PHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING SEED DEVELOPMENT IN RELATION TO GERMINATION IN CHICKPEA (*Cicer arietinum* L.)

By

CHARAN JEET MEHTA

Dissertation submitted to Chaudhary Charan Singh Haryana Agricultural University in partial fulfilment of the requirements for the degree of:

MASTER OF SCIENCE

IN

PLANT PHYSIOLOGY

COLLEGE OF BASIC SCIENCES AND HUMANITIES

CHAUDHARY CHARAN SINGH HARYANA AGRICULTURAL UNIVERSITY

HISAR

1992

DEDICATED

to the loving memory of my

father SH. D.R. MEHTA

without whose inspiration this

manuscript would have

remained only a dream

AND

Prof. G.S. PALIWAL

(My B.Sc. Advisor)

C E R T I F I C A T E - I

This is to certify that this dissertation entitled "Physiological and biochemical changes during seed development in relation to germination in chickpea (Cicer arietinum L.)", submitted for the degree of M.Sc. in the subject of Plant Physiology, of Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by Mr. Charan Jeet Mehta under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

15/5/92

(M.S. KUHAD)
Major Advisor

C E R T I F I C A T E - II

This is to certify that the dissertation entitled "Physiological and biochemical changes during seed development in relation to germination in chickpea (Cicer arietinum L.)", submitted by Mr. Charan Jeet Mehta to Chaudhary Charan Singh Haryana Agricultural University in partial fulfilment of the requirements for the degree of M.Sc. in Plant Physiology, has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

22/6/92

MAJOR ADVISOR

19/6/92

EXTERNAL EXAMINER

22/6/

HEAD OF THE DEPARTMENT

DEAN, POST GRADUATE STUDIES

ACKNOWLEDGEMENTS

I wish to express my most sincere and profound gratitude to my major advisor, Dr. M.S. Kuhad, Associate Professor, Department of Botany, for his benign, sagacious guidance and sustained encouragement during the present investigation and also for critically reviewing the manuscript.

I have a special word of thanks for Professor I.S. Sheoran (My Previous Major Advisor), Plant Physiologist, for their inexhaustible encouragement, cordial help, guidance and moral support during investigation.

My sincere thanks are due to Dr. M.R. Saharan, Associate Professor, Department of Chemistry and Biochemistry and Dr. R.K. Jain, Associate Professor, Department of Genetics, members of my Advisory Committee, for their help, gesture and precious suggestions.

I do hereby acknowledge Dr. Jai Parkash, Professor and Head, Department of Botany, for providing all the necessary facilities during my study and research work.

I highly thankful for the help and cooperation rendered by staff members and students of the Department of Botany during these studies.

I also thanks to Dr. A.L. Bhola, Plant Breeder, for his precious suggestions, comments and timely help during the entire course of studies.

I express my feeling of indebtedness to my benevolvent parents, brothers, Mr. Harish Mehta, Mr. Gautam Mehta, and Indu, Bhabis, Mrs. Anita Mehta and Suman Mehta, sister, lovely nephews, Gaurav and Yash and nice, Nidhi.

I duly acknowledge the timely and fruitful assistance, consistant cooperation and purmount love received from my fast friends viz. Ram Niwas, Balbir, Khatri, Balwant, Davinder, Satyabhan, Jagdish, Ishwar and Shallu.

I extend my thanks to the Department of Seed Technology for providing laboratory facilities during my research and to Mr. D.V. Hooda for clean typing.

HISAR
DATED: May, 1992

(CHARAN JEET MEHTA)

CONTENTS

CHAPTER - I

INTRODUCTION

Presently, the weakest link in agricultural progress of the country is very slow growth rate of pulse crops. These crops have a unique place in farming systems as they enrich soil fertility through biological nitrogen fixation. In our country, pusles constitute the only concentrated source of protein in the diet of most of the people, owing to economic and social reasons.

Among the pulses, chickpea is the major crop which accounts for 32 per cent of the area and 39 per cent of production of pulses in the country. A substantial part of agricultural production of the country comes from dryland agriculture and chickpea is the major crop of these areas thus, its improvement will have an impact on the agricultural growth of the country. The technological advances in the production of cereals has relegated the chickpea crop to more marginal lands thus the energy rich crop is being cultivated under conditions of energy starvation resulting in low productivity, 735 kg/ha (Anonymous, 1989).

Seed plays an important role in agricultural production and quality seed is the basic input for improving productivity and production.

"Good seed sown in good soil yields abundance" Manu.

The above statement indicates the awareness among the farmers about the use of quality seeds since antiquites. Thus the use of quality seeds is the basic component of achieving higher productivity of any crop. The quality of the seed is determined by its development and maturation.

Seed maturation refers to morphological, physiological and functional changes that occur from the time of fertilization until matured ovules are ready for harvest (Delouche, 1973). Normally this period is devided into two phases (i)

Seed development phase: the period between fertilization and maximum fresh weight accumulation (ii) Seed maturation phase: the period starting from the end of the development phase upto harvest (Abdul-Baki and Baker, 1973). Physiological maturity, normally understood as occuring when the seed reaches to maximum dry weight (Harrington, 1972), at this stage the nutrients flow from mother plant to seeds stop. At physiological maturity the vascular connection to the seed is broken due to formation of abscission layer (Eastin et al., 1973). Physiological and bio-chemical changes might set in, if the seeds are retained on the mother plant for longer duration after physiological maturity (Ovacharov and Kizilova, 1966) which would lead to the development of hard seeds or off-colour seeds in pulses (Dharmalingam and Ramakrishanan, 1978; Dharmalingam and Basu, 1989, 90). Physiological maturity of seed occured 52, 62 and 78 days after flowering in Cajanus cajan, Dolichos lablab and Stizolobium aterrimum respectively and at these times moisture content and germination were 11 and 80 per cent, 8 and 67 per cent and 45 and 80 per cent, respectively (Veira et al., 1988). However, such informations are not available in chickpea.

Many studies has been conducted on different aspects of seed development in legumes such as accumulation of carbohydrates, dry matter (Singh et al., 1980; Barratt, 1982; Geervani and Devi, 1988; Dharmalingam and Basu, 1989, 1990; Bharud and Patil, 1990) and accumulation of soluble and insoluble nitrogen, proteins, starch and ethanol soluble sugars (Singh and Jambunathan, 1982; Tripathi et al., 1987; Geervani and Devi, 1988). Most studies have examined only a single genotype except in few studies where more genotypes have been used. However, little emphasis has been given to the germination potential and its relationship with various physiological and biochemical parameters such as developmental age of seed, accumulation of protein, sugars, starch and dry matter. Therefore, present study was undertaken with following objectives:

1. To study the physiological and biochemical changes during seed development in chickpea cultivars differing in seed size
2. To study the development of germination potential in different chickpea cultivars and correlate with above parameters.

CHAPTER-II

REVIEW OF LITERATURE

Seed plays an important role in agricultural production and quality seed is the basic input for improving productivity and production. Seed production and quality is also affected by time of harvesting of seed, seed development period and seed maturation.

Seed maturation refers to morphological, physiological and functional changes that occur from the time of fertilization until matured ovules are ready for harvest (Delouche, 1973). These changes during seed maturation in chickpea were reported earlier by various scientists i.e. (Srivastava et al., 1981; Singh et al., 1981; Singh and Jambunathan, 1982; Franklin and Murry, 1982; Setia et al., 1984; Srivastava, 1987; Tripathi et al., 1987; Geervani and Devi, 1988; Sharma and Bhal, 1989; Bharud and Patil, 1990).

Seed development is the period between fertilization and maximum fresh weight accumulation and maturation begins at the end of seed development and continuous upto harvest (Abdul-Baki and Baker, 1973). In chickpea seed development period was 28 days after flowering (Singh et al., 1981; Singh and Jambunathan, 1982). However, Srivastava et al. (1981) reported 35 days period after flowering for seed development.

Physiological maturity is normally understood as occurring, when the seed reaches its maximum dry weight (Harrington, 1972). In chickpea seed, physiological maturity was reported 35 days after flowering (Srivastava et al., 1981; Singh et al., 1981; Singh and Jambunathan, 1981); 40 days after flowering (Tripathi et al., 1987) and 56 days after anthesis (Bharud and Patil, 1990).

Physiological and biochemical changes might set in, if the seeds retained on the mother plant for longer duration after physiological maturity (Oracharov and Kizilova, 1966) which would lead to the development of hard seeds or off-colour seeds in pulses (Dharmalingam and Ramakrishanan, 1978; Dharmalingam and Basu, 1989, 90).

The germination percent of seed is also affected by time of harvesting (Viera et al., 1988; Bharud and Patil, 1990). Some physiological and biochemical aspects of seed have correlation with germination percent also.

Fresh and dry weight of seed

The fresh weight of seed increased upto 28 days after flowering in chickpea during seed development and then a sharp decline was observed in all the cultivars, whereas rapid accumulation of grain matter was observed between 14 and 28 days after flowering. Dry weight of the seed increased upto 35 days after flowering, thereafter only a slight change recorded in all the cultivars. The cv. 850-3/27 attained maximum seed dry weight (Singh et al., 1981). Srivastava et al. (1981) reported that fresh weight of cotyledons increased during development until the 35 day stage and then decreased at maturity and dry weight of cotyledons increased throughout development. The accumulation of dry weight was maximum between 25 and 35 day stage. The initiation of seed maturation was indicated by the onset of desiccation as observed by a decrease in the fresh weight after 35 day stage. Fresh weight of seed increased upto 28 days after flowering in chickpea cultivar L-550 after that rapidly decreased and in cultivar G-130 maximum fresh weight was at 21 days after flowering and seed dry weight increased upto 28 days after flowering after that a slight decrease in both the cultivars (Singh and Jambunathan, 1982). The observation in chickpea suggest that pod and seed matured at different stage and rates agree with data of french and soyabean

(Ouebedeanx and Chollat, 1975). During the period 115-135 days after sowing seeds accumulated 92 per cent of their dry matter in chickpea (Hooda et al., 1986).

Tripathi et al. (1987) noted that the optimum dry matter accumulated at 40 days after flowering in chickpea seed. Most of the dry weight of seed, accumulated between 25 and 35 days after anthesis in chickpea (Srivastava, 1987). Whereas Bharud and Patil (1990) observed that dry matter accumulation in seed increased upto 56 days in the seed of Vikash and Vishwas cultivars of chickpea. Dry weight of seed in some legumes increased upto 7 fold in the last of the maturation (Geervani and Devi, 1988).

Fresh and dry weight of soyabean seed increased until about 50 days after flowering. Fresh weight, thereafter decreased rapidly but dry weight slightly (Ohmura and Howell, 1962; Bils and Howell,1963). Lin et al. (1984) found that fresh weight of the soyabean cv. Miles reached at maximum after 28 days after flowering, whereas the dry weight of seeds continued to increase upto 50 days after flowering. In case of soyabean fresh weight and dry weight increased upto maturity. Lowell and Kuo (1985) reported that fresh and dry weight of seed in soyabean increased upto full seed stage thereafter decreased in fresh weight and increased in dry weight. Fresh and dry weight were upto three fold and two fold higher respectively in seeds of Molvering than Willians 82 during development. Seed dry weight in soyabean during development increased upto 40 days after flowering, after that it become constant (Miles et al., 1988).

Singh et al. (1980) observed a large increase in seed size of 3 cultivars of pigeonpea during development between 14 and 28 days after flowering. Fresh weight per seed reached at maximum after 28 days after flowering, whereas dry weight per seed increased upto 35 days after flowering in all the three cultivars. After 35 days after flowering a slight increase in dry weight was noticed in case of Hy-3C and ICP-1 and did not change much in case of ST-1. The dry weight of seed of pigeonpea increased steadily and continously till the end of the maturation period (Balakrishanan et al., 1984). A continuous deposition of reserve food material have increased the dry weight of seed (Rao and Rao, 1975). Maximum increase in the dry weight was observed between 28 and 35 days of the development and this is in confirmity with the finding of Singh et al. (1980). There was significant difference among cultivars of pigeonpea. Among cultivars, CORG 11 was found to be superior in accumulation of reserve food material (Balakrishanan et al., 1984).

In case of pea fresh weight of seeds rose to a maximum after 30-33 day of flowering. The fresh weight of wrinkled peas was greater than that of round peas, but the dry weights of both varieties were almost similar (Stickland and Wilson, 1983). Fresh and dry weight of seed increased until 75 day after planting in pea, then the fresh weight decreased rapidly while dry weight increased, this is true for both varieties Finale and Solara (Deunff and Rachidian, 1988). Seed fresh weight increased until day 119 of planting and dry weight increased linearly between 106 and 120 days after planting by 13 g/day in pea (Rachidian and Deunff, 1986).

Seed dry weight was maximum at 16-18 days after flowering in cowpea after that it decreased slowly (Trammell, 1983; Kim et al., 1987). The volume of 100 dry seeds increased from 10-25 days after anthesis and subsequently it decreased in all the varieties of cowpea. The dry weight of 100 seeds differed significantly among the all varieties at all maturity stages in cowpea (Ramaiah, 1986). Hundred seed fresh weight of 22.6 g occurred on day 9, then declined and dry weight 8.0 g at maturity (day 24) in cowpea (Palanisamy et al., 1986).

Barratt (1982) noted the changes in fresh and dry weight of the developing cotyledons of the four cultivars of the fieldbean. He found that all the cultivars showed a similar pattern in their fresh weight changes, there was little accumulation for the first 25 days followed by a period of very rapid increase from approximately days 30-45. After this the fresh weight increased more gradually until about 60 days, then it declined as the seeds become dehydrated and matured. There were differences between the cultivars and lines, in the pattern of dry weight accumulation. The large seeded cv Minica increased in dry weight steadily after day 35 for the duration of the experiment, where the rapid increase started 3 days later in cv Felix proceeded at a faster rate but then ceased abruptly at 59. A some what similar result was observed with the two lines of Dacre and line D reahed its maximum weight at about day 60. Where as line A (as cv Minica) continued to increase even during the final dehydration stage. Two pauses in dry weight accumulation were observed between 35-38 and 48-52 for Dacre A and between 38-40 and 45-48 day for Dacre D. Minica had a single pause between 45 and 48 days but no pause in growth was observed with Felix. These apparent pauses may be equivalent to the 'lag' phase that have been reported during the seed development of Pisum sativum (Carr and Skene, 1966; Hedlay and Ambrose, 1980), Phaseolus vulgaris (Mutchler et al., 1980). Barratt and Pullen (1984) reported that the fresh weight increased rapidly in fieldbean at initial stages, whereas slower at later stages.

Mungbean seeds showed linear growth till maturity (28 DAA) (Setia et al., 1984). The fresh weight showed a rapid increase upto 15 days in case of seed and it abruptly fell on the 20th day and it decreased till maturation in mungbean (Dharmalingam and Basu, 1989, 90). In all the cultivars of urdbean, fresh weight of seed increased upto 16 days after anthesis and decreased at maturity, but dry weight of seed increased until maturity (Saha, 1987).

Genotypes of groundnut differed significantly in kernel filling percentage at 60 and 90 days after sowing, but all genotypes were on par at 115 days after sowing which indicate that the rate of kernel filling varies significantly at earlier stages of crop growth but reach more or less same level at the time of harvest (Habib et al., 1985). The dry weight of seed in podocarpus increased with increase stage of development of seed (Dodd et al., 1989).

The increase in weight was perceptible inspite of steady reduction in moisture content (Harrington, 1972; Delouche, 1973; Balakrishanan et al., 1984).

Fresh and dry weight of pod wall

Developing pod wall of legumes have been shown to be photosynthetically active and generally considered to perform a major role in carrying out the refixation of CO_2 released during either the respiration or the photo respiration and pod wall increased 20 per cent seed yield (Sheoran and Singh, 1988).

Fresh weight of pod wall of chickpea increased upto 21 days after flowering after that it decreased, until maturation in variety L-550 and in variety G-130 it decrease from 14 days after flowering. The dry weight of pod wall in L-550 increased upto 35 days after flowering after it becomes constant and in G-130 it increased upto 28 days after anthesis/after that it decreased (Singh and Jambunathan, 1982). The dry matter accumulation was faster in pod wall than the seeds in both the varieties of chickpea. The optimum dry matter accumulated on 30 days after flowering in the pod wall. In the pod wall 75-90 per cent of dry matter accumulated at 20 days after flowering compared with only 20-25 per cent in the seed. During 30-40 days the dry matter declined in the pod wall. This may be attributed to translocation of certain stored products to the seed or other storage organs or recycled into plant growth or maintenance and degradation losses due to respiration or other physiological process (Tripathi et al., 1987).

The husk weight of pigeonpea reached at maximum on 28th day after anthesis. The husk after reaching the maximum,started lossing weight which may be due to dehydration (McIlrath et al., 1963). There were significant difference among the cultivars of pigeonpea for the weight of husk during seed development (Balakrishanan et al., 1984).

The dry weight of husk per pod increased from 10-16 days after anthesis in cowpea and remained constant till 25 days after anthesis and at harvest/maturity, it slightly declined. The accumulation of dry matter in husk was complete after 22, 19, 22 days after anthesis in cvs C-152, S-448 and TUX-183660E, respectively (Ramaiah, 1986). Pod wall dry weight reached at maximum on 14 days after flowering in cowpea and in other it decreased (Kim et al., 1987).

Genotypes of groundnut differed significantly in percent shell dry weight of 60 and 90 days after sowing but all the genotypes were a par of 115 days after sowing, indicates that rate of percent shell weight varies siginficantly at earlier stages of crop growth but reach more or less same level at the time of harvest (Habib et al., 1985). The shell moisture content in mungbean decreased with the advancement in maturity. The decrease was 83.3 to 6.6 per cent in shells, on 25th days after anthesis and further decreased was at slow rate (Dharmalingam and Basu, 1989, 90).

Moisture content of seed

Seed physiological maturity is a critical event in seed development, characterized by a specific moisture content irrespective of species and environment, it is the result of slow habituation of desiccation. This event occurs in the fruit when an abrupt water deficient is induced by rupture of vascular connection with the mother plant.

Goteulina and Prikhod'ko (1982) reported that in some legumes 40 to 60 per cent moisture content was present when seed filling terminate. The seed moisture content of chickpea decreased from 79 to 14 per cent in cv Vikash and from 82 to 17 per cent in cv Vishwas (Bharud and Patil, 1990).

Moisture content per seed was similar in two cultivars of soyabean at each stage of development and moisture content decreased 80 to 15 per cent from first stage seed to last stage respectively (Lowell and Kuo, 1989). Moisture contents of seed of soyabean decreased steadily with increase in time of seed development (Miles et al., 1988).

The moisture content of pigeonpea seeds were observed and the moisture content of seed which was maximum on the 7th day after anthesis declined steadily with the development and maturation and reached the minimum between 20.0 and 24.0 per cent on 42nd day (Balakrishanan et al., 1989).

The moisture content of seed of pea decreased 84 to 14 per cent from initial stage to last stage of development (Rachidian and Deunff, 1986). Changes in seed moisture content in pea were similar in cv Solara with a slow dehydration from 61 day after planting to 75 days after planting (water loss 25 to 28 per cent in 14 days). At 75 days after planting, seed moisture content in the two varieties was identical at around 55 per cent. After maturity, seed showed intense dehydration to 14 per cent moisture content at harvest. Rapid desiccation continuous for 9 days in both varieties (Deunff and Rachidian, 1988).

Moisture content of seed of cowpea started to decrease at 8 days after anthesis to maturation (Kim et al., 1986). In seeds of mungbean decreased their moisture content 82.2 to 10 per cent with increase time of seed development 15 days to 35 days after flowering respectively (Dharmalingam and Basu, 1989, 90). Moisture content of seeds was maximum at 8 days after anthesis and declined steadily from about 12 per cent as the seed mature in urdbean (Saha, 1987).

Moisture content in podocarpus decreased 81.2 to 61.7 per cent from 6 weeks after fertilization to 24 week after fertilization respectively (Dodd et al., 1989). Rapid seed filling occurred in lupine seed when pod moisture content was 83 kg/ kg K.W. (Clapham and Barness, 1990). The loss of moisture in developing seed is a common phenomenon observed in many agricultural crop (Loewenberg, 1955).

Germination of seeds

The germination potential of dried seed of cvs of chickpea in Vikash and Vishwas were 3 and 11 per cent respectively on 21 days after anthesis. It increased gradually and attained the maximum on the 56 days after anthesis. Therefore, no further increase in germination was observed (Bharud and Patil, 1990).

In seeds of soyabean, when maximum dry weight get accumulated than the maximum germination was observed during seed development (Trammell, 1983). Fresh seed germination in soyabean was 75 per cent which occurred at 55 per cent of total dry weight of seed (Miles, 1986). Miles et al. (1988) reported that fresh soyabean seeds were viable very early in development as shown by seeds harvested at full seed stage and at this stage seeds had a moisture concentration of 740 g/kg F.W. These results contradicts previous reports showing little or no germination of fresh seed harvested at similar stage of maturity (Adams and Rinne, 1981; Dasgupta et al., 1982; Kermode and Bewely, 1985; Ozaki et al., 1956), but in support a report by Rosenberg and Rinne (1986) of 37 per cent viability for fresh seeds at a seed moisture of 787 g/kg.

In all cultivars of pigeonpea, the developing seed started germination from 21, attaining the maximum on 42nd days after anthesis (Balakrishanan et al., 1984). Although the developing seeds were capable for germination when they were physiologically mature and hence attained maximum dry weight (Delouche, 1973; Bishnoi, 1974; Manohar and Sachan, 1974). Germination percentage was higher in seeds obtained from mature pods of pigeonpea than the slightly green pods. The highest germintion i.e. 96 per cent was recorded in H76-19 and H77-208 in mature pod whereas seeds harvested from slightly green pods also gave, in majority of the cases germination percentage required for seed certification (Paroda et al., 1985).

Immature seeds of pea were able to germinate at very high moisture content (72-76%) and reached a first peak at 63 per cent seed moisture content and 183 to 198 mg DM. Hundred per cent germination reached at 34 to 43 per cent moisture content in dried seeds of pea (Rachidian and Deunff, 1986). At 58 and 61 days after planting in pea, seed have no germination percentage. After that increased steadly in germination in both the varieties and at 81 and 84 days after planting, showed 100 per cent germination in both the varieties (Deunff and Rachidian, 1988).

The developing seeds of cowpea were capable of germination from day 9, reaching a maximum of 95 per cent at day 24 (Palanisamy et al., 1986). The percentage of germination of developing seeds was 20.7 per cent after 13 days from anthesis and it gradually increased to 94 per cent (highest) at the physiological maturity in cowpea (Ramaiah, 1986). Germination percent of mungbean seed increased with increased in developmental period of seed from 5 to 25 days after flowering, it decreased at 30 to 35 days after flowering, by the presence of hard seeds, so the number of hard seeds increased at last stages of development (Dharmalingam and Basu, 1989, 90). Seed dry weight of Vigna radiata positively correlated with percent germination (Basu et al., 1990). Germination was highest at 94 and 101 days after emergence in peanut cultivars (Sombatsiri and Naun, 1987).

Sugars and starch

Seed has evolved a mechanism where by starch accumulated as a transient reserve materials which is utilized later in seed development and germination. This is somewhat unusual as reserve materials were frequently accumulate and do not turn during seed development (Bain and Mercer, 1966).

The percent of soluble sugars continuously decreased upto 28 days after flowering and then remained unchanged till the grain of chickpea matured. In starch content, a rapid increase was observed between 14 and 21 days after flowering. After reaching a maximum level at 28 days after flowering, the amount of starch in cvs L-550 and G-130 decreased between 28 and 35 days after flowering but remain constant in 850-3/27 (Singh et al., 1981). Starch content of seed in chickpea increased upto 28 days after flowering and then showed a slight decrease during the later stages of maturation in cv G-130 but in L-550 did not change until maturity. Although an increase in the accumulation of starch of seed was accompanied by decline in pod wall, it was not possible to say whether the starch of pod wall was mobilized and translocated to seed. The percentage of total soluble sugars declined during early stages of maturation indicating that those sugars were utilized for the accumulation of starch which increased during the same period in chickpea and reducing sugar decreased throughout the development (Singh and Jambunathan, 1982). Sugar concentration in chickpea seed at maturity were 0.59 to 1.0 mg/g with mean 0.84 mg/g (Sharma and Bahl, 1989).

Soyabean seed contained about 10 per cent dry weight of starch of mid-development (Yazdi-Samadi et al., 1977), but at maturity this had declined to approximately one per cent (Yazdi-Samadi et al., 1977; Wilson et al., 1978). Starch content in soyabean seed increased rapidly until approximately mid-development and thereafter declined sharply to a low level and alcohol soluble sugars increased continuously throughout development reaching a maximum value at maturity in soyabean (Adam et al., 1980).

Changes in the level of soluble sugars during maturation was seen in pigeonpea, as percent of the dry weight. These sugars continuously increased upto 14 days after flowering, then showed a sharp declined and thereafter remained more or less unchanged. Starch content in developing grains of pigeonpea was also determined. Dry weight of starch content at 14 days after flowering was 17.4 per cent in ST-1, 20 per cent in Hy-3C and 27 per cent in ICP-I and this increased to 61.9, 63.2 and 62.4 respectively (Singh et al., 1980). Rapid accumulation of starch in pigeonpea seed was observed between 21 and 28 days after anthesis, therefore a sharp declined was observed (Balakrishanan et al., 1984). The results were in conformity with finding of Singh et al. (1980).

In the developing pea seed, starch continuously accumulates upto maturity (Ryle et al., 1979). There was greater amount of sugars present during the first 40 days of development of wrinkled pea than the round pea (Stickland and Wilson, 1978). Carbohydrate concentration increased with increase in the developmental period of seed in cowpea (Kim et al., 1987).

The concentration of ethanol-soluble sugars in the cotyledons of field bean fell markedly between 30-45 days after flowering while that the starch increased most rapidly during a slightly earlier period, 27 to 62 days after flowering. Later on, the changes were more gradual. The small-seeded Dacre had a higher percentage of starch but a lower percentage of ethanol soluble sugars than the large seeded cultivars in all samples after day 40. Over the period 45 to 65 days Felix contains more starch and ethanol soluble sugars than Minica. The accumulation of starch extending for a major part of seed development is similar to that found in Cajanus cajan (Singh et al., 1980) and Cicer arietinum (Singh et al., 1981). In contrast Pisum arvense (Smith, 1973; Flinn and Pate, 1968) and Pisum sativum (Millerd and Spencer, 1974) have a shorter period of starch synthesis which is most completed by mid-development. Higher percentage of ethanol soluble sugars but lower percentage of starch in large seeded cvs compared to small seed cvs has been observed in fieldbean (Barratt, 1982). The general pattern of accumulation of different chemical constituents described in fieldbean resemble those descorded in earlier reports on seed of Vicia faba (Boulter and Davis, 1968; Manteuffel et al., 1976) and some of the legumes (Flinn and Pate, 1968; Mutchler et al., 1968; Singh et al., 1981;Hedley and Ambrose, 1980). In the both the cultivars of urd bean, soluble sugar increased upto 8 days after anthesis and showed a sharp decline at 12 days and decreased further till maturity. However, a rapid increase in starch content was observed from 8 days after anthesis to maturity. Since increase in seed dry weight was parallel to the accumulation of starch, it is assumed that starch accumulation in part might be responsible for increase in dry weight of seeds during development and maturation (Saha, 1987). Similar results were obtained with chickpea (Singh and Jambunathan, 1982) and soyabean (Bils and Howell, 1963).

Starch concentration increased with increase in stage of seed development in podocarpus upto 18 weeks after fertilization, after this it decreased. Sugar content increased upto 12 weeks after fertilization, after that rapidly decreased to 18 weeks after fertilization and after that it become constant (Dodd et al., 1989). Stachyose concentration increased as seed development progressed while raffinose, sucrose, glucose, fructose and inositol concentration decreased with largest decrease between 72 and 93 days after sowing in peanut (Ross and Mixon, 1989).

Protein content of seed

Protein accumulation in chickpea seeds during development was slower upto 25 days after flowering as only 14 per cent of the total cotyledons protein was deposited during this period, while in a much short period of 10 days from 25 to 35 days stage ~67 per cent of the total cotyledons protein was deposited. From 35 days to maturation, ~19 per cent of the total protein was deposited. The protein content in percent dry weight base increased 20.25 to 26.55 per cent from 15 to 45 days after flowering respectively. The protein percent also showed a simultaneous increase with the increase in dry weight from 25 to 35 day stage. This indicateda higher rate of protein accumulation compared to increase in dry weight (Srivastava et al. , 1981). The results of accumulation of protein N expressed as percent of seed dry weight. Percent of protein N increased slowly throughout the developmental stages in cvs G-130 and L-550 but in cv 850-3/27 it increased upto 28 days after flowering then remained constant till maturity in chickpea seed (Singh et al., 1981). Most of the proteins accumulated between

25 and 35 days after anthesis, in developing seed of chickpea (Srivastava, 1987). The pattern of N accumulation in seed was similar to that of dry matter in both the varieties of chickpea. BG-240 and L-550 showed similar rate of N accumulation in seed at 20 days after flowering, but decreased later in BG-240. Total N was maximum at 35 days after anthesis in both the varieties. Correlation coefficient between total dry matter and N accumulation at different days after flowering were high (r=0.9949) (Tripathi et al., 1987). In chickpea seed, protein content was ranging from 12.83 to 26.83 mg/g at maturity with mean 21.29 per cent (Sharma and Bahl, 1989). Hooda et al. (1986) reported that as increase in development period, protein concentration decreased in chickpea.

Percent protein in cotyledons of soyabean increased with increase in days of seed development (Bils and Howell, 1963). Protein content of soyabean accumulated 6 to 14 per cent of total seed dry weight upto 36 days after flowering, it was similar in 3 cultivars, but differed significantly at later stages of seed development. Protein content increased significantly throughout seed development but no significant increase in protein content at 66 days after flowering (Qiu et al., 1990).

The amount of protein in pigeonpea seed expressed as mg/seed, increased upto 35 days after flowering thereafter declined in case of ST-I and slightly increased in case of Hy-3C and ICP-I (Singh et al., 1980). The protein N which was high during early stages of development and maturation in seed of pigeonpea, decreased gradually as the seeds mature (Balakrishanan et al., 1984). These results are in conformity with finding of Singh et al. (1980). Accumulation of protein content did not show regular trends during seed development in cowpea (Kim et al., 1987).

The total N content of the cotyledons of field bean increased slowly at first, then from around day 35 there was a constant,rapid accumulated. until day 64, after which for Felix, Dacre A and D, no net increase was observed. Minica, however, maintained a steady increase in total N for the duration of experiment (Barratt, 1982). The cultivars Dacre D accumulated more total protein than Dacre B of field bean throughout the development of seed (Barratt and Pullen, 1984). Protein content increased with increase in development stages of seed in field bean (Barratt and Pullen, 1984).

Protein nitrogen in all the cultivars of Vigna radiata was very high at 4 days after anthesis then decreased upto 12 days and increased further upto maturity of the seed. Similar results were observed in the cultivars of Vigna mungo but protein nitrogen decreased continuously upto 16 days and increased further at maturity (Saha and Bera, 1990). These observations compared well with the results obtained in chickpea (Singh et al., 1981) and soyabean (Kapoor and Gupta, 1975; Sale and Campbell, 1980). Total protein content in seeds of all the cultivars of Vigna radiata and Vigna mungo were higher at 4 days after anthesis and decreased thereafter at 8 days but further increased in total protein was observed at 12 days after anthesis and this increased continued till maturity of seeds (Bera and Bera, 1990).

The content of protein increased with increase in developing stages of seed in common bean (Paredes et al., 1990). An increase in protein with in the cotyledons of Ricinus communis continued throughout of the seed development from 20-45 days after pollination, the most increased from 35 to 40 days after pollination after that it declined (Kermode and Bewely, 1985). Percent protein of seed in groundnut did not show much variation (28.8 to 31.2%) over the stage except for a minor reduction at 119 days sowing (Nagaraj et al., 1987). No regular pattern in accumulation of protein was found in podocarpus seed during development stages(Dodd et al., 1989).

The protein content increased on maturation in some legumes. However the proportional increase varied widely from 10 to 100 per cent. The change in nutrient composition and weight in last 10 days of maturation suggest that it is advantageous to harvest vegetable legumes when mature (Geervani and Devi, 1988). At maturity stage cotyledons are rich in protein content in some legumes (Sehgal et al., 1987).

Respiration

Oxygen uptake in seeds of soyabean on a unit weight basis was greatest in the early stages, declining throughout the development period. Whole seed 16 days after flowering consumed about 750 $\mu l\ O_2$/hr/100 mg dry weight. From 27 to 36 days, they consumed about 300 $\mu l O_2$/hr/100 mg dry weight and from 40 day until just before maturity about 50 µl/hr/100 mg dry weight. At maturity oxygen use was below the limit of the method (Bils and Howell, 1963).

Electrical conductivity

After physiological maturity of pea, the abrupt reduction in moisture content of pod wall, seeds and compartments, may correspond to an interruption of water supply to the pod. By measuring leakage of ions from the immature seed we can estimate inflow to the embryo of sap and solutes via testa (Wolswinkel and Ammerlaan, 1985; Rachidian and Deunff, 1986). In pea, conductivity is very high during the PI phase, concentrations in the sap being important in seed coat and liquid endosperm. At 61 days after planting, at the end of PI phase, conductivity is much reduces, afterwards it decreased slowly until 75 days after planting, then in second another fall lead to stabilization of conductivity, the seeds becoming dry. Determination of vascular connects show that at 77 days after planting in cv Finale, 25 per cent of all pods still have functional connections, while pods of cv Solara have none (Deunff and Rachidian, 1988).

C H A P T E R - III

MATERIALS AND METHODS

The investigation were carried out on chickpea (Cicer arietinum L.) cultivars, namely, Haryana Chana No. 1, Gaurav, L-550 and HK-88-232, differing in seed weight. Out of these, two cultivars were desi (Haryana Chana No. 1 and Gaurav) having approximately 17 to 19 g/100 seeds weight and another two were kabuli (L-550 and HK-88-232) having approximately 28-32 g/100 seeds weight.

Raising of crop

Chickpea crop was raised in Seed Technology Section and Pulses Section of Plant Breeding Department during the year 1990-91. Normal agronomic practices were adopted and plant protection measures were taken to harvest a good crop. At the time of flowering freshly opened flowers (anthesis) were tagged at March 7, 1991 onward.

Sampling

Pods were sampled at 15, 22, 29, 33, 37, 41 and 45 days after anthesis. The samples were always taken at 8.00 a.m. to avoid any possible variation in their chemical composition due to difference in sampling time. The pods were harvested randomly and brought to the laboratory in a polythene bag burried in the ice-bucket. The samples were divided into two sets. One set was referred to as fresh lot (A) and another set was kept at 35 ± 2°C for drying in an incubator and referred as dried lot (B). Various observations were recorded in these lots separately. There were four replicates for each observation.

Fresh and dry weight

Freshly harvested samples were separated into seed and podwall and their fresh weights were recorded. The number of seeds/pod were also recorded. After recording fresh weight, samples were dried in an oven at 75°C till constant weight and their dry weights were recorded.

Moisture content of seeds was determined as follow:

$$\text{Water content (\%)} = \frac{\text{Fresh weight of seed - Dry weight of seed}}{\text{Fresh weight of seed}} \times 100$$

Germination

These studies were carried out in the laboratory, under controlled conditions using glass petriplates (9 cm dia.). Petriplates were oven sterilized at 180 to 200° C for about 2 hours and then rinsed with absolute alcohol before use. The seeds were also surface sterilized with one percent, sodium hypochlorite solution for 2 to 3 minutes and washed with distilled water and dried on filter paper at room temperature. Ten healthy and uniform seeds from fresh and dry lots were sown separately in petriplate, each lined with Whatman filter paper No. 1. Five ml of distilled water was added to freshly harvested seeds and ten ml of distilled water to dry seeds. Petriplates were placed in a seed germinator, maintained at 25±1° C and RH 96 ± 2 per cent.

Germination counts were recorded on alternate days up to 14 days after sowing and percent germination calculated in each case. But the data on percent germination of 10 days after sowing has been discussed.

Total soluble sugars

Total soluble sugars of developing seeds (cotyledons) were estimated by the method of Yemm and Wills (1954).

Extraction was done according to Barnett and Naylor (1966). A known sample of cotyledon was homogenised in 80 per cent ethanol (v/v), refluxed for 15 minutes on a steam bath and centrifuged. The residue was further refluxed with 40 per cent ethanol. Thus, the extraction was repeated thrice. The supernatants from different extractions were pooled and final volume made to 15 ml with 80 per cent ethanol. The extract obtained was used for estimation of total soluble sugars and reducing sugars. The residue was set apart for starch estimation. Total soluble sugars were estimated by using the anthrone reagent.

Anthrone reagent

Anthrone (0.2 g) was dissolved in 100 ml concentrated sulphuric acid.

A 0.1 ml aliquot of the extract was evaporated to dryness in a test tube in a boiling water bath. On cooling, the residue was dissolved in one ml of glass distilled water and added to it 4.0 ml of anthrone reagent. Heat this mixture in a water bath for 15 minutes. After cooling absorbance was recorded at 620 nm on spectrophotometer (Backmen DO-64) against blank. Standard curve was prepared using graded concentration of glucose and data was expressed as mg/g on dry weight basis.

Reducing sugar

Reducing sugar of developing seed were estimated by Somogyi's modified method (Nelson, 1944; Somogyi's, 1945). A brief account of the method is given below.

Reagents

Copper reagent A

It was prepared by dissolving 25 g of anhydrous sodium carbonate, 25 g of potassium sodium tartarate, 20 g sodium bicarbonate and 200 g of anhydrous sodium sulphate in about 800 ml distilled water and diluted to one litre.

Copper reagent B

Fifteen g of copper sulphate was dissolved in 100 ml of distilled water containing two drops of HCl.

Arsenomolybedate reagent

Twenty five g of ammonium molybedate was dissolved in 450 ml distilled water and two ml of H_2SO_4 was then added with stirring. Three g of sodium hydrogen arsenate dissolved in 25 ml of distilled water was added with mixing and the solution was kept in incubator at 27°C for 24 hour. This reagent was stored in a glass stoppered brown bottle.

Copper reagents A and B were mixed in a ratio of 25:1 before use.

Procedure

One ml of test extract was taken in a test tube. Mixed copper reagent (1 ml) was added and then heated for 20 minutes in a boiling water bath. One ml of arsenomolybedate reagent was added and mixed thoroughly. A stable light blue or blue colour quickly appeared which was read at 520 nm against a suitable blank and result was expressed as mg/g on dry weight basis.

Standard curve was prepared by using graded concentration of dextrose.

Starch

Starch of developing seed was extracted and estimated according to Hassid and Neufeld (1964). The residue obtained after total soluble sugars extraction was suspended in 5 ml of chilled 26 per cent $HClo_4$, allowed to stand overnight and centrifuged for 15 minutes at 3000 rpm. The residue was further extracted twice with 26 per cent $HClo_4$. The supernatants were pooled and the volume made to 10 ml with 26 per cent $HClo_4$.

A 0.05 ml aliquot of the extract was used for starch estimation. Colour was developed by using 0.2 per cent anthrone reagent prepared in concentrated H_2SO_4 and absorbance recorded at 620 nm against blank. Standard curve was prepared using graded concentrations of glucose and data was expressed as mg/g on dry weight basis.

Protein

Protein of developing seeds were estimated by conventional micro Kjeldhal's method (A.O.A.C., 1970).

Reagents

1. Sodium hydroxide : 40 per cent
2. Concentrated sulphuric acid
3. Catalytic mixture : Potassium sulphate and crytalline copper sulphate were mixed in a ratio of 10:1.
4. Four per cent boric acid : 40 g of boric acid was dissolved in warm water and diluted to 1000 ml.
5. Methyl-bromocresol green indicator : Mixed one part of 0.2 per cent methyl red in ethanol with 5 parts of 0.2 per cent bromocresol green in ethanol.
6. 0.1 N H_2SO_4.

Procedure

One hundred mg of dried seed flour was taken in a hundred ml micro Kjeldhal's digestion flask. One g catalytic mixture was added to it followed by 10 ml of concentrationed H_2SO_4. The flasks were then kept in an inclined position on the burner in the digestion chamber and heated gently till the frothing subsides. This was followed by intense heating for one hour till the solution turns perfectly transparent giving a bluish green colour. After cooling, the content of flasks were transferred to 100 ml volumetric flask and volume made upto the mark with distilled water. Ten ml of this solution was taken for distillation in a micro Kjeldhal's apparatus and distilled after adding 10 ml of 40 per cent NaOH. The amount of ammonia absorbed by 4 per cent boric acid containing 1 to 2 drops of indicater was determined by titrating it with 0.1 N H_2SO_4. A blank sample was simply digested and distilled. The percent of N was calculated from 0.1 N H_2SO_4 used.

One ml of 0.1 N H_2SO_4 = 0.0014 g of nitrogen

$$\text{Percentage N} = \frac{0.0014 \times \text{volume made} \times 0.1\ \text{N}\ H_2SO_4\ \text{used}}{\text{Aliquot taken} \times \text{weight of sample}} \times 100$$

Nitrogen percentage, which was calculated from the ml of 0.1 N H_2SO_4 used, was converted to protein percentage by a conversion factor 6.25.

Total soluble sugars of seed leachate

Total soluble sugars of seed leachate after 16 hours of imbibation was estimated by anthrone method as described above for estimation of seed's total soluble sugars and result was expressed as mg/seed on dry weight basis.

Respiration

Fifty seeds of each cultivar were taken and placed in beaker containing 100 ml of distilled water. Beaker were placed in an incubator, set at 25±1°C and RH 96±2 per cent. After 16 hours seeds were taken and analysed for respiration.

Respiration rate of the seeds was measured with Gilson Differential Respirometer and expressed as μlO_2 consumed/hr/seed.

Procedure

Temperature of water bath was set at 25°C and 7 ml of distilled water was added in to the reference chamber and grease was applied at the top of centre well. Two ml of distilled water was added in to each flask and five seeds were placed. A 0.2 ml KOH solution (10%) was poured into central well along with a strip of filter paper (to increase the CO_2 absorbing area). Pressure in the two arms of the manometer was equalised by opening the valve and flasks were attached by rotation to greased joint and secured with the help of springs. Flasks were than lowered in the water bath and shaking motor was put on. Flasks were allowed to equilibiate to bath temperature for 20 to 30 min. During this period the digital micrometers were rotated counter clockwise and set to a valve of 200. The index line was fixed at the fluid level in each manometer. After equilibration, the valves were closed and shaking resumed. Oxygen uptake was recorded at regular intervals (10 min) upto 30 min by adjusting the fluid level to the indexline with the help of micrometers. Micrometers reading were recorded and respiration rate expressed as μlO_2 consumed/hr/seed. The respiration of seeds were recorded at 16 hr after soaking.

Electrical conductivity

Conductivity of the leachate solution, left after removing the seeds for respiration was monitored with conductivity meter (Type CM-82T). The conductivity was expressed as mmhos/cm/seed at 25°C.

C H A P T E R - IV

EXPERIMENTAL RESULTS

Investigation were carried out on chickpea (*Cicer arietinum* L.) cultivars namely, Haryana Chana No. 1, Gaurav, L-550 and HK-88-232. With a view to study the physiological and biochemical changes during seed development. Attempts were also made to study whether germination, had any correlation with physiological and biochemical changes during different stages of seed development.

Chickpea cultivars were raised with recommended agronomic practices under field conditions. Freshly opened flowers were tagged at flowering stage on March 7, 1991 onward.

Samples were collected at different stages of seed development i.e. 15, 22, 29, 33, 37, 41 and 45 days after anthesis for studying the physiological and biochemical changes associated with seed development and their correlation with germination potential. The data collected during seed development and on subsequent germination have been compiled, statistically analysed and described below under different heads.

Fresh weight of seed

A progressive increase in fresh weight of seed was observed upto 29 days after anthesis in V_1, 33 days after anthesis in V_2 and V_3 and 37 days after anthesis in cultivar V_4, which decline subsequently till 45 days after anthesis (Table 1). All cultivars studied, showed sharp increase in fresh weight during initial stage of seed development particularly at 22 days after anthesis. Among the cultivars, highest fresh weight of seed was observed in cultivar, V_4 (577 mg/ seed) at 37 days after anthesis and showed high fresh weight at all stages,

Table 1 Fresh weight (mg/seed) of seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	081	233	281	280	218	128	116
V_2 (Gaurav)	151	229	324	374	269	147	140
V_3 (L-550)	274	395	405	439	326	167	171
V_4 (HK-88-232)	122	403	531	562	577	374	267

	CD at 5 per cent
Cultivars	3
Stages	4
Cultivars x Stages	9

except at 15 days after anthesis. However, in the beginning i.e. 15 days after anthesis, cultivar V_3 exhibited highest fresh weight. Significant differences fresh weight of seeds were noticed among the cultivars at different stages of seed development.

Dry weight of seed

Seed dry weight increased sharply upto 22 days after anthesis in cultivars V_1 and V_4, but slowly in V_2 and V_3 (Fig. 1). Initially seed dry weight was more in V_3 followed by V_2 but at the time of harvesting V_4 had the maximum dry weight of seed followed by V_3 and V_2. The increase in dry weight continued upto 37 days after anthesis in all the cultivars except V_3, which exhibited dry matter accumulation upto 45 days after anthesis. After that decline in dry weight of seeds were observed in rest of the cultivars. Statistically significant differences in dry weight of seed were observed among the cultivars at various stages of development.

Fresh weight of pod wall

The data on fresh weight of chickpea pod wall is presented (Table 2). The fresh weight of pod wall was more in comparison to seed fresh weight on 15 days after anthesis in all cultivars except V_3 where values were comparable. Maximum fresh weight of pod wall were obtained on 22 days after anthesis in V_3 and V_4 and 29 days after anthesis in cultivars V_1 and V_2. Thereafter, gradual decrease in pod wall weight were observed till harvesting stage. Cultivar V_4 retained highest fresh weight of pod wall followed by cultivar V_3 at 45 days after anthesis.

Pod wall dry weight

Little changes were observed in the pod wall dry weight after 22 days after anthesis (Table 3). However varietal differences were significant, kabuli type cultivars had more pod-wall dry weight than desi type.

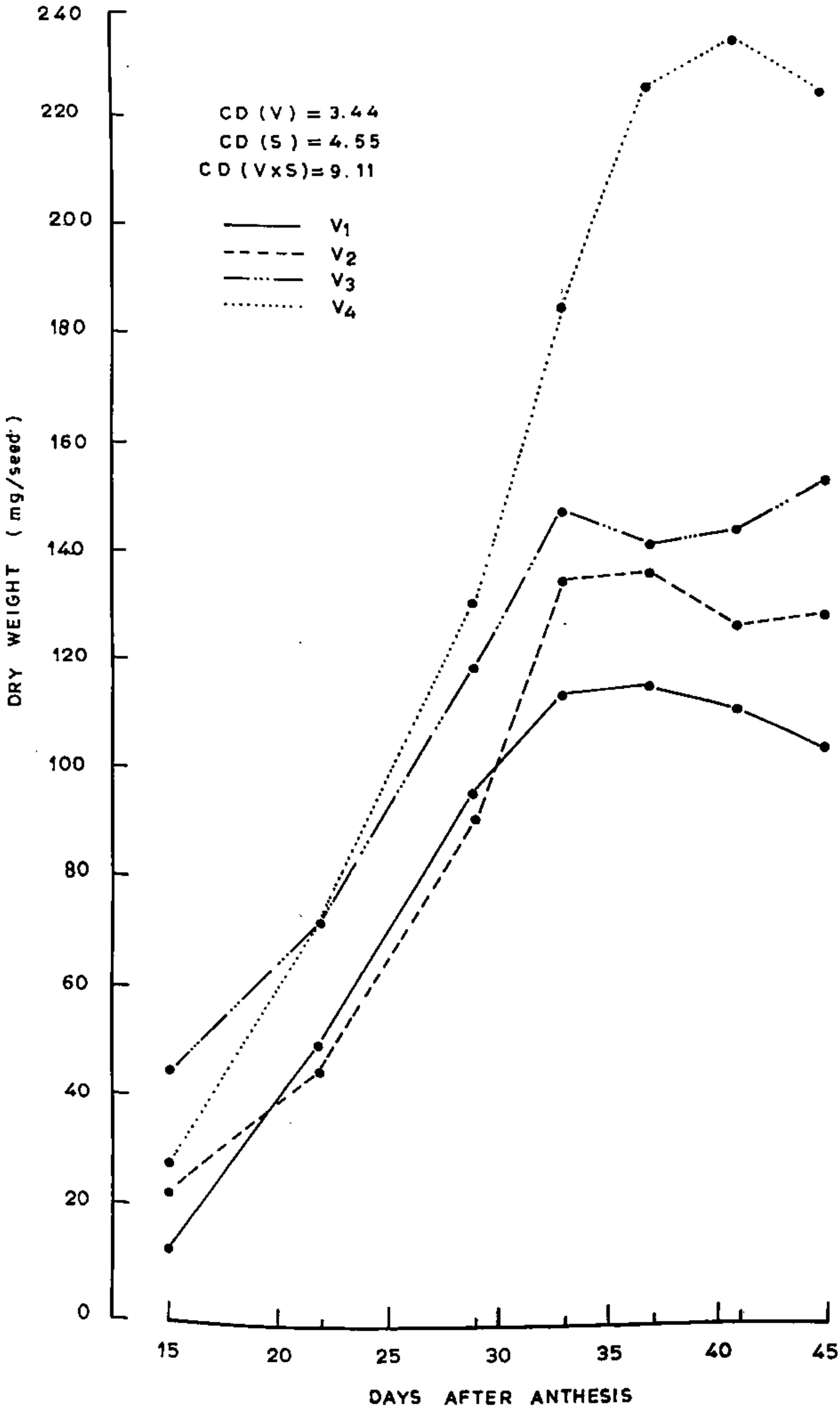

CD (V) = 3.44
CD (S) = 4.55
CD (VxS)=9.11
V_1
V_2
V_3
V_4
DRY WEIGHT (mg/seed)
DAYS AFTER ANTHESIS
0
20
40
60
80
100
120
140
160
180
200
220
240
15
20
25
30
35
40
45

Table 2 Fresh weight (mg/pod) of pod-walls of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	224	227	236	201	139	73	61
V_2 (Gaurav)	238	264	273	244	166	100	73
V_3 (L-550	267	296	291	283	184	92	88
V_4 (HK-88-232)	338	399	390	364	330	193	130

	CD at 5 per cent
Cultivars	6
Stages	8
Cultivars x Stages	17

Table 3 Dry weight (mg/pod) of pod-walls of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	44.66	67.31	66.30	60.00	63.00	59.00	57.39
V_2 (Gaurav)	54.33	69.00	67.66	69.00	74.00	70.00	67.00
V_3 (L-550)	66.00	94.60	82.00	75.66	91.00	74.34	77.30
V_3 (HK-88-232)	60.30	95.00	111.90	97.00	95.66	100.00	105.00

	CD at 5 per cent
Cultivars	2.39
Stages	3.17
Cultivars x Stages	6.34

Moisture content of seed

Seeds had the maximum moisture on 15 days after anthesis in all the cultivars. It decrease gradually upto 37 days after anthesis and thereafter the decrease was rapid except in cultivar V_4. Kabuli cultivars maintained higher moisture percentage as compared to desi cultivars at the time of harvest (Table 4). Statistically significant differences in moisture content of seed were observed not only among the cultivars but at different stage of seed development also.

Germination

Freshly harvested seeds

No germination was observed in 15 and 22 days old seeds in cultivars V_1 and V_4 (Fig. II). In freshly harvested seeds germination.was initiated on 22nd day in cultivars V_2 and V_3. Germination reached to 50 per cent level in cultivar V_1 on 29 days after anthesis. However, no germination was recorded in V_4 on this day. Complete germination was observed in desi cultivars on 37 days after anthesis whereas, kabuli cultivars still had lower germination. In these cultivars complete germination was recorded on 41 days after anthesis. There were significant differences in germination among the various cultivars.

Dry seeds

In dry seeds germination potential development was little late (Fig. III) as compare to freshly harvested seeds. Development of germination potential was gradual in all the cultivars except V_3, where the complete germination was attained within one week time i.e. from 33 to 37 days after anthesis. At harvesting stage (45 DAA), desi cultivars showed reduction in germination percentage (Fig. III).

Table 4 Moisture content (%) of seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	85.19	78.11	64.15	60.00	46.55	12.50	9.08
V_2 (Gaurav)	85.63	79.75	71.33	63.48	48.71	15.98	10.68
V_3 (L-550)	84.05	81.60	69.36	67.80	56.22	13.54	10.88
V_4 (HK-88-232)	85.85	81.80	74.91	57.08	60.91	62.19	17.49

	CD at 5 per cent
Cultivars	0.02
Stages	0.03
Cultivars x Stages	0.12

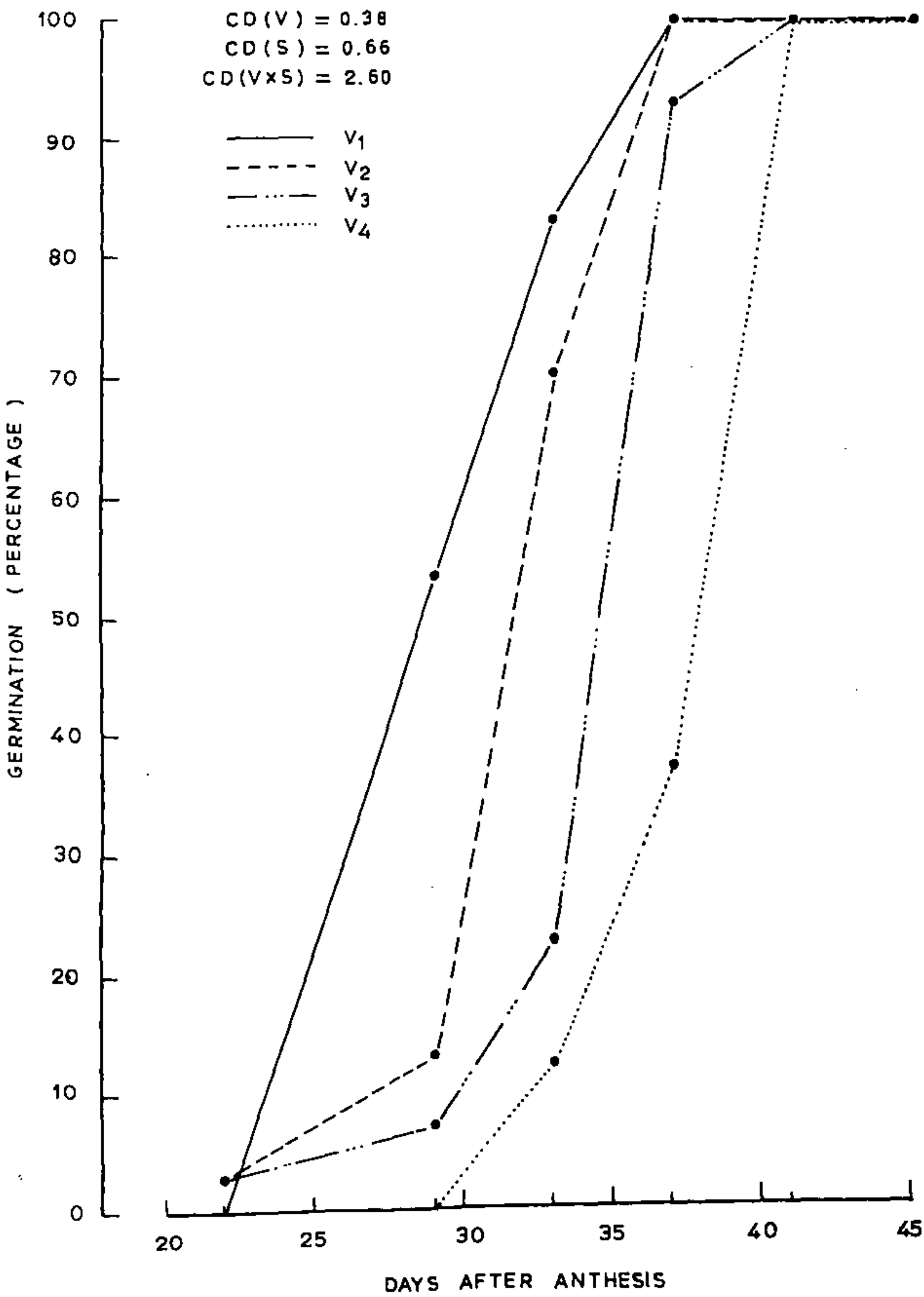
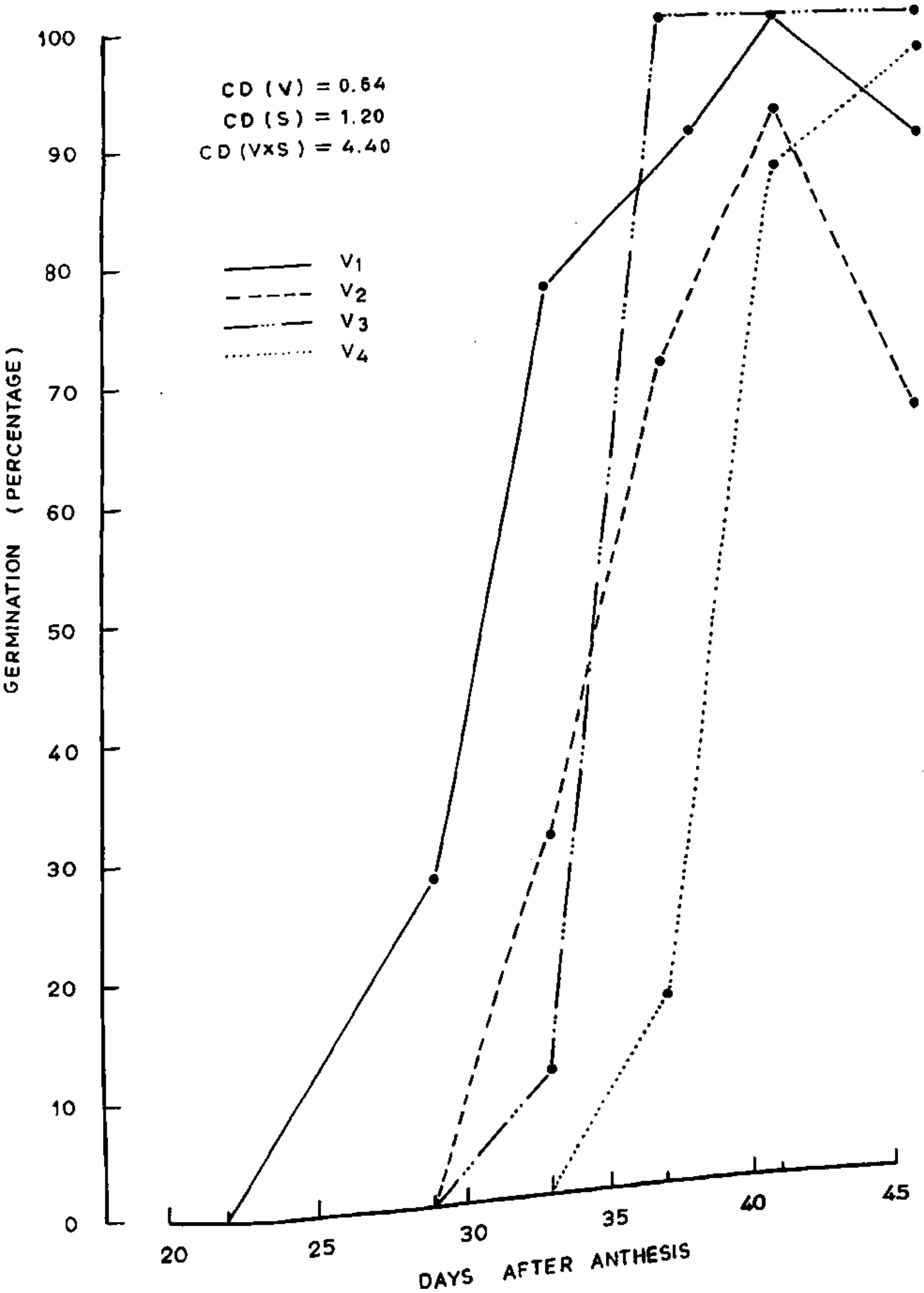

CD(V) = 0.38
CD(S) = 0.66
CD(V×S) = 2.60
V1
V2
V3
V4
GERMINATION (PERCENTAGE)
DAYS AFTER ANTHESIS
0
10
20
30
40
50
60
70
80
90
100
20
25
30
35
40
45

Fig. II Germination (%) of freshly harvested seeds of chickpea cultivars at different stages of seed development

Fig. III Germination (%) of dry seeds of chickpea cultivars at different stages of seed development



CD (V) = 0.64
CD (S) = 1.20
CD (V×S) = 4.40
V1
V2
V3
V4
GERMINATION (PERCENTAGE)
100
90
80
70
60
50
40
30
20
10
0
20
25
30
35
40
45
DAYS AFTER ANTHESIS

Total soluble sugars of seed

Total soluble sugar content was maximum at first stage i.e. 15 days after anthesis (Table 5). A rapid decrease was observed in cultivars V_1 and V_2 from 15 days after anthesis to 22 days after anthesis. However, this decrease was relatively slow in kabuli cultivars. Interestingly, minimum sugar content was observed on 29 days after anthesis in all the cultivars. Thereafter a slight (though significant) increase was recorded in all cultivars. Kabuli cultivars maintained relatively higher sugar content than desi cultivars at all the development stages till harvest (Table 5).

Reducing sugar of seed

In general, reducing sugar content was very low in all the cultivars of chick-pea (Table 6). All the cultivars showed significant difference among themselves at 15 and 22 days after anthesis. Thereafter, not much difference was observed among the cultivars. Maximum reducing sugar content was observed in all the cultivars on first sampling i.e. 15 days after anthesis. Which decline subsequently to lowest value on 29 days after anthesis. Again, a considerably high accumulation in reducing sugar was noted on 37 days after anthesis in all cultivars except in cultivar V_4 which had such value on 45 days after anthesis.

Starch content of seed

At initial stage i.e. 15 days after anthesis, all cultivars showed minimum starch content (Table 7). Thereafter an increase in starch content was observed, but the rate of increase and stage vary among the cultivars. Cultivars V_1 and V_3 had more starch than V_2 and V_4 at first stage. Rapid increase in starch content was observed in V_1 upto 22 days after anthesis, in V_2 and V_3 upto

Table 5 Total soluble sugars (mg/g) of seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	161.33	60.66	20.66	37.33	26.35	31.31	31.34
V_2 (Gaurav)	190.33	73.01	10.10	49.00	25.03	26.30	41.66
V_3 (L-550)	208.67	107.00	27.66	47.10	33.00	52.67	60.65
V_4 (HK-88-232)	220.33	169.33	13.33	44.33	33.66	45.30	57.30

	CD at 5 per cent
Cultivars	1.37
Stages	1.81
Cultivars x Stages	3.62

Table 6 Reducing sugar content (mg/g) of seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	19.89	6.63	2.34	2.41	10.42	10.00	6.12
V_2 (Gaurav)	12.27	8.65	3.36	3.86	8.53	4.90	5.74
V_3 (L-550)	11.67	7.05	4.31	5.08	8.16	8.12	8.22
V_4 (HK-88-232)	14.99	11.19	4.59	5.53	5.67	6.99	7.54

	CD at 5 per cent
Cultivars	4.17
Stages	5.52
Cultivars x Stages	11.04

Table 7 Starch content (mg/g) of seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	211	435	484	476	497	509	554
V_2 (Gaurav)	171	483	601	533	479	417	512
V_3 (L-550)	230	444	551	550	524	420	523
V_4 (H-88-232)	144	351	493	579	603	568	573

	CD at 5 per cent
Cultivars	7.00
Stages	9.00
Cultivars x Stages	18.00

29 days after anthesis in V_4 upto 37 days after anthesis, thereafter little change in starch content was observed. Cultivar V_4 had significantly higher starch content compared to other cultivars at the time of harvest.

Protein content of seed

Different cultivars of chickpea showed significant difference in seed protein content (Table 8). Maximum protein content (21.77%) was recorded in cultivar V_4 on 33 days after anthesis and minimum (16.93%) in cultivar V_1 on 15 days after anthesis. Not much change in protein content was observed with the stages of seed development. All the cultivars showed a slight increase in protein content from 15 days after anthesis to 22 days after anthesis. Thereafter some cultivars showed a decrease, whereas other showed an increase in protein content (Table 8).

Total soluble sugars of seed's leachate

A large quantity of soluble sugars leached out from seeds in all the cultivars (Table 9). This leaching continues to increase upto 29 days after anthesis in all the cultivars and thereafter, it decreased in all the cultivars. Significant difference in total soluble sugar of seed's leachate were observed among the cultivars and stages of development. Leaching was relatively less in V_3 cultivar compares to other cultivars (Table 9).

Cultivar V_3 exhibited least leaching of total soluble sugars and cultivar V_2 showed the maximum leaching of total soluble sugars from seeds at 45 days after anthesis.

Respiration of seed

Significant differences were observed in respiration rate of chickpea cultivars seed at different stages of development (Fig. IV). It increased gradually from 15 days after anthesis upto 22 days after anthesis in all the cultivars. Thereafter,

Table 8 Protein content (%) *of seeds of chickpea* cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	16.93	17.06	17.93	20.13	18.39	17.88	18.38
V_2 (Gaurav)	17.32	18.80	18.84	19.74	19.54	18.85	20.47
V_3 (L-550)	18.07	20.30	17.06	17.10	17.14	17.24	17.24
V_4 (HK-88-232)	18.18	20.42	21.48	21.77	17.36	18.79	18.79

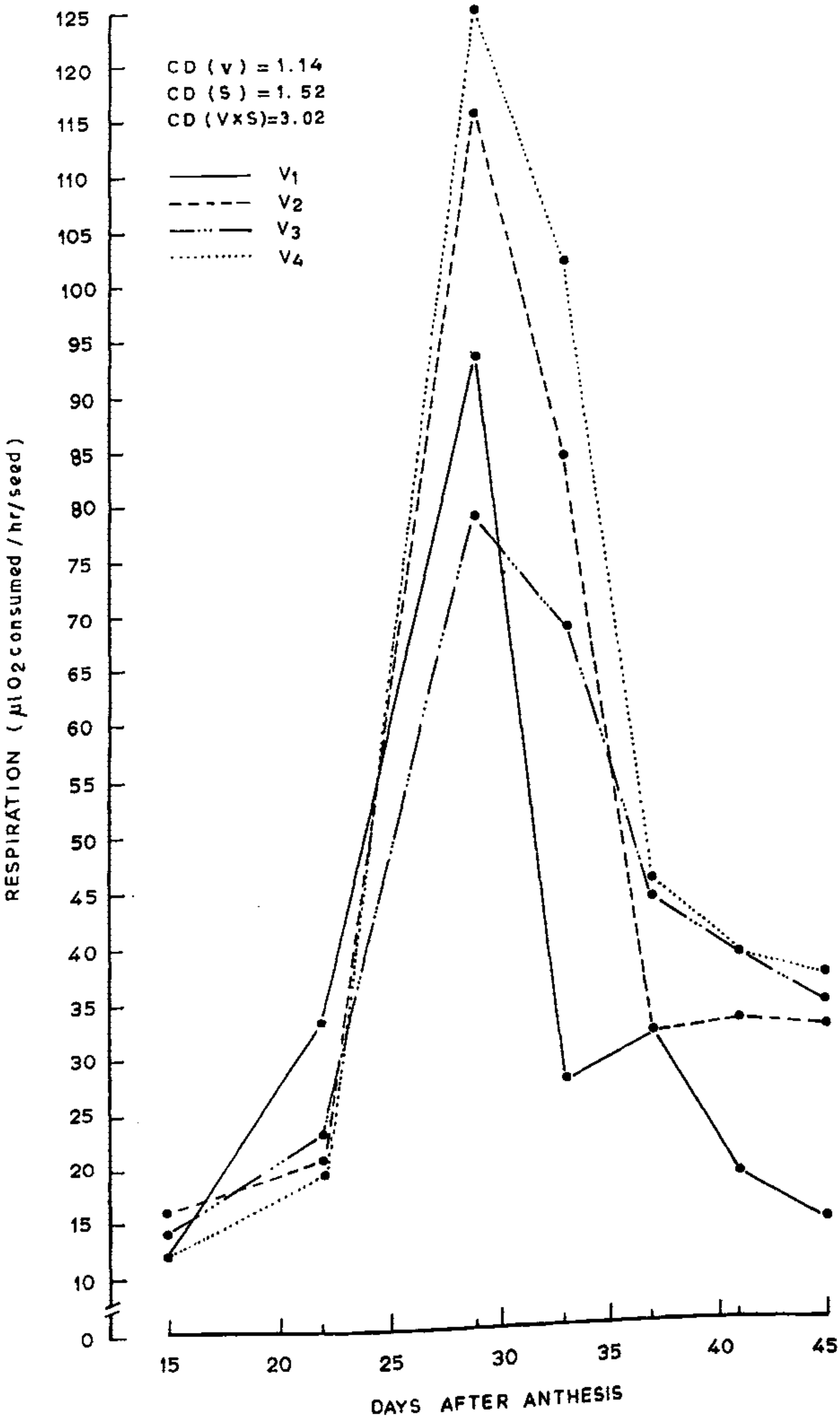
	CD at 5 per cent
Cultivars	0.13
Stages	0.18
Cultivars x Stages	0.36

Table 9 Total soluble sugars content (mg/seed) in leachates of dry seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	285	827	922	259	166	165	574
V_2 (Gaurav)	340	885	991	426	248	210	283
V_3 (L-550)	341	344	585	534	495	272	140
V_4 (HK-88-232)	282	430	994	981	747	404	304

	CD at 5 per cent
Cultivars	15
Stages	20
Cultivars x Stages	40

Fig. IV Respiration rate ($\mu l O_2$ consumed/hr/seed) of seeds of chickpea cultivars at different stages of seed development



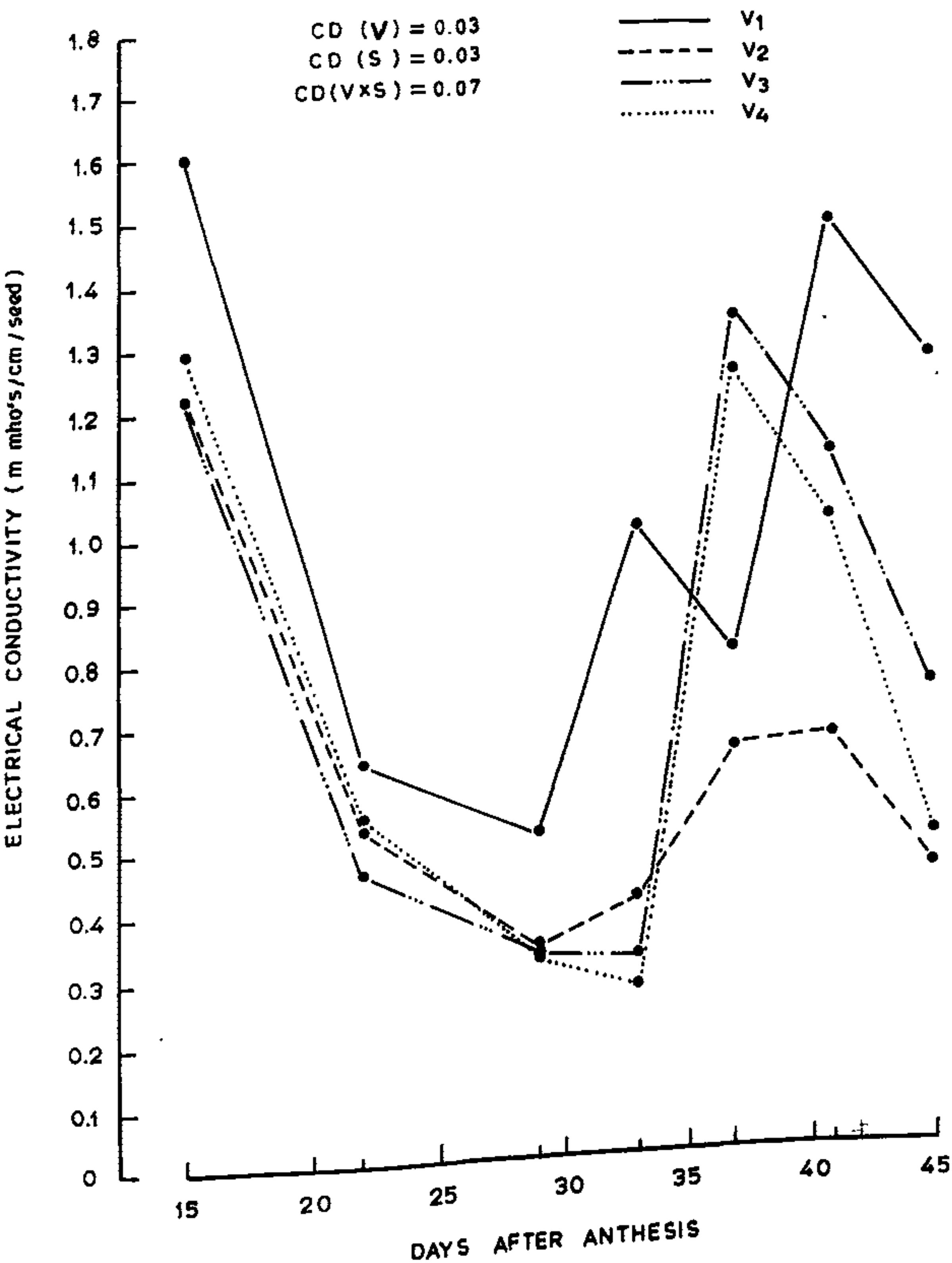
CD (v) = 1.14
CD (S) = 1. 52
CD (VxS)=3.02
V_1
V_2
V_3
V_4
RESPIRATION (μl O₂ consumed / hr / seed)
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
0
15
20
25
30
35
40
45
DAYS AFTER ANTHESIS

increase was rapid upto 29 days after anthesis (Fig. IV). All the cultivars showed a peak of respiration rate at 29 days after anthesis and maximum respiration rate was recorded in cultivar V_4 and minimum in cultivar V_3 at this stage. Later on, respiration rates decrease in all the cultivars upto the last stage i.e. 45 days after anthesis. Kabuli cultivars showed more respiration than desi cultivars after 33 days after anthesis till harvesting stage (Fig. IV).

Electrical conductivity of seed

Maximum leaching of electrolytes were observed on 15 days after anthesis in all the cultivars (Fig. V). Thereafter, conductivity of the leachate decrease upto 29 days after anthesis in all the cultivars. At this stage, kabuli cultivars showed lesser conductivity than desi cultivars. After this stage, a rapid increase in electrolyte concentration was observed, whereas, this increase was observed on day 37 in cultures V_3 and V_4. Cultivar V_2 showed a slightly less increase in electrical conductivity (Fig. V). At the time of harvest, electrical conductivity decreased in all the cultivars, but it was still more that than the value recorded at 29 days after anthesis (Fig. V). Maximum leaching was observed in cultivar V_1 and minimum in V_2.

Fig. V. Electrical conductivity (mmho's/cm/seed) of seeds of chickpea cultivars at different stages of seed development



CD (V) = 0.03
CD (S) = 0.03
CD(V×S) = 0.07
V1
V2
V3
V4
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ELECTRICAL CONDUCTIVITY (m mho's/cm / seed)
15
20
25
30
35
40
45
DAYS AFTER ANTHESIS

Table 10 Correlation among the parameters at different stages of seed development

Parameters	Starch	Reducing sugar in seed	Sugars of seed	Sugars in seed leachate	Fresh weight of seed	Dry weight of seed	Moisture (%) in seed	Electrical conductivity	Germination of freshly harvested seed	Germination of dry seeds	Protein of seed	Dry weight of pod-wall	Fresh wt. of pod-wall	Respiration in seed
Starch	1.0000	-0.6937	-0.8767	0.3580	0.4760	0.7666	-0.4087	-0.4387	0.4858	0.4044	0.1181	0.4941	-0.1770	0.4882
Reducing sugar in seed		1.0000	0.7617	-0.4249	-0.4113	-0.5362	0.3001	0.5578	-0.3034	-0.2137	-0.2208	-0.3181	0.0835	-0.6068
Sugars of seed in seed			1.0000	-0.3146	-0.2654	-0.6475	0.5023	0.3936	-0.5726	-0.4674	-0.1082	-0.2751	0.3364	-0.5150
Sugars in seed leachate				1.0000	0.4517	0.0620	0.2396	-0.4997	-0.3545	-0.4397	0.2865	0.2628	0.4115	0.6353
Fresh weight of seed					1.0000	-0.4923	0.4005	-0.4989	-0.3130	-0.3800	0.3674	0.7559	0.7026	0.6315
Dry weight of seed						1.0000	-0.4721	-0.2417	0.5955	0.5191	0.1371	0.6822	-0.1874	0.3304
Moisture (%) in seed							1.0000	-0.0963	-0.8551	-0.8597	0.0726	0.0288	0.8470	0.1711
Electrical conductivity								1.0000	0.1489	0.3008	-0.4927	-0.4246	-0.3070	-0.6319
Germination of freshly harvested seeds									1.0000	0.9601	-0.0717	0.0168	-0.8332	-0.1593
Germination of dry seeds										1.0000	-0.1531	0.0082	-0.8472	-0.2755
Protein in seed											1.0000	0.3717	0.3113	0.2843
Dry weight of pod-wall												1.0000	0.3765	0.4145
Fresh weight of pod-wall													1.0000	0.4153
Respiration in seed														1.0000

CHAPTER - V

DISCUSSION

The changes in fresh weight of seed and pod-wall for four cultivars have been shown in Table 1 & 2 and dry weight of seed and pod-wall in Fig. I & Table 4. We observed two phases of pod development in Haryana Chana No. 1 (i) Pod-wall development phase-which was upto 22 days after anthesis and (ii) Seed development phase which was from 22 days after anthesis to 37 days after anthesis (Fig. I), whereas Sheoran and Hooda (1988) reported two phases in cultivar H75-35. First phase upto 15 days after anthesis and second from 15 to 42 days after anthesis. So, it point out to varietal differences in phase development to occur during pod development of chickpea. Kabuli cultivars had more fresh weight in seed than desi cultivars at each stage (Table 1). The differences between kabuli and desi cultivars were probably due to variation in the time taken for seeds to maturity, in addition to genetic factor. Moisture percent of seed decreased with increase in time of seed development in all the cultivars (Table 4). Earlier, similar trend in moisture content decrease, during progressive seed development were reported in chickpea (Bharud and Patil, 1990), in pigeonpea (Balakrishanan et al., 1984), in cowpea (Kim et al., 1986), in urdbean (Saha, 1987), in pea (Deunff and Rachidian, 1988), in soyabean (Miles et al., 1988; Lowell and Kuo, 1989), in mungbean (Dharmalingam and Basu, 1989, 90) and in podocarpus (Dodd et al., 1989). Decrease in moisture content was gradual upto 33 days after anthesis in all cultivars followed by a rapid decrease except HK-88-232. So decrease in fresh weight at later stages seems to be due to decrease in moisture content of seed. It may be attributed to break up of vascular connection and faster dehydration towards physiological maturity.

All cultivars showed maximum fresh weight at 33 days after anthesis in seed excep HK-88-232, where such value were obtained at 37th day, in this. Thereafter progressive decrease in fresh weight was observed till maturity (Table 1). However, different worker reported variations in days after anthesis to attain the maximum fresh weight during seed development viz. 28 days after flowering in cultivar G-130 (Singh et al., 1981), 35 days after anthesis in cultivar L-550 and 21 days after flowering in cultivar G-130 (Singh and Jambunathan, 1982). Maximum fresh weight observed were in pigeonpea cultivar ST-1 at 28 days after anthesis (Singh et al., 1980), in soyabean cultivar Miles at 28 days after anthesis (Lin et al., 1984), at 15 days after anthesis in urd bean (Saha, 1987), in mungbean at 16th day (Dharmalingam and Basu, 1989, 90) and at maturity in soyabean (Barratt and Pullen, 1989).

Accumulation of dry matter increased upto 37 days after anthesis in seed of desi cultivars and upto 41 days after anthesis in HK-88-232, whereas cultivar L-550 did not show regular pattern in dry weight accumulation (Fig. I). Here again, variation in days after flowering for getting maximum dry matter accumulation were reported upto 40 days after anthesis by Tripathi et al. (1987), upto maturity (Srivastava et al., 1981; Bharud and Patil, 1990) and after that it decreased. We called this stage, as physiological maturity stage, after this stage supply from mother plant to seed get ceased due to breaking up of vascular connections, therefore dry weight of seed decreased in seeds due to utilization of stored material for various physiological processes like respiration and due to the breaking of vascular connection. Variation in increase in dry matter in other crops have been reported by Trammell (1983) in cowpea increased upto 10 to 18 days after flowering, upto 35 days after anthesis in soyabean (Lin et al., 1987), until maturity in urdbean (Saha, 1987) in pigeonpea (Rao and Rao, 1975) and in mungbean (Setia et al., 1989).

Dry weight of seeds have positive correlation with starch (r=0.7666) and negative correlation with total soluble sugars (r = -0.6475) and with reducing sugar (r = -0.5362) (Table 10). Starch proved to be a major constituent for dried seed and showed a progressive increase with time of development. Increase starch content during seed development seemed to be contributed mainly by total soluble sugars and reducing sugar because starch showed negative correlation with these parameter.

After attaining the maximum dry weight of pod wall at 22 days after anthesis in desi cultivars and 37 days after anthesis in L-550 declined continuously upto maturity.

This may be attributed to translocation of certain stored products to the seed or other storage organ or recycled into plant growth or maintenance and degradation losses due to respiration or other physiological processes. Dry weight of pod wall showed positive correlation with fresh weight and dry weight of seed (r = 0.7559 and 0.6822, respectively).

Desi cultivars showed early and relatively more germination percent at all stages than kabuli in freshly harvested seeds (Fig. II). However reverse was true in dry seed at last stage i.e. 45 DAA (Fig. III). Maximum germination in dry seeds was obtained at 41 DAA in desi cultivars followed by decrease at last stage (Fig. III). In mungbean germination percentage of dry seeds also decreased at later stages of seed development (Dharmalingam and Basu, 1989, 90). These decrease may be due to development of hard seeds at later stages of seed development.

Seed obtained from initial stages of development, did not show germination (Fig. II & III). It may be due to lack of sufficient metabolites required for germination.

The germination potential increased with progressive development of seed (Fig. II & III) except at 45 DAA in dry seeds of desi cultivars. Similar conclusion were drawn earlier observed (Bharud and Patil, 1990). Germination was sufficient at 37 and 41 days after anthesis in desi and kabuli cultivars respectively of freshly harvested seeds. However, in dried seeds it was at 41 and 45 days after anthesis except cultivar L-550 having such germination at 37 days after anthesis (Fig. II & III). So it appear that we can harvest the crop before maturity for early sowing of next crop. In pigeonpea, seed harvested from slightly green pods also gave, in majority of the cases germination percentage required for seed certification (Paroda et al., 1985).

The percentage of germination of seeds increased with the increase in days of seed development and dry matter accumulation. It was interesting to observed a positive correlation (r = 0.5955 and 0.5191) with dry matter accumulation in both cases, i.e. freshly harvested and dry seeds. In seeds of soyabean also maximum germination percentage was occured, when maximum dry matter accumulated during seed development (Trammell, 1983). Germination of freshly harvested seeds in soyabean was 75 per cent which occured at 55 per cent of total dry weight of seed (Miles, 1986). It may be due to considerable accumulation of starch which act as a raw material for germination. Moreover, germination also had positive correlation with dry matter and starch content of seed (Table 10).

Germination of freshly harvested, as well as dry seeds had more negative correlation with moisture content i.e. r = -0.8555 and -0.8597, respectively. Immature seeds of pea were able to germination at very high moisture content (72-76%), reached a first peak at 63 and 100 per cent germination reached at 34-43 per cent moisture content in dried seed so the moisture content had negative correlation with germination (Rachidian and Deunff, 1986).

Germination of freshly harvested seeds had negative correlation with total soluble sugars of seed (r = -0.5726) and with fresh weight of pod-wall (r = -0.8332) and germination of dry seeds had negative correlation with fresh weight of pod-wall (r = -0.8472) (Table 10).

Kabuli seeds showed increase in starch concentration upto 37 days after anthesis and after that decreased till harvesting, where as cultivar Gaurav did not show a regular pattern in starch concentration and in Haryana Chana No. 1, starch concentration increase throughout the seed development (Table 7). Soluble sugars decreased rapidly upto 29 days after anthesis after that no regular pattern was observed (Table 5). Reducing sugar decreased upto 33 days after anthesis, followed by a marked increase at 37th day and thereafter it become constant in all the cultivars (Table 6). Whereas, Singh et al. (1981) reported that the percent of soluble sugars continuously decreased upto 28 days after flowering and then remained unchanged, till the grain of chickpea matured. In starch content, a rapid increase was observed between 14 and 21 days after flowering. After reaching a maximum levels at 28 days after flowering, the amount of starch decreased. Same pattern was observed in pigeonpea by Balakrishanan et al (1984) and in podocarpus by Dodd et al. (1989).

Although an increase in the accumulation of starch of seed was accompanied by decline in starch of pod-wall, it was not possible to say whether the starch of pod-wall was mobilized and translocated to seed.

Singh and Jambunathan (1982) earlier reported that in chickpea the percentage of total soluble sugars and reducing sugar declined during early stages of maturation and this indicating that those sugars were utilized for the accumulation of starch which showed marked increase during the same period. We also observed that starch content have negative correlation with total soluble sugars (r = -0.8767) and with reducing sugars (r = -0.6937) (Table 10). In contrast to this, in soyabean, total soluble sugars increased continuously throughout seed development reaching a maximum value at maturity and starch content increase rapidly until mid-development and thereafter declined sharply to low level (Adams et al., 1980).

Starch content of seed was higher in kabuli cultivars than desi cultivars in chickpea (Table 7). Whereas, in fieldbean, small seeded cultivars had higher concentration of starch than bold seeded cultivars (Barratt, 1982).

At early stages upto 29 days after anthesis, desi cultivars showed more concentration of protein than kabuli and after that kabuli cultivars had more protein content than desi till maturity (Table 8). Whereas, in fieldbean cultivar Dacre D accumulated more total protein than Dacre B, with progressive development of seed (Barratt and Pullen, 1986). Little changes in protein concentration were observed at different stages of development in all cultivars (Table 8). However, Srivastava et al. (1981) observed increase in protein content from 20.25 to 26.55 from 15 to 45 days after flowering respectively. Protein content did not had correlation with all parameters studied except electrical conductivity

(Table 10). Whereas, Tripathi et al. (1987) reported that potein content of seed had positive correlation (r = 0.9949) with dry matter accumulation.

There was no regular pattern in accumulation of protein in chickpea seed (Table 8). Same was true in soyabean (Kim et al., 1987), whereas protein content in cotyledons of soyabean increased with progressive development of seed (Bils and Howell, 1963).

Kabuli cultivars had more amount of sugars then desi cultivars in seed leachate. Sugars of seed leachate increased upto 29 days after anthesis and after that decrease till maturity in desi cultivars (Table 9). Sugars of leachate had positive correlation with respiration (r = 0.6353)and negative with electrical conductivity (r = -0.4997). Not even a single infromation is available in literature regarding this parameter in legumes.

Respiration rate of seed increased rapidly till 29 days after anthesis, followed by a sharp decline upto 33 days after anthesis, after that it declined gradually (Fig. IV). Whereas in soyabean the oxygen uptake in seeds decreased with progressive seed development and at maturity, oxygen uptake was below the limits of method (Bils and Howell, 1963). Respiration rate of seed had positive correlation with sugars of leachate (r=0.6353) and fresh weight of seed (r = 0.6315) whereas negative correlation with reducing sugar (r = -0.6068), total soluble sugars (r = -0.5150) and electrical conductivity (r = -0.6319) (Table 10). The negative correlation with electrical conductivity with respiration in developing seeds, may due to, respiration maintaining integrity of membrane, resulting in less leaching of ions, thereby maintaining low electrical conductivity.

In general electrical conductivity decreased upto 29 days after anthesis in all the cultivars and after that increased upto 37 days after anthesis followed by decline in all cultivars, except Haryana Chana No. 1, where the peak was at 41 days after anthesis (Fig. V), whereas in pea, conductivity was very high during PI phase. At 61 days after planting, at the end of PI phase, conductivity was much reduced afterwards, it declined slowly until 75 days after planting, then in second another fall lead to stabilization of conductivity, the seed becoming dry (Deunff and Rachidian, 1988).

Electrical conductivity had positive correlation with reducing sugar (r = 0.5578) and negative with sugars of seed leachate (r = -0.4997), with fresh weight of seed (r = -0.4989), with protein (r = -0.4927) and with respiration (r = -0.6319).

Fresh weight of seed had positive correlation with respiration because of availability of substrats and water in fresh weight of seed increase the rate of respiration resulting in more ATP production which may be responsible for better uptake of water and maintaining membrane permeability.

Conclusion

1. Fresh weight of seed and pod-wall, dry weight of seed and pod-wall and moisture content of seed were more in kabuli cultivars then desi cultivars at all stages of seed development.
2. Germination of seeds were early and relatively more in freshly harvested seeds as well as dried seeds in desi cultivars in comparison to kabuli cultivars except L-550.
3. Physiological maturity was observed at 37 days after anthesis in desi cultivars and at 41 days after anthesis in HK-88-232.

4. Thirty three days after anthesis, kabuli cultivars contained more amount of starch than desi, whereas total soluble sugars were more at all stages of seed development.
5. Fresh weight of seed increased upto 33 days after anthesis in all cultivars except HK-88-232 but in case of fresh weight of pod-wall increased upto 22 and 29 days after anthesis in kabuli and desi cultivars, respectively.
6. The germination percentage increased with progressive seed development in freshly harvested and dry seeds except at last stage of desi cultivars in dry seeds.
7. Total soluble sugars of seed decreased rapidly upto 29 days after anthesis and after that no regular pattern was observed where as in protein content of seed, there was no regular pattern of accumulation throughout the seed development in all the cultivars.
8. Desi cultivars had more concentration of sugars in seed leachate than kabuli upto 29 days after anthesis, but reverse was true at subsequent stages except in Haryana Chana No. 1 at last stage.
9. Germination of freshly harvested seeds had positive correlation with dry weight of seed and starch content of seed and negative correlation with total soluble sugars of seeds, moisture content of seed, and with fresh weight of pod-wall.
10. Germination of dry seeds had positive correlation with dry weight of seed and negative correlation with total soluble sugars of seed, moisture content of seed and fresh weight of pod-wall.

CHAPTER - VI

SUMMARY

The investigation were carried out on chickpea (Cicer arietinum) cultivars, namely, Haryana Chana No. 1, Gaurav, L-550 and HK-88-232 out of which first two are desi cultivars and last two are kabuli cultivars; to study the physiological and biochemical changes during seed development in relation to seed germination.

The crop was raised under field using recommended agronomic practices. The pods were harvested at 15, 22, 29, 33, 37, 41 and 45 days after anthesis. The pods were divided into two sets. One set was referred to as fresh lot and another set was kept at 35 ± 2°C for drying in an incubator and referred as dried lot. Various observations were recorded in these lots separately. Following observations such as, change in fresh and dry weight of seed, fresh and dry weight of pod-wall, moisture content in seed, germination of freshly harvested seeds and dry seeds, total soluble sugars of seed, reducing sugar of seed, starch of seed, protein of seed, total soluble sugars of seed leachate, respiration of seed and electrical conductivity of seed in each cultivar at each stage of seed development were collected and statistically analysed to find the critical difference (CD) and correlation among various parameters.

Total soluble sugars of seed and seed leachate were estimated by the method of Yemm and Wills, reducing sugar by Nelson-Somogyi method, starch estimated according to Hassid and Neufeld (1964) and protein content by micro Kjeldhal method. Respiration rate was recorded by Differential Respirometer and electrical conductivity by Conductivity Meter. Germination of freshly harvested and dry seeds studies were carried out in petri plates lined with Whatman filter paper No. 1, using the seeds, obtained from various stages of seed development.

With the progressive development of seed, the dry weight of seed increased upto a limit stages, while the moisture content decreased. Percent germination in freshly harvested seeds as well as dry seeds increased with progressive development of seed except in dried seeds of desi cultivars at last stage. Haryana Chana No. 1 showed two phases of pod development (i) pod-wall development phase (ii) seed development phase, such phases were not observe in other cultivars. Germination percent had positive correlation with dry weight of seed, starch content of seed, while the negative correlation with total soluble sugars of seed, reducing sugar of seed and moisture content of seed.

Total soluble sugars and reducing sugar decreased and starch content increased during the corresponding period of seed development. So the starch content had negative correlation with total soluble sugars of seed and reducing sugar of seed, while positive correlation with dry weight of seed. Kabuli cultivars maintained more concentration of starch then desi cultivars upto mid-development after that reverse trend was observe. Differences in protein content had no regular pattern during seed development. Moreover protein content did not have any correlation with any parameters except electrical conductivity during seed development.

The total soluble sugars of seed leachate were more in all cultivars at initial stages and had positive correlation with respiration, and negative with electrical conductivity. The rate of respiration increased at initial stages, followed by a considerable decrease. Respiration had positive correlation with total soluble sugars of seed leachate and fresh weight of seed and negative correlation with reducing sugar of seed. The electrical conductivity decreased at initial stages and after that increased upto 41 days after anthesis, followed by a decrease at last stage of seed development. Electrical conductivity had positive correlation with reducing sugar and negative correlation with sugars of seed leachate, fresh weight of seed and protein content of seed.

LITERATURE CITED

LITERATURE CITED

Abdul-Baki, A.A. and Baker, J.E. (1973). Are changes in cellular organelles or membranes related to vigour loss in seeds. Seed Sci. & Technol. 1: 89-125.

Adams, C.A. and Rinne, R.W. (1981). Seed maturation in soyabean (Glycine max L. Merr) is independent of seed mass and of parent plant is necessary for production of viable seeds. J. Exp. Bot. 32: 615-620.

Adams, C.A.; Rinne, R.W. and Fjerstad, M.C. (1980). Starch deposition and carbohydrase activities in developing and germinating soyabean seeds. Ann. Bot. 45: 577-582.

Annonymous (1976). International rules for seed testing. Seed Sci & Technol. 4: 3-49.

Anonymous (1989). Agriculture Situation in India. 43: 920.

A.O.A.C. (1970). Association of official analytical chemists. Official methods of analysis, Washington, D.C.

Bain, J.M. and Mercer, F.V. (1966). Sub-cellular organization of the developing cotyledons of Pisum sativum L. Aust. J. Biol. Sci. 19: 49-67.

Balakrishanan, K.; Vanangamudi, K.; Mallika; Mrs. and Natarajaratnam, N. (1984). Seed development and maturation studies in pigeonpea. Madras Agric. J. 71(6): 387-390.

Barnett, N.M. and Naylor, A.W. (1966). Aminoacid and protein metabolism in Bermuda grass during water stress. Pl. Physiol. 41: 1222-1230.

Barratt,, D.H.P. (1982). Changes during development in the nitrogen, uncombined amino acid and carbohydrate content of cotyledons from different cultivars and lines of field bean (Vicia faba). Ann. Bot. 49: 761-768.

Barratt, D.H.P. and Pullen, Anu (1984). Control of seed protein accumulation in field bean (Vicia faba L.). Ann. Bot. 53: 683-689.

Basu, B.C.; Muralidharan, V.; Rani, M.S.C.; Nagrajan, M.; Rangaswamy, S.R. and Perumul, R.K.P. (1990). Effect of seed size on germination and seedling growth in green gram (Vigna radiata) and blackgram (Vigna mungo) cultivars. J. Agron. Crop Sci. 164(3): 213-216.

Bera, A.K. and Bera, Anita (1990). Pattern of total and fractional protein content of developing seeds of mungbean (Vigna radiata L.) and urdbean (Vigna mungo L.). Legume Res. 13(2): 69-72.

Bharud, R.W. and Patil, R.B. (1990). Studies on physiological maturity of gram (Cicer arietinum L.) seeds. Seed Res. 18(2): 160-162.

Bhor, S.B.; Thete, R.Y.; Patil, R.B. and Bharud, R.W. (1988). Effect of seed size on growth, yield, yield attributes and seed quality of gram. Seed Res. 16(2): 143-147.

Bils, R.F. and Howell, R.W. (1963). Biochemical and cytological changes in developing soyabean cotyledons. Crop Sci. 3: 304-308.

Bishnoi, U.R. (1974). Physiological maturity of seeds in Triticola hexaploid L. Crop Sci. 14: 819-821.

Boulter, D. and Davis, O.J. (1968). Nitrogen metabolism in developing seeds of Vicia faba. New Phytol. 67: 935-946.

*Carr, D.L. and Skene, K.G.M. (1961). Diauxic growth curves of seeds with special reference to frenchbean (Phaseolus vulgaris). Aust. J. Biol. Sci. 14: 1-12.

Clapham, V.M. and Barness, S.L. (1990). Development and maturation of white lupine seeds. Agron. J. 82(4): 707-710.

Dasgupta, U.; Bewely, J.D. and Yeung, F.C. (1978). Desiccation tolerant and desiccation intolerant stages during development and germination of Phaseolus vulgaris seeds. J. Exp. Bot. 33: 1045-1057.

Delouche, J.C. (1973). Seed maturation. Seed production manual. NSC and Rockfeller foundation pp. 162-165.

Deunff LE Vyon and Rachidian Z. (1988). Interruption of water delivery of physiological maturity is essential for seed development, germination and seedling growth in pea (Pisum sativum). J. Exp. Bot. 39: 1221-1230.

Dharmalingam, C. and Basu, R.N. (1989). Seed development and maturation studies in mungbean. Seed Res. 17(2): 103-108.

Dharmalingam, C. and Basu, R.N. (1990). Seed development *and maturation* studies in mungbean. Seed Res. 18(1): 1-6.

Dharmalingam, C. and Ramakrishnan, V. (1978). Seed quality in relation to seed size and seed coat colour variation in black gram (Vigna radiata L. Hepper). Seed Res. 6: 101-109.

Dodd, M.C.; Staden, J.V. and Smith, M.I. (1989). Seed development in Podocarpus henkelii : an ultra structural and biochemical study. Ann. Bot. 64: 297-310.

Eastin, J.D.; Hultquist, J.H. and Sullivan, C.Y. (1973). Physiological maturity in grain sorghum. Crop Sci. 13: 173-178.

Flinn, A.M. and Pate, J.S. (1968). Biochemical and physiological changes during maturation of fruit of the field pea (Pisum arvense L.). Ann. Bot. 32: 479-495.

*Franklin, V. and Murray, D.R. (1982). Z. Pelanzehphysiol. 106(5): 447-452.

*Gateulina, G.G. and Prikhod'ko, V.A. (1982). Period of fruit and seed development in leguminous crops. S-Khakad. 4: 25-38.

*Geervani, P. and Devi, U. (1988). Effect of maturation on nutrient composition of selected vegetable legumes. J. Sci. Fd. & Agric. 46(2): 243-248.

Habib, A.F.; Syed Sadaquat, Skeikh; Kulkarni, G.K. and Nadaf, H.L. (1985). Kernal filling as an index of maturity in bunch groundnut. Seed Res. 13(3): 142-144.

*Harrington, J.F. (1972). Seed Biology. II (ed) T.T. Kozlowski. pp. 145-245. Academic Press, New York.

*Hassid and Neufeld (1964). Quantitative determination of starch in plant tissues. In: Methods in carbohydrate chemistry (Whistler, R.L. ed.). Vol. IV, New York, U.S.A. pp. 33-36.

Hedley, C.L. and Ambrose, M.J. (1980). An analysis of seed development in Pisum sativum L. Ann. Bot. 46: 89-105.

Hooda, R.S.; Rao, A.S.; Luthra, Y.P.; Sheoran, I.S. and Singh, R. (1986). Partitioning and utilization of carbon and nitrogen for dry matter and protein production of chickpea. J. Exp. Bot. 37: 1492-1502.

Dharmalingam, C. and Basu, R.N. (1990). Seed development and maturation studies in mungbean. Seed Res. 18(1): 1-6.

Dharmalingam, C. and Ramakrishnan, V. (1978). Seed quality in relation to seed size and seed coat colour variation in black gram (Vigna radiata L. Hepper). Seed Res. 6: 101-109.

Dodd, M.C.; Staden, J.V. and Smith, M.I. (1989). Seed development in Podocarpus henkelii : an ultra structural and biochemical study. Ann. Bot. 64: 297-310.

Eastin, J.D.; Hultquist, J.H. and Sullivan, C.Y. (1973). Physiological maturity in grain sorghum. Crop Sci. 13: 173-178.

Flinn, A.M. and Pate, J.S. (1968). Biochemical and physiological changes during maturation of fruit of the field pea (Pisum arvense L.). Ann. Bot. 32: 479-495.

*Franklin, V. and Murray, D.R. (1982). Z. Pelanzehphysiol. 106(5): 447-452.

*Gateulina, G.G. and Prikhod'ko, V.A. (1982). Period of fruit and seed development in leguminous crops. S-Khakad. 4: 25-38.

*Geervani, P. and Devi, U. (1988). Effect of maturation on nutrient composition of selected vegetable legumes. J. Sci. Fd. & Agric. 46(2): 243-248.

Habib, A.F.; Syed Sadaquat, Skeikh; Kulkarni, G.K. and Nadaf, H.L. (1985). Kernal filling as an index of maturity in bunch groundnut. Seed Res. 13(3): 142-144.

*Harrington, J.F. (1972). Seed Biology. II (ed) T.T. Kozlowski. pp. 145-245. Academic Press, New York.

*Hassid and Neufeld (1964). Quantitative determination of starch in plant tissues. In: Methods in carbohydrate chemistry (Whistler, R.L. ed.). Vol. IV, New York, U.S.A. pp. 33-36.

Hedley, C.L. and Ambrose, M.J. (1980). An analysis of seed development in Pisum sativum L. Ann. Bot. 46: 89-105.

Hooda, R.S.; Rao, A.S.; Luthra, Y.P.; Sheoran, I.S. and Singh, R. (1986). Partitioning and utilization of carbon and nitrogen for dry matter and protein production of chickpea. J. Exp. Bot. 37: 1492-1502.

Kapoor, A.C. and Gupta, Y.P. (1975). Changes in protein and amino acid in the developing soyabean seed and effects of phosphorus nutrition. J. Fd. Sci. & Agric. 28: 113-120.

Kermode, A.R. and Bewely, J.D. (1985). The role of maturation drying in the transition from seed development to germination. I. Acquisition of desiccation and germibility during development of Ricinus Communis L. seeds. J. Exp. Bot. 36: 1906-1915.

Kermode, A.R.; Wilson, R. and Bewely, J.D. (1986). The role of maturation drying in the transition from seed development to germination. J. Exp. Bot. 37: 1887-1898.

*Kim, S.D.; Cha, Y.H.; Cho, J.T.; Kwun, K.C.; Son, S.G. and Park, S.I. (1987). Changes in development and nutrient composition of pod after flowering in cowpea (Vigna unguiculata L.). Korean J. Crop Sci. 31(1): 68-73.

Kooistra, E. (1962). On the differences between smooth and three types of wrinkled pea. Euphytica. 11: 357-373.

Lin, P.C.P.; Egli, B.; Dennis, Li, M.G. and MeeKel, L. (1984). Polyamine titer in the embryonic axis and cotyledons of Glycine max (L) during seed growth and maturation. Pl. Physiol. 76: 366-371.

Loewenberg, J.B. (1955). The development of bean seeds (Phaseolus vulgaris L.). Pl. Physiol. 30: 244-249.

Lowell, A. and Kuo, M.T. (1989). Oligosaccharide metabolism and accumulation in developing soyabean seed. Crop Sci. 29(2): 459-465.

Madison, J.T.; Thompson, J.E. and Muenster, A.M.E. (1976). Deoxyribonuclic acid, ribonuclic acid, protein and uncombined amino acid content of legume seed during embrogeny. Ann. Bot. 40: 745-756.

*Manohar, M.S. and Sachan, S.C.P. (1975). Pod development and germination studies on pea (Pisum sativum L.). Veg. Sci. 1: 22-30.

*Manteuffel, R.; Muntz, K.; Puchel, M. and Scholz, G. (1976). Phase development changes of DNA, RNA and protein accumulation during the ontogenesis of broadbean (Vicia faba L.) seeds. Biochem. Physiol. Pflanzen. 169: 595-605.

*Mayer, A.M. (1973). The control of the initial stages of germination. Some biochemical investigation. Seed Sci. & Technol. 1: 51-72.

McIlrath, W.J.; Abrol, Y.P. and Hellingman, J. (1963). Dehydration of seeds in intact tomato fruits. Sci. 142: 1681.

*Miles, D.F. (1986). Effect of the stage of development and desiccation environment on soyabeanseed quality and respiration during germination. Dissertation Abstarcts International B (Sci. & Engin.). 46(9): 2884-2885.

Miles, D.F.; Tekrony, D.M. and Egli, D.B. (1988). Changes in viability, germination and respiration of freshly harvested soyabean seed during development. Crop Sci. 28: 700-704.

*Millerd, A. and Spencer, D. (1974). Changes in RNA synthesising activity and template activity in nuclei from cotyledons of developing pea seeds. Aust. J. Pl. Physiol. 1: 331-341.

Mutchler, M.A.; Bliss, F.A. and Hall, T.C. (1980). Variation in the accumulation of seed storage protein among genotypes of Phaseolus vulgaris. Pl. Physiol. 65: 627-630.

Nagaraj, S.; Chauhan, S. and Ravindra, V. (1987). Influence of maturity on oil, protein content and yield in three spanish bunch groundnut varieties. Seed & Farms. 13(8): 53-54.

*Nelson, N. (1944). J. Biol. Chem. 153: 375 (In Methods in Enzymology, 1957 Vol. III (Eds.) S.P. Colowick and N.O. Kaplan. p. 55. Academic Press, New York.

Ohmura, T. and Howell, R.W. (1962). Respiration of developing and germinating soyabean seeds. Physiol. Plantarum. 15: 341-350.

Ouebedeanx, B. and Chollat, R. (1975). Growth and development of soyabean (Glycine max L.) pods. Pl. Physiol. 55: 745-748.

Ovacharov, K.K. and Kizilova, K.G. (1966). Differences in seed quality and plant productivity. In Physiological Basis of Seed Germination. 54-140. Amerind Publishing, New Delhi.

*Ozaki. K.; Saito, M. and Nita, K. (1956). Studies on the seed development and germination of soyabean plants at various ripening stages. Hokkaido Natl. Exp. Stn. Res. Bull. 70: 6-14.

*Palanisamy, V.; Vanagamudi, K. and Jayabarathi, M. (1986). Seed development and maturation in cowpea (Vigna sinensis L.). Trop. Grain Legume. Bull. 33.

*Paredes, L.O.; Guevara, L.F.; Barba, D.L. and Rosa, A.D. (1990). Biosynthesis of lactins in developing seeds of common bean. Fd. Chem. 35(3): 237-243.

Paroda, R.S.; Tomer, R.P.S. and Malik, B.P.S. (1985). Variation in germination in different genotypes of pigeonpea (Cajanus cajan) at maturity. Seed Res. 13(2): 157-158.

Qiu, L.J.; Wang, J.L. and Mong, Q.X. (1990). Preliminary study on accumulation characteristics of protein and oil in developing soyabean seeds. Scienti Agril. Sinica. 23(5): 28-32.

Ramaiah, H. (1986). Studies on seed development, physiological maturity and their relationship on seed quality in cowpea (Vigna ungiculata L.). Mysore J. Agric. Sci. 20: 339-340.

*Rachidian, Z. and Deunff, Y. Le (1986). Germination capacity of pea seeds (1) Pod and seed development : implication for seed germination with or without drying. Agronomie. 6(5): 463-468.

Rao, M.K.V. and Rao, R.G. (1975). Growth, respiration and endogenous auxin of developing and germination seeds of pigeonpea (Cajanus indicus). Seed Res. 3: 1-10.

Rosenberg, L.A. and Rinne, R.W. (1980). Moisture loss as a peruisite for seedling growth in soyabean seeds (Glycine max L. Merr). J. Exp. Bot. 37: 1663-1674.

*Ross, L.F. and Mixon, A.C. (1989). Changes in soluble carbohydrates in developing seed from florunner peanuts (Arachis hypogea L.). J. Fd. Comp. & Anal. 2(2): 157-160.

Ryle, G.L.A.; Powell, C.E. and Gordon, A.J. (1979). The respiratory costs of nitrogen fixation in soyabean, cowpea and white clower. II. Nitrogen fixation and the respiratory of the noduled roots. J. Exp. Bot. 30: 135-153.

Saha, Anita (1987). Physiology of seed development in urdbean. Indian J. Pl. Physiol. 2: 199-201.

Saha, Anita and Bera, A.K. (1990). Nitrogen fractions in mungbean (Vigna radiata) and urdbean (Vigna mungo) seeds during development and maturation. Legume. Res. 13(3): 133-135.

Sale, P.W. and Campbell, L.C. (1980). Changes in physical characteristic and composition of soyabean seed during crop development. Field Crop Res. 3: 147-155.

Sehgal, C.B.; Gandhi, V. and Varma, B. (1987). Histological and biochemical studies on the cotyledons of some legume (II). Reserve metabolites during seed development. Cytologia. 52(4): 847-858.

Setia, N.; Malik, C.P. and Setia, R.C. (1984). Comparative histological studies in growing pod of chickpea and mungbean. Phytomorphol. 34(1-4): 204-208.

Sharma, S. and Bhal, A. (1989). Variability for biochemical characters in Cicer arietinum L. Legume Res. 12(1): 179-182.

Sheoran, I.S. and Singh, R. (1988). Photosynthetic contribution of pod wall in seed development of chickpea. Proc. Indian Natn. Sci. Acad. B 53: 531-534.

Singh, U. and Jambunathan, R. (1982). Changes in starch, oligosaccharides and soluble sugars in developing pod wall and seed of chickpea. Phytochem. 21(2): 297-299.

Singh, U.; Jambunathan, R. and Naryanan, A. (1980). Biochemical changes in developing seeds of pigeonpea (Cajanus cajan). Phytochem. 19: 1291-1295.

Singh, U.; Jambunathan, R. and Saxena, N.P. (1981). Changes in carbohydrates, amino acids and proteins in developing seed of chickpea. Phytochem. 20: 373-378.

Smith, D.L. (1973). Nucleic acid, proteins and starch synthesis in developing cotyledons of Pisum arvense L. Ann. Bot. 37: 795-804.

Sombatsiri, T. and Naun, S. (1987). Seed quality of peanut (Arachis hypogea L.) from harvest at different stages. Seed. Sci. & Technol. 15(3): 613-616.

Somogyi (1945). A new reagent for the determination of sugars. J. Biol. Chem. 160: 61-69.

*Srivastava, K.N. (1987). Starch and protein content and enzyme levels in developing cotyledons of chickpea. Ann. Agric. Res. 8(1): 86-94.

Srivastava, K.N.; Mehta, S.L.; Naik, M.S. and Eggum, B.O. (1981). Protein accumulation and protein quality of bengal gram (Cicer arietinum) cotyledons during development. J. Agric. Fd. Chem. 29: 24-27.

Stickland, R.C. and Wilson, K.E. (1983). Sugar and starch in developing round and wrinkled pea seeds. Ann. Bot. 52: 919-921.

*Trammell, C.A. (1983). Influence of maturity level on variety response of southernpea to dessication by glyphosate. Dissertation Abstracts International B 44(3): 660.

Tripathi, A.; Chandra, S. and Bhattacharya, A. (1987). Accumulation of dry matter and nitrogen in developing pods and their bearing on desirable plant type of chickpea. Indian J. Agric. Sci. 52(12): 909-911.

*Turner, D.H. and Turner, J.F. (1957). Physiology of pea fruits. III. Changes in starch and phosphorylase in the developing seed. Aust. J. Biol. Sci. 10: 302-309.

*Turner, J.F.; Turner, D.H. and Lee, J.B. (1957). Physiology of pea fruits IV. Changes in sugars in the developing seed. Aust. J. Bio. Sci. 10: 407-413.

*Veira, R.D.; Veira, R.V.; Carvalho, N.M.; Numes, D.L.G. and Da, S. (1988). Seed development in pigeonpea (Cajanus cajan), nyacinthbean (Dolichos lablab) and velvatbean (Stizolobium aterrimum). Cientifica 16(1): 125-131.

Yazdi-Samadi, B.; Rinne, R.W. and Seif, R.D. (1977). Components of developing soyabean seeds, oil, proteins, sugars, starch, organic acids and amino acids. Agron. 69: 481-486.

*Yemm, E.W. and Wills, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57: 508-514.

*Wilson, L.A.; Birmingham, V.A.; Moon, D.P. and Snyder, H.E. (1978). Isolation and characterization of starch from mature soyabeans. Cer. Chem. 55: 661-670.

Wolswinkel, P. and Ammerlaan, A. (1985). Effects of potassium on sucrose and amino acid release from the seed coat of developing seeds of Pisum sativum. Ann. Bot. 56: 35-43.

---

* Original not seen

APPENDICES

Appendix I Dry weight (mg/seed) of seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	12.00	51.00	98.00	115.00	116.00	112.00	105.00
V_2 (Gaurav)	22.00	46.00	93.15	136.03	137.00	127.50	129.03
V_3 (L-550)	44.06	73.00	121.10	148.00	141.03	145.05	153.05
V_4 (HK-88-232)	17.00	73.01	133.00	185.70	225.07	233.01	223.10

	CD at 5 per cent
Cultivars	3
Stages	4
Cultivars x Stages	9

Appendix II Germination (%) of freshly harvested seeds of chickpea cultivars at *different* stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	000	000	053	083	100	100	100
V_2 (Gaurav)	000	003	013	070	100	100	100
V_3 (L-550)	000	003	007	023	093	100	100
V_4 (HK-88-232)	000	000	000	013	037	100	100

	CD at 5 per cent
Cultivars	0.38
Stages	0.65
Cultivars x Stages	2.60

Appendix III Germination (%) of dry seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	000	000	027	077	087	100	090
V_2 (Gaurav)	000	000	000	030	070	092	066
V_3 (L-550)	000	000	000	010	100	100	100
V_4 (HK-88-232)	000	000	000	000	017	087	097

	CD at 5 per cent
Cultivars	0.76
Stages	1.00
Cultivars x Stages	4.00

Appendix IV Respiration rate ($\mu l O_2$ consumed/hr/seed) of seeds of *chickpea* cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	11.87	33.33	92.80	27.49	30.53	18.00	13.60
V_2 (Gaurav)	16.27	20.13	115.47	84.13	31.36	32.00	31.60
V_3 (L-550)	14.00	22.40	78.40	68.53	43.60	37.73	33.60
V_4 (HK-88-232)	14.20	19.07	125.33	102.00	45.00	37.63	36.53

	CD at 5 per cent
Cultivars	1.14
Stages	1.51
Cultivars x Stages	3.02

Appendix V Electrical conductivity (mmhos/cm/seed) of seeds of chickpea cultivars at different stages of seed development

Cultivars	Days after anthesis						
	15	22	29	33	37	41	45
V_1 (Haryana Chana No. 1)	1.59	0.63	0.51	1.00	0.79	1.47	1.26
V_2 (Gaurav)	1.22	0.52	0.33	0.40	0.64	0.65	0.44
V_3 (L-550)	1.22	0.45	0.31	0.31	1.33	1.11	0.73
V_4 (HK-88-232)	1.29	0.54	0.31	0.26	1.24	1.00	0.49

	CD at 5 per cent
Cultivars	0.03
Stages	0.03
Cultivars x Stages	0.07