was reduced to about 45%, whereas on 48th day of anthesis the activity of GPT was increased to about 45%. The activity in the 60th day mature embryo showed only less than half the activity of the 48th day embryo.

The activity of GOT on fresh tissue basis showed gradual increase from 12th day and registered a peak on 36th day and again gradually decreased with maturity. On a dry tissue basis, the peak activity was on 24th day in the case of both aminotransferases. When the activity was expressed on a whole tissue basis, the pattern of changes was same as that on a per gram fresh tissues (Table 13).

A comparison of GPT and GOT at a particular stage of development showed that the level of GOT was much higher in all stages except day 24th. By the 60th day the aminotransferases registered comparatively lower values both on fresh and dry tissue basis.

(d) Testa

The testa was analysed from 18th day onwards. In testa also, there was appreciable amounts of activity especially during earlier days of development. Alanine aminotransferases showed highest activity on 18th day whereas, that of aspartate aminotransferase was on 24th day on a fresh and dry tissue basis. On a whole tissue basis, maximum activity of both aminotransferases was registered on 24th day (Table 13). In testa also aspartate aminotransferase showed much higher level of activity than alanine aminotransferase throughout.

On a per gram fresh tissue basis at a particular stage of development, leaf tissue had relatively higher GPT activity than the other tissues. This was true before

development of the embryo (upto 12 days). By the time embryo development was sufficient to permit sampling this tissue became more active in the GPT concentration. In the later stages of development the leaf tissue was comparatively low in activity than embryo.

GOT on the other hand had a different profile. In the early stages, pod had more activity or as active as the leaf. At later stages, though embryo still accounted for the highest activity, the other tissues had activities which were higher than GPT in the corresponding tissues.

Changes in protein

The protein was estimated in the crude homogenates as described earlier and the values are represented on the basis of concentration and contents and presented in table 14 and figures 33 and 34.

Table 14

Figures 33 and 34.

Concentration

(a) Leaves

In leaves the protein concentration were analysed upto 48th day after anthesis. The protein level on zero day of anthesis was 30.83 mg on a fresh weight basis which slightly decreased on 3rd day of anthesis. On 6th day the amount of protein increased by more than 50% of the 3rd day sample. The maximum concentration of protein was on day 18 and decreased in subsequent stages. The 48th day protein concentration was negligible when compared with other stages. The pattern of changes was similar on a dry tissue basis also.

Table 14 : Changes in Protein and Specific activity of GPT and GOT in different plant parts following anthesis in the winged bean

Days after Anthesis	Tissue Analysed	Protein, mg.			Specific activity	
		Per g. fresh tissue	Per g. dry tissue	Per whole tissue	GPT	GOT
0	Leaf	30.83±0.56	155.34±2.85	17.73±0.32	2.94±0.10	2.67±0.20
	Pod	23.22±0.70	287.06±8.68	1.07±0.03	2.24±0.16	7.40±0.30
3	Leaf	27.71±0.88	138.38±4.44	17.76±0.56	7.74±0.40	4.17±0.35
	Pod	19.13±1.33	204.37±14.22	2.39±0.16	1.31±0.18	6.52±0.21
6	Leaf	43.42±4.93	215.41±24.47	29.87±3.39	6.91±0.09	3.24±0.34
	Pod	22.37±2.22	197.64±19.67	6.98±0.69	0.85±0.07	6.83±0.15
12	Leaf	36.13±2.24	172.88±10.73	25.14±1.56	8.11±0.93	3.69±0.30
	Pod wall	6.88±1.06	54.01±4.10	52.83±8.06	2.66±0.51	13.17±2.17
	Embryo	32.52±1.45	142.03±6.33	2.86±0.12	1.74±0.19	3.37±0.12
18	Leaf	50.62±1.87	232.94±8.64	36.19±1.34	2.04±0.23	2.82±0.30
	Pod wall	7.72±0.65	54.71±4.65	145.80±12.41	8.17±0.40	10.75±1.85
	Embryo	24.83±2.54	77.79±8.05	6.91±0.70	3.52±0.39	7.51±0.75
	Testa	26.91±0.82	98.78±3.02	2.90±0.09	1.40±0.26	2.67±0.32
24	Leaf	34.19±1.86	142.43±7.78	30.23±1.65	6.26±0.51	4.12±0.45
	Pod wall	10.71±1.23	68.72±7.90	313.30±36.06	3.26±0.40	9.03±1.75
	Embryo	53.80±2.34	147.45±6.42	17.05±0.74	5.88±0.89	4.31±0.49
	Testa	28.18±1.54	86.39±4.75	3.80±0.20	1.11±0.16	5.84±0.89
36	Leaf	15.46±2.59	60.99±10.22	15.41±2.58	1.97±0.28	8.77±1.56
	Pod wall	10.87±1.67	42.63±6.56	353.81±54.50	1.95±0.32	6.00±1.04
	Embryo	73.19±4.53	153.02±9.48	49.18±3.04	1.95±0.17	3.38±0.28
	Testa	11.79±1.21	35.22±3.63	1.93±0.20	1.00±0.18	3.18±0.56
48	Leaf	1.20±0.10	2.60±0.23	0.79±0.07	2.06±0.29	4.43±0.68
	Pod wall	4.85±0.38	8.76±0.68	90.74±7.12	2.10±0.40	4.75±0.82
	Embryo	250.68±2.89	296.32±3.42	95.76±1.10	0.79±0.09	0.93±0.18
	Testa	8.38±0.42	9.93±0.50	0.47±0.02	1.47±0.23	3.12±0.51
60	Embryo	277.77±4.63	310.77±5.18	100.55±1.67	0.33±0.03	0.47±0.04
	Testa	6.49±0.76	7.32±0.86	0.34±0.04	1.04±0.05	1.48±0.20

(The values are the mean of a minimum of 6 individual determinations)

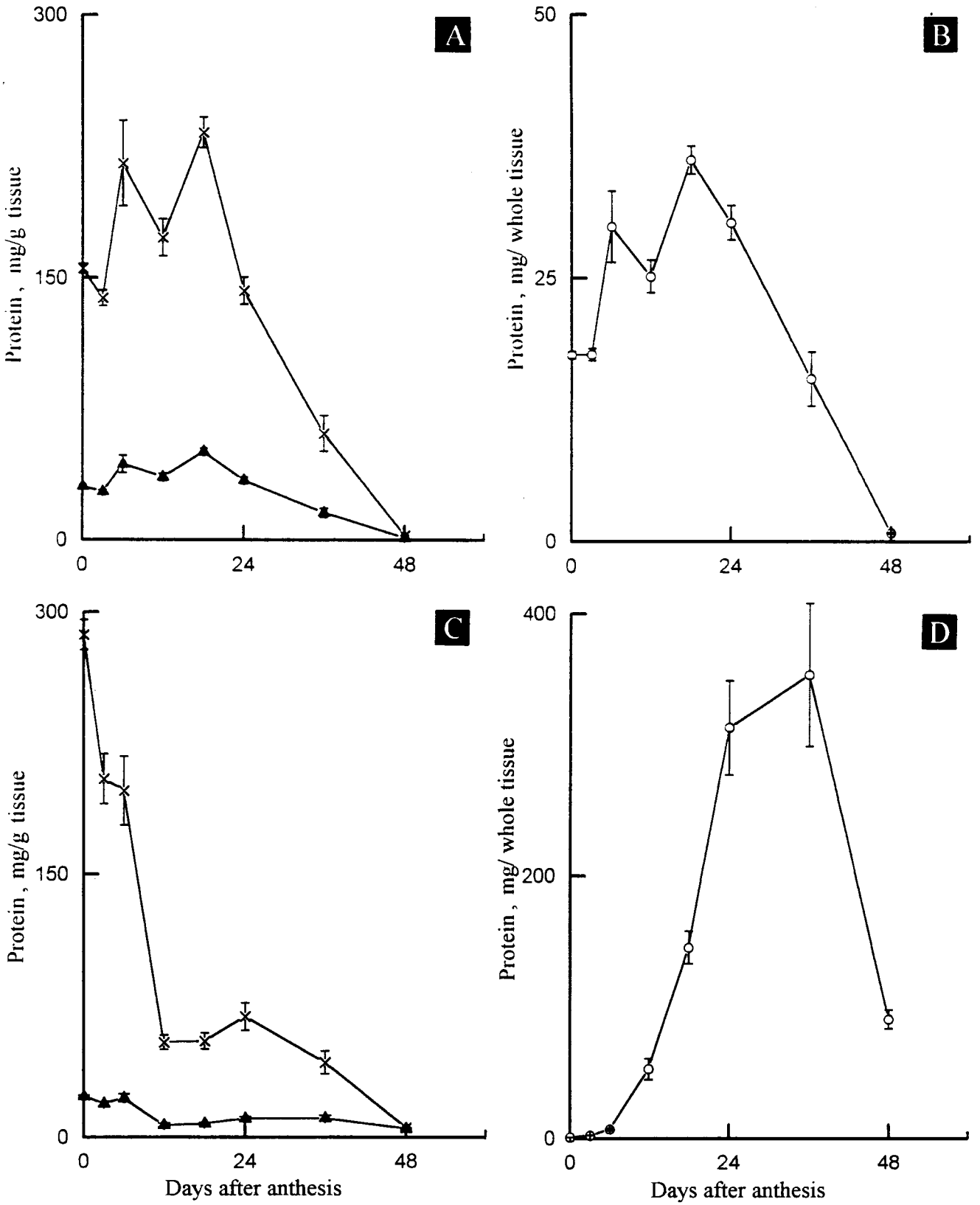


Figure 33. Changes in protein in leaf and pod/pod wall following anthesis in the winged bean. -▲-Fresh tissue -×- Dry tissue -○-Whole tissue.

A&B-Leaf C&D-Pod/Pod wall

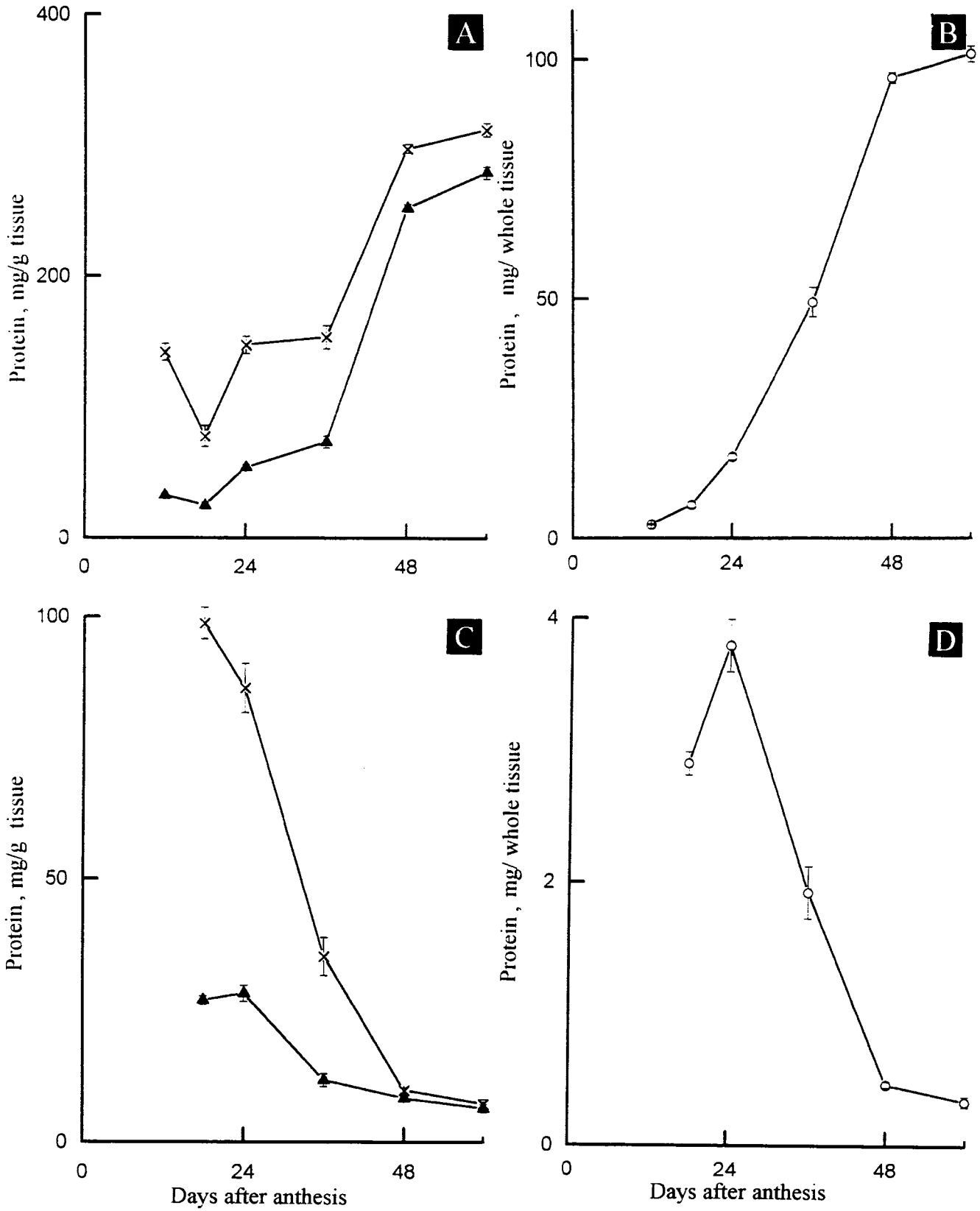


Figure 34. Changes in protein in embryo and testa following anthesis in the winged bean. –▲– Fresh tissue –×– Dry tissue –○– Whole tissue.

A&B-Embryo C&D-Testa

(d) Pod/pod wall

The protein concentration was high during the early stages of developing pods. The 12th day pod wall showed a marked decrease, to reach a value of less than one third of the protein of 6th day samples. Proteins showed only marginal increase from 12th day to 36th day. A decrease by half on 48th day to reach the lowest level was noticed, on a fresh weight basis. On dry tissue basis, the concentration of protein decreased gradually upto 18th day and then increased slightly on 24th day, which decreased afterwards to register the minimum value by the 48th day.

(c) Embryo

The seeds on 12th day showed higher protein level than the pods of previous stages on a fresh tissue basis. On 18th day, the protein concentration showed a slight drop. But from 18th day onwards, the protein concentration increased gradually and attained maximum value on 60th day. The increase in concentration upto 36th day was gradual and afterwards very steep.

(d) Testa

The maximum concentration of protein in testa was on day 24th on a fresh tissue basis whereas, on a dry weight basis the amount of protein was higher on 18th day and decreased gradually till 60th day.

Content

(a) Leaf

The protein content on zero and third day was equal and showed a steep increase by more than 50% on 6th day which slightly decreased on 12th day of

anthesis. The maximum protein content was on 18th day which was reduced marginally on 24th day with a further reduction by half on 36th day. The 48th day protein was very low when compared with other stages.

(c) Pod/pod wall

In the pod/pod wall, the increase in protein content was slow and gradual during the earlier stages of development. But from 12th day onwards, the increase was fast and attained highest amount on 36th day of anthesis. The 48th day protein content was only one fourth of the 36th day pod wall protein.

(c) Embryo

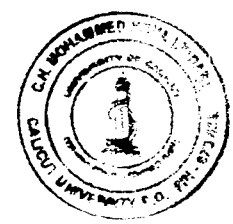
The increase in protein content was gradual in the embryo and highest amount was reported on 60th day of anthesis. From a minimum value on day 12 protein increased almost 6 fold by day 24. By the 36th day a nearly 3 fold further increase was seen. Forty eight day had nearly double this value and was nearly the same as on day 6th.

(d) Testa

The protein content of the testa was analysed from 18th day onwards. The increase was gradual and maximum amount was reported on the 24th day and then decreased gradually in subsequent stages.

Relative protein concentration among tissues during development

Comparing the concentration of protein among the tissues at a particular stage showed that on a fresh tissue basis both leaf and pod had nearly equal protein values. By day 3 the level was lower in the pod. Leaf overtook by the 6th day to almost double the level. On day 12 both leaf and embryo had nearly identical



values but pod wall had comparatively very low values. Leaf maintained the high level by 18th day followed by embryo and testa. Pod wall was low in protein. By 24th day, embryo had higher protein concentration than all other tissues, pod wall maintaining the low level. By the 36th day leaf, pod wall and testa had nearly the same levels of protein but embryo had 5-6 time higher values. The most conspicuous stage was 48th day with the highest value in embryos and negligible value in other tissues.

Specific activity

(a) Leaf

The specific activity of alanine aminotransferase was highest in the 12th day leaf whereas, that of aspartate aminotransferase was on the 36th day leaf after anthesis.

(b) Pod/pod wall

In the pod/pod wall, the highest specific activity of alanine aminotransferase was on 18th day while aspartate aminotransferase showed highest activity on 12th day.

(c) Embryo

The specific activity of alanine aminotransferase was highest on day 24. Aspartate aminotransferase showed highest specific activity on 18th day and decreased gradually.

(d) Testa

In the testa, the highest specific activity of alanine aminotransferase was on day 18 while that of aspartate aminotransferease was on 24th day.

Discussion

Fresh weight and Dry weight percentage

The fresh weight of the axillary (source) leaves increased with maturity and attained maximum on 36 day of anthesis and subsequently decreased. In dicot leaves, most cell divisions stop well before the leaf is fully grown and the 80% of the final leaf expansion is caused solely by the growth of preformed cells and this growth occurs for the entire leaf area (Dale, 1988). The gradual increase in leaf fresh weight observed here during development of fruit may be due to this growth and expansion the lamina with maturity and very high photosynthetic activity. The dry weight percentage was almost uniform upto 18 day of anthesis and then increased. The increase was most prominent after 36 day due to the rapid decrease in moisture content associated with maturity and senescence.

In winged bean, it was observed that the axillary leaf (source leaf) expands simultaneous with the flower / fruit development and got abscised before the pods started to ripen. Woomer *et al.* (1978) experimenting in Hawaii, observed that the leaves of a particular variety of winged bean became chlorotic during pod filling as the leaves of all the other varieties remained green. According to Kelly and Davies (1988), as reproductive structures became strong sinks, vegetative organs, somehow also become weaker ones. In roots, loss of sink strength is accompanied by decreasing transport of mineral nutrients and cytokinins upward through the xylem. It is quite likely that a decreased cytokinins supply to leaves is partly responsible for the start of leaf senescence.

The increase in fresh weight of pods was slow during the earlier stages, but the pod wall showed rapid increase after 12 day and attained 90% of its total fresh weight by the end of 24 day probably due to very high sink activity, while the

embryo on that day showed only below 50 % of its total fresh weight. The fresh weight of the pod (pod wall + seeds) was maximum on 36 day of which 82% fresh weight was attained on 24 day. After 36 day of anthesis, the fresh weight showed a rapid decrease due primarily to loss of moisture accompanying maturity. According to Bisson and Jones (1932) and Flinn *et al.(*1977), working on garden peas, the early increase in fresh weight of the fruit was due almost entirely to the growth of fleshy pod wall, which reached their maximum fresh weight while the peas were still very small. In a report of the National Academy of Sciences (Anonymous, 1975) and studies of Pospisil *et al.* (1978), the development of pods and winged bean is very fast and occurs in two stages. The longitudinal growth of pod lasts 16 – 20 days in the course of which the pods attained their full length; and additional 45 days or so is needed for the pods to mature and the seeds to ripen and dry. Data and Pratt (1980) also studied the pattern of pod growth and development at various ages after anthesis in winged bean. Pod growth in length, width and fresh weight and seed growth in length, width and fresh weight showed sigmoid growth patterns as observed in snap beans by Mitchel *et al.* (1951) and Watada and Morris (1967). Studies by the present author on V16 variety also agrees with the sigmoid pattern of growth curve (Figure. 17)

There was a lag period in fresh weight increase both in pod wall and embryo during development. This period was between 24 to 36 day in pod wall and 18 to 24 days in the embryo. Carr and Skene (1961) called this phenomenon as 'diauxic' meaning bi phasic. Data and Pratt (1980) reported this phenomenon in winged bean seeds. An interpretation by Smith (1973) was that the lag marks basic changes in cellular activity and coincides with the end of cell division in the cotyledons and with a transition from a phase of expansion dominated by solute accumulation

to a non-expansive one in which insoluble reserves start to accumulate.

The growth of seed coat is completed considerably in advance of that of the embryo (Pate and Flinn, 1977; Bewley and Black, 1994). Here the seed coat showed 83% of its total fresh weight by the end of the 24 day. According to Copeland and Mc Donald (1995), after fertilization more fresh weight is in the seed coat and later only the embryo surpasses the testa weight.

The drying-out of the pod wall in its later life causes major losses in fresh weight. According to Salisbury and Ross (1991) nitrogenous products such as amino acids and amides, dry matter and minerals also decrease due to extensive transport associated with desiccation so that more than a loss of water is involved. But, water losses in the seeds are partly compensated for by dry weight gains due to deposition of reserve materials in the cotyledons. The dry weight of embryo on 36 day was about 48% which almost doubled on 48 day, while in the pod wall only 55% dry weight was noted on 48 day. According to Copeland and Mc Donald (1995), when the seed possess its maximum dry weight, the seed attains 'Physiological maturity'; desiccation starts only then.

Changes in Total Sugars

Sucrose, the major form of carbon translocated in plants is the principal product of photosynthesis and can account for a large proportion of the total CO_2 absorbed by a plant during photosynthesis. More than 75% of the final seed carbon usually comes from direct transfer of current photosynthate from the source leaves (Ho, 1988; Peoples and Gifford, 1990). And for this smooth translocation of the photosynthates, a highly specialized cell layer, called the 'Para Venal Mesophyll' (PVM) is present in winged bean leaves which is presumably associated intimately with assimilate transport and compartmentation (Giaquinta, 1983).

In the present study it was seen that the concentration and content of total sugars in the leaves increased gradually with development and reached the maximum on 24 day after anthesis and steeply decreased afterwards reaching a minimum by the 48 day. According to Salisburry and Ross (1991), as leaves grow, their ability to photosynthesize increases until they are fully expanded; then it begins to decrease slowly due to senescence ie, chlorophyll breakdown and loss of functional chloroplast. Gan and Amasino (1997) suggested that leaf senescence is not simply a degenerative process, but is also a recycling process in which nutrients are translocated from the senescing cells to the developing seeds or storage tissues.

The developing fruit is almost solely dependent on current photoassimilates from the source leaves; *in situ* photosynthesis by the green tissues of fruit appears to be of uncertain significance. However, its main contribution is apparently through the refixation of the respiratory CO_2 of the developing seeds and the pod tissue itself. (Pate and Flinn, 1973; Oliker *et al.*, 1978 *b* ; Sheoran *et al.*, 1987).

The concentration and content of total sugars in the pod / pod wall increased gradually upto 24 days after anthesis and decreased. This decrease may probably be due to the lesser availability of photosynthates from the senescing source leaves. The drastic decrease in total sugars in the pod wall after 36 day of anthesis may be mainly associated with pod wall senescence. Bisson and Jones (1932) experimenting with garden peas and found maximum sugar concentration (dry weight basis) on 24 day pod wall which declined afterwards. According to him this was probably due almost entirely to translocation to the peas rather than transformation into other classes of carbohydrates in the pods. Data and Bautista (1983) studied the chemical changes in winged bean (C.V. TPT – 2) pods / seeds during development and found that total sugars in the pericarp increased upto 25 days after anthesis

mainly due to an increase in non reducing sugars and subsequent decrease after 25 days was due to decrease in both reducing and non reducing sugars.

The higher content of total sugars on 24 and 36 day was due to the higher fresh weight of the massive pod wall. Developing legume fruits seem to represent a well balanced system in which the two process of phloem unloading from the seed coat and solute uptake by cotyledons co-operate in making possible the growth of a developing seed. It would seem that the part played by leaf and pod wall in the supply of nutrient to the developing seed increases as the seed develops, becoming more important after cotyledon initiation. Hardham (1976) noticed a large increase in the amount of xylem and phloem and in the number of phloem transfer cells in the funicle of *Pisum sativum* after the endosperm has been assimilated. The studies of Wolswinkel and Ammerlaan (1983) in *Vicia faba* proved that after the release of assimilates into the seed coat apoplast from the unloading sites, sucrose is more intensively taken up by seed coat tissues than amino acids. From the results of V16, it is evident that the seed coat was highly active in supplying nutrients upto 36 day after anthesis and afterwards its activity decreased due to the lesser availability of photosynthates and also due to its own senescence. It is known that the seed coats of *Pisum sativum* acquire resources of starch, protein and minerals which are mobilized as the seed coats senesce and as the embryo matures (Murray and Collier, 1977; Hocking and Pate, 1977).

In the embryo of V16, the concentration of total sugars was high during the earlier stages and attained maximum by day 18 which showed more or less the same concentration on 24 day and decreased after wards reaching 8.4 % on 60 day on a dry weight basis. It has been observed that in garden pea free sugars achieve an early maximum in seed growth but then fall slowly once starch synthesis gets

underway (Pate and Flinn, 1977). During development of pea and other leguminous seeds, there is a rapid rise in starch content accompanied by a fall in sugars. (Bisson and Jones, 1932; Mc Kee *et al.*, 1955; Sehgal *et al.*, 1987). Data and Bautista (1983) found that starch content in winged bean (C.V. TPT 2) seeds continued to increase upto 40 days after anthesis but declined then reaching about 7.5% on 60 day after anthesis. Contrary to the findings of above authors, Garcia and Palmer (1980) found no starch in the seeds at full maturity whereas, Kamaladevi (1985) reported 0.28% starch in mature dry winged bean seeds of PT 3.

According to Kadam *et al.* (1982) starch and sugars are transient reserve materials, which are utilized later during seed maturation. Significant accumulation of protein and lipids occurs in winged bean during this period. Possibly, in the later stages of seed maturation, winged bean does not receive adequate supplies of carbohydrates from the parent plant, and sugars and starch are utilized for the synthesis of protein and lipids. Data and Bautista (1983) found accumulation of fat during development in the winged bean seeds and reported about 18% on a dry weight basis at the end of 60 days of anthesis.

Pospisil *et al.* (1971) also reported about 15-20% lipids in mature dry winged bean seeds. The sharp fall in total sugars after 24 day in V 16 may be due to the conversion of sugars into lipids. The storage of fatty acid as a seed reserve rather than carbohydrate is a way of maximizing the quantity of stored energy in a small volume of tissue (Slack and Browse, 1984).

Changes in Total Phenolics

In the leaf and embryo, the concentration and content of total phenolics decreased with maturity whereas in pod / pod wall and testa, the concentration and content of total phenolics increased. Kadam *et al.* (1982) followed the changes

in chemical composition of winged bean embryos at different stages of seed maturity. They found that reduction of polyphenol content was observed at all stages of developing winged bean embryos. In the present study it was found that in the leaf and embryos, the concentration and content of total phenolics decreased with maturity and its presence in the mature embryo was least when compared to the other parts studied.

Phenolic compounds play a defensive role in plants (Haslam, 1979; Harborne, 1988). Kozlowska *et al.* (1983) and Iwanowska *et al.* (1994) studied its histological localization in the hulls of *Brassica napus* (L.) during different stages of embryo genesis and seed maturation and suggested that this confers protection on the maturity of the seeds until germination. As phenolic compounds in seed coat act as allelopathic agents against microorganisms, they may act as inhibitors to fungal growth and seed germination under humid storage condition (Halloin, 1986; Gadzala and Zobel, 1993; Mohamed-Yasseen *et al.*, 1994 and Graven *et al.* 1996). Winged bean plants, fruits and seeds are free from serious diseases and pests (Anonymous, 1975). The resistance against pests and diseases may be due to the presence of phenolic compounds especially in the seed hull, fruit wall and leaves.

Seed coat imposed dormancy is a characteristic feature of the leguminous seeds and it is well established in the winged bean seeds (Tran and Cavanagh, 1984). Several authors have studied the relation between seed coat-imposed dormancy and the presence of phenolic compounds in the seed coat. According to Gillikin and Graham (1991), the impermeably thick seed coats are created by the oxidation of phenolic compounds by polyphenol oxidase or peroxidase and majority of peroxidase activity in soybean seed was localized in the seed coat. This is in close agreement with the variety V16, where the concentration of total phenolics was

highest in the seed coat. Here the percentage of imbibing seeds was only 32% while delayed and scarified seeds constituted 20 and 48% respectively {Table 3).

Free Amino acid Spectrum

Fruit development and growth are dependent on photosynthetic carbon dioxide fixation in leaves and translocation of sucrose, amino acids and organic acids to the immediate fruit cells. The changes in protein composition and amino acid distribution in the fruit will be, therefore, reflected in amino acids in the leaf (Ashley, 1972; Ho, 1988). Amino acid synthesis is not confined to leaves, but occurs in other tissues as well as transfer among tissues can occur. Roots are capable of synthesising a number of amino acids (Nagl, 1979). The non-photosynthetic fixation of carbon dioxide by enzymes in legume roots and nodules appears to play a key role in the synthesis of carbon skeletons for amino acid synthesis, respiratory substrates etc. (Peoples and Gifford, 1990). In addition to leaf, pod is committed to the nourishment of the enclosed developing seeds (Pate, 1975). Raacke (1957) suggested that nitrogenous compounds translocated from leaves were assimilated into pod proteins which in turn served as a nitrogen source for the enclosed seed.

In general, the changes in concentration of most of the amino acids was not consistent in each stage of each tissue. Also, the data for free amino acids gave no clear indication that changes in their distribution pattern confirmed to definite families of amino acids or to intra familial transformations. Glutamic acid was the most abundant amino acid at all stages in leaf, pod wall, embryo and testa. The concentrations of aspartic acid, alanine, glycine, threonine, leucine(s), tyrosine, phenylalanine + tryptophan and lysine were also very high. The aromatic amino acids and phenolic compounds arise from a common pathway, the shikimic acid

pathway (Gilchrist and Kosuge, 1980). The higher concentration of phenolics and the much better presence of aromatic amino acids in most of the stages of all the tissues clearly justifies it (Table 11A, B and 12). Asparagine + glutamine, proline, arginine and the sulphur amino acids were the limiting amino acids and the failure to detect these amino acids in some cases could be interpreted as being due only to the lack of their build up in concentration sufficient to be detected by the technique employed; they were no doubt available for tissues since they enter the composition of proteins. Ekpenyong and Borchers (1982) analysed the amino acid profile of winged bean variety TPt – 2 and found that all the protein amino acids are present in fairly good concentration in young and mature leaves, pod wall and embryo.

Variation in the proportion and composition of free amino acids was seen with the age of the leaf. Aging of the leaves and senescent changes in amino acid pool of the leaf is the functional relationship between the leaf and sink organs.

The V 16 variety studied here showed differences in amino acid composition especially between the seed, pod and embryo. Murray (1983) reported that in garden peas asparagine was prominent throughout in the pod wall while alanine, glutamine and valine were prominent in earlier days and then declined. In the young embryos, alanine and glutamine were the most prominent while seed coat showed alanine, glutamine and glutamate as the prominent amino acid in the earlier days. In an earlier investigation of Murray (1979) extensive and selective metabolism of amino acids take place in the seed coat prior to the release of amino acids. Murray and Kennedy (1980) and Peoples and Gifford (1990) suggested that the translocated substances can be transferred during the passage across the seed coats. A range of amino acids can be synthesised in the seed coat. The compounds

separated from the seed coat inner surface are, therefore, derived from, but not identical to the material unloaded from the sieve tubes into the seed coat tissue. This may reflect the differences in the metabolic activity of the seed coat, which could affect both conversion of incoming amino acid as well as amino acid released from protein break down in the seed coat. Wolswinkel (1992) commended that amino acid composition of the amino fraction released from the seed coat depends on the species and, to some extent as the stage of development.

Total free amino acids

As leaves grow, their ability to photosynthesize increases until they are fully expanded; then it begins to decrease slowly due to senescence. Breakdown of protein during senescence of the leaves could produce amino acids for translocation to the developing seed. Pate *et al.* (1974) observed that the amino acid level increased in the phloem sap during leaf senescence.

The concentration of total amino acid was higher in the earlier stages in leaf, pod / pod wall and testa which decreased with maturity. While it was highest in the 12 day embryo (testa + embryo) it slightly decreased on 18th day but again gradually increased reaching a maximum by 36th day. On a content basis, the total free amino acid showed gradual increase in pod wall upto 24 th day and for embryo upto 36 th day while in testa it was maximum on 18 th day and decreased. The leaf showed gradual decrease upto 12th day and then increased on 18 th day which again decreased afterwards.

Storey and Beevers (1977) found that in *Pisum sativum*, the protein and amino acid content of leaf and pod wall in the earlier stages was higher which decreased in aging leaf and pod wall while it increased in the developing seed. According to them free amino acids did not accumulate in the senescing leaf or

pod wall when protein was degraded in each organ. It was suggested that these amino acids were quickly metabolised *in situ* or translocated to the developing seed.

Murray (1979) while studying the nutritive role of seed coat of garden pea found that the free amino acid content of seed coat was higher during the early stages, reaching a maximum by the 15 th day of anthesis. Similarly, the maximum free amino acid content of cotyledons occurs shortly after that of the seed coats, just preceding a phase of exponential increase in net synthesis of proteins. Developing pea cotyledons are the site of depositions of large amounts of proteins. These proteins are synthesised over a relatively short period of time thus creating a demand for rapid supply of amino acids (Basha and Beevers, 1976). The rapid decrease in amino acid concentration and content after 36 th day in leaf, pod wall and testa and the rapid increase in protein after 36 th day (Table 14) in embryo clearly justifies the above authors' view.

Changes in Protein

During development, seeds characteristically synthesize relatively large quantities of food reserves which are mobilized following germination and their catabolites are used to support the growth of the seedling until it can established itself as a photosynthesizing, autotrophic plant. Thus, during development, the metabolic mode of the seed is largely anabolic, and following germination, catabolic; interpolated between these two events is maturation drying (Bewley, 1995).

The biosynthesis of storage protein in seeds occurs during the last two thirds of their development commencing after cell division and formation of the embryo body is completed and ceasing during the later stages of maturation drying (Larkins,

1981; Higgins, 1984). A quantitative analysis of m-RNA levels during seed development in soybean cotyledons showed that its levels are low in amounts during early stages which rise to peak at the mid maturation stages, the time of maximum protein deposition and finally the m-RNA levels declined as the seed begins to mature and dry out (Bewley and Greenwood, 1990).

The increased protein content of the developing seed is largely at the expense of nitrogen derived from the senescing mother plant, including the pod, although nitrogen fixation by root nodules during reserve deposition may increase this supply (Bewley and Black, 1983). Pate *et al.* (1974) observed that the increase in amino acid levels in phloem sap of legumes during leaf senescence is consistent with this concept. According to Storey and Beevers (1977) the accumulation of protein in the developing cotyledon proceed simultaneously with depletion of proteins in the subtending leaf and the pod. They found a close relationship between protein depletion and proteolytic activity in the pod and suggested that in the developing embryo, protein mobilization may be controlled by the level of proteolytic enzymes.

In the present studies in V 16 variety showed gradual accumulation of proteins in the embryo, attaining more than 95% by day 48th. Simultaneous with accumulation of proteins, the leaf, pod wall and testa showed gradual depletion in their protein level which was faster after 36th day. As mentioned earlier, the free amino acid levels of the leaf, pod wall and testa also showed decrease, which was faster after 36th day, but showed an increase in the embryo. Data and Bautista (1983) studied the chemical changes in developing pod of winged bean and found that in the embryo, the percentage protein upto 25 days after anthesis was more or less the same which slowly increased in the later stages of development while the

protein content (%) of the pericarp decreased rapidly.

It would seem that the part played by leaf and pod wall in the supply of nutrient to the developing seed increases at the seed matures, becoming more important after cotyledon initiation. Hardham (1976) noticed a large increase in the amount of xylem and phloem and in the number of phloem transfer cells in the funicle of *Pisum sativum* after the endosperm has been assimilated. Murray (1979) found that in *Pisum sativum* the protein content of the seed coat was maintained at a higher value throughout the period of rapid cell expansion (earlier stages) in embryo. It is known that the seed coat acquire reserves of starch and protein which are mobilized as the seed coat senesce.

It was found that in V 16 variety, the content of protein was much higher in the leaf (upto 24 days) and pod wall (upto 36 days) than the embryo and testa. The protein content of the pod wall on 24 th and 36 th day was about 20 times higher than the embryo but the percentage content was higher in the embryo. Martin (1978) in Puerto Rico reported by analysing 32 accessions of winged bean, that bulk of the dry weight was in the pod wall and not in the seeds. In the young whole pod (10 cm) and maturing pod (14 to 19 cm), much more protein was present in the pod wall than in seeds contained there in, although the percentage content of protein was higher in the seed than in the wall.

Alanine and aspartate aminotransferases activities in the winged bean plant parts following anthesis

In winged bean both alanine aminotransferase and aspartate aminotransferase activities were assayed in axillant leaves, pod / pod wall, embryo and testa.

In an investigation of enzyme and protein changes occurring during seed

development in *Lolium temulentum* , Hedley and Stoddart (1972 *b*) reported that alanine and aspartate aminotransferases showed similar pattern of activity which corresponded closely to the changes observed in protein synthesis during maturation of the seed. Both enzymes showed two peaks of high activity at the second and fourth week stages after anthesis and these coincided with the periods of maximum protein synthesis. In view of this close relationship, which was also observed in a similar study of enzyme and protein changes in developing *Lolium* leaves (Hedley and Stoddart (1972 *a*), these workers have suggested that alanine and aspartate aminotransferases may exert an important role in regulation of protein synthesis and some constituents for which some amino acids serve as precursors. When a comparison was made between the aminotransferases and protein contents of the embryo upto 36th day, the activity of both GPT and GOT were very high in embryo and the protein content of the embryo at this corresponding developmental stages exhibited only a slight increase probably due to the high metabolism of the amino acids resulting in low accumulation of proteins. However, after 36th day, despite the low activity of these enzymes, proteins get accumulated significantly indicating comparatively reduced metabolism and the tissue become storage in function and accumulation of storage proteins. On the other hand high activity of both GPT and GOT and a concomitant increase in proteins occur upto 24 days. During this period increased metabolic changes are expected because both source and sink are very active. So, after 24 days, the activity and protein content are considerably reduced showing negligible role of storage function. However, according to Murray (1987), the testa plays a critical role for the nutrition of the seed for the in early stages of development and later only as a protective covering.

Contradictory to the findings of Hedley and Stoddart (1972b) in *Lolium*, winged bean pods, embryo and testa showed more activity in aspartate aminotransferase (GOT) compared to less activity of GPT during development upto 24 days after anthesis (Fig 30, 31 and 32). However, corresponding changes were not observed in the concentration of aspartic acid, glutamic acid and alanine.

On a per gram fresh and dry tissues and on whole tissue basis, leaf tissue had higher GPT activity than GOT during the earlier days. The concentration of alanine and glutamic acid are found to be significantly reduced during the corresponding intervals probably due to translocation from the source tissue (axillant leaf) to the actively growing fruit (sink). However, the concentration of glutamic acid maintained its higher level. The photosynthetic origin of this amino acid and its immediate transamination due to high activity of GPT in these tissues cannot be ruled out in this context in winged bean. In *Vicia faba* high activity of alanine and aspartate of aminotransferases resulted in the contribution of large amount of alanine and aspartate from the abundant glutamic acid in leaf tissue as was reported by Kirk and Leech (1972) and according to these authors, alanine and aspartic acid are involved in the synthesis of all the other protein amino acids.

Murray and Kennedy (1980) studied the enzymes of nitrogen metabolism in two cultivars of pea seeds (c.v. Telephone and c.v. Melbourne Market) and reported that in the seed coat, maximum activities of alanine and aspartate aminotransferases were during the earlier stages of development. While in the cotyledons alanine aminotransferase activity showed maximum in the earlier stages aspartate amino transferase showed maximum activity only after 24th day of anthesis in both cultivars. In winged bean variety V 16, the embryo showed more GOT activity than GPT throughout the stages of development (except on day 24 th)

on a per gram fresh and dry and whole tissue basis (Figure. 31). Also, the maximum activities of both aminotransferases in the embryo clearly exceeded the seed coats.

In *Phaseolus vulgaris* when the purified GPT was assayed, it did not use pyruvate as the oxoacid, but supported transfer of the amino group of phenylalanine to oxoglutarate (Anderson and Beardall, 1991). This aromatic amino acid serve as precursor for biosynthesis of phenolics. So it is inferred that the GPT activity may result in the transamination of phenylalanine and may indirectly involve in the phenolic production. However, in the present study, direct correlation cannot be drawn between the GPT activity and phenolic content. Notwithstanding, in pod wall and testa, maximum GPT activity follows a significant increase in the phenolic content especially during the late stages of development.

In Bengal gram increase in the activities of both GOT and GPT are reported during post anthesis development (Ghildiyal and Sinha, 1971). The activity of these enzymes in leaf also were very high. However, after 13 days seeds showed lesser activity of GPT and GOT. In winged bean difference in GPT and GOT activity during early days in leaf and pod was observed. In leaf the activity of GPT was higher than GOT, whereas, the reverse was the case with pods.

The high activity of both aminotransferases in the developing pods / embryos (Figures 30 and 31) upto 36 days, which results in the synthesis of alanine and aspartic acid is understandable because of the increased requirement of various amino acids for protein synthesis and some constituents for which some amino acids serve as precursors.

The specific activity of the enzymes in the leaf was higher in the early of stages(Table 14). The pod/pod wall showed higher GOT specific activity. In the case of embryo the specific activity of both GPT and GOT was higher upto 36th day

of anthesis and declined afterwards. Testa also showed high specific activities in the earlier stages which may be due to the enzymatically active proteins since considerable enzyme activity existed in these tissues. The decrease in both specific activities in the embryo in the late stages was mainly due to storage protein synthesis.

CHAPTER 7

Biochemical Studies in Three Types of Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) Seeds During Germination.

CONTENTS

Biochemical Studies in Three Types of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC) Seeds During Germination

Introduction

Winged bean seeds contain very high protein and they are comparatively rich in lipids. Starch and soluble carbohydrates are low. Hildebrand *et al.* (1981) reported in 20 genotypes of mature winged bean seeds that contained 2.8% starch. Microstructure studies on winged bean seeds (Saio *et al.*, 1983) revealed that starch granules were frequent in early stages of development, but with maturity of the seeds, the starch disappeared.

A number of studies exist on seed germination and reserve mobilization during germination and seedling growth. Mobilization of cotyledonary reserve protein is a major event during the germination of legume seeds. The amino acids generated during proteolysis of reserve proteins are translocated to and utilized by the embryonic axis. Following transport of a selected group of amino acids and amides to the growing seedling, further interconversion is required to provide the full range of amino acids and nitrogen components necessary for protein synthesis and growth. Dry seeds contain very little free amino acids. The growth of the embryo in the germinating seed is dependent on the supply of amino acids for its protein synthesis. The amino acid pool increases during germination. The main source of these amino acids is the storage protein, but its amino acids need not necessarily be the same as that of the newly synthesized seedling proteins and apparently interconvesion of the amino acids occur. Amino acid formation and transformation, therefore, constitute an important aspect of the metabolism of germinating seeds. An important reaction involving amino acids in plants is

transamination. Using preparation from seedlings, a number of amino acids have been shown to be transaminated in the presence of ketoacids (Forest and Wightman, 1972*a*)

The main objectives of this study is to examine the development of transaminase activity (GOT and GPT) in the non growing (cotyledons) and growing (plumule and radicle) tissues during germination of the 3 types of seeds and attempt to correlate the changes in enzyme activities with the changing composition of the free amino acid fraction and proteins from each organ. Also, total sugars are estimated simultaneously.

Material and methods

(These were as described in Materials and Methods)

Results

Changes in fresh weight in three types of winged bean seeds during germination

The values are represented in table 15 and figure. 35.

Table 15

Figure 35

Imbibing seeds

(a) Cotyledon

The fresh weight showed gradual increase upto 6 h, and then steeply increased on 12 and 24 h, of imbibition. After 24 h, the increase in fresh weight was slow. About 90% increase from the control was recorded at the end of 24 h, which reached almost 100% on 48 h. The increase in fresh weight at the end

Table 15: Changes in fresh weight in 3 types of winged bean seeds during germination

Time of imbition, h	Tissue analysed	Fresh weight, mg/tissue.		
		Imbibing	Delayed	Scarified
0	Cotyledon	252.25±7.76	252.25±7.76	252.25±7.76
2	Cotyledon	259.67±4.50	252.25±7.76	258.89±1.08
4	Cotyledon	265.00±2.44	257.57±6.12	278.22±4.67
6	Cotyledon	282.00±3.26	272.72±4.16	315.63±11.28
12	Cotyledon	406.01±2.45	320.68±2.81	418.50±7.10
24	Cotyledon	481.40±1.14	375.35±8.12	453.26±2.93
	Embryonic axis	11.00±0.69	11.00±0.18	12.00±0.31
48	Cotyledon	500.75±2.06	433.95±2.89	465.92±5.28
	Embryonic axis	13.80±0.28	13.00±0.50	15.70±0.14
72	Cotyledon	513.20±1.48	544.71±9.91	493.64±6.80
	Plumule	9.23±0.09	8.50±0.09	9.71±0.20
	Radicle	10.50±0.63	9.89±0.11	11.01±0.27

(The values are the mean of a minimum of 6 individual determinations)

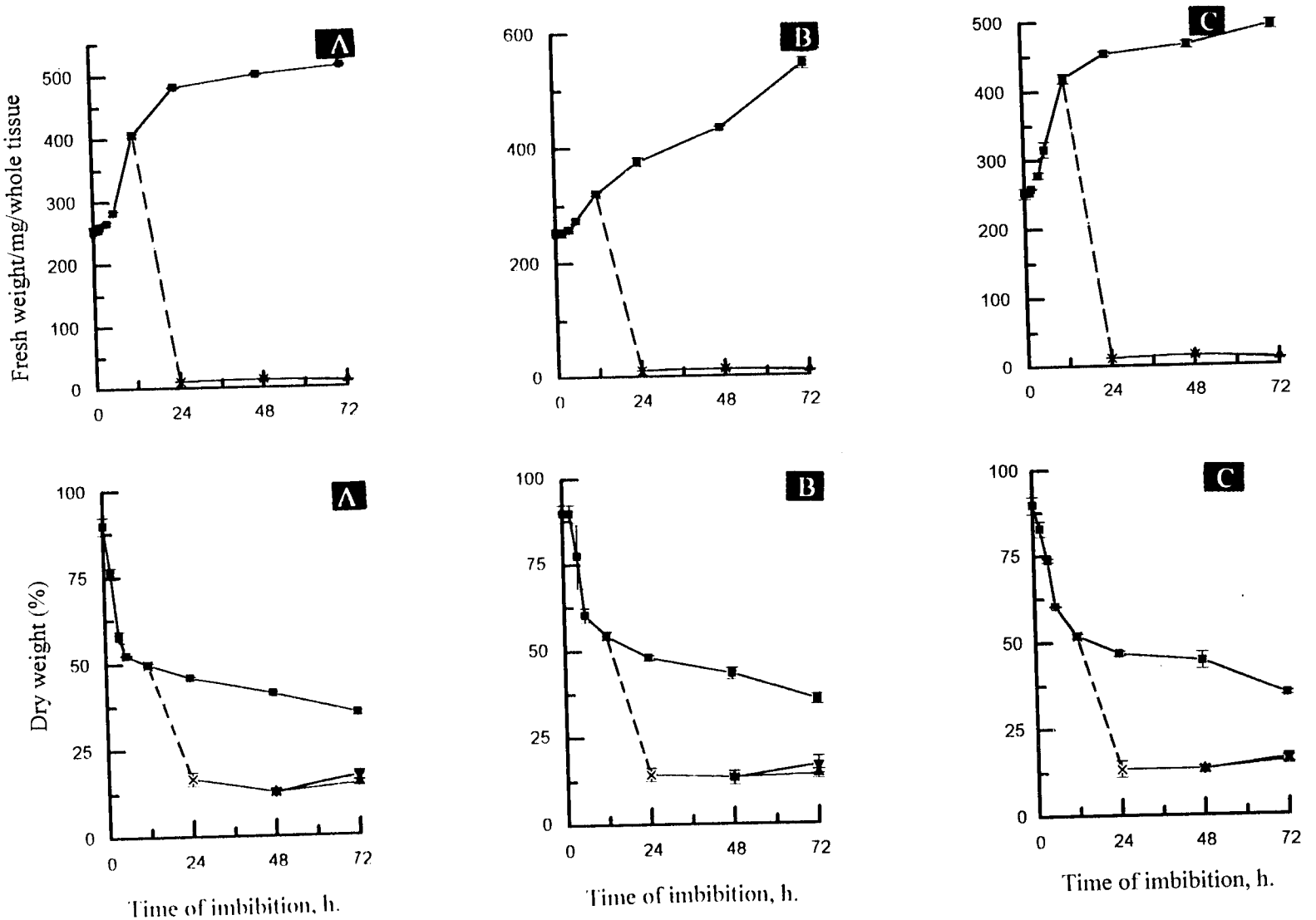


Figure 35. Changes in fresh weight (whole tissue) and dry weight (%) in three types of winged bean seeds during germination. A-Imbibing seeds, B-Delayed seeds and C-Scarified seeds.

-■- -Cotyledon -✱- -Embryonic axis -▲- -Plumule -▼- -Radicle

of 72 h was only marginal.

(b) Embryonic axis

The fresh weight of the embryonic axis was very negligible when compared with the cotyledons. The 48 h, embryonic axis showed about 25% increase in fresh weight from the previous stage.

(c) Plumule and Radicle

The fresh weight of the plumule was slightly lower than the radicle by about 15%. The total fresh weight of the plumule + radicle showed more than 40% increase than the 48 h embryonic axis.

Delayed seeds

(a) Cotyledon

The increase in fresh weight of the delayed seeds was slower than the imbibing seeds especially during the earlier hours. But after 12 h, the increase became faster and steep. More than 100% increase was noted at the end of 72 h when compared to the zero hour cotyledons.

(b) Embryonic axis

The fresh weight of the embryonic axis of the 24 h was same as that of the imbibing seeds, which increased to about 20% on 48 h. The fresh weight of the 48 h embryonic axis was lower than that of the imbibing types.

(c) Plumule and Radicle

In the case of delayed seeds also, the fresh weight of the plumule was slightly lower than the radicle by more than 15%. The total fresh weight of the plumule + radicle showed more than 40% increase from the 48 h embryonic axis.

Scarified seeds

(a) Cotyledon

The increase in fresh weight of the cotyledons was gradual upto 6 h and then steeply increased at the end of 12 h which again gradually increased in subsequent stages. At the end of 72 h, about 95% over all increase in fresh weight was noticed. The value was the lowest among the 3 types of seeds. The fresh weight increase in the cotyledons exceeded that of the delayed seeds in all stages except 72 h.

(b) Embryonic axis

The fresh weight of the embryonic axis on 24 and 48 h was higher in the scarified seeds than that of the imbibing and delayed types. The 48 h embryonic axis showed about 30% increase than the 24 h embryonic axis.

(c) Plumule and Radicle

In the case of scarified seeds also the plumule fresh weight was slightly lower (about 15%), than the radicle. The fresh weight of plumule and radicle together showed more than 30% increase from the 48 h embryonic axis.

Changes in dry weight in three types of winged bean seeds during germination

The values are represented in table 16 and figure 35.

Table 16

Figure 35.

Table 16: Dry weight percentage of 3 types of winged bean seeds during germination

Time of imbition, h	Tissue analysed	Dry weight (%)		
		Imbibing	Delayed	Scarified
0	Cotyledon	89.85±2.53	89.85±2.53	89.85±2.53
2	Cotyledon	76.18±1.60	89.85±2.53	82.91±2.21
4	Cotyledon	57.69±1.85	77.42±9.23	73.73±0.52
6	Cotyledon	52.43±0.20	60.33±2.05	60.30±0.15
12	Cotyledon	49.71±0.47	54.36±1.22	51.76±0.60
24	Cotyledon	45.78±0.44	47.97±0.75	46.76±0.71
	Embryonic axis	16.56±2.00	14.36±1.94	13.45±2.45
48	Cotyledon	41.09±0.36	43.33±1.68	44.74±2.42
	Embryonic axis	12.59±1.09	13.35±2.01	13.36±1.04
72	Cotyledon	35.12±0.62	35.62±1.41	34.99±0.51
	Plumule	14.90±0.86	14.23±1.38	15.80±1.18
	Radicle	17.29±1.34	16.85±2.47	16.49±1.28

(The values are the mean of a minimum of 6 individual determinations)

Imbibing seeds

(a) Cotyledons

The dry weight percentage of the cotyledons decreased corresponding with the progress of water uptake. On 24 h after contact with water, the dry weight of the cotyledon reduced to 50% with further reduction in subsequent stages and reached about 40% at the end of 72 h.

(b) Embryonic axis

The embryonic axis showed higher dry weight (nearly 25%), during 24 h of contact with water than 48 h embryonic axis.

(c) Plumule and Radicle

The dry weight percentage of the plumule was slightly lower than the radicle by about 16%. The total dry weight percentage of plumule and radicle together was more than 150% higher when compared to the 48 h embryonic axis.

Delayed seeds

(a) Cotyledons

The cotyledons of delayed seeds showed no change in dry weight at the end of 2 h of water contact. From 4 h onwards, the dry weight percent started decreasing gradually and about 30% reduction in dry weight was observed at the end of 12 h, which on 24 h was almost 50% and at the end of 72 h, the dry weight of the cotyledon was reduced to about 40%, the value being almost equal to that of the cotyledons of imbibing seeds.

(b) Embryonic axis

The dry weight percentage of the embryonic axis on 24 h was slightly

lower than that of the imbibing seeds. On 48 h, the dry weight percentage was slightly lower than the 24 h embryonic axis.

(c) Plumule and Radicle

The dry weight percentage of the plumule was almost equal to that of the imbibing seeds and was slightly lower than that of the radicle by about 20%. The dry weight percentage of the plumule and radicle together was about 130% higher than the 48 h embryonic axis.

Scarified seeds

(a) Cotyledons

The decrease in dry weight of the cotyledons of the scarified seeds was comparatively faster than the delayed seeds during the earlier hours. The dry weight percentage gradually decreased and attained about 50% at the end of 24 h. It further reduced on 48 h and 72 h. The 72 h dry weight was reduced to about 40% of the zero hour cotyledons.

(b) Embryonic axis

The embryonic axis on 24 h showed comparatively lower dry weight percentages than that of imbibing seeds, but was almost same as the delayed seeds. The dry weight of 48 h showed practically no changes from the 24 h embryonic axis and was equal to that of the delayed seeds.

(c) Plumule and Radicle

The plumule of 72 h showed higher dry weight than the plumule of imbibing and delayed seeds. Here the dry weight percentage of plumule and radicle was almost same. The dry weight percentage of plumule and radicle

together was more than 140% higher than the embryonic axis of 48 h.

Changes in total sugar in three types of winged bean seeds during germination

The values are represented in table 17 and figure 36.

Table 17

Figure 36

Concentration

Imbibing seeds

(a) Cotyledons

The concentration of total sugars in the cotyledons was high during the earlier stages of imbibition, which gradually decreased when imbibition progressed. On a per gram fresh tissue basis, the concentration of total sugars gradually decreased and at the end of 2 h, it was reduced to about 50% of its original concentration. A slight increase in concentration was noticed on 24 h and decreased gradually afterwards, reaching about one third of its original concentration at 72 h. On per gram dry tissue basis, the concentration of total sugars showed a gradual reduction upto 6 h and showed a slight increase at 12 h, which again increased on 24 h and decreased after wards. The 24 h sugar concentration was slightly higher than that of zero h sample.

(b) Embryonic axis

The total sugar concentration of the embryonic axis was slightly lower (20%) in 48 h than that of 24 h embryonic axis. But when calculated on the basis of per gram dry tissue there was practically no change in the concentration

Table 17. Changes in total sugar in 3 types of winged bean seeds during germination.

Time of Imbibition, h	Tissue analysed	Total sugars, mg								
		Imbibing			Delayed			Scarified		
		Per g fresh tissue	Per g dry tissue	Per whole tissue	Per g fresh tissue	Per g dry tissue	Per whole tissue	Per g fresh tissue	Per g dry tissue	Per whole tissue
0	Cotyledon	67.86±0.99	75.52±1.10	17.09±0.25	67.86±0.99	75.52±1.10	17.09±0.25	67.86±0.99	75.52±1.10	17.09±0.25
2	Cotyledon	54.96±3.16	72.15±4.16	14.28±0.82	67.86±0.99	75.52±1.10	17.09±0.25	60.51±0.78	72.98±0.95	15.66±0.20
4	Cotyledon	41.15±1.60	70.76±3.56	10.91±0.42	57.00±1.07	73.63±1.38	14.64±0.27	51.66±1.93	70.07±2.61	14.37±0.53
6	Cotyledon	35.73±1.25	68.16±2.40	10.07±0.35	40.12±2.24	66.50±3.73	10.94±0.61	39.80±2.21	66.10±3.63	12.56±0.69
12	Cotyledon	34.80±1.45	70.02±2.91	14.12±0.58	34.22±0.59	62.94±1.09	10.97±0.34	30.06±1.84	58.08±3.55	12.58±0.77
24	Cotyledon	37.12±3.00	81.07±6.54	18.26±1.47	36.81±1.36	/6./4±2.84	13.81±0.51	35.97±1.70	76.92±3.63	16.30±0.77
	Embryonic axis	5.43±0.19	32.82±1.14	0.05±0.00	4.38±0.53	30.49±3.72	0.05±0.00	3.56±0.45	26.57±3.6	0.04±0.00
48	Cotyledon	29.08±1.02	70.77±2.48	13.98±0.49	30.06±0.62	69.38±1.44	13.04±0.26	29.99±0.89	67.03±1.99	13.97± 0.41
	Embryonic axis	4.20±0.10	33.35±0.79	0.05±0.01	4.62±0.61	34.65±4.59	0.06±0.00	4.86±0.75	36.44±5.56	0.07±0.00
72	Cotyledon	21.00±0.95	59.80±2.72	10.77±0.48	22.07±1.19	62.36±4.00	12.02±0.64	21.29±1.17	60.86±3.35	10.50±0.57
	Plumule	3.96±0.17	26.59±1.20	0.04±0.00	3.63±0.35	25.53±2.52	0.03±0.00	4.27±0.70	27.05±4.48	0.04±0.00
	Radicle	3.37±0.05	19.48±0.30	0.04±0.00	2.96±0.13	17.58±0.80	0.03±0.00	3.20±0.66	19.45±4.06	0.03±0.00

(The values are the mean of a minimum of 6 individual determinations)

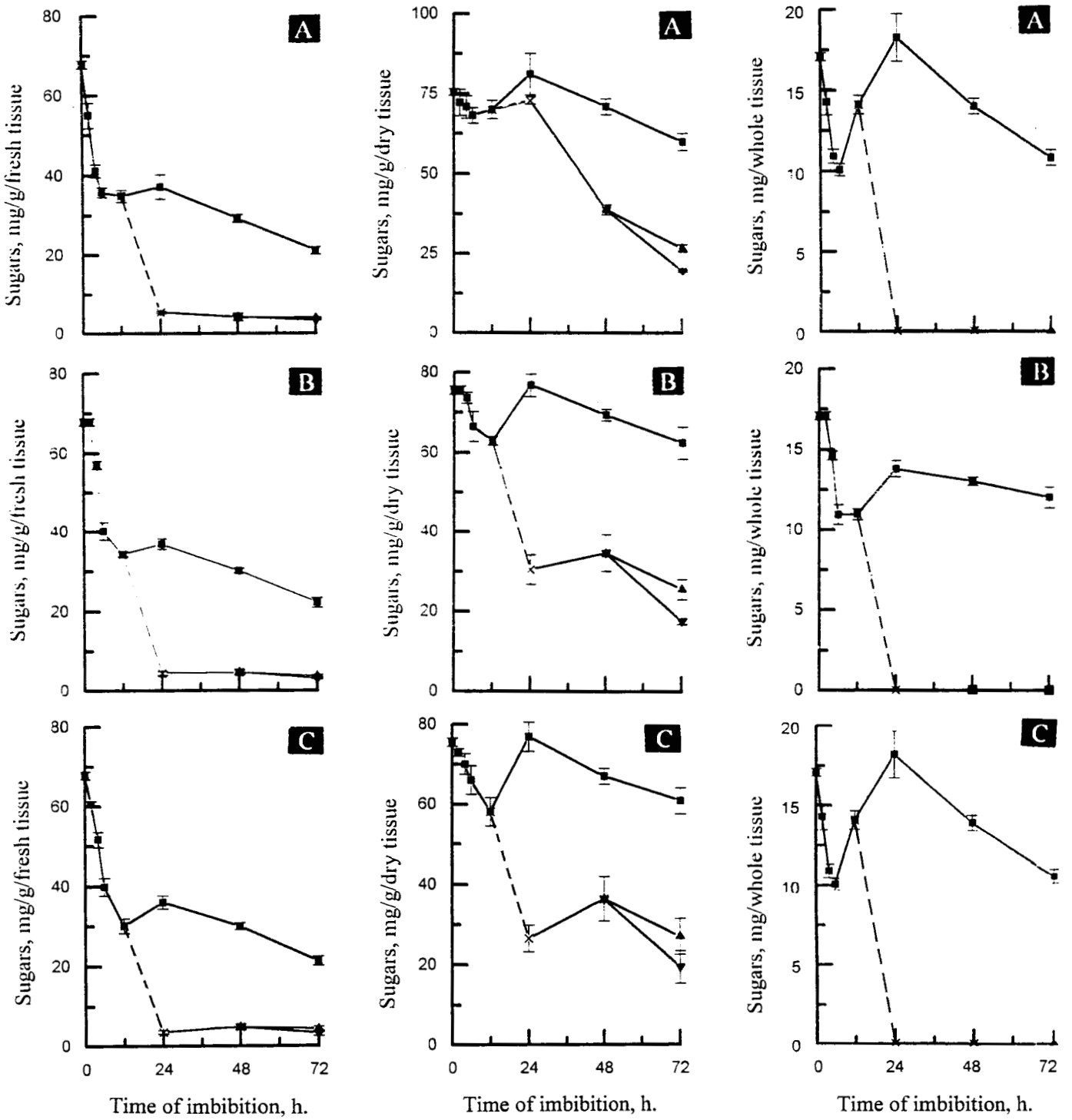


Figure 36. Changes in total sugars in three types of winged bean seeds during germination. A-Imbibing seeds, B-Delayed seeds and C-Scarified seeds.

-■- -Cotyledon -✳- -Embryonic axis -▲- -Plumule -▼- -Radicle

of total sugars in the 48 h embryonic axis.

(c) Plumule and Radicle

The concentration of total sugars of the plumule was about 15% higher than in the radicle on a fresh weight basis. The plumule and radicle together showed an increase of about 75% in total sugar concentration from the 48 h embryonic axis. On a per gram dry tissue basis, the plumule showed about 40% higher concentration of total sugars than the radicle. Similarly, about 40% increase in sugar concentration occurred in the plumule + radicle from the 48 h embryonic axis.

Delayed seeds

(a) Cotyledons

The cotyledons of delayed seeds followed the same pattern of total sugar changes observed in imbibing seeds. The concentration of total sugars gradually reduced and about 50% reduction in total sugar was registered at the end of 12 h of imbibition. It showed a slight increase on 24 h and again decreased in the subsequent stages, reaching about one third of its original concentration. On a per gram dry tissue basis, the concentration at 24 h was almost the same as that of the initial concentration, whereas the 72 h cotyledon showed about 20% reduction than the control.

(b) Embryonic axis

On a fresh weight basis, the embryonic axis of the delayed seeds at 24 h showed slightly lower concentration (about 25%) of total sugars with respect to imbibing seeds. The concentration of total sugars of the 2 h and 48 h embryonic axis was same in the delayed seeds. On a per gram dry tissue basis about

15% increase was observed in the 48 h embryonic axis from 24 h.

(c) Plumule and Radicle

In the case of delayed seeds also, the plumule showed more than 15% higher concentration of total sugars than the radicle on a fresh weight basis. But on a dry tissue basis, more than 30% increase in concentration was observed in the plumule. The plumule + radicle showed more than 40% increase in total sugar concentration from the 48 h embryonic axis on a fresh weight basis, while on a dry weight basis the increase was only about 25%.

Scarified seeds

(a) Cotyledons

The scarified seeds also followed the same pattern of total sugar changes as in imbibing and delayed seeds. The concentration of total sugars decreased gradually upto 12 h and showed a slight increase on 24 h and decreased in further stages. The 24 h sugar concentration was reduced to about 50% of the original. On a per gram dry tissue basis, the concentration showed a gradual decrease upto 12 h and then a rise at 24 h, which was almost same as that of the zero h cotyledons. About 20 % reduction in total sugars was noticed at the end of 72 h.

(b) Embryonic axis

The embryonic axis at the end of 48 h of imbibition showed an increase in sugars of more than 35% than the 24 h embryonic axis and was the highest among the 3 types of seeds. On a per gram dry tissue basis, the increase in the concentration of total sugars was about 25%.

(c) Plumule and Radicle

In the case of scarified seeds, the sugar concentration in the plumule was higher than the radicle on fresh weight basis which on a dry weight basis was more than 25%. The plumule and radicle together showed more than 50% increase in total sugars than the 48 h embryonic axis on a fresh tissue basis. On a dry tissue basis it was only about 30%.

Content

Imbibing seeds

(a) Cotyledon

The cotyledons of imbibing seeds showed a gradual decrease in total sugar, and became reduced to about 60% of the zero hour value at the end of 6 h. But on 12 h, the content of total sugars slightly increased and on 24 h, it was almost same as the total sugar content of the dry seed cotyledons. The content of total sugars decreased on 48 h and at the end of 72 h of imbibition, the content of total sugars was reduced to about 60% of the zero hour cotyledons.

(b) Embryonic axis

When calculated on a whole tissue basis the amount of total sugars in the embryonic axis was very negligible, since the tissue weight was too low. The total sugar content of both 24 h and 48 h embryonic axis was the same.

(c) Plumule and Radicle

The plumule and radicle showed the same amount of total sugars. About 60% increase in total sugar content was noted in the plumule and radicle together when compared to the 48 h embryonic axis.

Delayed seeds

(a) Cotyledon

The content of total sugar in the cotyledons of delayed seeds started decreasing gradually from 4 h, which on 24 h showed a slight increase and again gradually decreased in subsequent stages.

(b) Embryonic axis

The content of total sugar in the 24 h embryonic axis was same as that in the imbibing seeds. The 48 h embryonic axis showed 20% increase over the 24 h embryonic axis.

(c) Plumule and Radicle

The total sugar content of the plumule and radicle was 25% lower than that of the imbibing seeds. In the delayed seeds also, the content of total sugars was the same in the plumule and radicle. The total sugar content of the plumule and radicle together was also the same as that of the 48 h embryonic axis.

Scarified seeds

(a) Cotyledons

The cotyledons of scarified seeds was similar to the other two types of seeds in the content of sugars. On 24 h, the amount of total sugar showed an increase, but was lower than the dry cotyledons and decreased afterwards. Here also the total sugar content of the 72 h cotyledons was reduced to about 60%.

(b) Embryonic axis

The total sugar content of the 24 h embryonic axis was about 20% lower than that of imbibing and delayed seeds. On 48 h of imbibition, the total sugar

content of the embryonic axis became almost 3- fold and was the highest among the 3 types of seeds.

(c) Plumule and Radicle

The total sugar content of the plumule of scarified seeds was equal to that of the imbibing seeds, but higher than the delayed seeds. The content of total sugars of the radicle was only 75% of the plumule. The total sugar content of the plumule and radicle together was equal to the 48 h embryonic axis.

Changes in free amino acids

The values for individual amino acids and total amino acids (sum of individual amino acids) are represented in table 18 A to I.

Table 18 A to I

The most abundant amino acid was glutamic acid in all the tissues analysed at all stages. Detectable amount of methionine was present only in a few stages. Leucine and isoleucine were not clearly separated from each other on the chromatogram and were determined as a pair. For the same reason, phenylalanine+ tryptophan and asparagine+glutamine were determined together. The total free amino acids were also expressed by adding up the individual amino acids of each tissue of a particular stage.

Concentration

Imbibing seeds

(a) Cotyledon

In the cotyledons of dry seeds (zero hour, the control), glutamic acid was the most abundant amino acid followed by aspartic acid. Lysine closely followed

Table 18A. Changes in individual free amino acids in 'imbibing' seeds during germination

Time of imbibition, h	Tissue analysed	Amino acids, μ moles per g. fresh tissue																Total
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	2.53	1.12	0.61	3.66	1.96	2.27	2.14	0.31	1.89	ND	1.82	2.96	1.17	2.74	2.51	1.38	2.12	31.19
2	Cotyledon	2.55	1.16	0.65	3.78	1.95	2.30	2.25	0.32	1.96	ND	1.91	2.85	1.20	2.75	2.55	1.35	1.98	3151
4	Cotyledon	2.98	1.20	0.84	4.12	1.56	2.31	2.28	0.30	1.82	ND	2.15	3.17	1.24	2.84	2.60	1.27	1.61	32.29
6	Cotyledon	2.92	1.20	0.88	4.46	1.58	2.50	2.44	0.35	1.81	T	2.46	3.34	1.23	2.61	2.72	1.08	1.85	33.43
12	Cotyledon	2.63	1.11	0.73	4.68	1.69	2.47	2.82	0.40	1.83	T	2.66	3.39	1.58	2.72	3.06	1.15	2.03	34.95
24	Cotyledon	3.46	0.82	0.55	5.32	1.85	2.69	3.11	0.62	2.05	T	2.87	3.48	0.86	2.93	3.14	1.09	1.88	36.72
	Embryonic axis	0.31	0.14	T	0.54	T	0.30	0.42	T	ND	ND	0.34	0.18	T	T	0.26	ND	T	2.49
48	Cotyledon	3.60	0.71	1.22	6.85	2.32	1.18	3.48	1.39	1.12	0.65	3.41	2.88	1.53	3.85	2.89	0.21	2.58	39.87
	Embryonic axis	0.39	0.38	T	0.87	0.15	0.46	0.66	T	ND	ND	0.63	0.52	0.26	T	0.37	T	T	4.69
72	Cotyledon	4.31	0.61	1.74	7.82	2.14	0.85	4.89	1.62	1.37	1.80	4.43	3.52	1.14	2.84	3.11	0.23	3.67	46.09
	Plumule	0.62	0.28	0.42	1.33	T	T	0.54	ND	ND	T	0.77	0.52	T	0.37	0.48	T	ND	5.33
	Radicle	0.79	0.37	0.39	1.70	T	0.21	0.72	T	T	T	0.92	0.68	T	0.53	0.46	ND	T	6.77

(Values are the mean of two individual determinations. T - trace, ND – not detected)

Table 18B. Changes in individual free amino acids in 'imbibing' seeds during germination

Time of imbibition, h	Tissue analysed	Amino acids, μ moles per g. dry tissue																	Total
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	2.81	1.24	0.67	4.07	2.18	2.52	2.38	0.34	2.10	ND	2.02	3.29	1.30	3.04	2.79	1.53	2.35	34.63
2	Cotyledon	3.34	1.52	0.85	4.96	2.55	3.01	2.95	0.42	2.57	ND	2.50	3.74	1.57	3.60	3.34	1.77	2.59	41.28
4	Cotyledon	5.16	2.08	1.45	7.14	2.70	4.00	3.95	0.52	3.15	ND	3.72	5.49	2.14	4.92	4.50	2.20	2.79	55.91
6	Cotyledon	5.56	2.28	1.67	8.50	3.01	4.76	4.65	0.66	3.45	T	4.69	6.37	2.34	4.97	5.18	2.05	3.52	63.66
12	Cotyledon	5.29	2.23	1.46	9.41	3.39	4.96	5.67	0.80	3.68	T	5.35	6.81	3.17	5.47	6.15	2.31	4.08	69.23
24	Cotyledon	7.55	1.79	1.20	11.62	4.04	5.87	6.79	1.35	4.47	T	6.26	7.60	1.87	6.40	6.85	2.38	4.10	80.14
	Embryonic axis	1.87	0.84	T	3.26	T	1.81	2.53	T	ND	ND	2.05	1.68	T	T	1.57	ND	T	15.01
48	Cotyledon	8.76	1.72	2.96	16.67	5.64	2.87	8.46	3.38	2.72	1.58	8.29	7.01	3.72	9.36	7.03	0.51	6.27	96.95
	Embryonic axis	3.09	3.01	T	6.91	1.19	3.65	5.24	T	ND	ND	5.00	4.13	2.06	T	2.93	T	T	37.21
72	Cotyledon	12.27	1.73	4.95	22.26	6.09	2.42	13.92	4.61	3.90	5.12	12.61	10.02	3.24	8.08	8.85	0.65	10.44	131.16
	Plumule	4.16	1.87	2.81	8.92	T	T	3.62	ND	ND	T	5.16	3.48	T	2.48	3.22	T	ND	35.72
	Radicle	4.56	2.13	2.25	9.83	T	1.21	4.16	T	T	T	5.32	3.93	T	3.06	2.66	ND	T	39.11

(Values are the mean of two individual determinations. T - trace, ND – not detected)

Table 18C. Changes in individual free amino acids in 'imbibing' seeds during germination

Time of imbibition h	Tissue analysed	Amino acids, μ moles per whole tissue																	Total
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	0.63	0.28	0.15	0.92	0.49	0.57	0.53	0.07	0.47	ND	0.45	0.74	0.29	0.67	0.63	0.34	0.53	7.78
2	Cotyledon	0.66	0.30	0.16	0.98	0.50	0.59	0.58	0.08	0.50	ND	0.49	0.74	0.31	0.71	0.66	0.35	0.51	8.12
4	Cotyledon	0.78	0.31	0.22	1.09	0.41	0.61	0.60	0.07	0.48	ND	0.56	0.84	0.32	0.75	0.68	0.33	0.42	8.47
6	Cotyledon	0.82	0.33	0.24	1.25	0.44	0.70	0.68	0.09	0.51	T	0.69	0.94	0.34	0.73	0.76	0.30	0.52	9.34
12	Cotyledon	1.06	0.45	0.29	1.90	0.68	1.00	1.14	0.16	0.74	T	1.07	1.37	0.64	1.10	1.24	0.46	0.82	14.12
24	Cotyledon	1.66	0.39	0.26	2.56	0.89	1.29	1.49	0.29	0.98	T	1.38	1.67	0.41	1.41	1.51	0.52	0.90	17.61
	Embryonic axis	0.003	0.001	T	0.005	T	0.003	0.004	T	ND	ND	0.003	0.001	T	T	0.002	ND	T	0.02
48	Cotyledon	1.80	0.35	0.61	3.43	1.16	0.59	1.74	0.69	0.56	0.32	1.70	1.44	0.76	1.92	1.44	0.10	1.29	19.90
	Embryonic axis	0.005	0.005	T	0.01	0.002	0.006	0.009	T	ND	ND	0.008	0.007	0.003	T	0.005	T	T	0.06
	Cotyledon	2.21	0.31	0.89	4.01	1.09	0.43	2.50	0.83	0.70	0.92	2.27	1.80	0.58	1.45	1.59	0.11	1.88	23.57
72	Plumule	0.005	0.002	0.003	0.01	T	T	0.004	ND	ND	T	0.007	0.004	T	0.003	0.004	T	ND	0.04
	Radicle	0.008	0.003	0.004	0.01	T	0.002	0.007	T	T	T	0.009	0.007	T	0.005	0.004	ND	T	0.05

(Values are the mean of two individual determinations. T - trace, ND – not detected)

Table 18 D. Changes in individual free amino acids in winged bean 'delayed' seeds during germination

Time of imbibition, h	Tissue analysed	Amino acids, μ moles per g. fresh tissue																	Total
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	2.53	1.12	0.61	3.66	1.96	2.27	2.14	0.31	1.89	ND	1.82	2.96	1.17	2.74	2.51	1.38	2.12	31.19
2	Cotyledon	2.53	1.12	0.61	3.66	1.96	2.27	2.14	0.31	1.89	ND	1.82	2.96	1.17	2.74	2.51	1.38	2.12	31.19
4	Cotyledon	2.50	1.07	0.64	3.86	1.78	2.42	2.11	0.47	1.92	ND	1.98	2.83	1.18	2.70	2.64	1.30	2.08	31.48
6	Cotyledon	2.68	1.10	0.66	4.04	1.75	2.47	2.31	0.45	1.71	T	2.11	2.96	1.21	2.91	2.63	1.18	1.55	31.72
12	Cotyledon	3.08	0.96	0.83	4.32	1.80	2.59	2.38	0.52	1.75	T	2.25	3.10	1.28	2.90	2.61	0.95	1.68	33.20
24	Cotyledon	2.94	0.95	0.92	4.58	1.86	2.61	2.57	0.55	1.84	T	2.53	3.28	1.49	3.09	2.93	1.18	2.11	35.43
	Embryonic axis	0.34	T	ND	0.46	T	0.25	0.36	T	ND	ND	0.37	0.24	T	T	0.20	ND	ND	2.22
48	Cotyledon	3.44	1.15	1.38	4.95	2.28	1.73	3.16	0.97	1.29	0.72	3.51	3.12	1.87	4.08	2.68	0.31	2.26	38.90
	Embryonic axis	0.45	0.13	T	0.73	0.16	0.43	0.61	T	T	ND	0.59	0.55	0.18	T	0.38	T	T	4.15
72	Cotyledon	4.12	0.82	1.81	7.56	1.96	0.97	3.93	1.69	1.65	1.46	4.21	3.61	1.70	2.81	3.30	0.68	2.81	45.09
	Plumule	0.58	0.19	0.50	1.28	ND	T	0.51	ND	ND	T	0.74	0.54	ND	0.25	0.49	T	ND	5.08
	Radicle	0.64	0.39	0.31	1.63	T	0.23	0.68	ND	ND	T	0.89	0.62	T	0.45	0.44	T	T	6.28

(Values are the mean of two individual determinations. T - trace, ND – not detected)

Table 18 E. Changes in individual free amino acids in winged bean 'delayed' seeds during germination

Time of imbibition, h	Tissue analysed	Amino acids, μ moles per g. dry tissue																	Total
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	2.81	1.24	0.67	4.07	2.18	2.52	2.38	0.34	2.10	ND	2.02	3.29	1.30	3.04	2.79	1.53	2.35	34.63
2	Cotyledon	2.81	1.24	0.67	4.07	2.18	2.52	2.38	0.34	2.10	ND	2.02	3.29	1.30	3.04	2.79	1.53	235	34.63
4	Cotyledon	3.22	1.38	0.82	4.90	2.29	3.12	2.72	0.60	2.47	ND	2.55	3.65	1.52	3.48	3.40	1.67	2.68	40.47
6	Cotyledon	4.44	1.82	1.09	6.69	2.90	4.09	3.82	0.74	2.83	T	3.49	4.90	2.01	4.82	4.35	1.76	2.56	53.31
12	Cotyledon	5.66	1.76	1.52	7.94	3.31	4.76	4.77	0.95	3.21	T	4.13	5.70	2.35	5.83	4.80	1.74	3.09	60.62
24	Cotyledon	6.12	1.98	1.91	9.54	3.87	5.44	5.35	1.40	3.83	T	5.27	6.83	3.10	6.44	6.10	2.45	4.39	73.76
	Embryonic axis	2.36	T	ND	3.20	T	1.74	2.50	T	ND	ND	2.57	1.67	T	T	1.39	ND	ND	15.53
48	Cotyledon	7.93	2.65	3.18	11.42	5.26	3.99	7.29	2.23	2.97	1.66	8.10	7.20	4.31	9.41	6.18	0.71	5.21	89.70
	Embryonic axis	3.37	0.97	T	5.46	1.19	3.22	4.56	T	T	ND	4.41	4.11	1.34	T	2.39	T	T	31.02
72	Cotyledon	11.56	2.30	5.08	21.22	5.50	2.72	11.03	4.74	4.63	4.09	11.81	10.13	4.77	7.88	9.26	1.90	7.88	126.50
	Plumule	4.07	1.33	3.51	8.99	ND	T	3.58	ND	ND	T	5.20	3.79	ND	1.75	3.44	T	ND	35.66
	Radicle	3.79	2.31	1.83	9.67	T	1.36	4.03	ND	ND	T	5.28	3.67	T	2.67	2.61	T	T	37.22

(Values are the mean of two individual determinations. T - trace, ND - not detected)

Table 18F. Changes in individual free amino acids in winged bean 'delayed' seeds during germination

Time of imbibition h	Tissue analysed	Amino acids, μ moles per whole tissue																	Total
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	0.63	0.28	0.15	0.92	0.49	0.57	0.53	0.07	0.47	ND	0.45	0.74	0.29	0.69	0.63	0.34	0.53	7.78
2	Cotyledon	0.63	0.28	0.15	0.92	0.49	0.57	0.53	0.07	0.47	ND	0.45	0.74	0.29	0.69	0.63	0.34	0.53	7.78
4	Cotyledon	0.64	0.27	0.16	0.99	0.45	0.62	0.54	0.12	0.49	ND	0.50	0.72	0.30	0.69	0.67	0.33	0.53	8.02
6	Cotyledon	0.73	0.29	0.17	1.10	0.47	0.67	0.62	0.12	0.46	T	0.57	0.80	0.32	0.79	0.71	0.32	0.42	8.56
12	Cotyledon	0.98	0.30	0.26	1.38	0.57	0.83	0.76	0.16	0.56	T	0.72	0.99	0.41	0.92	0.83	0.30	0.53	10.50
24	Cotyledon	1.10	0.35	0.34	1.71	0.69	0.97	0.96	0.20	0.69	T	0.94	1.23	0.55	1.15	1.09	0.44	0.79	13.20
	Embryonic axis	0.003	T	ND	0.005	T	0.002	0.003	T	ND	ND	0.003	0.002	T	T	0.002	ND	ND	0.02
48	Cotyledon	1.49	0.49	0.59	2.14	0.98	0.75	1.37	0.42	0.55	0.31	1.52	1.35	0.81	1.77	1.16	0.13	0.98	16.81
	Embryonic axis	0.005	0.001	T	0.009	0.002	0.005	0.007	T	T	ND	0.007	0.007	0.002	T	0.004	T	T	0.05
72	Cotyledon	2.24	0.44	0.98	4.11	1.06	0.52	2.14	0.92	0.89	0.79	2.29	1.96	0.92	1.53	1.79	0.37	1.53	24.48
	Plumule	0.004	0.001	0.004	0.01	ND	T	0.004	ND	ND	T	0.006	0.004	ND	0.002	0.004	T	ND	0.04
	Radicle	0.006	0.003	0.003	0.01	T	0.002	0.006	ND	ND	T	0.008	0.006	T	0.004	0.004	T	T	0.05

(Values are the mean of two individual determinations. T - trace, ND – not detected)

Table 18G. Changes in individual free amino acids in winged bean 'scarified' seeds during germination

Time of imbibition h	Tissue analysed	Amino acids, μ moles per g. fresh tissue																	Total
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	2.53	1.12	0.61	3.66	1.96	2.27	2.14	0.31	1.89	ND	1.82	2.96	1.17	2.74	2.51	1.38	2.12	31.19
2	Cotyledon	2.50	1.15	0.63	3.70	1.90	2.20	2.11	0.29	1.94	ND	1.96	2.91	1.10	2.79	2.67	1.39	2.058	31.39
4	Cotyledon	2.63	1.18	0.78	3.98	1.67	2.28	2.31	0.36	1.80	ND	2.08	3.12	1.28	2.89	2.65	1.16	1.82	31.99
6	Cotyledon	2.95	1.33	0.91	4.28	1.65	2.41	2.47	0.41	1.69	ND	2.22	3.15	1.14	2.88	2.74	0.97	1.96	33.16
12	Cotyledon	3.06	1.08	0.70	4.84	1.65	2.40	2.68	0.48	1.65	T	2.39	2.96	1.20	3.08	2.91	0.84	2.14	34.06
24	Cotyledon	3.69	0.78	0.58	5.61	1.90	2.55	2.95	0.79	1.91	0.46	2.68	3.30	1.05	3.11	2.98	1.26	1.75	37.35
	Embryonic axis	0.33	T	ND	0.59	T	0.21	0.35	T	T	ND	0.35	0.16	0.13	T	0.23	T	T	2.35
48	Cotyledon	3.92	1.24	0.91	6.52	2.40	1.68	3.46	1.16	1.18	0.58	3.60	3.09	1.45	3.62	2.85	0.34	2.29	40.29
	Embryonic axis	0.41	0.33	T	0.93	0.16	0.53	0.61	T	T	ND	0.65	0.50	0.28	T	0.33	T	T	4.20
72	Cotyledon	4.38	0.85	1.36	8.10	2.27	1.12	4.60	1.46	1.55	1.60	4.71	3.87	1.96	2.89	3.14	0.18	3.34	46.38
	Plumule	0.65	0.20	0.38	1.38	T	T	0.58	ND	ND	T	0.74	0.48	T	0.34	0.46	T	T	5.21
	Radicle	0.77	0.24	0.41	1.79	T	0.15	0.67	T	T	0.24	0.77	0.63	T	0.65	0.47	T	T	6.89

(Values are the mean of two individual determinations. T - trace, ND – not detected)

Table 18II. Changes in individual free amino acids in winged bean 'scarified' seeds during germination

Time of imbibition h	Tissue analysed	Amino acids, μ moles per g. dry tissue																	Total
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	2.81	1.24	0.67	4.07	2.18	2.52	2.38	0.34	2.10	ND	2.02	3.20	1.30	3.04	2.79	1.53	2.35	34.63
2	Cotyledon	3.01	1.38	0.75	4.46	2.29	2.65	2.54	0.34	2.33	ND	2.36	3.50	1.32	3.36	3.22	1.37	2.47	37.65
4	Cotyledon	3.56	1.60	1.05	5.39	2.26	3.09	3.13	0.48	2.44	ND	2.82	4.23	1.73	3.91	3.59	1.57	2.46	43.31
6	Cotyledon	4.89	2.20	1.50	7.09	2.73	3.99	4.09	0.67	2.80	ND	3.68	5.23	1.89	4.77	4.54	1.60	3.25	59.68
12	Cotyledon	5.91	2.08	1.35	9.35	3.18	4.63	5.17	0.92	3.18	T	4.61	5.71	2.31	5.95	5.62	1.62	4.13	69.72
24	Cotyledon	7.89	1.66	1.24	11.99	4.06	5.45	6.30	1.68	4.08	0.98	5.73	7.05	2.24	6.65	6.37	2.69	3.74	79.80
	Embryonic axis	2.45	T	ND	4.38	T	1.56	2.60	T	T	ND	2.60	1.18	0.96	T	1.71	T	T	17.44
48	Cotyledon	8.76	2.77	2.03	14.57	5.36	2.50	7.73	2.59	2.63	5.29	8.04	6.90	3.24	8.09	6.37	0.75	5.11	18.73
	Embryonic axis	3.06	2.47	T	6.96	1.19	3.96	4.56	T	T	ND	4.86	3.74	2.09	T	2.47	T	T	39.36
72	Cotyledon	12.51	2.42	3.88	23.14	6.48	3.20	13.14	4.17	4.42	4.57	30.46	11.06	2.74	8.25	8.97	0.51	9.54	132.46
	Plumule	4.11	1.26	2.40	8.73	T	T	3.67	ND	ND	T	4.68	3.03	T	2.15	2.91	T	T	32.94
	Radicle	4.66	2.06	2.48	10.85	T	0.90	4.06	T	T	1.45	4.66	3.82	T	3.94	2.85	T	T	41.73

(Values are the mean of two individual determinations. T - trace, ND – not detected).

Table 18 I. Changes in individual free amino acids in winged bean 'scarified' seeds during germination

Time of imbibition,h	Tissue analysed	Amino acids, μ moles per whole tissue																	Total
		Asp	Thr	Ser	Glt	Pro	Gly	Ala	Cys	Val	Met	Asn Gln	Leu Iso	Tyr	Phe Trp	Lys	His	Arg	
0	Cotyledon	0.63	0.38	0.15	0.92	0.49	0.57	0.53	0.07	0.47	ND	0.45	0.74	0.29	0.69	0.63	0.34	0.53	7.78
2	Cotyledon	0.64	0.29	0.16	0.95	0.49	0.56	0.54	0.07	0.50	ND	0.50	0.75	0.28	0.73	0.67	0.35	0.53	8.02
4	Cotyledon	0.73	0.32	0.21	0.10	0.46	0.63	0.64	0.10	0.50	ND	0.57	0.86	0.35	0.80	0.73	0.32	0.50	8.82
6	Cotyledon	0.93	0.41	0.28	1.35	0.52	0.76	0.77	0.12	0.53	ND	0.70	0.99	0.35	0.90	0.86	0.30	0.61	10.38
12	Cotyledon	1.28	0.45	0.29	2.02	0.69	1.00	1.12	0.20	0.69	T	1.00	1.23	0.50	1.28	1.21	0.35	0.89	14.20
24	Cotyledon	1.67	0.35	0.26	2.54	0.86	1.15	1.33	0.35	0.86	0.20	1.21	1.49	0.47	1.40	1.35	0.57	0.79	16.85
	Embryonic axis	0.003	T	ND	0.007	T	0.002	0.004	T	T	ND	0.004	0.001	0.001	T	0.002	T	T	0.02
48	Cotyledon	1.82	0.57	0.42	3.03	1.11	0.17	1.61	0.54	0.54	0.27	1.67	1.43	0.67	1.68	1.32	0.15	1.06	18.67
	Embryonic axis	0.006	0.005	T	0.01	0.002	0.008	0.009	T	T	ND	0.01	0.007	0.004	T	0.005	T	T	0.06
72	Cotyledon	2.16	0.41	0.67	3.99	1.12	0.55	2.27	0.72	0.76	0.78	2.32	1.91	0.47	1.42	1.55	0.08	1.64	22.82
	Plumule	0.006	0.001	0.003	0.01	T	T	0.005	ND	ND	T	0.007	0.004	T	0.003	0.004	T	T	0.04
	Radicle	0.008	0.003	0.004	0.02	T	0.001	0.007	T	T	0.002	0.008	0.006	T	0.007	0.005	T	T	0.07

(Values are the mean of two individual determinations. T - trace, ND – not detected)

aspartic acid. Proline, glycine, alanine, valine, asparagine + glutamine, leucine + isoleucine, phenylalanine + tryptophan, lysine and arginine were also present in appreciable amounts. Methionine could not be detected in this stage.

A marginal increase in concentration occurred in a few amino acids at the end of 2 h of imbibition. Others maintained their concentration except slight decrease in leucine+isoleucine and arginine. Here also glutamic acid maintained its highest level followed by aspartic acid. Methionine could not be detected in this sample also.

The concentration of most of the amino acids were slightly increased at the end of 4 h of imbibition. Glutamic acid showed the highest concentration followed by aspartic acid. The concentrations of serine, asparagine+glutamine, leucine + isoleucine were increased slightly while proline, valine, histidine and arginine showed slight decrease. Other amino acids maintained its previous level. Methionine could not be detected.

In the cotyledons after 6 h of imbibition, glutamic acid and aspartic acid maintained its highest position. The concentration of glycine, alanine, cysteine, asparagine + glutamine, leucine + isoleucine, lysine and arginine were increased while aspartic acid, phenylalanine +tryptophan and histidine showed decrease. Threonine, serine, proline, valine and tyrosine maintained its previous level. The concentration of methionine was increased to trace.

In the 12 h cotyledon, glutamic acid was the most abundant amino acid followed by lysine, alanine and aspartic acid. Proline, alanine, cysteine, asparagine + glutamine, tyrosine, phenylalanine + tryptophan, lysine, histidine and arginine showed increase in their concentration while aspartic acid threonine and serine showed decrease from the previous stage. Other amino acids

maintained its previous level.

Glutamic acid maintained its highest position in the 24 h cotyledons. Aspartic acid again became the second most abundant followed by lysine and alanine. The concentration of proline, glycine, cysteine, valine, asparagine + glutamine, leucine + isoleucine, phenylalanine + tryptophan etc. were increased while threonine, serine, tyrosine, histidine and arginine were decreased. Methionine maintained its trace level.

In the cotyledons of imbibing seeds after 48 h of imbibition, glutamic acid and aspartic acid maintained their previous positions. Alanine was the third highest followed by lysine. The concentration of serine and cysteine were increased to more than 2-fold. The concentration of histidine was reduced one fifth, glycine was reduced to less than half and that of valine was about half. Significant increases in concentration were noticed in the case of proline, alanine, asparagine + glutamine, tyrosine, phenylalanine + tryptophan and arginine. The level of methionine was increased to appreciable level in this stage.

In the 72 h cotyledons, glutamic acid showed the highest concentration followed by alanine and aspartic acid. Arginine was next to aspartic acid. The concentration of most of the amino acids were increased while that of threonine, proline, glycine, tyrosine and phenylalanine + tryptophan were decreased.

(b) Embryonic axis

When compared with the cotyledons, the concentration of most of the amino acids were very low in the embryonic axis. In the 24 h embryonic axis, glutamic acid showed the highest concentration followed by alanine, aspartic acid and glycine. Serine, proline, cysteine, tyrosine, phenylalanine + tryptophan and

arginine were only in traces. Valine, methionine and histidine were not detected. Other amino acids were present in assayable concentration.

The concentration of most of the amino acids were increased in the 48 h embryonic axis. Here also glutamic acid showed the highest concentration followed by alanine and glycine. Serine, cysteine, phenylalanine + tryptophan and arginine maintained the trace level while the concentration of histidine was increased to trace level. Similarly, the concentration of proline and tyrosine were increased to assayable amount from trave level. Here also valine and methionine could not be detected.

(c) Plumule

In the plumule, the concentration of glutamic acid was much higher than other amino acids. Aspartic acid was the second highest amino acid followed by alanine. Proline, glycine, methionine, tyrosine and histidine were present only in traces. Cysteine and valine could not be detected. Appreciable amounts of other amino acids were present.

(d) Radicle

The free amino acid concentration of the radicle was slightly higher than the plumule. Here also glutamic acid was in the highest position followed by glycine and alanine. Proline, cysteine, valine, methionine, tyrosine and arginine were only in traces. Histidine could not be detected. The concentrations of threonine, asparagine + glutamine, leucine + isoleucine and phenylalanine + tryptophan were also present in higher level than the plumule.

Delayed seeds

(a) Cotyledon

In the cotyledons of delayed seeds, the concentration of free amino acids after 2 h of imbibition was same as that of the zero hour cotyledons. In the 4 h cotyledons, glutamic acid showed the highest concentration followed by lysine and aspartic acid. Glycine was in the fourth position. Threonine, proline, alanine, leucine + isoleucine, phenylalanine + tryptophan, histidine and arginine showed marginal decrease in their concentrations while others showed slight increase. Methionine could not be detected.

In the cotyledons after 6 h of imbibition, glutamic acid was again in the highest position followed by aspartic acid and lysine. The concentration of aspartic acid, glutamic acid, alanine, asparagine+glutamine, leucine + isoleucine, phenylalanine + tryptophan were slightly increased from the previous level while that of valine, histidine and arginine were decreased. Others maintained their level. Here the concentration of methionine was increased to trace level.

After 12 h of imbibition, glutamic acid maintained its highest position followed by aspartic acid, lysine and glycine. The concentration of most of the amino acids were increased, while that of threonine and histidine decreased. Methionine, phenylalanine + tryptophan and lysine maintained their previous level.

The concentration of most of the amino acids were increased on the 24 h cotyledons with glutamic acid in the highest position followed by aspartic acid and lysine. Here aspartic acid showed slight decrease in its concentration from the previous level. Threonine, glycine, cysteine and methionine maintained their

previous level.

Glutamic acid and aspartic acid maintained their highest position in the 48 h cotyledons also. Alanine was the third highest followed by lysine. Here the concentration of most of the amino acids showed much increase except valine, leucine + isoleucine, lysine and histidine. Of these, the concentration of histidine was reduced to one fourth from the previous level. Also the concentration of methionine was increased to assayable level.

The concentrations of most of the amino acids had shown much increase at the end of 72 h of imbibition. Glutamic acid was the highest followed by aspartic acid and alanine. The concentration of methionine and histidine were doubled from the previous level. Threonine, proline, glycine, tyrosine and phenylalanine + tryptophan showed decrease in their concentration.

(b) Embryonic axis

In the 24 h embryonic axis, only a few amino acids were recovered in assayable level. Among these, glutamic acid was highest in concentration followed by alanine and aspartic acid. Other amino acids recovered in assayable level were glycine, asparagine + glutamine, leucine+isoleucine and lysine. Threonine, proline, cysteine, tyrosine, phenylalanine + tryptophan were present only in traces. Serine, valine, methionine, histidine and arginine could not be detected. The concentration of most of the amino acids were much better in the 48 h embryonic axis. Several amino acids not detected in the 24 h embryonic axis were increased to trace level. Glutamic acid showed the highest position followed by alanine and aspartic acid. Glycine was next to aspartic acid. The concentration of asparagine + glutamine and leucine+isoleucine were also fair. Methionine could not be detected. Serine, valine, histidine and arginine not

detected in the 24 h embryonic axis were increased to trace level.

(c) Plumule

Glutamic acid showed much higher concentration in the plumule also. The second highest was aspartic acid. Serine, alanine and lysine showed almost same concentration and were in the third position. The concentrations of asparagine + glutamine, leucine + isoleucine were also fair. Glycine, methionine and histidine were present only in traces while proline, cysteine, valine, tyrosine and arginine could not be detected.

(d) Radicle

In the radicle, most of the amino acids showed higher concentration than the plumule. Here also glutamic acid was in the highest position followed by alanine and aspartic acid. The concentrations of asparagine + glutamine, leucine + isoleucine, phenylalanine + tryptophan and lysine were also fair. Proline, methionine, tyrosine, histidine and arginine were present only in traces while cysteine and valine could not be detected.

Scarified seeds

(a) Cotyledons

In the scarified seeds, the cotyledons after 2 h of imbibition showed only marginal changes in a few amino acids. Here glutamic acid was highest in concentration followed by lysine and aspartic acid. Appreciable concentration of all other amino acids were present. Methionine could not be detected. The concentrations of serine and cysteine were very low, but in assayable level.

The concentration of glutamic acid slightly increased from the previous

level and was the highest after 4 h of imbibition. Here also lysine and aspartic acid were next to glutamic acid. The concentrations of glycine, alanine, asparagine + glutamine, leucine + isoleucine, phenylalanine + tryptophan etc. were fairly good. Methionine could not be detected in this stage also.

The 6 h cotyledons showed increased concentrations of most of the amino acids from the previous stage. Here also glutamic acid was highest in concentration followed by aspartic acid and lysine. Proline, leucine + isoleucine, phenylalanine + tryptophan maintained their previous level, while valine, tyrosine and histidine showed slight decrease in concentration from the previous level. Methionine could not be detected in this stage also.

The concentration of glutamic acid, aspartic acid and lysine were increased and maintained their highest position in the 12 h cotyledons also. Alanine, cysteine, asparagine + glutamine, tyrosine, phenylalanine + tryptophan, lysine and arginine showed slight increase in their concentrations whereas, threonine, serine, leucine + isoleucine and histidine showed decrease in concentrations from previous stage. Proline, glycine and valine maintained their level. The concentration of methionine was increased to trace level.

The concentrations of glutamic acid further increased and was the highest in the 24 h cotyledons also. Aspartic acid was the second highest followed by lysine and alanine. The concentrations of glycine, valine, asparagine + glutamine, leucine+ isoleucine, phenylalanine + tryptophan, histidine and arginine were also generous. The concentration of threonine, serine, tyrosine and arginine were decreased from the previous level while methionine concentration was increased to assayable level.

In the cotyledons of scarified seeds at the end of 48 h of imbibition, most

of the amino acids showed increased level in their concentrations from the previous stage except glycine, valine, leucine + isoleucine, lysine and histidine. The concentrations of histidine was reduced to one fourth. Here also glutamic acid was highest in concentration followed by aspartic acid and alanine.

In the 72 h cotyledons, the concentrations of most of the amino acid were increased. Glutamic acid showed highest concentration followed by aspartic acid and alanine. Arginine was the fourth highest followed by lysine. The concentrations of threonine, proline, glycine, tyrosine, phenylalanine + tryptophan and histidine were reduced. The concentration of histidine was reduced to half whereas that of methionine was doubled from the previous stage

(b) Embryonic axis

In the embryonic axis of 24 h of imbibition, glutamic acid was highest in concentration. Alanine was the second highest and aspartic acid closely followed alanine. Assayable amounts of glycine, asparagine + glutamine, leucine+isoleucine, tyrosine and lysine were also present. Threonine, proline, cysteine, valine, phenylalanine + tryptophan, histidine and arginine were present only in traces. Serine and methionine could not be detected.

The concentration of free amino acids increased in the 48 h embryonic axis. Glutamic acid showed the highest concentration followed by alanine and glycine. Aspartic acid was in the fourth position. The concentrations of glycine and tyrosine were increased to more than double and leucine + isoleucine showed 3 fold increase in the 48 h embryonic axis. Cysteine, valine, phenylalanine + tryptophan, histidine and arginine maintained the trace level while the concentration of serine was increased to trace level. Also the concentrations of threonine and proline were increased to fairly good level. Methionine could not

be detected here also.

(c) Plumule

In the plumule also glutamic acid maintained its highest position followed by aspartic acid and alanine. The concentration of asparagine + glutamine were also generous. Proline, glycine, methionine, tyrosine, histidine and arginine were present only in traces. Cysteine and valine could not be detected.

(d) Radicle

In the radicle also glutamic acid was present in higher concentration than the other amino acids. Aspartic acid was the second highest followed by alanine. The concentration of asparagine + glutamine, leucine + isoleucine and phenylalanine + tryptophan were also good. Proline, cysteine, valine, tyrosine, histidine and arginine were present only in traces.

Total free amino acids

The total free amino acids were arrived at by summing up the individually determined values of the amino acids by thin layer chromatography. The values are represented in tables 18 A to I.

Concentration

The values are represented in tables 18 A, B, D, E, G and H

Imbibing seeds

(a) Cotyledons

The concentration of total amino acids gradually increased and reached the maximum value at 72 h of imbibition. On a fresh tissue basis, more than 10% increase in total free amino acids occurred at the end of 12 h which

increased to more than 15% after 24 h of imbibition. After 48 h, the increase in free amino acids concentration was about 25% which on 72 h reached to about 50%. When calculated on a dry tissue basis, the increase in free amino acids after 12 h of imbibition was 2-fold when compared with the 0 h dry cotyledons. The concentration further increased to more than 2-fold on 24 h which on 48 h was nearly 3-fold. At the end of 72 h of imbibition, the increase in concentration reached 4-fold.

(b) Embryonic axis

The concentration of total free amino acids on a fresh tissue basis in the embryonic axis was low when compared with the cotyledons. The concentration almost doubled in the 48 h from the 24 h embryonic axis. On a dry tissue basis, the concentration of 48 h was more than double than the 24 h embryonic axis.

(c) Plumule and Radicle

The concentration of total free amino acids in the plumule was slightly lower than the radicle. The concentration of the plumule and radicle together was about 2.5-fold of the 48 h embryonic axis on a fresh tissue basis. But on a dry tissue basis, the concentration of plumule and radicle together was only about 2-fold the 48 h embryonic axis.

Delayed seeds

(a) Cotyledons

In the cotyledons of delayed seeds, the increase in total free amino acids was gradual but slower than the imbibing seeds especially during the earlier hours of imbibition. Here the pattern of increase in amino acids same as the imbibing seeds on a fresh and dry tissue basis, but the percentage increase was slightly

lower in all stages.

(b) Embryonic axis

The concentrations of total free amino acids on a fresh tissue basis almost doubled in the 48 h embryonic axis from the 24 h embryonic axis. The same pattern occurred on a dry tissue basis too.

(c) Plumule and Radicle

In the case of delayed seeds also the concentration of total free amino acids in the plumule was slightly lower than the radicle on a fresh and dry tissue basis. The plumule and radicle together on a fresh and dry tissue basis showed nearly 3-fold increase in total free amino acids from the 48 h embryonic axis. On a dry tissue basis also, the concentration of total amino acids registered nearly 3-fold increase.

Scarified seeds

(a) Cotyledons

In the cotyledons of scarified seeds, the increase in total free amino acids on a fresh and dry tissue basis was gradual. On a fresh tissue basis, about 10% increase in total amino acid concentration was noticed at the end of 12 h of imbibition and about 20% and 30% increase was noticed at 24 h and 48 h of imbibition. At the end of 72 h, the increase was about 50%. But on a per gram dry tissue basis, nearly 2-fold increase occurred at the end of 12 h which increased to more than 2-fold after 24 h of imbibition. After 48 h, the increase in total free amino acids was more than 2.5-fold which on 72 h was nearly 4-fold.

(b) Embryonic axis

On a fresh and dry tissue basis, the concentration of total free amino acids in the 48 h embryonic axis was almost double than 24 h embryonic axis.

(c) Plumule and Radicle

In the case of scarified seeds also, the concentration of total free amino acids in the plumule was slightly lower than the radicle. Here, on a fresh weight basis the plumule and radicle together registered about 3-fold increase in total free amino acid concentration from the 48 h embryonic axis. But only 2-fold increase was registered on a per gram dry tissue basis.

Content

The values are represented in tables 18 C, F and I

Imbibing Seeds

(a) Cotyledons

The content of free amino acids increased gradually from 0 h cotyledons and reached the highest value on 72 h. More than 80% increase in total amino acid content was registered at the end of 12 h of imbibition, which after 24 h was more than 2-fold. After 72 h of imbibition, the total amino acid content was above 3-fold in cotyledons.

(b) Embryonic axis

Quantitatively, the content of total free amino acids in the embryonic axis, plumule and radicle were very low as the total weight of these tissues were very low. The 48 h embryonic axis recorded 3-times increase in total free amino acid content than the 24 h embryonic axis.

(c) Plumule and Radicle

The plumule and radicle together showed about 1.5-times total free amino acid content than the 48 h embryonic axis. The radicle showed marginally higher content than the plumule.

Delayed seeds

(a) Cotyledons

In the cotyledons of delayed seeds, the total amino acid content started increasing only after 2 h of imbibition. It gradually increased and after 12 h of imbibition, more than 30% increase was recorded in total content. A near doubling was seen after 24 h from the 0 h cotyledons which after 48 h of imbibition was more than 2-fold and increased to more than 3-fold after 72 h of imbibition.

(b) Embryonic axis

The 48 h embryonic axis showed about 2.5-fold increase in total amino acid content than the 24 h embryonic axis.

(c) Plumule and Radicle

The plumule and radicle together registered about 2-fold increase in total free amino acid content from the 48 h embryonic axis.

Scarified seeds

(a) Cotyledons

The content of total amino acids gradually increased from 0 h and a near doubling of the value occurred after 12 h of imbibition. After 24 and 48 h of imbibition, more than 2-fold increase were registered. After 72 h of contact with

water, the content of total amino acids registered nearly 3-fold increase.

(b) Embryonic axis

A three fold increase in total amino acid content was registered in the 48 h embryonic axis from the previous stage.

(c) Plumule and Radicle

The plumule and radicle together constituted a near doubling of the value from 48 h embryonic axis. The value was nearly double in the radicle when compared to plumule. Among the various tissues analysed from the three types of seeds, the increase in total amino acid content was slow in the delayed seeds. In the cotyledons only 80% increase was noted after 12 h of imbibition while a near doubling was seen in the other two types of seeds. A 2-fold increase was noted after 48 h in the delayed seeds, while the other two types showed more than 2-fold increase. But after 72 h of imbibition, the delayed seeds showed maximum content in total free amino acids. Imbibing seeds closely followed delayed seeds. The increase in total amino acid content was faster in the scarified seeds during the earlier hours than the other two types.

In the embryonic axis, almost identical pattern followed in all the three types of seeds, but the combined values of plumule and radicle showed slightly higher value in total amino acid content in the scarified seeds than the imbibing and delayed seeds.

Alanine and aspartate aminotransferases activity in three types of winged beans seeds during germination

The values are expressed on a per gram fresh and dry tissue and on a whole tissue basis. The analytical data are reported in tables 19 A, B and C and figures

37, 38 and 39.

Tables 19 A, B and C and figures 37, 38 and 39

Imbibing seeds

(a) Cotyledons

In the cotyledons of imbibing seeds, both alanine and aspartate amino transferases were highly active in all stages during germination. Aspartate aminotransferase activity exceeded alanine aminotransferase activity in all stages during germination. When imbibition commenced, i.e., 2 h after contact with water, the activity of both GPT and GOT showed a significant rise but again during the 6 h, the activity of GPT slightly increased and then decreased gradually upto 24 h, then again gradually increased and reached maximum activity at the end of 72 h. In contrast at 6 h imbibition GOT showed a decrease in activity when compared with 4 h cotyledons. In the 12 h, the activity of GOT slightly increased and afterwards decreased gradually upto 48 h of contact with water. The highest activity of GOT was recorded at the end of 72 h of imbibition which was over 30% higher than the 0 h value.

On a per gram dry tissue basis, the activities of both GPT and GOT showed gradual increase from 0 h cotyledons and showed maximum activity at the end of 72 h. In the case of whole tissues, the activity of GPT showed gradual increase upto 72 h, though an insignificant drop observed during the 4 h of imbibition. In the case of GOT, a rise in activity was noticed at the end of 2 h, and then gradually decreased upto 6 h, which again increased gradually upto 72 h, where the maximum activity occurred.

Table 19A: Activities of alanine and aspartate aminotransferases in 3 types of winged bean seeds during germination.

Time of imbibition,h	Tissue analysed	Transaminase activity, units/g fresh tissue					
		Imbibing		Delayed		Scarified	
		GPT	GOT	GPT	GOT	GPT	GOT
0	Cotyledon	157.89±17.68	181.29±11.54	157.89±17.68	181.29±11.54	157.89±17.68	181.29±11.54
2	Cotyledon	178.82±21.75	228.98±28.03	157.89±17.68	181.29±11.54	160.71±07.18	182.13±13.18
4	Cotyledon	172.35±05.49	207.03±08.77	157.17±07.38	178.46±08.82	149.11±07.18	201.93±02.32
6	Cotyledon	176.33±11.80	188.38±09.04	147.09±04.57	167.18±07.30	167.62±10.08	196.62±07.08
12	Cotyledon	168.48±09.04	213.83±27.60	143.26±05.97	194.22±14.41	197.29±13.57	255.02±07.94
24	Cotyledon	162.77±23.50	196.05±24.08	140.49±08.59	169.89±14.86	167.31±16.49	194.44±20.08
	Embryonic axis	317.18±23.17	216.57±18.16	267.11±15.26	176.35±11.18	324.28±20.21	231.90±21.73
48	Cotyledon	170.44±21.31	194.01±31.49	154.10±15.10	169.54±12.93	137.09±21.89	159.55±17.67
	Embryonic axis	280.80±27.04	187.35±24.27	253.82±12.23	198.21±11.45	258.22±09.46	204.71±16.11
72	Cotyledon	192.98±17.59	244.34±16.06	176.19±19.05	220.74±14.17	203.58±08.76	266.30±21.61
	Plumule	139.47±20.78	431.09±48.76	113.79±09.69	418.18±17.56	171.18±15.18	466.14±16.38
	Radicle	174.99±20.23	348.95±33.78	131.41±11.71	323.14±14.33	177.95±07.87	365.12±23.83

(The values are expressed as units of enzyme activity ± S.D. on a per gram fresh tissue basis)

Table 19 B. Activities of alanine and aspartate aminotransferases in 3 types of winged bean seeds during germination.

Time of imbibition,h	Tissue analysed	Transminases activity. units /g dry tissue					
		Imbibing		Delayed		Scarified	
		GPT	GOT	GPT	GOT	GPT	GOT
0	Cotyledon	176.65±19.78	202.83±12.91	176.65±19.78	202.83±12.91	176.65±19.78	202.83±012.91
2	Cotyledon	234.73±28.55	300.58±36.80	176.65±19.78	202.83±12.91	193.83±8.66	219.67±15.90
4	Cotyledon	298.75±9.51	358.86±15.20	229.34±10.76	260.41±12.87	202.23±9.73	273.87±3.14
6	Cotyledon	336.31±22.52	359.29±17.24	243.80±7.57	277.10±12.10	277.97±16.71	326.10±11.74
12	Cotyledon	338.92±18.18	430.15±55.52	263.53±10.98	357.28±26.50	381.16±26.21	492.69±15.34
24	Cotyledon	355.54±51.33	456.46±52.59	292.87±17.90	354.15±30.97	357.80±35.26	415.82±42.94
	Embryonic axis	1915.33±139.91	1307.78±109.66	1860.09±106.26	12228.06±77.85	2411.01±150.26	1724.16±161.56
48	Cotyledon	414.79±51.86	472.13±76.63	355.64±34.84	391.27±29.84	306.41±48.92	356.61±39.49
	Embryonic axis	2230.34±214.77	1488.08±192.77	1901.27±91.61	1484.71±85.76	1932.78±70.80	1532.26±120.58
72	Cotyledon	549.48±50.08	695.72±45.73	494.63±53.48	619.70±39.78	581.82±25.03	761.07±61.76
	Plumule	936.04±139.46	2893.22±327.24	799.64±68.09	2938.72±123.40	1083.41±96.07	2950.25±103.67
	Radicle	1012.08±117.00	2018.21±195.37	780.00±69.49	1917.74±85.04	1079.13±47.72	2214.19±144.51

(The values are expressed as units of enzyme activity ± S.D. on a per gram fresh tissue basis

Table 19 C. Activities of alanine and aspartate aminotransferases in 3 types of winged bean seeds during germination

Time of imbibition, h	Tissue analysed	Transaminase activity, units per whole tissue					
		Imbibing		Delayed		Scarified	
		GPT	GOT	GPT	GOT	GPT	GOT
0	Cotyledon	39.78±4.45	45.68±2.90	39.78±4.45	45.68±2.90	39.78±4.45	45.68±2.90
2	Cotyledon	46.49±5.65	59.53±7.28	39.78±4.45	45.68±2.90	39.37±1.75	44.62±3.22
4	Cotyledon	45.67±1.45	54.86±2.32	40.39±1.89	45.86±2.26	41.45±1.99	56.13±0.64
6	Cotyledon	49.72±3.32	53.27±2.55	40.00±1.24	45.47±1.98	52.80±3.99	61.94±2.23
12	Cotyledon	68.40±3.67	86.80±11.20	45.93±1.91	62.15±4.61	86.46±5.67	106.59±3.31
24	Cotyledon	78.29±11.30	94.30±11.58	52.68±3.22	63.70±5.57	75.79±7.67	88.08±9.09
	Embryonic axis	3.48±0.25	2.38±0.19	2.93±0.16	1.93±0.12	3.89±0.24	2.78±0.26
48	Cotyledon	85.39±10.67	97.19±15.77	66.72±6.53	73.41±5.59	63.74±10.17	74.19±8.21
	Embryonic axis	3.87±0.37	2.58±0.33	3.29±0.15	2.57±0.14	4.05±0.14	3.21±0.25
72	Cotyledon	98.99±9.02	125.34±23.45	95.84±10.36	120.00±7.70	100.36±4.31	131.28±10.65
	Plumule	1.28±0.19	3.97±0.45	0.96±0.08	3.55±0.14	1.66±0.14	4.52±0.15
	Radicle	1.83±0.21	3.66±0.35	1.29±0.11	3.19±0.14	1.95±0.08	4.01±0.25

(The value are expressed as units of enzyme activity ± S. D. on a whole tissue basis)

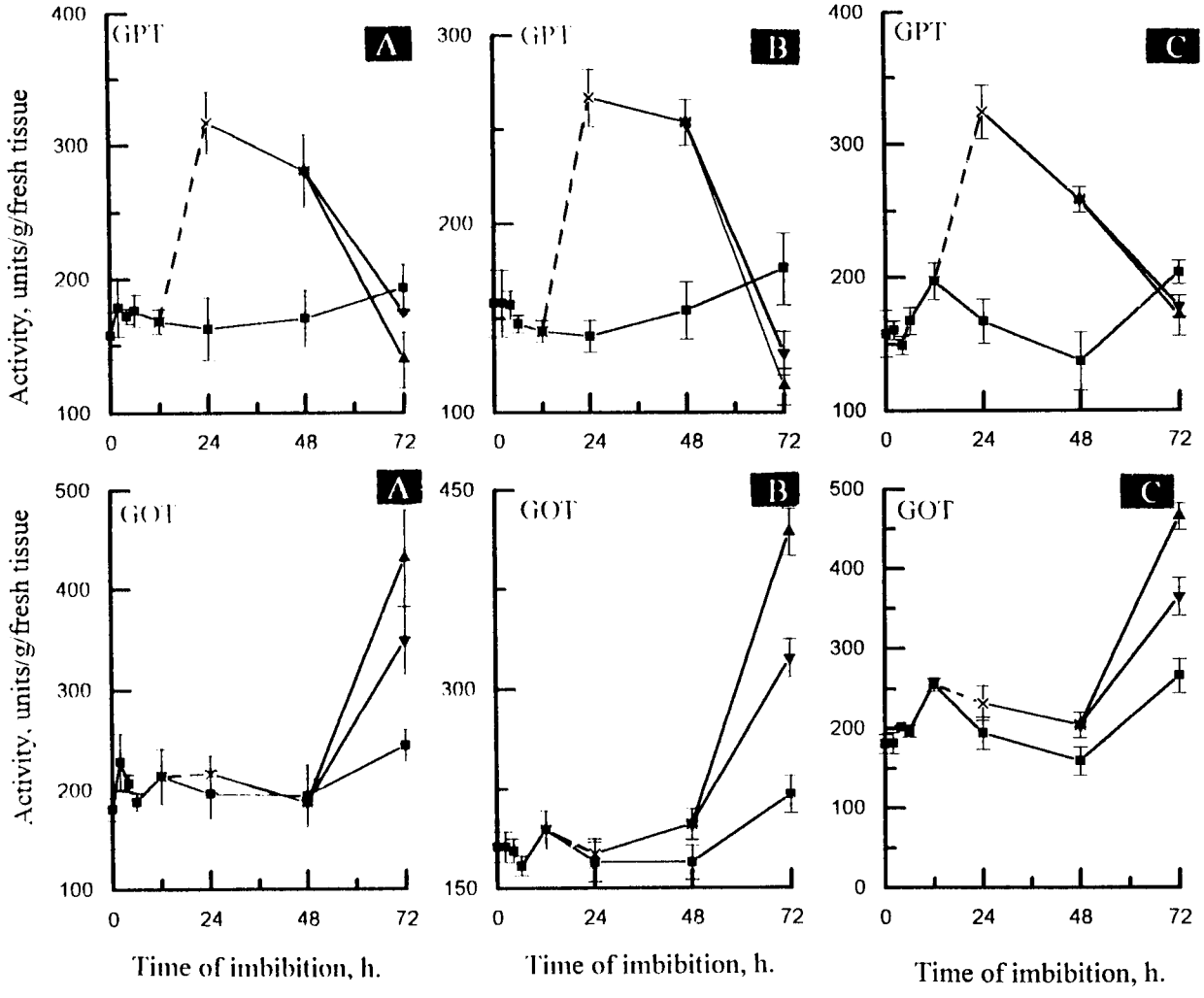


Figure 37. Activities of alanine and aspartate aminotransferases in three types of winged bean seeds during germination. A-Imbibing seeds, B-Delayed seeds and C-Scarified seeds.

-■- -Cotyledon -✳- -Embryonic axis -▲- -Plumule -▲- -Radicle

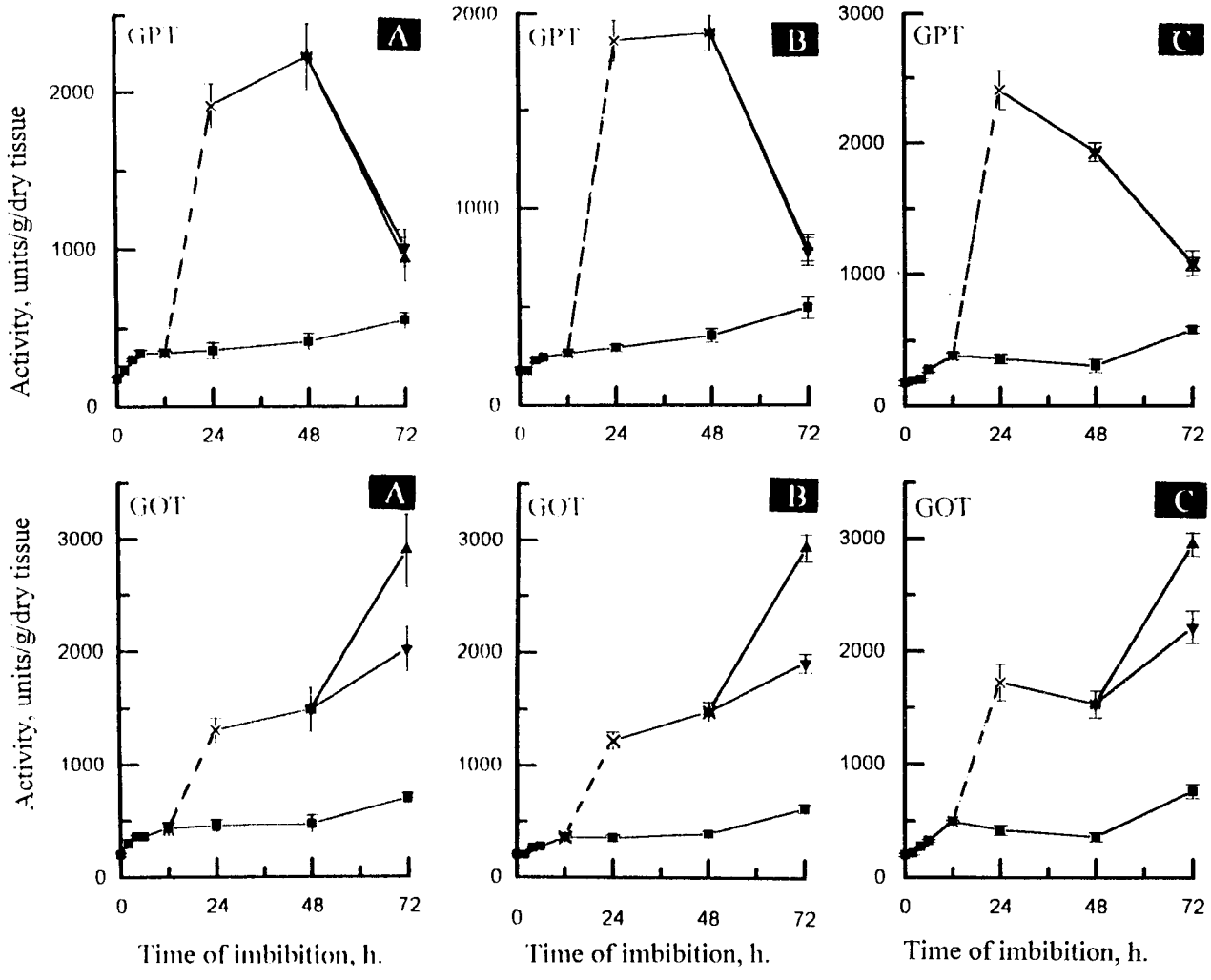


Figure 38. Activities of alanine and aspartate aminotransferases in three types of winged bean seeds during germination. A-Imbibing seeds, B-Delayed seeds and C-Scarified seeds.

—■— -Cotyledon —✳— -Embryonic axis —▲— -Plumule —▼— -Radicle

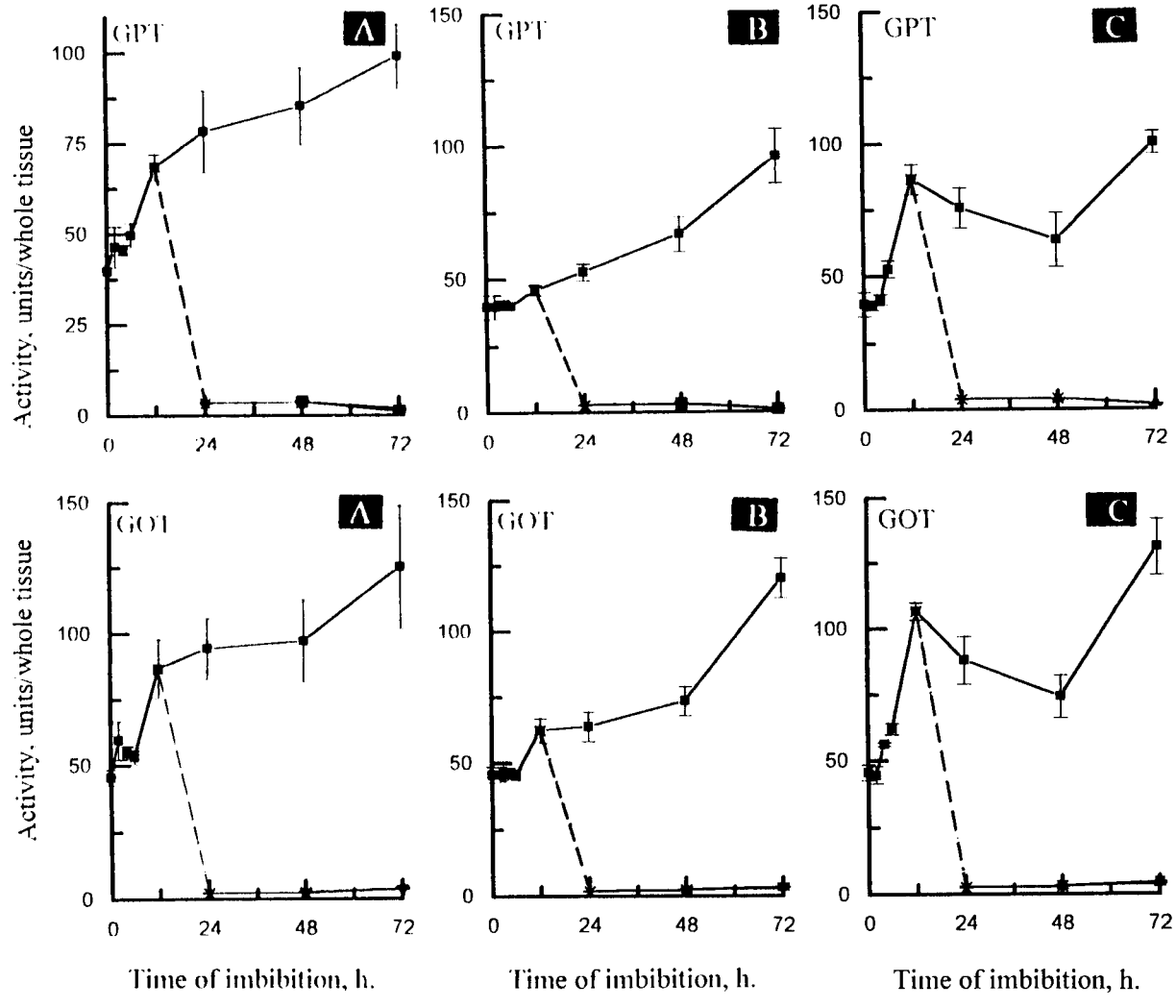


Figure 39. Activities of alanine and aspartate aminotransferases in three types of winged bean seeds during germination. A-Imbibing seeds , B-Delayed seeds and C-Scarified seeds.

-■- -Cotyledon -✳- -Embryonic axis -▲- -Plumule -▼- -Radicle

(b) Embryonic axis

The activities of alanine and aspartate aminotransferases were observed in the embryonic axis from the 24 h onwards and both aminotransferases showed higher activity than their respective cotyledons, except in 48 h GOT. Contrary to the higher activity of GOT in the cotyledons, the embryonic axis showed higher activity of GPT. The activity of both aminotransferases were higher during the 24 h which slightly decreased on 48 h. On a per gram dry tissue and per whole tissue basis, both alanine and aspartate aminotransferases showed higher activity on 48 h of contact with water.

(c) Plumule

In the plumule, the activity of aspartate aminotransferase was 3- times higher than alanine aminotransferase. The same pattern followed on a per gram dry and per whole tissue basis.

(d) Radicle

The activity of aspartate aminotransferase in the radicle was double than that of alanine aminotransferase. The same pattern followed in the case of per gram dry and per whole tissue basis.

The radicle showed higher activity of GPT (25%) than that of plumule, while the activity of GOT was lower by 20% than the plumule. On a per gram dry tissue basis, the GPT activity of the radicle was slightly higher than the plumule while that of GOT was more than 30% lower. On a whole tissue basis, the GPT activity of the radicle was more than 40% higher than the plumule and that of GOT of the radicle was slightly lower than the plumule.

The GPT activity of plumule and radicle together was slightly higher than

the 48 h embryonic axis while that of GOT was 4 times higher on a fresh tissue basis. On a per gram dry and whole tissue basis, the combined GPT activity of plumule and radicle was lower than the 48 h embryonic axis, whereas, the activity of GOT of plumule and radicle together was more than 3-fold the 48 h embryonic axis.

Delayed seeds

(a) cotyledon

In the case of delayed seeds, on a fresh weight basis, the activity of alanine aminotransferase gradually decreased upto 24 h of contact with water and afterwards gradually increased forming a peak activity at the end of 72 h. In the case of aspartate aminotransferase, the activity gradually decreased upto 6 h of imbibition and showed a rise in activity during 12 h and again showed a drop in activity during 24 h and practically no change at 48 h, but again showed an increase at 72 h of imbibition and was the peak in activity.

In the case of delayed seeds also, aspartate aminotransferase activity exceeded alanine aminotransferase. Also, the activities of both aminotransferases in cotyledons of delayed seeds were comparatively lower than that of the cotyledons of imbibing seeds.

Contrary to the changes in enzyme activities found in fresh tissue of delayed seeds, the activities on a per gram dry tissue and whole tissue showed gradual increase in activities during zero to 72 h of water uptake of both aminotransferases.

(b) Embryonic axis

The activities of both aminotransferases in the embryonic axis was higher than that of the cotyledons. As in the case of imbibing seeds, here also embryonic

axis showed a decrease in activity during 48 h in the case of alanine aminotransferase. But the case was different in aspartate aminotransferase activity of the 48 h embryonic axis, which showed higher activity than 24 h embryonic axis.

On a per gram dry tissue basis and per whole tissue basis, the activities of both aminotransferases was higher during 48 h when compared with 24 h embryonic axis. The embryonic axis of delayed seeds showed lower activity than the embryonic axis of imbibing seeds.

(c) Plumule

In the plumule, the activity of aspartate aminotransferase was about 4 times higher than that of alanine aminotransferase. The activities of both aminotransferases were slightly lower than that found in imbibing seeds and scarified seeds. The same pattern followed on a per gram dry and whole tissue basis.

(d) Radicle

The radicle also showed higher aspartate aminotransferase activity, than alanine aminotransferase activity. The same pattern followed on a per gram dry and whole tissue basis. The alanine aminotransferase activity of radicle was slightly higher than that found in plumule, whereas the aspartate aminotransferase activity was more than 20% lower than the plumule on a fresh weight basis. On a dry weight basis, the GPT activity of the radicle was marginally lower than the plumule, but GOT activity of the radicle showed more than 30% decrease. On a whole tissue basis, the GPT activity of the radicle showed more than 30% higher activity than the plumule while that of GOT was slightly lower.

The combined GPT activity of the plumule + radicle was same as the 48 h embryonic axis while that of the GOT was 4 times higher than the 48 h embryonic axis on a per gram fresh tissue basis. On a per gram dry tissue basis, the GPT activity of the plumule + radicle was slightly lower than the 48 h embryonic axis and that of the GOT was more than 3 times higher. On a whole tissue basis, the GPT activity of plumule + radicle was about 30% lower than the 48 h embryonic axis while, that of the GOT was slightly lower than the 48 h embryonic axis.

Scarified seeds

(a) Cotyledons

The cotyledons of scarified seeds showed a slight increase in activities of GPT and GOT on the 2 h of water uptake. The activity of GPT showed a rise in activity. On the 6 h, the activity of GPT increased while that of GOT slightly decreased. The cotyledons of 12 h showed a rise in activity of both aminotransferases which gradually decreased upto 48 h of contact with water. At 72 h of imbibition, both aminotransferases showed a rise in activity, the GPT showed about 50% increase and GOT showed about 60% increase; these were the peak activities on a per gram fresh tissue basis.

On a per gram dry tissue basis, and per whole tissue basis, both the activities of GPT and GOT gradually increased from zero hour upto 12 h and then gradually decreased upto 48 h. At the end of 72 h, the activities were almost doubled in the case of per gram dry tissues and in the case of whole tissues, the GPT showed above 50% increase and GOT was almost doubled when compared with the 48 h cotyledons.

(b) Embryonic axis

As reported in imbibing seeds, the embryonic axis of the scarified seeds also showed a decrease in activity of both aminotransferases from 24 to 48 h of imbibition, about 20% reduction in GPT and about 10% reduction in GOT. The same pattern followed on a per gram dry tissue basis. But in the case of whole tissues, the activities of 48 h were slightly higher than that of the 24 h embryonic axis.

(c) Plumule

In the plumule, on a fresh weight basis, the activities of both aminotransferases ware slightly higher than that found in imbibing and delayed seeds. Here also the activity of GOT exceeded GPT by almost 3 times. The pattern of change was same in the case of per gram dry and per whole tissues.

(d) Radicle

In the radicle, the activity of GPT was slightly higher than that found in plumule on a fresh weight basis. The GOT activity was more than double than that of GPT on a per gram fresh and dry tissues and on a whole tissue basis. The radicle also showed higher activities of both aminotransferases when compared with imbibing and delayed seeds. On a fresh weight basis, the alanine aminotransferase activity of the plumule was almost same as that of the radicle. But the aspartate aminotransferase activity of radicle was more than 20% lower than the plumule. The pattern was same as a per gram dry tissue basis also. On a whole tissue basis, the GPT activity of the radicle was more than 15% higher than the plumule, while that of the GOT was 10% lower than the plumule.

Changes in protein in three types of winged bean seeds during germination

The values are expressed on a per gram fresh and dry tissue and on a whole tissue basis . The analytical data are reported in table 20 and figure 40.

Table 20 and Figure 40

Concentration

Imbibing seeds

(a) Cotyledons

Maximum concentration of proteins were observed in the dry cotyledons on a fresh weight basis. About 15 percent reduction in concentration of proteins was observed after 2 h of imbibition which gradually decreased and at the end of 24 h of imbibition, the concentration decreased to about one half and further reduced to one third at the end of 72 h.

Contrary to the changes in fresh tissue, when calculated on a per gram dry tissue basis, the concentration of proteins showed gradual increase and at the end of 12 h, more than 10% increase occurred forming a peak and decreased in subsequent stages. About 10% reduction in proteins were noticed at the end of 72 h when compared with the initial protein concentration.

(b) Embryonic axis

The concentration of protein in the embryonic axis was negligible when compared with the cotyledons. The embryonic axis after 48 h of contact with water showed a 10% increase in total protein over the 24 h embryonic axis. The concentration on a per gram dry tissue basis showed about 50% increase at 48 h over the 24 h embryonic axis.

Table 20. Changes in Protein in 3 types of winged bean seeds during germination

Time of imbibition, h.	Tissue analysed	Protein, mg								
		Imbibing			Delayed			Scarified		
		Per g. fresh tissue	Per g. dry tissue	Whole tissue	Per g. fresh tissue	Per g. dry tissue	Whole tissue	Per g. fresh tissue	Per g. dry tissue	Whole tissue
0	Cotyledon	301.58±13.94	335.64±15.51	75.99±3.51	301.58±13.94	335.64±15.51	75.99±3.51	301.58±13.94	335.64±15.51	75.99±3.51
2	Cotyledon	258.59±14.77	339.42±19.38	67.23±3.84	301.58±13.94	335.64±15.51	75.99±3.51	282.03±11.29	340.16±13.62	72.84±2.92
4	Cotyledon	196.97±05.78	341.43±10.02	341.43±10.02	235.94±11.03	304.75±14.24	60.77±2.84	258.77±10.24	350.96±13.88	71.93±2.84
6	Cotyledon	184.90±04.00	352.67±07.63	52.14±1.12	195.29±05.80	323.69±09.61	53.11±1.57	225.70±05.76	374.29±09.55	71.09±1.81
12	Cotyledon	186.69±04.33	375.56±08.70	75.89±1.76	188.05±12.99	345.93±23.89	60.17±4.15	204.59±09.38	395.25±18.12	85.51±3.92.
24	Cotyledon Embryonic axis	163.86±04.81 9.01±00.88	358.01±10.59 54.40±05.31	78.81±2.31 0.09±0.00	173.01±07.21 7.82±00.88	360.74±15.01 54.42±06.14	64.87±2.70 0.08±0.01	166.66±08.60 8.07±00.54	356.42±17.25 60.06±14.03	75.49±3.65 0.09±0.00
48	Cotyledon Embryonic axis	139.88±13.03 9.99±00.47	340.43±31.73 79.40±03.80	70.07±6.52 0.13±0.00	152.21±08.97 10.04±0.67	351.28±20.70 75.20±05.01	66.05±3.89 0.13±0.01	150.66±14.74 10.05±00.68	336.74±32.95 76.06±04.85	70.19±6.86 0.10±0.01
72	Cotyledon Plumule Radicle	105.46±03.35 24.36±01.57 24.17±01.75	300.30±09.54 163.45±10.60 140.03±10.42	54.10±.71 0.22±0.01 0.25±0.01	114.81±03.99 23.41±01.02 23.65±01.46	322.33±11.21 164.49±07.14 140.40±0871	62.53±2.17 0.19±0.01 0.23±0.01	105.60±03.64 27.35±01.00 25.37±01.89	301.79±1.40 173.09±06.33 153.87±11.49	52.06±1.79 0.26±0.01 0.27±0.02

(The values are the mean of a minimum of 6 individual of determinations)

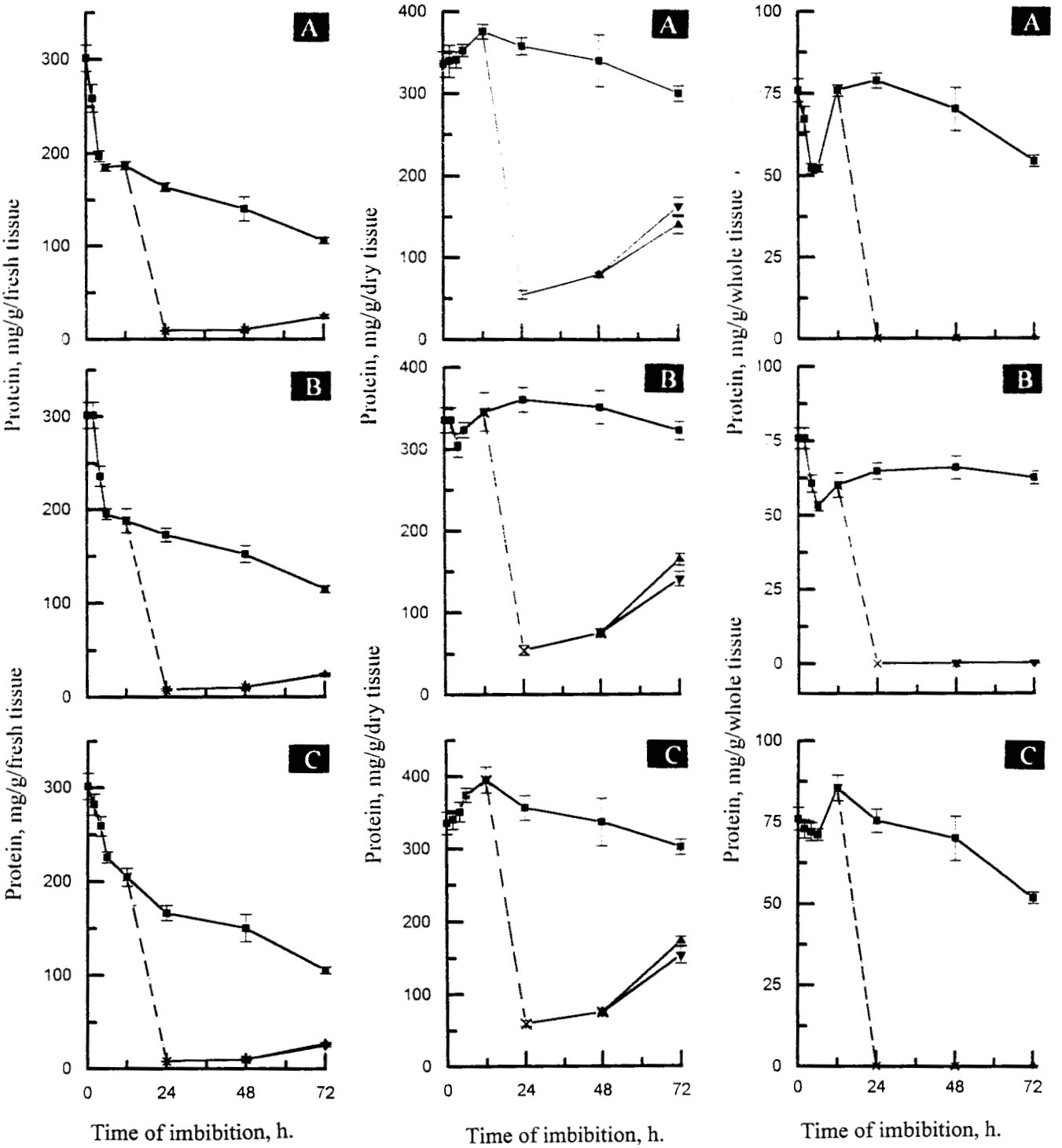


Figure 40. Changes in protein in three types of winged bean seeds during germination. A-Imbibing seeds, B-Delayed seeds and C-Scarified seeds.

—■— -Cotyledon —✳— -Embryonic axis —▲— -Plumule —▼— -Radicle

(c) Plumule and Radicle

The concentration of protein in the plumule and radicle was same on a per gram fresh tissue basis, while on a per gram dry tissue basis the plumule showed about 15% higher concentration than the radicle.

The concentration of protein of the plumule plus radicle was four times higher than the 48 h embryonic axis, which on a dry tissue basis was 3- times higher than the 48 h embryonic axis.

Delayed seeds

(a) Cotyledons

In the cotyledons of delayed seeds, on a per gram fresh tissue basis, there was a sharp decrease in the protein concentration at the end of the 4 h of contact with water which decreased gradually and at the end of 24 h, was reduced to about 60% and further reduced to one half after 48 h and finally reached to about 40% after 72 h of imbibition. But when calculated on the basis of per gram dry tissue, the concentration of protein showed a slight decrease at the end of 4 h and then increased gradually and attained a maximum at the end of 24 h (about 10% increase) but again reduced on 48 and 72 h. The 72 h cotyledon showed only about 5% decrease in total protein when compared against the zero hour proteins concentration. The cotyledons of delayed seeds showed slightly higher concentration of protein in most of the stages when compared with the imbibing seeds (Table 20).

(b) Embryonic axis

The protein concentration of the embryonic axis of the delayed seeds at the end of 24 h was lower than that of the imbibing seeds. The concentration slightly

increased on 48 h on a per gram fresh tissue basis. The concentration of protein at the end of 24 h on a dry weight basis was same as that of the imbibing seeds which showed about 40% increase on 48 h of imbibition.

(c) Plumule and Radicle

The protein concentration of the plumule was same as the radicle on a per gram fresh tissue basis. On a per gram dry tissue basis, the protein concentration of the plumule was higher by about 15% than the radicle.

On a per gram fresh tissue basis, concentration of protein of the plumule and radicle together was about 5 times higher than the 48 h embryonic axis while on a per gram dry tissue basis was only 4- times.

Scarified seeds

(a) Cotyledons

The concentration of protein in the cotyledons of scarified seeds showed gradual decrease upto 72 h of contact with water. The 48 h protein concentration showed 50% decrease over the control, which again reduced to one third after 72 h. But when calculated on the basis of per gram dry tissue, the concentration of total protein showed gradual increase to about 20% at the end of 12 h, and then gradually decreased in subsequent stages. The concentration of protein on zero hour and 48 h were almost same. But a 10% reduction from the initial concentration was recorded at the end of 72 h of imbibition and was equal to that of the imbibing seeds.

(b) Embryonic axis

In the embryonic axis of the scarified seeds, the protein concentration of

48 h showed 25% increase than the 24 h embryonic axis on a per gram fresh and dry tissue basis. The concentration of proteins in the embryonic axis of the delayed and scarified seeds at the end of the 48 h were almost equal.

(c) Plumule and Radicle

The protein concentration of the plumule was more or less same as the radicle on a per gram fresh tissue basis. On a dry weight basis, the concentration of protein in the plumule was more than 10% higher than the radicle.

The concentration of protein of the plumule and radicle together was 5 times higher than the 48 h embryonic axis on per gram fresh tissue basis, which on a per gram dry tissue basis was more than 4 times higher.

Content

Imbibing seeds

(a) Cotyledons

The content of protein in the dry cotyledons gradually decreased upto 6 h of contact with water. On 12 h, the amount of protein increased to the initial level which again slightly increased on 24 h, forming the maximum content and gradually decreased to about 70% of the initial level at the end of 72 h of imbibition.

(b) Embryonic axis

The content of protein in the 24 h embryonic axis was negligible since the embryonic axis itself was very small at that stage. The embryonic axis at the end of 48 h showed about 50% increase in content of total protein.

(c) Plumule and Radicle

The protein content of the radicle was about 15% higher than the plumule. The plumule and radicle together showed more than 3 times higher content than the 48 h embryonic axis

Delayed seeds

(a) Cotyledons

The cotyledons of delayed seeds showed about 20% decrease in content of protein at the end of 4 h and about 30% decrease at the end of 6 h, but started increasing gradually and attained a supplementary peak at the end of 48 h which again slightly decreased at the end of 72 h contact with water. The protein content of 48 h showed more than 10% decrease than the initial level which at the end of 72 h was reduced by almost 20%. When compared with the imbibing seeds and scarified seeds, the content of protein was much higher in the delayed seeds at the end of 72 h of imbibition.

(b) Embryonic axis

In delayed seeds also the protein content of the embryonic axis was very low during the 24 h of imbibition. On 48 h, the content of protein in the embryonic axis showed an increase of about 60% and was equal to that of the imbibing seeds.

(c) Plumule and Radicle

In the delayed seeds, the radicle showed more than 20% higher protein content than the plumule. The content of protein of the plumule and radicle together was more than 3 times higher than the 48 h embryonic axis.

Scarified seeds

(a) Cotyledons

The protein content of the cotyledons of the scarified seeds showed gradual decrease corresponding with the progress of imbibition upto 6 h. A 10% increase in the content of protein was noticed in the 12 h cotyledons and was the peak content which then decreased in subsequent stages. In 72 h cotyledon, the content of protein was reduced to about 70% of the 0 h cotyledons.

(b) Embryonic axis

The embryonic axis at the end of 48 h showed about 10% more protein than that on 24 h. The 48 h protein content was low when compared with imbibing and delayed seeds.

(c) Plumule and Radicle

The content of protein of the plumule was almost same as the radicle. The plumule and radicle together showed more than 5 times higher protein content than the 48 h embryonic axis.

Specific activity

The analytical data are reported in table 21

Table 21

Imbibing seeds

(a) Cotyledons

The specific activity of both aminotransferases gradually increased and attained maximum at the end of 72 h of imbibing. A slight drop in activity was

Table 21. Specific activities of alanine and aspartate aminotransferases in 3 types of winged bean seeds during germination

Time of Imbibition, h	Tissue analysed	Specific activity					
		Imbibing		Delayed		Scarified	
		GPT	GOT	GPT	GOT	GPT	GOT
0	Cotyledon	0.52±0.05	0.60±0.03	0.52±0.05	0.60±0.05	0.52±0.05	0.60±0.03
2	Cotyledon	0.69±0.08	0.88±0.08	0.52±0.05	0.60±0.05	0.56±0.02	0.64±0.04
4	Cotyledon	0.87±0.02	1.05±0.04	0.66±0.03	0.75±0.04	0.57±0.02	0.78±0.00
6	Cotyledon	0.95±0.06	1.02±0.06	0.75±0.02	0.85±0.04	0.74±0.04	0.87±0.03
12	Cotyledon	0.90±0.04	1.14±0.14	0.76±0.03	1.03±0.07	0.96±0.06	1.24±0.03
24	Cotyledon	0.99±0.14	1.19±0.14	0.81±0.04	0.98±0.08	1.00±0.09	1.16±0.12
	Embryonic axis	35.20±2.70	24.03±2.01	34.15±1.95	22.55±1.43	40.18±2.50	28.73±2.69
48	Cotyledon	1.21±0.15	1.38±0.22	1.01±0.09	1.11±0.18	0.91±0.14	1.06±0.11
	Embryonic axis	28.10±2.70	18.75±2.42	25.28±1.21	19.74±1.14	25.69±0.94	20.36±1.60
72	Cotyledon	1.83±0.16	2.31±0.15	1.53±0.16	1.92±0.12	1.92±0.08	2.52±0.20
	Plumule	5.72±0.85	17.69±2.00	4.86±0.41	17.86±0.75	6.25±0.55	17.04±0.59
	Radicle	7.23±0.83	14.43±1.39	5.55±0.49	13.66±0.60	7.01±0.31	14.39±0.93

(The values are the mean of minimum of 6 individual of determinations)

noted during 12 h in the case of GPT and at 4 h in the case of GOT. At all stages of the imbibition, the GOT specific activity exceeded the GPT specific activity.

(b) Embryonic axis

The embryonic axis on 24 h of imbibition showed higher specific activities for both aminotransferases than the 48 h embryonic axis. The GPT specific activity exceeded the GOT specific activity.

(c) Plumule and Radicle

The specific activity of alanine aminotransferase in the radicle was more than 25% higher than that of the plumule while the specific activity of aspartate aminotransferase in the radicle was lower by 20% than the plumule.

The specific activity of plumule and radicle together was more than 50% lower than the 48 h embryonic axis in the case of alanine aminotransferase. In the case of aspartate aminotransferase, the specific activity of plumule and radicle together was more than 70% higher than the 48 h embryonic axis.

Delayed Seeds

(a) Cotyledons

The specific activity gradually increased from zero to 72 h. The GOT showed higher specific activity than GPT at all stages. The specific activities of both aminotransferases was slightly lower than the imbibing seeds at all stages.

(b) Embryonic axis

The specific activities of the 48 h embryonic axis were reduced to about 70% over the 24 h in the case of GPT, while that of GOT was only slightly lower. The GPT specific activity exceeded that of the GOT specific activity.

(c) Plumule and Radicle

In the delayed seeds, GPT of the radicle was slightly higher than (about 15%) the plumule, while in the case of GOT, it was about 25% lower than plumule. The combined specific activities of plumule and radicle together was more than 50% lower than the 48 h embryonic axis, while in the case of GOT, it was 60% higher than the 48 h embryonic axis. Here also as in imbibing seeds, the GOT specific activity exceeded the GPT specific activity.

Scarified Seeds

(a) Cotyledons

The specific activities increased gradually from zero hour and reached maximum on 72 h of imbibition. When compared with the cotyledons of delayed seeds, the scarified seeds showed highest specific activity.

(b) Embryonic axis

The specific activities of both aminotransferases were highest during the 24 h than the other 2 types of seeds and about 60% reduction in specific activity occurred in the case of GPT and about 70% reduction occurred in the case GOT of 48 h embryonic axis. In both stages, the specific activity of GPT exceeded that of GOT.

(c) Plumule and Radicle

The GPT specific activity of the radicle of the scarified seeds was also higher (more than 10%) than the plumule while that of the GOT was about 20% lower than the plumule. The combined specific activities of plumule and radicle were lower by about 50% than the 48 h embryonic axis in the case of GPT while

that of GOT was more than 50% higher than the 48 h embryonic axis. Here also specific activity of GOT of both plumule and radicle exceeded that of the GPT.

Discussion

Changes in fresh weight and dry weight during germination

By definition, "germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of embryonic axis" (Bewley and Black, 1994). The visible sign that germination is complete is usually the penetration of the structures surrounding the embryo by the radicle. Subsequent events, including the mobilization of the major storage reserves are associated with growth of the seedling (Bewley, 1997).

As mentioned earlier, uptake of water by a dry mature seed is triphasic with a rapid initial uptake (phase I) followed by a plateau phase (phase II), A further increase in water uptake occurs only after germination is completed, as the embryonic axis elongates. Because dormant seeds do not complete germination, they cannot enter phase III. According to Bewley (1997), phase I and II represent germination and phase III represents post germination events. In the present study it can be assumed that upto 24 h of water uptake is phase I, 24-30 h is phase II and the phase III commences after 30 h.

In the studies by the author using V16 variety, the 3 types of seeds followed the triphasic mode of water uptake. At the end of 24 h of imbibition, the imbibing and scarified seeds registered more than 90 and 80% increase in fresh weight respectively, whereas, the delayed seeds showed only 50% increase due to the slow water uptake during the earlier hours. The final hydration level was higher in the delayed seeds which were 10 and 20% higher than the imbibing and scarified

seeds respectively. But there was no difference in dry weight percentage at the end of 72 h among the 3 types of seeds. As pointed out earlier, the differences in final hydration level among seed types of intra and inter varieties may be due to the size, shape, structure and composition of the testa as well as the embryo.

On day 3, the radicle showed higher fresh weight and dry weight percentage than the plumule in all the 3 types of seeds. As already known, it is the radicle which extends and expands first during the process of germination.

It was observed that the testa breaking near the micropylar region and radicle penetration started between 24 – 30 h in seeds of V16 variety (Table-4). Spruny (1973) found that the root tip of pea seeds bursts through the testa by 25 hours after contact with water. Radicle extension through the structures surrounding the embryo is the event that terminates germination and marks the commencement of seedling growth. This extension may or may not be accompanied by cell division (Bewley, 1997). He is of opinion that there are three possible reasons for the commencement of radicle growth. During germination the osmotic potential of the radicle cells become more negative due to the accumulation of solutes, would lead to increased water uptake and the resulting increase in turgor would drive cell extension.

A second possibility is that extensibility of the radicle cell walls allows for their elongation.

A third possibility is that seed tissues surrounding the radicle tip weaken, thus allowing the tip to elongate. In many germinating seeds, the testa splits during imbibition, and it is only the rigidity of the radicle cell walls that restrains growth (Schopfer and Plachy, 1985).

At the end of 72 h of imbibition in the present study about 20% dry matter loss was observed in the imbibing types, 15% loss in the delayed types while the scarified types showed about 25% loss in the testa -freed oven dried seeds (Data not shown in table). Contrary to this finding, Kamaladevi (1985) reported that in PT 3 variety, the cotyledon pair on day 9 contained 85.8% of the dry matter in the testa-freed oven dried seeds and the embryonic axis on day one was found to constitute only 0.61% of the weight of the imbibed seed in the dried condition. In V 16 variety the percentage of embryonic axis on day one constituted 0.82, 0.87 and 0.76 % respectively for imbibing, delayed and scarified seeds on a dry weight basis.

It is known that GA and cytokinins are involved in inducing the formation of hydrolytic enzymes which regulate the mobilization of reserves (Mayer and Poljakoff-Mayber, 1989). In ten days, following seed sowing in compost and germination under glass house conditions, the cotyledons of runner bean and pea (both hypogeal) lost respectively 71 and 74% of dry weight; under similar conditions, the cotyledons of french bean (epigeal) suffered 85% loss in weight (Lovell and Moore, 1970). Kamaladevi (1985) suggested that the slow mobilization of reserves may be due to the inadequacy of the amount of stimulant transmitted from the winged bean axis tissue or that the production is not sustained. Also, because of the unusually small bulk of the sink tissue (axis) and hence limited structural and energy requirements for metabolites, products of reserve mobilization are not transferred sufficiently fast from the source (cotyledons).

The slightly higher loss of dry weight content in the case of scarified seeds may be due to the enhanced leakage of solutes due to scarification as reported by Simon (1984).

Changes in total sugars during germination

Rarely are sugars the predominant storage carbohydrates in seeds. A well known exception is *Acer saccharum*, which contains 64% sucrose and 5.2% of other sugars, with no starch (Crocker and Barton, 1957). Kamaladevi (1985) reported 8.9 percent total sugar on a dry weight basis in the testa-freed cotyledons of PT 3 variety. The data of Rockland *et al.* (1979) indicated 12% soluble sugars for the seed of an unspecified winged bean line. The winged bean seed with 7.5% soluble sugars on a dry weight basis, as found in the present study using V16 variety qualified it to be classified as a sugar accumulating seed, particularly in the light of the low concentration of starch.

There were no significant quantitative differences in the level of total sugars observed between the 3 types of seeds during the period of germination and post-germination stages upto 72 h. In all the 3 types of seeds, both content and concentration of total sugars showed gradual decrease upto 12 h. Respiration during initial water uptake requires a supply of readiliy - available substrate other than that derived from hydrolysis of the major stored reserves, since the latter only become available after embryo growth has commenced (Bewley and Black, 1983). The decline in sugar concentration (and content) may be attributed to the possibility that it served as the readily available respiratory substrate after contact with water. According to Mayer and Shain, (1974), the substrates consumed in respiratery metabolism during the early period of germination are sugars or oligosaccharides.

At the end of 24 h of contact with water the concentration and content of sugars increased and were almost the same as that of the 0 h cotyledons on a dry tissue basis. After 72 h, the cotyledons of 3 types of seeds showed about 20%

reduction in total sugar than the 0 h cotyledons - control. But the total concentration of sugars of the cotyledons + plumule and radicle on a dry weight basis showed around 50% increase than the 0 h cotyledons in all the 3 types of seeds.

Studying the reserve mobilization in winged bean during post germination stages Nabeesa Salim and Harikumar (1994) found that starch increased 3-fold while total sugars and lipids showed more than 20% decrease in the cotyledons after 3 days. According to these authors, the increased starch content in the winged bean seeds during germination may be attributed to gluconeogenesis from the lipid reserves. According to Beevers (1980), during seedling development (post germination) acetyl Co A of lipid origin is used for carbohydrate synthesis via the glyoxylate cycle. Sucrose is generated through intermediate formation of fructose 6- phosphate by the activity of fructose 1, 6-bisphosphatase by reversal of glycolytic reactions. An increased rate of fuctose 1, 6-bisphosphatase activity has been reported in winged bean seedling development by Kamaladevi and Madhusudanan (1989). The fructose 6- phosphate is further converted to sucrose by the sucrose phosphate synthase reaction. The sucrose generated in the cotyledons is transported in the phloem and metabolized to other sugars or converted to starch. Starch synthesis during germination presumably from lipids has been reported in soybean (Adams *et al.*, 1980). In castor bean the major product of lipid mobilization is sucrose which is taken up by active transport into the cotyledons. More than 80% of this sucrose is re-distributed to the growing axis (Bewley and Black, 1983).

Free amino acids

The amino acids showed variations in the concentration and content with the progress of imbibition. Glutamic acid was the most abundant amino acid followed by aspartic acid, alanine, glycine in almost all stages. Methionine was not detected in the earlier stages but increased to detectable amounts in late stages. Most of the amino acids showed elaboration of its concentration and content in all stages while threonine, glycine and histidine showed gradual decrease. The pattern of variation was not the same for different amino acids. As has been suggested by Derbyshire *et al.* (1976), this could be attributed to the variation in the free amino acid composition of the storage proteins. When compared with the cotyledons, the concentration and content of free amino acids were much lower in the embryonic axis, plumule and radicle. Also, only a few amino acids were present in assayable amounts in the embryonic axis and at the end of 72 h, the concentration and content of most of the amino acids increased in the plumule and radicle. Valine, methionine and histidine were not detected in the embryonic axis.

Mobilization of storage proteins involves proteolysis; the liberated amino acids may then undergo considerable inter conversion leading, in particular, to synthesis of amides. Following transport of a selected group of amino acids and amides to the growing seedling, further interconversion is required to provide the full range of amino acids and nitrogenous components necessary for protein synthesis and growth (Lea and Joy, 1983).

In contrast to the more balanced composition of average cytoplasmic proteins in the seedling, the seed proteins often have a great predominance of a few amino acids (Sodek and Wilson, 1973). In barley proteins, glutamic acid

plus proline account for 60% of the amino acid residues. Over a half of protein nitrogen in legumes such as pea or soybean are in the form of glutamic acid, aspartic acid, arginine and leucine (Sosulski and Holt, 1980). Ekpenyong and Borchers (1982) also reported that the major amino acids of the winged bean (variety TPt – 2) seed proteins are glutamic acid, aspartic acid, alanine, leucine, lysine and arginine. The observation by the present author are also in consonance with the above report.

Glutamine and asparagine that occur in most proteins are formed from glutamic acid and aspartic acids. They represent especially important forms in which nitrogen is transported form one part of the plant to another in which surplus nitrogen can be stored (Salisbury and Ross, 1991). According to Bewley and Black (1983), variations between species is considerable. In pea it was found that, the major N carrier was a non-protein amino acid homoserine. Only very little aspartate or glutamate is transported to the axis. Pate (1989) reported that in temperate legumes, the amides asparagine and glutamine make considerable contributions, whereas in some tropical legumes like winged bean, cow pea, soybean etc., the ureides, allantoin and allantoic acid are the chief N transport compounds involved. Xylem exudates from detached nodules of winged bean and cow pea show total ureide levels as high as 40 – 60 mM.

Total amino acids

The concentration of total free amino acids on a dry weight basis showed progressive increase from the 2 h onwards and registered a four-fold increase after 72 h in the cotyledons. The embryonic axis of 48 h showed more than a two-fold increase from that of the 24 h embryonic axis. The amino acid concentration of the radicle was slightly higher than the plumule. The plumule

and radicle together showed a five-fold increase from the 48 h embryonic axis. The pattern of change was almost same in all the 3 types of seeds. On a whole tissue basis also, the pattern of changes were almost same as that of the concentration. The abundance of free amino acids was in the cotyledons than the embryonic axis (Table 18 A to I).

According to Capdevila and Dure (1977) the amino acid pool increases during germination. When cotton seed embryos were germinated for 3 days, the total amino acid pool of cotyledon showed a five-fold increase. Madhusudanan *et al.* (1982) found in germinating cow pea seeds that the values for total free amino acids showed an increase in 12 h, a doubling in 24 h, a further doubling in 48 h followed by a halving in 72 h. The main source of these amino acids is the storage proteins, but their amino acid ratio need not necessarily be the same as that of the newly synthesized seedling protein and apparently interconversion of amino acids occurs. The main pathways for such interconversions are transamination and deamination reactions. It has been suggested that amino acids rapidly provide keto acids to allow establishment of respiratory cycles in early stages of germination and even to sugar synthesis in gluconeogenesis (Collins and Wilson, 1975).

Protein changes

The dry seed constituted 33.56% protein on a testa-free dry weight basis which registered a depletion of about 10 % after 72 h of imbibition in all the 3 types of seeds. According to Kamaladevi (1985), winged bean cotyledons (PT 3) after 9 days of germination showed only 15% loss in proteins. In contrast to this, in mung bean seeds germinating in the dark, only about 5% of proteins was left in the cotyledons at the end of 6 days (Mina mikawa, 1979). The slow rate at which

proteins decreased in the cotyledons may be due to slow proteolysis, either because of low production of proteases or regulation by protease inhibitors. If the elaboration of the digestive protease in the winged bean seed is retarded due to insufficient hormonal stimulant production by the small sized axis, protein mobilization will be slowed down in the cotyledons. Winged bean seeds contain protease inhibitors (Kortt, 1979), but it is not known whether these inhibitors have any effect during germination on the hydrolysis of reserve proteins held in the protein bodies. An interesting observation in a study from this laboratory (Khaleel, unpublished) was that the vascular strand distribution in winged bean was very sparse in cotyledon during germination. The reserves (both lipids and proteins) of the cotyledonary cells showed degradation only in a few layers of the cells around the vascular strands during germination. This might account for the slow mobilisation of reserves from the winged bean cotyledons during germination.

When compared with the cotyledons, the concentration of proteins of 24 h embryonic axis on a dry weight basis was more than 15% irrespective of the seed types. But the concentration in the plumule and radicle together showed several fold increase and was same as that of the respective cotyledons after 72 h of imbibition. On a whole tissue basis also the same pattern followed though the protein content of the embryonic axis was very negligible when compared with the cotyledons. According to Bewley (1997), protein biosynthetic reactions are characteristic of the developing axis tissue and in the cotyledons of germinating seeds. All the components necessary for the resumption of protein synthesis upon imbibition are present within the cells of mature embryos, although polysomes are absent (Bewley, 1997). Initial protein synthesis is dependent on extant

ribosomes, but newly synthesized ribosomes are produced and used within hours of initial polysome assembly (Dommer and Van der Walle, 1990). New m-RNAs are transcribed as germination proceeds. The majority of these are likely to encode proteins essential for the support of normal cellular metabolism, that is growth maintenance reactions (Bewley and Marcus, 1990). Studies with embryonic axis of *Phaseolus vulgaris* have shown that germinating seeds can utilize ribosomes formed during the maturation phase (Walbot, 1971). In peas also there is evidence for the preservation of functionally active ribosome during seed desiccation (Paulson and Beevers, 1973).

Amino acids formed by degradation of storage proteins or free amino acids present in the dry seed could be reutilized for synthesis of metabolically active proteins. It was also possible that cotyledonary tissues brought about the synthesis of amino acids from nitrate nitrogen, for which evidence exists in developing legume seeds (Schlesier and Muntz, 1974). It was, however, not clear why free amino acids and other non-protein nitrogenous material should be utilized in the elaboration of significant quantities of protein in the cotyledons, when the primary purpose was amino acid transfer to axis.

The metabolic form of proteins in the cotyledons of winged bean seed may constitute only a small part of the total protein. According to Millerd (1975) and Miege (1982), upto 80% of the total proteins in legume seeds are constituted of reserve proteins. The axis proteins are constituted essentially of metabolic and structural proteins; immunoelectrophoretic studies have, however, indicated the presence of storage proteins also in the axis tissue of legumes (Millerd, 1975).

Alanine and aspartate aminotransferases activities in three types of winged bean seeds during germination

Germination studies on the three types of winged bean revealed that the activities of both alanine and aspartate aminotransferases were active in the dry seed which gradually increased during germination. Embryonic axis, plumule and radicle exhibited very high activity than the cotyledons. In the cotyledons, plumule and radicle, the activity of GOT exceeded GPT while in the embryonic axis, the reverse was the case. According to Wightman and Forest (1978), the activity of different aminotransferases may vary quantitatively during seed germination and seedling growth. In general, these enzymes increase significantly in total activity in the developing organs of the young plant.

In early investigations (Albaum and Cohen, 1943; Smith and Williams, 1951), a marked increase in activity of glutamate oxaloacetate and glutamate pyruvate aminotransferases was found in the embryo during the first few days of germination in *Hordeum vulgare, Zea mays* and *Cucurbita* seeds.

Madhusudanan *et al.* (1982) studied the changes in aspartate and alanine aminotransferases in *Vigna unguiculata* seeds upto 72 h of germination and found that at all stages during germination, alanine aminotransferase activity exceeded aspartate amino- transferase. But there was not consistent increase in activity upto 72 h of imbibition.

Nabeesa Salim (unpublished) found that by forcing of cotyledonary axillary bud growth during seed germination in winged bean, the GOT activity in cotyledons, plumule and radicle was prominently higher than GPT upto 10 days

of germination.

The presence of transaminases in a variety of seeds has been shown by Smith and Williams (1951). In most cases, the activity of GOT was higher than that of GPT. However, in peas the reverse was the case and in corn both enzymes seemed to increase at about the same rate during germination. These authors also showed that no fixed relation existed between increase in protein in the seeds and increase in transaminase activity, and concluded that there was no evidence to show that the two processes were directly correlated. In contrast to the results of Smith and Williams, Albaum and Cohen (1943) found fairly good correlation between increase in proteins, transaminase activity and soluble nitrogen content especially during seedling growth. In the present study also, no such correlation can be drawn between protein changes and aminotransferases activity in the cotyledons. But in the embryonic axis, plumule and radicle, the activity of both GPT and GOT increased with protein concentration and content (Table 19 A, B, C and 20).

The total free amino acid pool of the cotyledons, embryonic axis, plumule and radicle gradually increased simultaneous with enzyme activity during imbibition in the three types of seeds. Comparatively very high activity of GPT and GOT in the embryonic axis, plumule and radicle indicates that mobilized reserves from the cotyledons undergo considerable metabolic changes. Gradual increase in activity of the cotyledons probably indicates interconversion of amino acids formed by degradation of proteins, appropriate aminotransferases reactions or from intermediates of fat and carbohydrate metabolism mainly pyruvate and glutamate. Lipid degradation in winged bean cotyledons during germination was reported by Mathew (1997) and Nabeesa Salim and Harikumar, (1994).

There was not much difference in activity of GPT and GOT between the three types of seeds. According to Bewley (1997), this shows that the metabolic status of the embryos are similar, the difference being only in the seed coat hardness.

The specific activity of GPT and GOT of the cotyledons also increased gradually and reached maximum after 72 h of imbibition (Table 21). Aspartate aminotransferases showed higher specific activity than alanine aminotransferases in the cotyledons, plumule and radicle. The reverse was the case with embryonic axis. The embryonic axis, plumule and radicle showed much higher specific activity than the cotyledons. Also, the specific activity of both GPT and GOT of the plumule and radicle was lower than the embryonic axis (Table 21). The gradual increase in specific activity of the cotyledons may be attributed to the increase in enzyme activity since the protein concentration of the cotyledons showed not much variation during the 72 h period of water contact. The higher specific activity of embryonic axis, plumule and radicle is mainly due to enzymatically active protein in these fast growing tissues.

GENERAL CONCLUSIONS

General conclusions

Contents

GENERAL CONCLUSIONS.

From the author's investigations on the physiology and biochemistry of winged bean, with special reference to seed/pod development and germination, some important observations emerged. These are summarised below:

Imbibition Studies and Field Emergence

The degree of seed coat imposed dormancy as evaluated by water uptake pattern showed intra and inter verietal differences. Correlation can be drawn between testa thickness and permeability. PT51 *a* and 51 *b* are 100 % imbibers; V4, EC 28886, PT 15 and PT62 are composed of imbibing and scarified seeds, whereas, V5, V8, VI6 and selection 12 are constituted by imbibing, delayed and scarified seeds. The percentage of dry testa in PT 51 *a* and 51 *b* were very low when compared with other verities. Also, the time of testa breaking was between 18-24 h. In all the other varieties, the testa breaking occurred between 24-30 h.

The studies indicated that no correlation existed between imbibition pattern and seed size and weight. However, dark coloured seeds were found to be more impermeable than light coloured seeds.

The difference in final hydration level among the seed types of intra and inter varieties may be due to seed size, shape, structure and composition of the testa as well as the embryo.

All the 10 varieties showed 100% germination in Petri dish conditions in the laboratory. Contrary to this, in the field, maximum germination percentage varied in the range 97% (V16) and 50% (V4). The variation in germination between the two environmental conditions may be attributed to the soil atmosphere which may inhibit germination to some extent.

V5, V8, VI6, selection 12 and PT 62 were found to be more adapted for survival since their germination was spread over several days due to the different levels of hardness of the seed types present.

Pod / seed developmental studies

The source leaves got abscised well before the pods started to ripen probably due to strong sink effect. Pods attained about 90% of the maximum fresh weight at the end of 24 day of anthesis while the embryo showed only 50% of its total fresh weight.

The pods of V16 variety showed sigmoid pattern of growth. Both pod wall and embryo showed diauxic increase in fresh weight. It was found that the axillary source leaf matures first followed by pod wall, testa and finally the embryo. The increase in dry weight of the embryo was rapid after 36 day of anthesis.

The concentration and content of total sugars, amino acids and proteins exhibited decrease in late stages of development in axillary source leaf, pod wall and testa. A significant accumulation of protein in the embryo during late stages of development with simultaneous decrease in total sugars, amino acids and proteins in other tissues may be due to utilization of these metabolites for the production of reserve proteins and lipids in the embryo in a relatively short period of time.

The pod wall and testa showed maximum phenolics in the late stages as seeds matured. The presence of phenolic compounds in the seed coat is known to contribute to the impermeability of the seed. The seeds of variety V16 in the present studies, in fact, showed only 32% imbibing seeds, the remaining constituted of delayed and scarified seeds.

Glutamic acid was the most abundant amino acid at all stages in leaf, pod wall, embryo and testa, followed by aspartic acid, alanine, glycine, threonine, leucine(s), tyrosine, phenylalanine + tryptophan. Qualitative and quantitative changes in the free amino acids distribution was more prominent in

the later stages of development. This probably reflects the sink - source relationship during development.

The seed coat plays a dominant role in the nutrition of the developing embryo. The comparatively high levels of alanine and aspartate aminotransferases in the seed coat during seed development clearly justifies this. Extensive and selective metabolism of amino acids take place in the seed coat prior to the release of amino acids.

The activities of GPT and GOT were very high in the embryo upto 36 days after anthesis and the protein content of the embryo at this stage exhibited only a slight increase due to the high metabolism of the amino acids resulting in low accumulation of proteins. The low activity of these enzymes after 36 days shows that the tissue (embryo) functioned as a storage site with rapid accumulation of proteins.

The leaf tissue showed higher GPT activity than GOT during the early days development. The concentration of alanine and aspartic acid was found to be significantly reduced during the corresponding intervals while the concentration of glutamic acid maintained its higher level. The photosynthetic origin of these amino acids and its immediate transamination due to high activity of GPT in these tissues cannot be ruled out. The pod / pod wall, embryo and testa showed more activity of GOT than GPT upto 24 days. However, corresponding changes were not observed in the concentration of aspartic acid, glutamic acid and alanine.

The high activity of both aminotransferases in the developing pods / embryo upto 36 days which results in the synthesis of alanine and aspartic acid is understandable because of the increased requirement of various amino acids for protein synthesis and some constituents for which some amino acids serve as precursors.

Biochemical Studies During Germination

The percentage of embryonic axis (sink) was very low when compared with the cotyledons (source). The unusually small bulk of the sink tissue and hence its minimal structural and energy requirements for metabolites, limits efficient mobilization of products of reserves from the sources.

The winged bean seed with 7.5% soluble sugars on a dry weight basis, as found in the present study using V16 variety qualified it to be a sugar accumulating seed, particularly in the light of low concentration of starch.

An increase in total concentration of sugars at the end of 72 h in cotyledons, plumule and radicle were attributed to carbohydrate synthesis via glyoxylate cycle.

During germination also, glutamic acid was the most abundant amino acid followed by aspartic acid, alanine, glycine and lysine. The pattern of variation for different amino acids during germination was not identical, possibly due to variation in the amino acid composition of the storage proteins.

The total amino acid pool increased during germination. The amino acid concentration of the radicle was slightly higher than that of plumule. The pattern of changes was the same for the 3 types of seeds. The increased amino acid pool during germination provides keto acids to allow establishment of respiratory cycles in early stages of germination and even to sugar synthesis in gluconeogenesis.

The slow rate at which protein decreased in cotyledons may be due to slow proteolysis either because of low production of proteases or due to regulation by protease inhibitors.

The increase in protein concentration form 24h embryonic axis to 72h plumule and radicle is due to *de novo* synthesis of metabolically active proteins.

Both alanine and asparate aminotransferases were very active in the dry seed which gradually increased during germination. Embryonic axis, plumule and radicle exhibited very high activity than the cotyledons.

No fixed relationship existed between increase in protein in the cotyledons and increase in transaminase activity. But in the embryonic axis, plumule and radicle, the activity of both GPT and GOT increased with protein concentration and content.

The very high activity of GPT and GOT in the embryonic axis, plumule and radicle indicate that mobilized products form the cotyledons undergo considerable metabolic changes.

The gradual increase in activity of the cotyledons probably indicates interconversion of amino acids formed by degradation of proteins, appropriate aminotransferases or from intermediates of fat and carbohydrate metabolism, mainly glutamate and pyruvate.

There was not much difference in activity of GPT and GOT between the 3 types of seeds showing that the metabolic status of the embryos is similar, the difference being only in the seed coat hardness.

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