BIOCHEMICAL ANALYSIS OF CHICKPEA CULTIVARS IN RELATION TO DROUGHT TOLERANCE

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Dedication

Affectionately dedicated

to my parents

who inspired me

at every mode of life

Jyoti

BIOCHEMICAL ANALYSIS OF CHICKPEA CULTIVARS IN RELATION TO DROUGHT TOLERANCE

by

MISS JYOTI VINODRAO RAUT
(Reg. No. 20123)

A Thesis Submitted

in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE (AGRICULTURE)
in
BIOCHEMISTRY

Approved by :

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CERTIFICATE

This is to certify that the thesis entitled **"Biochemical Analysis of Chickpea Cultivars in Relation to Drought Tolerance"**, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra); in partial fulfillment of the requirements for the award of degree of **Master of Science (Agriculture) in Biochemistry,** is a record of bona fide research work carried out by **Miss Jyoti Vinodrao Raut** under my guidance and supervision and that no part the thesis has been submitted for any other degree or diploma.

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

Place : M.P.K.V. Rahuri

Date : 25/06/02

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Date : 27/06/2002

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Miss J. V. Raut

CONTENTS

CANDIDATE'S DECLARATION iii

CERTIFICATES

1. Research Guide iv
2. Associate Dean (PGI) v

ACKNOWLEDGEMENT vi

LIST OF TABLES x

LIST OF FIGURES xi

ABSTRACT xii

1. INTRODUCTION 1
2. REVIEW OF LITERATURE 5
   - 2.1 Nutritional composition and utilization 5
   - 2.2 Effect of water stress on growth and yield of crop plants 7
   - 2.3 Effect of water stress on some biochemical constituents 8
     - 2.3.1 Proline 8
     - 2.3.2 Free amino acids 10
     - 2.3.3 Soluble proteins 11
     - 2.3.4 Enzymatic changes 12
     - 2.3.5 Total and reducing sugars 12
     - 2.3.6 Total polyphenols 13
     - 2.3.7 Chlorophyll 14
     - 2.3.8 Minerals 14
3. MATERIAL AND METHODS 15
   - 3.1 Material 15
     - 3.1.1 Seeds 15
     - 3.1.2 Pots and soil 15
     - 3.1.3 Chemicals 15

3.2 Methods 16

3.2.1 Proline 16

3.2.2 Free amino acids 17

3.2.3 Soluble proteins 20

3.2.4 Reducing sugars 22

3.2.5 Total sugars 24

3.2.6 Total polyphenols 24

3.2.7 Chlorophyll 28

3.2.8 Chlorophyll stability index 28

3.2.9 Phosphorous 29

3.2.10 Potassium 30

3.2.11 Statistical analysis 31

4. RESULTS AND DISCUSSION 32

4.1 Effect of water stress on proline 32

4.2 Effect of water stress on free amino acids (FAA) 36

4.3 Effect of water stress on soluble proteins 38

4.4 Effect of water stress on reducing sugars and total sugars 40

4.5 Effect of water stress on total polyphenols 44

4.6 Effect of water stress on chlorophylls 47

4.7 Effect of water stress on phosphorous and potassium. 52

4.8 Correlations 55

5. SUMMARY AND CONCLUSIONS 58

6. LITERATURE CITED 62

7. VITA 72

LIST OF TABLES

Sr. No.	Title	Page
1.	Effect of water stress on the content of proline in leaves of chickpea cultivars (fresh wt. basis)	33
2.	Effect of water stress on the content of free amino acids in leaves of chickpea cultivars (fresh wt. basis)	37
3.	Effect of water stress on the content of soluble proteins in leaves of chickpea cultivars (fresh wt. basis)	39
4.	Effect of water stress on the content of reducing sugars in leaves of chickpea cultivars (fresh wt. basis)	41
5.	Effect of water stress on the content of total sugars in leaves of chickpea cultivars (fresh wt. basis)	42
6.	Effect of water stress on the content of total polyphenols in leaves of chickpea cultivars (fresh wt. basis)	45
7.	Effect of water stress on the content of chlorophyll a in leaves of chickpea cultivars (fresh wt. basis)	48
8.	Effect of water stress on the content of chlorophyll b in leaves of chickpea cultivars (fresh wt. basis)	49
9.	The chlorophyll stability index in leaves of chickpea cultivars (fresh wt. basis)	51
10.	Effect of water stress on the content of phosphorus in leaves of chickpea cultivars (fresh wt. basis)	53
11.	Effect of water stress on the content of potassium in leaves of chickpea cultivars (fresh wt. basis)	54
12.	Correlation coefficient of proline accumulation with some biochemical constituents.	56

LIST OF FIGURES

Sr. No.	Title	Page
1.	Standard curve for estimation of proline	18
2.	Standard curve for estimation of free amino acids	21
3.	Standard curve for estimation of soluble proteins	23
4.	Standard curve for estimation of reducing sugars	25
5.	Standard curve for estimation of total polyphenols	27
6.	Increase in proline (folds) in different chickpea cultivars in response to water stress	35
7.	Increase in total sugars (folds) in different chickpea cultivars in response to water stress	43
8.	Increase in polyphenols (folds) in different chickpea cultivars in response to water stress	46

ABSTRACT

BIOCHEMICAL ANALYSIS OF CHICKPEA IN RELATION TO DROUGHT TOLERANCE

by

MISS JYOTI VINODRAO RAUT

A candidate for the degree of

MASTER OF SCIENCE (AGRICULTURE)

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Research Guide	:	Dr. J.K. Chavan
Department	:	Biochemistry

The present investigations were undertaken to evaluate the promising chickpea cultivars for drought tolerance based on the accumulation of certain biochemical constituents in response to artificial drought. An attempt was also made to identify the additional / alternate biochemical parameter to assess the drought tolerant character in chickpea.

Eight promising chickpea cultivars showing differential reaction to drought condition were obtained from the Senior Pulses Breeder of the University and grown in a glass house in pots. The water stress was applied at 40 DAS and the leaf sample both of normal and stressed plants of each cultivar were analyzed for changes in proline and other biochemical constituents by the standard procedures.

An application of artificial stress resulted in an increase in mean leaf proline content from 76 mg to 1000 mg/ 100 g in eight cultivars.The cultivars that are known to be drought tolerant accumulated 20-50 fold. The higher leaf proline due to water stress. Confirmed the role of proline

Phule G-96006, Vijay, Phule G-97110, BG-256 and BG-362 were evaluated as drought tolerant cultivars based on spectacular increase in leaf proline upon water stress.

Among the other biochemical constituents studied viz. free amino acid, soluble proteins, reducing and total sugars, polyphenols, chlorophylls, phosphorous and potassium, only the accumulations of total and reducing sugars and polyphenols were found to exhibit a positive relationship while the soluble proteins were found to be negative relationship with proline accumulation in leaves upon water stress. These results indicated that the accumulation of total sugars, polyphenols and potassium contents while a decrease in soluble proteins in response to water stress can be used as additional parameter to evaluate drought tolerance in chickpea. However a large number of chickpea cultivars with differential reaction to drought need to be analyzed to confirm these observations.

Pages 1 to 72

Chapter Opener Page

INTRODUCTION

1. INTRODUCTION

The nutritional importance of food legumes as an economic source of proteins, carbohydrates, minerals, and vitamins has been recognized throughout the world (Milner, 1972; Salunkhe, 1982). It is more economical to consume grain legumes directly instead of converting them into animal proteins. Legumes has been considered as leading candidates in protein supply to the malnourished areas of the world. The seeds contain 2 to 3 times more proteins than cereals. The combination of cereals and legumes provide a good balance of amino acids. Considerable resources and efforts have been diverted to improve grain yield, nutritional quality, storage, and processing technology of grain legumes.

On the global basis, chickpea (*Cicer arientinum L.*) is the third most important pulse crop after dry beans (*Phaseolus vulgaris L.*) and dry peas (*Pisum sativum L.*). The area under this crop is about 11.1 million hectares and production is about 8.5 million tonnes in the world (FAO, 1998). The important chickpea growing countries are India, Pakistan, Ethiopia, Myanar and Turkey. The area under chickpea in India is about 8.41 million ha and production 6.68 million tonnes with a productivity of 794 kg/ha. In Maharashtra, it is second important pulse crop next to pigeonpea, occupying an area of 9.33 lakh ha with total production of 5.00 lakh tonnes and productivity of 643 kg/ha (Anonymous, 1999).

Food legumes are the important source of dietary proteins, particularly for the vegetarian diets. Legume seeds provide 2-3 times higher protein than cereals. The chemical composition of chickpea is (g/100 g) : protein 12.4 to 30.0, carbohydrates 50.6 to 70.9 , ash 2.04 to 4.67, lipids 3.1 to 7.42 and crude fiber 1.2 to 8.5. It is also rich in iron, calcium, potassium and phosphorus (Agarwal and Bhattacharya, 1980;

Angela *et al.*1987; Fernandez and Berry, 1988 and Samman *et al.*, 1999).

Chickpea is mainly cultivated as rainfed crop in rabi season on residual soil moisture. Although India is the largest producer of this crop, its productivity is low when it is compared with other countries like Italy, Sudan, Turkey. It is due to the environmental biotic and abiotic factors. The biotic factors are the occurrence of diseases, pests, insects, nematodes and parasitic weeds while the abiotic factors are the drought, temperature, salt, heat or cold stress etc.

The drought is one of the major environmental factors which affects the productivity of chickpea to a great extent. The physiological processes in plant are affected by the soil moisture stress and thereby resulting in reduced plant growth. It is mainly due to the development of the high osmotic pressure in the roots and shoots. The data on extent of yield losses in field crops, particularly in chickpea due to drought condition are meager. However, a considerable information is available on biochemical changes in leaves in response to water stress to a varying degrees. Attempts have been made to identify and characterize the specific biochemical constituent(s) associated with drought tolerant behavior of the field crops including chickpea.

Gorbanli *et al.*, (1998) observed a decrease in soluble protein while an increase in total and in each amino acid such as aspartic acid, glutamic acid, proline, leucine and arginine in chickpea leaves upon water stress. Sharma *et al.*, (1986) reported that salinity increased the pool of free amino acids and proteolytic activity in embryonic axis and cotyledons of germinating chickpea.

Bates *et al.*, (1973) showed that proline increases proportionately faster than other amino acids in plants under water stress. It has been suggested as an evaluating parameter for selecting drought tolerant

varieties. Singh and Rai (1983) compared two cultivars of chickpea for their soluble sugar content. The total and non-reducing sugars accumulate with increased water stress. Thus, higher content of non-reducing and total soluble sugar was characteristics of shoots of the tolerant cultivars.

Gupta *et al.*, (1997) showed that water stress increased the proline, amino acids and total soluble sugar content in leaves but decreased the soluble proteins and starch content and suggested that water stress induced increase in amino acids particularly of proline and soluble sugars might be responsible for osmotic adjustment. Munoj et. al., (1998) observed a cDNA encoding proline rich-protein (PRP) from *Cicer arietinum*, changes in expression during development and abiotic stresses and concluded that the protein encoded by the PRP clone participates in the drought tolerance. Singh and Singh (1999) studied the effect of salinity on Na, K and proline content of chickpea genotypes and reported that the tolerant genotypes had lower sodium, higher potassium and higher proline content in shoots than the susceptible genotypes.

These reports indicated that water / salt stress markedly influence the biochemical changes in chickpea leaves and roots. The increase or decrease of certain biomolecules in the leaves is a physiological adaptation of the plant with adverse/stress conditions. Certain cultivars may have higher physiological efficiency in such adaptation. Hence, information on definite association of one or more biomolecules in such adaptation will be quite useful in further improvement of the crop.

The chickpea is known to be a drought tolerant crop. However considerable genetic variation has been observed among the different varieties for water requirements. Certain varieties give maximum yield under irrigated conditions while some give good performance under rainfed cultivation. Among the rainfed cultivars Phule G-96006 has been

recognized as national check for evaluating the drought tolerant cultivars in chickpea. During the recent past, several high yielding cultivars have been developed at the Pulse Research Project of this University. It is therefore decided to evaluate these varieties for various biochemical constituents involved in drought tolerance. Keeping in view these facts, an experiment was planned to study the changes in various biochemical constituents related to drought tolerance in eight promising chickpea cultivars. The objectives of the study were :

1 To analyze promising chickpea cultivars showing variable drought tolerance behavior for various biochemical constituents under normal and stressed condition.
2 To identify the biochemical constituent(s) involved in drought tolerance in chickpea.

Chapter Opener Page

REVIEW
OF
LITERATURE

2. REVIEW OF LITERATURE

Exhaustive reviews are available on the chemistry, technology and utilization of chickpea (Chavan *et al.*, 1986). However, the information on biochemical constituents associated with drought tolerance in chickpea is limited. The available literature on these aspects of chickpea is briefly reviewed in these sections.

2.1 Nutritional Composition and Utilization

The protein content of chickpea in a range of genetic cultivars has been reported to range from 18 to 28 per cent (Angela *et al.*, 1987., Fernandez and Berry, 1988., Singh *et al.*, 1981,Chavan *et al.* , 1993, Chaudhari *et al.* , 2001). The methionine, tryptophan , threonine, and valine are the limiting amino acids in chickpea protein. (Rao and Subramanian, 1970; FAO, 1970). The globulin constitutes the major storage protein (56 %) in chickpea seeds followed by glutelin (18.1 %) and albumin (12 %) with least proportion of prolamin (Singh and Jambunathan, 1982). The globulin is the most deficient in methionine and cysteine, while the albumin and glutelin contained higher amounts of these amino acids. Hence, globulin fraction is mainly responsible for the poor protein quality of chickpea proteins for human and other monogastric animals (Millerd ,1975).

Chickpea seeds contain 52.4 to 70.9 per cent total carbohydrates of which major proportion is contributed by starch (Lineback and Ke, 1975., Aman, 1979). Starch is principal component of chickpea seed. The starch content in whole seed samples of chickpea ranges from 37.2 to 50.8 per cent (Agarwal and Bhattacharya, 1980). The dehusked *dhal* contains 55.3 to 58.1 per cent starch (Jambunathan and Singh, 1978). Desi cultivars contain lower amount of starch than the kabuli cultivars. The

amylose content of chickpea starch ranges from 31.8 to 45.8 per cent of the total starch, remainder being amylopectin. The reducing and non-reducing sugars and crude fiber constitute most of the other carbohydrates of chickpea seeds. The soluble sugars range from 4.8 to 9 per cent. The non-reducing sugars comprise most of the soluble sugars in chickpea. The kabuli cultivars are reported to contain slightly higher amounts of soluble sugars than the desi types (Jambunathan and Singh, 1978). The crude fiber content in chickpea ranges from 7.1 to 13.5 per cent of which cellulose and hemicellulose are major components.

The total lipid content in chickpea ranges from 3.1 to 6.9 per cent (Sharma and Goswami, 1971., Jambunathan and Singh, 1978). The triglycerides are the major components of neutral lipids, whereas lecithin is the major component of polar lipids. Among the fatty acids, unsaturated fatty acids constitute 67.13 per cent while the saturated fatty acids make up 10.42 per cent (Salunkhe *et al.*, 1982). The linoleic and linolenic in unsaturated fatty acid group and palmitic in saturated fatty acid group are the principal fatty acids. The unsaturated fatty acids of legume lipids have been implicated in lowering blood serum and liver cholesterol levels (Jaya and Venkataraman, 1979) and physiological development and functions of the brain and retina (Lamptey and Walker, 1976). The proteins and lipids of chickpea lowered the serum cholesterol level in rats (Murthy and Urs, 1985). These observations indicate that crude fiber, lipids and proteins of chickpea have hypocholesterolemic effects in rats.

Chickpea is a good source of dietary minerals such as calcium, phosphorous, magnesium, iron, and potassium (Pak and Barja, 1974., Tiwari *et al.* , 1977, Jambunathan and Singh, 1981). Chickpea contains considerable amount of ascorbic acid (Chandra and Arora, 1968). The

chickpea seeds contained lower trypsin inhibitor activity than most other legumes but higher levels of galactose-containing oligosaccharides such as raffinose, stachyose, and verbascose which are responsible for flatulence (Rackis, 1975).

The chickpea seeds are processed into a variety of products before consumption. The dehusking into *dhal*, milling of *dhal* into flour, use of flour to prepare variety of traditional products, cooking *dhal* or whole seeds with spices, sprouting, fermentation, use of green seeds as a part of "mixed vegetables", and canning are some of the major processing technologies employed in utilization of chickpea as food in various parts of the world. The chickpea flour has also been used to manufacture weaning foods and bakery products (Chavan *et al.*, 1986).

2.2 Effect of Waters Stress on Growth and Yield of the Crop Plants

Drought stress during vegetative stages of growth alone does not appear to cause a significant loss in chickpea and pea yield. (Davies *et al.*, 1985; Chandrashekhariah *et al.*, 1986). However, Farah *et al.*, (1988) reported that flowering is the most sensitive stage to drought. It is probable that high sensitivity to drought during the reproductive stage is due to the lack of new root growth, as reported for pea (Davies *et al.*, 1985) and faba bean (Salter and Drew, 1965). Some of the early formed roots also begin to senesce by this time.

Faba bean (French and Legg, 1979; Day and Legg, 1983; Hussain *et al.* , 1983) and pea (Wilson *et al.*, 1985) can be equally sensitive to drought at all growth stages. In the past, most attention in drought research has been on the obviously important reproductive crop growth stages, which coincide with drought occurrence in receding soil moisture conditions. However, research on the effects of and responses to drought

during early crop establishment phases remain neglected and requires greater attention.

Although reliable estimates are not available, the reduction in plant growth and grain yields due to occurrence of drought particularly at flowering stage can be as high as 50 to 90 per cent. Hence there is a need to develop cultivars tolerant to drought. The major biotic stress factor of chickpea worldwide is drought. Two breeding approaches are suggested to reduce the problem of drought. One has been to breed for short duration varieties that can escape the problem; the other to breed and select genuinely drought resistant plants. Several short duration cultivars like ICCV-2, ICCV-88201, KCV–88202, has been developed and found to escape the drought at flowering and grain forming stage.

For drought resistant breeding a well proved resistant material is used in crossing programme. Such material should also have good agronomical properties. Hence, there is a need to standardize one or two well-defined biochemical parameters associated with drought tolerance in chickpea. This will facilitate breeders for appropriate selection of material for crossing work.

2.3 Effect of Water Stress on Some Biochemical Constituents.

2.3.1 Proline

Steawart *et al.,* (1966) reported that proline formation required available carbohydrates. Even through proline is synthesized from its precursor, it did not accumulate in bean leaves during water stress due to its utilization through oxidation. The oxidation of proline was only prevented when there was sufficiently high level of carbohydrates. This lack of sufficient levels of carbohydrates may result in decrease in proline content in bean leaves during water stress (Steawart, 1972).

Bates *et al.*, (1973) showed that proline, which increases proportionately faster than other amino acids in plants under water stress has been suggested as an evaluating parameter for selecting drought tolerant varieties.

Jirali *et al.*, (1989) reported that increase in plant age results in decrease in leaf moisture content and increase in free proline content in chickpea. They also observed that high N-content throughout the growth and concluded that free proline has negative correlation with leaf moisture content and positive correlation with yield.

Singh and Kumar (1992) soaked the chickpea genotypes for 24 h in water, 0.1 ppm $ZnSO_4$ or 0.1 ppm $CuSO_4$ or were not soaked before germination and then germinated at 0, -2.2 and –3.0 bar moisture stress. They observed increase in sugar concentration in embryo axis in untreated seeds. They also observed, due to water stress, there was decrease in amino acid concentration in the embryo axis and colyledons where as there was increase in proline content in the axis. Treated plants showed higher protein concentration in axis and cotyledons.

Yadav and Khare (1995) carried out an experiment with chickpea and observed that leaf proline and seed proline content were higher in rainfed plants than irrigated one. They also reported that high leaf proline at the vegetative stage was associated with high yield under rainfed condition.

Mukane *et al.*, (1996) carried out an experiment with pigeonpea to assess biochemical markers for drought tolerance. They reported that there was increase in free proline and a carbohydrate in leaves and thus had a positive linear relationship with water stress. They also advised to use these parameters as biochemical markers for drought tolerance in pigeonpea.

Randeva and Ilieva (1998) studied the relationship between the free proline content and productivity of spring forage pea cultivars grown under drought stress. A drought condition contributed to an increase in the free proline content. This increase being higher in the reproductive phase of the crop.

Sivakumar *et al.*, (1998) studied the effect of drought hardening on proline content of tree seedlings. They observed that the drought hardening increased the proline content in tamarind, *Albizia, Azadirachta* and Eucalyptus with maximum content at 180 days after sowing. Highest content was observed in *Azadirachta* followed by eucalyptus and tamarind. Thus, they concluded that proline accumulation may serve as one of the promising indices for evaluation of drought tolerance in plants.

Gruda and Schnitzler (2000) studied the effect of water supply to seedlings cultivated in different substrates and raising systems on the bio-morphological and plant physiological parameter of lettuce and reported that the difference in proline content between well-watered and stressed seedlings was smaller.

2.3.2 Free amino acids

Malik (1986) showed that decrease in free amino acids due to water stress has been reported in maize and in cotyledons and embryo of germinating chickpea (Ivanov, 1989).

Sharma *et al.* , (1986) showed that the salt stress induced changes in protein degradation and proteolytic activity in germinating chickpea seed and reported that salinity increased the pool of free amino acids and proteolytic activity in embryonic axis and cotyledons.

Mehrotra and Bhist (1990) studied the changes in free amino acid in susceptible and tolerance cultivars of chickpea under iron deficiency stress and reported that the recovery treatment succeeded in reversing the

effect of the deficiency. However, glutamic acid (that is deficiency tolerant) and glycine, theronine and lysine (that is deficiency susceptible) continued to decrease.

Frechilla *et al.*, (2000) studied the source of nitrogen nutrition (N-fixation or nitrate assimilation) as a major factor involved in pea response to moderate water stress and showed than intercellular CO_2 concentration decreased with drought and the decrease was more gradual in N-fixing plants. Water deficit increase water use efficiency, glutamine synthetase [glutamate : ammonia ligase] activity, the levels of total soluble sugars (in both roots and leaves), free amino acids and proline content in N-fixing plants. This concluded that N source has a major effect on the response of pea plants to water stress.

2.3.3 Soluble proteins

Gupta *et al.*, (1997) showed that water stress increased the proline, amino acids and total soluble sugars content in chickpea leaves but decreased the soluble protein and starch content and suggested that water-stress induced increase in amino acids particularly of proline and soluble sugar might be responsible for osmotic adjustments. It was concluded that the treatment with benazladehyde and GA could partly alleviate the effect of water stress in both susceptible and moderately tolerant cultivars.

Gorbanli *et al.*, (1998) conducted an experiment to study the effect of water stress on the soluble proteins and amino acids content of chickpea. They observed decrease in soluble protein and increase in amino acids during the stress in roots and shoots of both the cultivars. They also observed that there was increase in proline under drought stress.

2.3.4 Enzymatic changes

Hasio (1973) noted that alpha-amylase formation in germinating seeds was depressed by water stress. Sheoran *et al.*, (1981) reported that the water stress at flowering stage (70 days) of pigeonpea resulted in decreased water potential in nodules, roots and leaves. The decreased water potential in nodules resulted in decreased activities of nitrogenase, glutamine synthetase, glutamine dehydrogenase and urease. In roots, the activity of glutamine synthetase and glutamine dehydrogenase decreased under stress, where as in leaves, these activities were not affected.

2.3.5 Total and reducing sugars

Wadleigh and Ayers, (1945) and Woodhams and Kozlowski, (1954) showed that sugar content did not increase in all species upon drought. The decrease in starch content in plants subjected to severe water stress over a period of time, and there was reduced starch, total sugars and reducing sugars, and total carbohydrates in tomato and bean plants which took place before the appearance of the first signs of wilting. When the plants were irrigated, a very rapid and marked increase in starch was noted, but no corresponding increase in reducing and non-reducing sugars was apparent. Finally, after several droughts, none of which was sever enough to bring the soil moisture to permanent wilting point, there was a marked decrease in sugars and starch content of roots, stems and leaves. Total carbohydrates were less at the end of eight weeks of growth than at any other stage of the experiments indicating that recurrent water deficits greatly reduced photosynthesis.

Vaadia *et al.*, (1961) showed an increase in the content of sugars during water stress attribute to the hydrolysis of starch by enhanced amylase activity. Other products may also synthesize sugars.

Singh and Rai (1983) compared two cultivars of chickpea for their soluble sugar content. The total and non-reducing sugars accumulated with increased levels of water stress. Thus, higher content of non-reducing and total soluble sugars was characteristics of shoots of the tolerant cultivars. Starch and soluble sugar decreased in germinating chickpea by altering the metabolism due to water stress (Malik *et al.*, 1986).

Koundal and Chopra (1989) in the outdoor pot trails on chickpea after 5 and 7 days of water stress investigated that NR, amino-N and sugar contents increased in stressed nodules as compared with controls. Upon rewatering, activities of the nitrogen assimilating enzymes and respiration increased while nitrate reductase activity, sugar and amino N-contents of the nodules decreased compared with controls.

2.3.6 Total polyphenols

Water deficit could alter the levels of phenolic compounds in several ways. A water stress could also lead to the increased levels of phenolic compounds, on the other hand, by reducing the rate of growth leading to a build up of lignin precursor, which are then metabolically diverted to various phenolics (Del, 1972). Sinha (1979) demonstrated that the phenol content of leaves as well as nodules increased by 1.5 to 2.0 fold over the control under stress conditions at all growth stages.

A decrease in phenolic production might result from a decrease in the activity of key enzymes in phenolic biosynthesis, such as phenylalanine ammonia lyase or from the reduction in the supply of substrates due to inhibition of photosynthesis (Bradford and Hsiao 1982.)

2.3.7 Chlorophyll

The chlorophyll is an important photosynthetic unit of plant cells. The decline in chlorophyll pigment in the plants due to water stress has been reported in maize (Sharma *et al.*, 1990) and in groundnut (Krishna *et al.*, 1989). Chen *et al.*, (1964) reported that both chlorophyll a and chlorophyll b decreased with increasing in water stress in barley.

2.3.8 Minerals

Singh and Singh (1999) studied the effect of salinity on Na, K and proline content of chickpea genotypes and reported that the tolerant genotypes had lower sodium, higher potassium and higher proline content in shoots than the susceptible genotypes.

Chapter Opener Page

MATERIAL AND METHODS

3. MATERIAL AND METHODS

3.1 Material

3.1.1 Seeds

The mature dry seeds of following eight chickpea cultivars of 2001 harvest with varying levels of drought tolerance characteristics were obtained from the Senior Pulse Breeder, All India Coordinated Pulse Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri.

1. BG-391
2. BG-362
3. BG-256
4. Vijay
5. Phule G-97110
6. Phule G-9222-2
7. Phule G-96006
8. Phule G-5

The seeds were cleaned and stored in refrigerator at 4°C until used for sowing in a pot culture experiments.

3.1.2 Pots and soil

Earthen pots (9″ X 12″), which were perforated at bottom to allow drainage, were dipped in 0.5 percent copper sulfate solution for ten minutes and made free from microorganisms. These pots were filled with 10 kg proportionate mixture of black soil and well-decomposed organic manure for conducting a pot culture experiments.

3.1.3 Chemicals

All the chemicals used for the analyses were of analytical grade. They were obtained from Sarabhai Chemicals, Baroda, E. Merck (India), or Qualigen, Mumbai.

3.2 Methods

Five seeds of each cultivar were sown in a separate earthen pots, in duplicate, containing about 10 Kg soil and the crop were raised in a glass house condition. The plants in both the sets were irrigated regularly until 40 days. Later, one set of plants were continued to irrigate beyond 40 days while other set of plants irrigation was discontinued at 40 days until plants went in stress condition. The stressed condition was observes after 50 days of sowing as indicated by a temporary wilting symptoms of growing pants. The leaf samples of unstressed and stressed plants were obtained and held at 4°C, brought do the laboratory and analyzed immediately for following parameters.

3.2.1 Proline

Proline content in the 50 day old chickpea leaves subjected to stress as well as the control, was determined by using acid ninhydrin reagent by the method of Bates *et al.*, (1973). The proline content was expressed in terms of mg per 100 g fresh weight basis.

Reagents

1. **Acid ninhydrin** : Ninhydrin (1.25 g) was dissolved in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid with agitation.
2. **Sulphosalicylic acid (3%)** : Three grams of sulphosalicylic acid were dissolved in 100 ml of distilled water.
3. **Standard L-proline** : Proline 100 mg, were dissolved in 100 ml distilled water. This solution contained 1000 μg of proline per ml. From this stock solution, 1 ml was again diluted to 100 ml with distilled water. This diluted solution contained 10 μg of proline per ml.

Procedure

The leaf sample of 1 g was homogenized with 15 ml of 3% aqueous solution of sulphosalicylic acid and the homogenate was filtered through Whatman No. 1 filter paper. The filtrate was used for the estimation of proline.

One millilitre of the filtrate was mixed with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in the test tube for one hour at 100 ^{0}C and the reaction mixture was extracted with 4 ml toluene and mixed vigorously for 15 to 20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis.

Calibration of standard curve

The standard working solution of 10 µg per ml of proline was prepared. The series of levels of standard solution was pipetted out corresponding to 0, 1, 2,...., 10 ug concentration and colour was developed as above. The absorbance was measured at 520 nm and the graph of extinction against µg of proline was plotted (Fig. 1).

3.2.2 Free amino acids

The free amino acids from the leaves were extracted with 80 percent (v/v) ethanol (Yemm and Willis, 1954) and determined by ninhydrin method (Rosen, 1957). The values were expressed in terms of mg L-leucine per 100 g fresh weight basis.

Reagents

1. **Sodium cyanide** (0.1 M) : Exactly 0.49 g sodium cyanide were

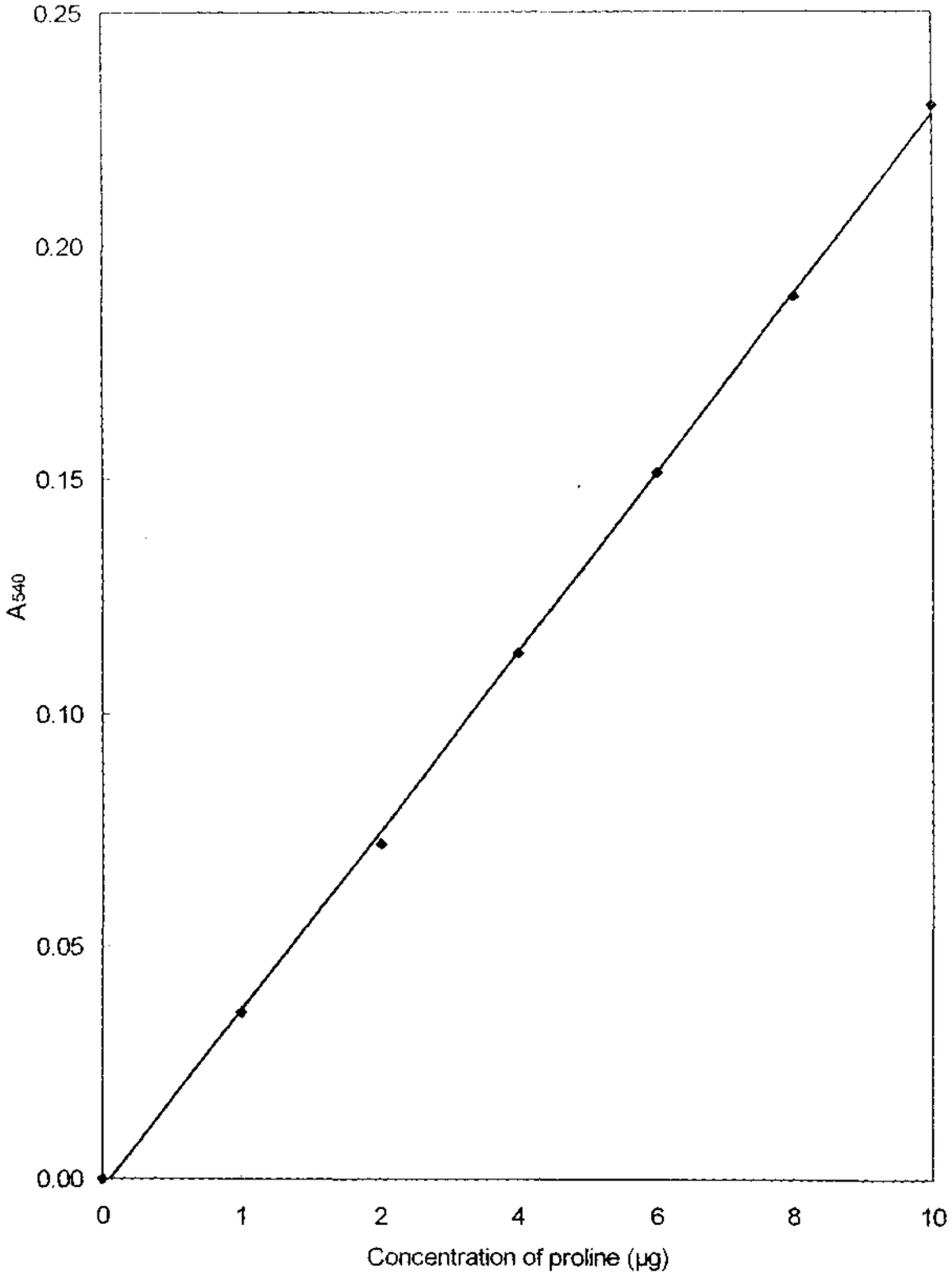

Fig. 1 Standard curve for estimation of proline

2. **Acetate buffer** : Sodium acetate (446.62 g) was dissolved in distilled water containing 41.23 ml of glacial acetic acid. The pH was adjusted to 5.4 and the volume made to 1000 ml with distilled water.
3. **Acetate cyanide buffer** : It was prepared by mixing 20 ml of sodium cyanide (0.1 M) with 980 ml of acetate buffers.
4. **Ninhydrin (3%)** : Ninhydrin powder (3 g) was dissolved in 100 ml of 2-methoxy ethanol.
5. **Standard L-leucine** : Standard L-leucine (10 mg) was dissolved in 100 ml of distilled water. This solution contained 100 µg of L-leucine per ml.
6. **Diluent :** Isopropyl alcohol and distilled water were mixed in the ratio of 1:1(v/v).

Procedure

One gram of the leaf sample of each cultivar were separately macerated and extracted in 25 ml of 80 per cent boiling ethanol, successively thrice, centrifuged and the supernatants pooled. The extract was decolorized by charcoal treatment and evaporated to about 5 ml. This was further diluted to 50 ml with water, filtered and used for analysis of free amino acids.

Two ml of extract were pipette into the test tube and 0.5 ml ninhydrin reagent added to it. The tubes were heated for 15 min in a boiling water bath. Immediately after removing the sample tubes from water bath, 5 ml of diluent was added to each test tube and shaken vigorously on vortex mixer. After cooling to room temperature, the colour intensity was read at 570 nm on Specromic-20. The content of the total free amino acids was calculated from the standard calibration curve

prepared in the same way, using L-leucine and expressed as mg free amino acids per 100 g fresh weight basis. (Fig. 2).

3.2.3 Soluble proteins

The leaves (1 g) were homogenized with 10 ml distilled water, centrifuged (5000 X g, 30 min) at 4 ^{0}C and the clear supernatant was used for the determination of soluble proteins by the procedure of Lowry *et al.*, (1951).

Reagents

1. **Alkaline sodium carbonate solution (2%)**: It was prepared by dissolving 2 g Na_2CO_3 in 100 ml of 0.1N NaOH solution.
2. **Copper sulfate (0.5 %)** : It was freshly prepared by dissolving 0.5 g $CuSO_4$ in 1 per cent Na-K tartarate solution.
3. **Alkaline solution** : It was prepared on the day of use by mixing 50 ml of solution 1 above, with 1 ml of solution 2.
4. **Folin-ciocalteau :** It was prepared by diluting commercial grade reagent with equal volume of water on the day of use.
5. **Standard protein (Bovine serum albumin)**: It was prepared by dissolving 100 mg bovine serum albumin in 100 ml distilled water. From the stock solution, 10 ml were diluted to 100 ml with distilled water. The solution contained 100 µg bovine serum albumin per ml.

Procedure

One ml of test solution after proper dilution was mixed with 5 ml of alkaline solution and kept for 10 min at room temperature. Diluted Folin-ciocalteau reagent (0.5 ml) was rapidly added with immediate mixing and color intensity were measured after 30 min at 750 nm against

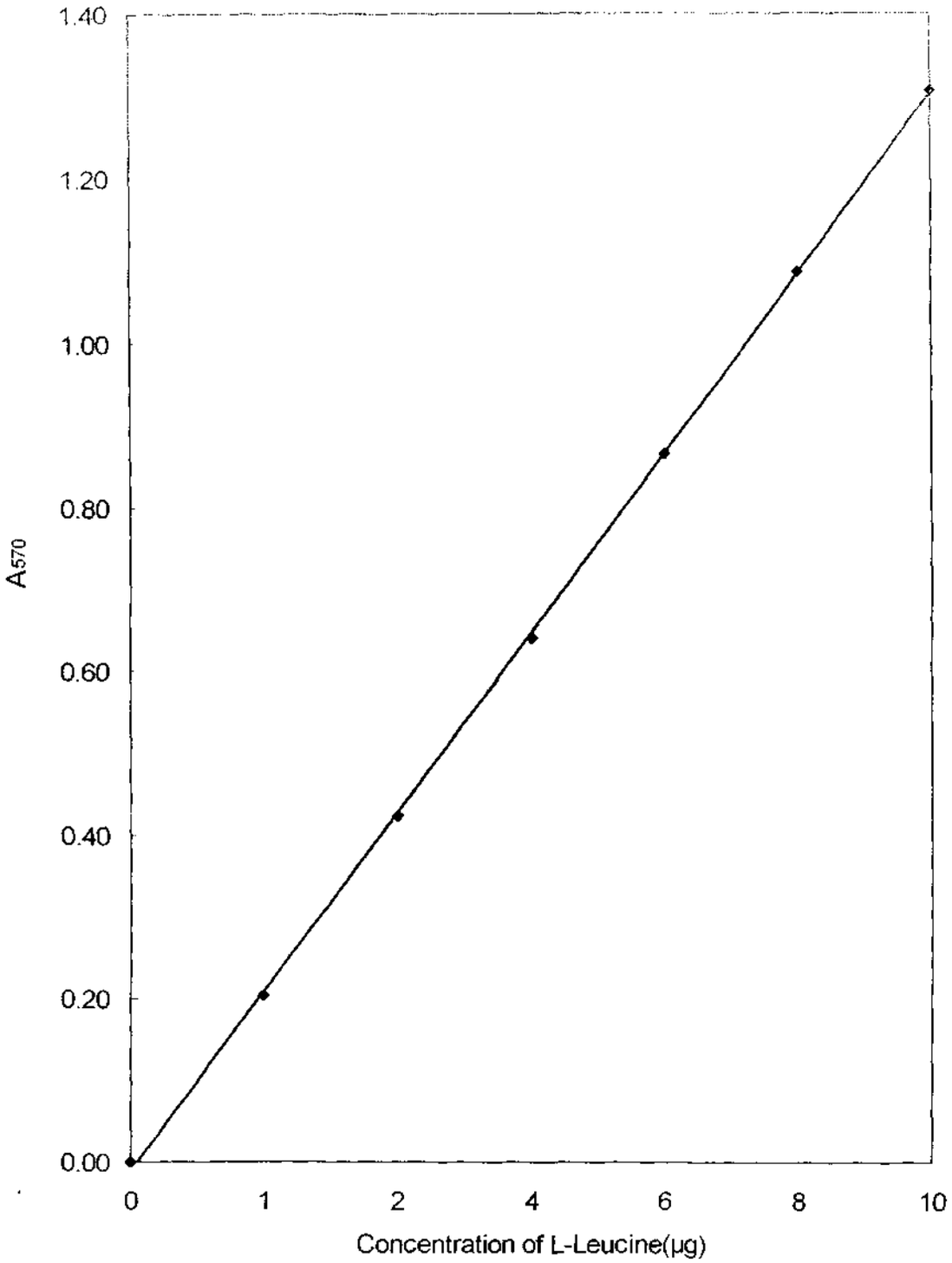

Fig. 2 Standard curve for estimation of free amino acids

blank. The protein content was calculated from standard curve prepared by using different concentration of BSA (Fig. 3).

3.2.4 Reducing sugars

The aqueous extract prepared for the determination of free amino acids was used to determine the total and reducing sugars by the arsenomolybdate reagent, as per the method of Nelson (1944).

Reagents

1. **Reagent A :** This solution contained 25 g Na_2CO_3 (anhydrous), 25 g Rochelle salt, 20 g $NaHCO_3$ and 200 g Na_2SO_4 in 100 ml distilled water.
2. **Reagent B :** This was prepared by dissolving 15 g $CuSO_4$ containing 1-2 drops of concentrated H_2SO_4 per 100 ml.
3. **Copper sulfate reagent :** It was a mixture of 25 parts of reagent A and one part of reagent B (v/v).
4. **Arsenomolybdate reagent:** It was prepared by dissolving 25 g ammonium molybdate in 450 ml distilled water. To this was added 21 ml of conc. sulfuric acid and 3 g of sodium arsenate in 25 ml distilled water. The contents were mixed and incubated at 37 ^{0}C for 48 hr.
5. **Standard D-glucose solution :** D-glucose (100 mg) were dissolved in 100 ml of distilled water. This solution contained 1 mg of D-glucose per ml. From this stock solution, 10 ml was further diluted to 100 ml with water. This diluted solution contained 100 μg D-glucose per ml.

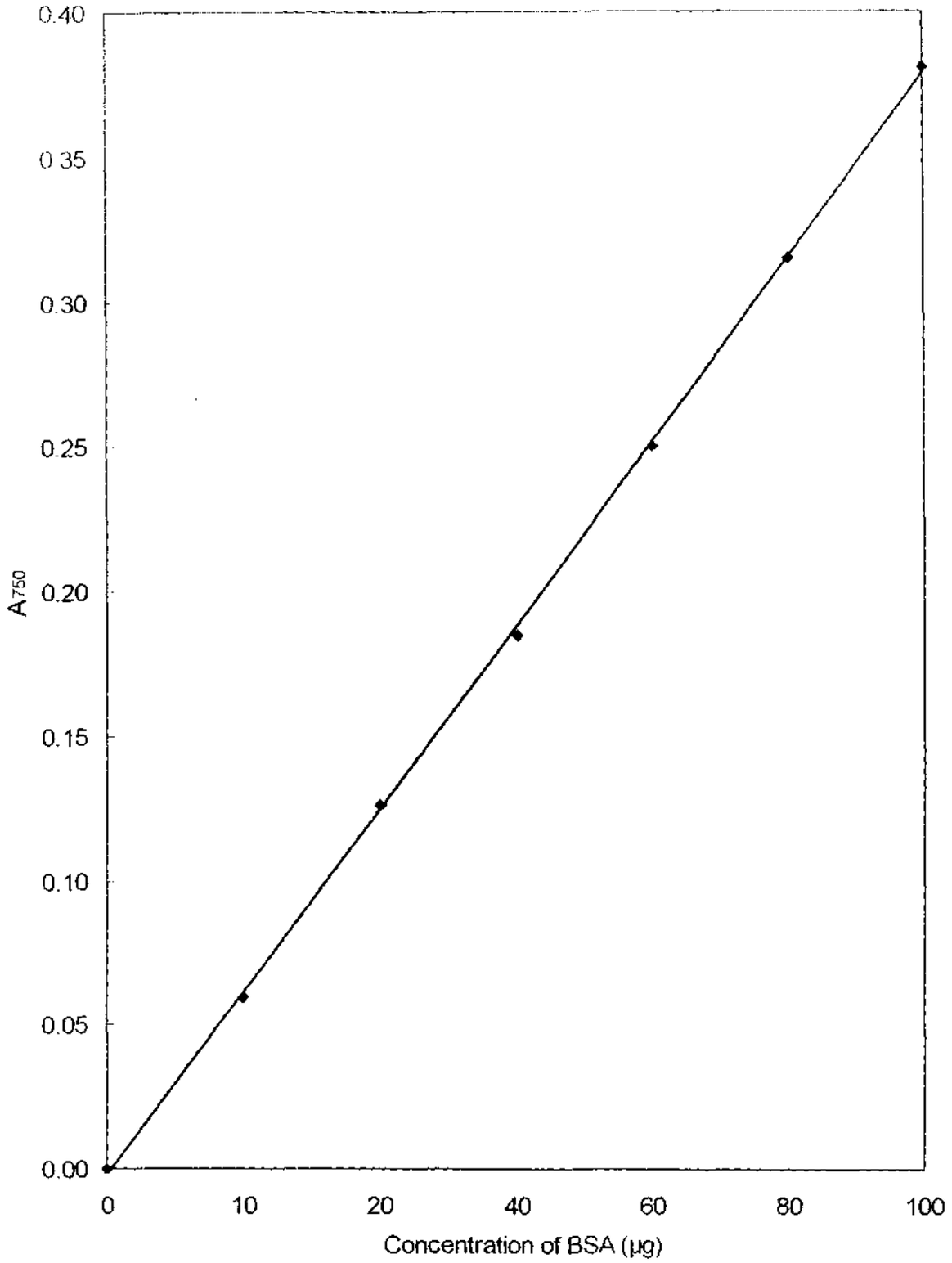

Fig 3 Standard curve for estimation of soluble proteins

Procedure

One millilitre of diluted sample extract was added to 1 ml of copper sulfate reagent, mixed and heated for 20 min in a boiling water bath. The tubes were cooled and 1 ml of arsenomolybdate reagent was added to it. The mixture was diluted to 8 ml and absorbance was measured at 520 nm. The reducing sugars were calculated from the standard curve prepared by the same procedure using D-glucose at 0 to 100 μg concentration. The results are expressed as mg per 100 g sample (Fig 4).

3.2.5 Total Sugars

To an aliquot 1 ml of ethanol extract, 5.0 ml diluted hydrochloric acid (5 N) were added and kept for 24 h for inversion. It is neutralized with 5 N sodium hydroxide using pH meter. The extract is cleared with saturated lead acetate and deleaded with sodium phosphate (Loomis and Shull, 1937). The sugars in this extract were estimated as total sugar by Nelson Somogyi method as described above and result expressed as mg per 100 g (Nelson, 1944).

3.2.6 Total polyphenols

Total polyphenol content in fresh leaves were determined by using Folin-Denis reagent as described by Swain and Hillis, (1959).

Reagents

1. **Folin-Denis reagent:** Sodium tungstate (100 g) and phosphomolybdic acid (20 g) were dissolved in 750 ml water. To it, 50 ml phosphoric acid were added. The contents were refluxed for 2 h and made to 1000 ml.

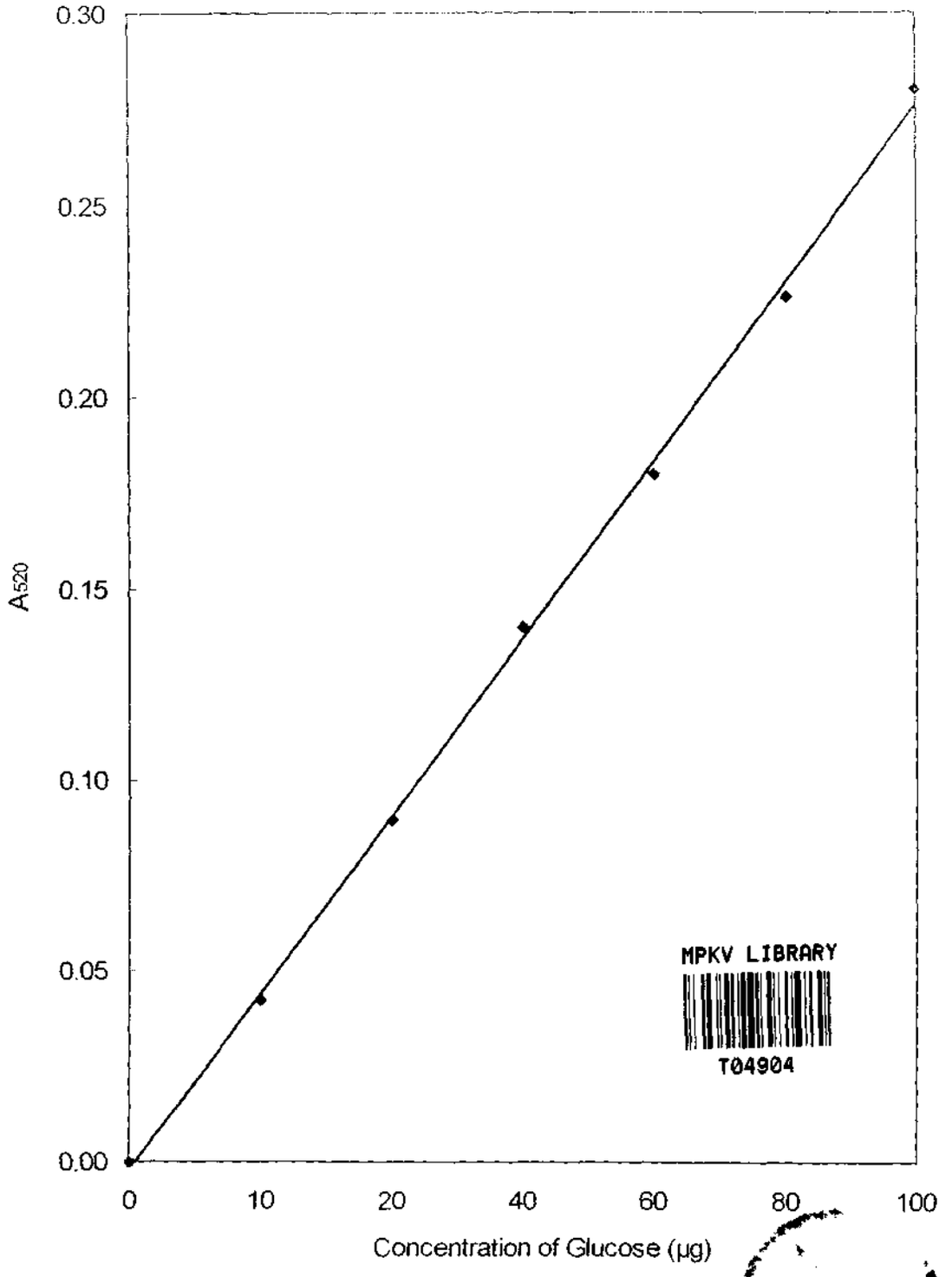

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Fig 4 Standard curve for estimation of reducing and total sugars

2. **Alkaline reagent** : This was prepared by dissolving 350 g of sodium carbonate in 1000 ml water at 80 °C. The solution was allowed to stand overnight and filtered through glass wool.
3. **Standard tannic acid solution (0.1 %)** : One hundred mg of tannic acid was dissolved in 100 ml of distilled water. Then 10 ml of this solution was diluted to 100 ml with distilled water. One ml of this solution contained 100 µg of tannic acid.

Extraction

One gram of fresh leaves of each cultivar were separately macerated in mortar and pestle in 10 ml hot water, extracted for about 30 min and the contents centrifuged. The extraction was repeated and the pooled supernatants were diluted to 25 ml.

Colour development

Half a millilitre of extract was mixed with about 7 ml water and 0.25 ml Folin-Denis reagent. After 10 min, 1 ml of alkaline reagent was added in each tube and contents mixed thoroughly. After 20 min, the contents were made to 10 ml and extinction measured at 650 nm on spectronic-20.

Standard curve

It was prepared by using 10, 20, 40, 80 and 100 µg of tannic acid concentrations (0.1 to 1ml of std. solution) in separate test tubes, in duplicate. The colour was developed in a total of 10 ml volume by same procedure as described for samples. The concentration of total phenolics in leaf samples was calculated from std. curve and expressed as mg per 100 g fresh weight basis (Fig 5).

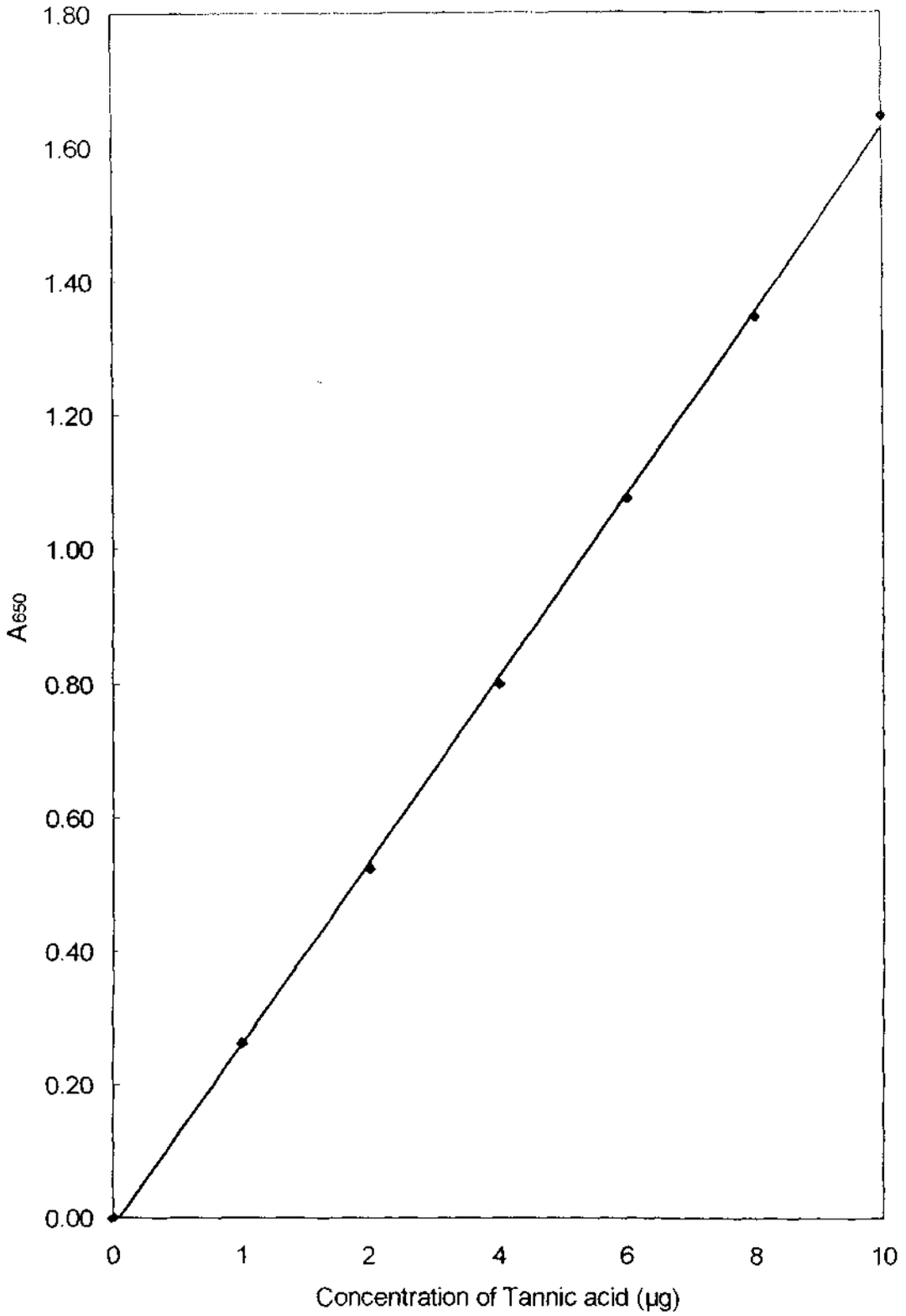

Fig.5 Standard curve for estimation of total polyphenols

3.2.7 Chlorophyll

The chlorophyll in the leaves of chickpea under control as well as stress condition was extracted by 80% acetone as described by Arnon(1949).

Procedure

One gram of leaves were macerated in mortar and pestle and extracted in 20 ml of 80 per cent acetone. The contents were centrifuged (5000 x g, 10min) and the supernatants collected. The procedure was repeated until the residue became colourless. The final volume of the extract was made to 100 ml. The extinction of the chlorophyll extract was recorded at 645 and 663 on spectronic-20, separately.

Calculations

The amount of chlorophyll present in the extract as mg chlorophyll per g tissue was calculated using the following equations.

mg chlorophyll a/g tissue = $12.7(A_{663})-2.69(A_{645})$ x V/1000 x W

mg Chlorophyll b/g tissue = $22.9(A_{645})-4.68(A_{663})$ x V/1000 x W

Where A = absorbance at specific wavelength.

V = Final volume of chlorophyll extract in 80 % acetone

W = Fresh weight of tissue extracted, g

3.2.8 Chlorophyll stability index

It was determined by calculating the difference in extinction at 652 nm of chlorophyll extracts of leaves subjected to heat treatment. For one leaf sample (0.5 g fresh) in 50 ml water was subjected to heating at 56 ^{0}C for 30min before extraction of chlorophyll as

described earlier. The other leaf sample without heat treatment served as control. The difference in two readings was defined as chlorophyll stability index for each variety calculated separately.

Calculation :

ΔR = Reading without heating – Reading after heating at 56 ^{0}C.

Where, ΔR = Chlorophyll stability index.

3.2.9 Phosphorous

The content of phosphorous in the leaf of chickpea in control as well as stressed plants was determined by the procedure of Jackson (1975).

Reagents

1. **Ammonim molybdate :** Dissolved 25 g of ammonium molybdate in 400 ml of distilled water.
2. **Ammonium vanadate in HNO_3 :** Dissolved separately 1.25 g of ammonium metavanadate in 300 ml of boiling distilled water and cooled to room temperature. Then 250 ml of concentrated HNO_3 and vanadate solution were added to ammonium molybdate solution and made to 1000 ml.
3. **Standard phosphorous solution :** 0.2195 g of analytical grade potassium dihydrogen phosphate were dissolved in distilled water diluent to 1000 ml. This solution contained phosphorus 50 mg per 1000 ml.
4. **Preparation of standard curve:** A standard graph was prepared by using 50 to 1000 µg concentrations of phosphorus. The standard solution (1 to 20 ml) was used to develop colour in a final volume of 50 ml and read at 470 nm after 30min. The curve

was prepared by plotting absorbance on Y-axis and phosphorous concentration in μg per ml solution on X-axis.

Procedure

One millilitre of aliquot of plant diacid extract taken in 50 ml volumetric flask, mixed with 10 ml of vanadate molybdate reagent diluted to 50 ml with distilled water and mixed well. The colour was read after 30 min at 470 nm. The phosphorus concentrations were calculated using the std. curve expressed as per cent.

3.2.10 Potassium

The content of potassium in the leaves of chickpea under control as well as stressed plants was determined by the method of Chapman and Pratt, (1961).

Reagents

1. **Standard stock solution of potassium** : Dissolved 1.907 g of potassium chloride in distilled water and made up to 1000 ml. This solution contained 1000 mg potassium. A 100 ml of this stock solution was diluted to 1000 ml. This working solution contained 100 mg of potassium.
2. **Preparation of standard curve:** Pipetted 0, 0.5, 1, 2, 4 and 5 ml of stock solution of 100 mg per litre separately into 100 ml volumetric flask. The volume was made with distilled water and shaken well. This contained 0, 0.5, 2, 3, 4 and 5 mg of potassium per litre, respectively and read on flame photometer. A standard curve plotting flame photometer reading on Y-axis and concentration of potassium mg per litre on X-axis was then prepared.

Procedure

The plant extract (diacid digested) was directly read on flame photometer or after appropriate dilution so that final concentration range between 0 to 50 mg potassium per litre. A blank without sample was also run simultaneously.

Result was calculated using a standard reading from potassium solution and expressed as per cent potassium.

3.2.11 Statistical analysis

The data obtained on various biochemical parameters on eight chickpea cultivars were analyzed for standard error and correlation with proline by the procedure of Panse and Sukhatme (1967).

Chapter Opener Page

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

The chickpea cultivars having differential reaction towards water stress were analyzed for various biochemical constituents as influenced by artificial drought in a pot culture experiment. An increase in leaf proline content by several folds in response to drought has been reported in many crops. Thus, increase in leaf proline content during water and salt stress is now been accepted as biochemical parameter to evaluate the drought tolerant character of a crop plant. However there is a need to identify additional parameters to screen the germplasm for drought tolerance. The chickpea is known to be a drought tolerant crop. However it is cultivated under rainfed and irrigated condition. The rainfed crop is usually grown on residual soil moisture. Hence the crop grows on limited water. The cultivars with a drought tolerant character will give higher yield. Hence four known drought tolerant cultivars along with four other cultivars were studied in relation to changes in various biochemical constituents including proline. A several fold increase in proline in response to artificial drought was considered as a conferment of drought tolerant cultivar and this criteria was used to evaluate the cultivars for any additional parameter for drought tolerance in chickpea.

The seedlings were raised in pot culture experiment and the leaves were analyzed for various biochemical constituents both at controlled and stressed condition. The results obtained are presented and briefly discussed in this section.

4.1 Effect of Water Stress on Proline

The leaf proline content in eight chickpea cultivars as influenced by water stress is presented in Table 1. The leaf proline

Table 1. Effect of water stress on the content of proline in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants (mg/100g)	Stressed Plants (mg/100g)	Increase(+)/ Decrease(-), %
Phule G-5	96	81	-15.6
BG-391	135	252	+86.6
PhuleG-9222-2	135	402	+198
BG-256	57	1050	+1742
BG-362	54	1224	+2167
Phule G-97110	50	1512	+2924
Vijay	36	1320	+3567
Phule G-96006	42	2160	+5043

Range	36-135	81-2160
Mean	76	1000
S.E. ±	14	235

content in normal plant (18.7 % soil moisture) was found to range from 36-135 mg/100 g fresh leaves. The application of water stress to the plants at 45 days of plant growth (8 % soil moisture) resulted in a marked increase (81 to 2160 mg/100 g) in leaf proline content. In some varieties, which are known to be tolerant to drought such as BG-256, BG-362, Phule G-97110, Vijay, Phule G-96006 recorded highest proline in the leaves. The leaf proline content in these cultivars was found to range between 1050 to 2160 mg/100 g. This increase in proline content was 1742 to 5043 per cent higher than the control plants. The increase in proline content in this cultivars in response to drought was found to the about 18 to 50 folds (Fig 6). Among the varieties, a well known cultivar for drought tolerance namely Phule G-96006 recorded the highest increase in proline content which confirmed the content of proline as a biochemical criteria for judging the drought tolerant character in chickpea. The cultivar Phule G-5 an irrigated one showed a decrease in proline content as subjected to drought. Thus, the results indicated that a leaf proline content is a good biochemical parameter for evaluating the drought tolerant nature in chickpea cultivars.

Several investigators have reported positive relationship between free proline content of leaves with drought tolerance in chickpea (Bates *et al.,* 1973; Singh and Kumar, 1992; Yadav and Khare 1995; Mukane *et al.,* 1996; Randeva and Ilieva 1998; Sivakumar, *et al.,* 1998). The higher proline content in the leaves has been suggested an evaluating parameter for selecting drought tolerant varieties. (Bates *et al.,* 1973). The proline content has been found to be negatively associated with leaf moisture content and positively associated with grain yield in chickpea (Jirali *et al.,* 1989). Yadav and

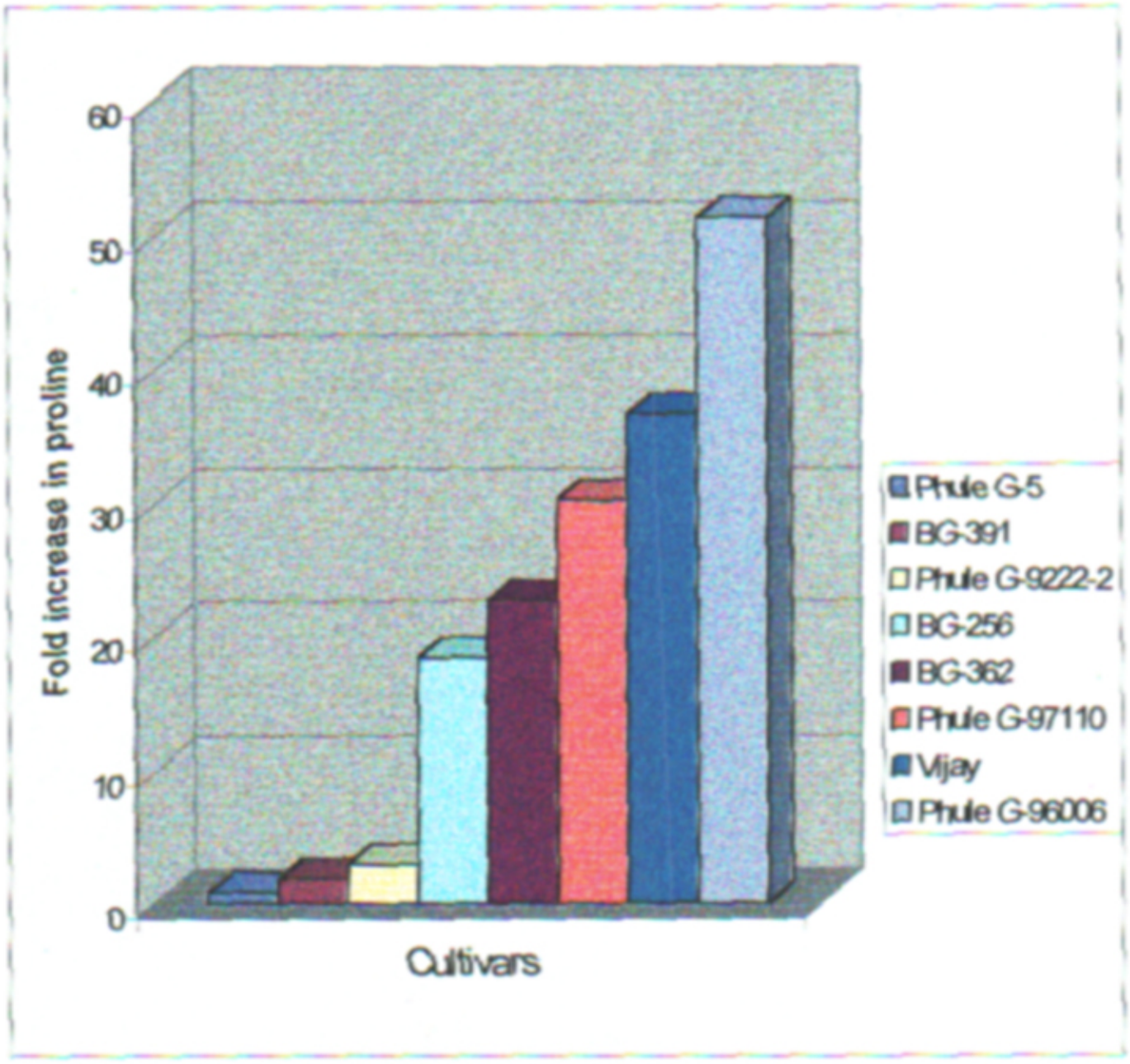

Fig 6. Increase in proline (folds) in different chickpea cultivars in response to water stress.

Khare (1995) observed the higher leaf proline content and seed yield in rainfed plants of chickpea than irrigated one. Similar results have been reported in pigenpea (Mukane *et al.*, 1996). Randeva and Hieva (1998) reported an increase in free proline content in pea leaves in response to drought condition. Similarly, a drought hardening has been found to increase the proline content in Tamarind, *Azardirachta* and Eucalyptus (Shivkumar, *et al.*, 1998).

They concluded that proline accumulation might serve as one of the promising indices for evaluating the drought tolerance in plants. All these reports clearly indicated that higher proline accumulation in response to drought in chickpea is a well-documented parameter. The accumulation of proline in the leaves is a physiological adaptation of plant against the water deficits. This facilitates to maintain the osmotic conditions in the cell and to protect the activity of the compounds like enzymes, so that metabolic activity is continued at decreased water tension. Similar results have been obtained in present study. The cultivars known to be drought tolerant was found to accumulate several fold higher proline content in the leaves during water deficits. The cvs. Phule G-5, BG-391 or Phule G-9222-2 were found to be drought susceptible as these did not accumulate proline in response to drought.

4.2 Effect of Water Stress on Free Amino Acids (FAA)

The changes in total free amino acids content after water stress in eight cultivars of chickpea are presented in Table 2. The FAA contents in the leaves of normal plants of eight cultivars were found to range from 80 to 160 mg/100 g and after water stress it was found to vary from 80 to 140 mg/100 g. These results indicated that water

Table 2. Effect of water stress on the content of free amino acids in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants (mg/100g)	Stressed plants (mg/100g)	Increase(+)/ Decrease(-), %
Phule G-5	100	105	+5.00
BG-391	140	80	-42.8
Phule G-9222-2	110	136	+23.6
BG-256	100	140	+36.6
BG-362	80	122	+52.5
Phule G-97110	160	135	-15.6
Vijay	130	125	-3.84
Phule G-96006	120	119	-0.83

Range	80-160	80-140
Mean	117	120
S.E.±	8	7

stress does not affect the accumulation of FAA markedly in chickpea on an average basis. However, it is interesting to note that the FAA content was found to decrease slightly upon water stress in drought tolerant cultivars, such as Vijay (-3.84%), Phule G-96006 (-0.83%) and Phule G-97110 (-15.6 %). These observations indicated that the FAA content of leaves is not significantly affected due to water stress.

The changes in FAA in response to water stress have been studied in maize (Malik, 1986), germinating chickpea (Sharma *et al.*, 1986) and in the chickpea under iron deficiency condition (Mehrotra and Bhist, 1990). The FAA contents were found to decrease due to water stress in maize (Malik 1986) and chickpea (Mehrotra and Bhist, 1990). Similar observations have been recorded in the present investigation although the decrease in FAA was not very significant.

4.3 Effect of Water Stress on Soluble Proteins

The data on changes in leaf soluble proteins content in response to water stress in chickpea cultivars are presented in Table 3. The soluble proteins in the leaves of normal plants were found to range from 215 to 300 mg/100 g with a mean of 253 mg/100 g while it varied from 230 to 350 mg/100 g with a mean of 368 mg/100 g in water stressed plants. Thus, the soluble proteins were found to increase due to water stress in some chickpea cultivars. However, such increase in soluble proteins was not significant order. The cvs. Vijay and Phule G-5 however, exhibited a decrease in soluble proteins upon the water stress. Although there is a slight increase in the soluble proteins in response to water stress, the magnitude of increase observed does not warrant drawing the positive relationship.

Gupta *et al.*, (1997) have observed a decrease in soluble protein

Table 3. Effect of water stress on the content of soluble proteins in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants (mg/100g)	Stressed plants (mg/100g)	Increase(+)/ Decrease(-), %
Phule G-5	235	230	-2.12
BG-391	305	340	+11.47
Phule G-9222-2	215	390	+81.3
BG-256	230	360	+56.5
BG-362	225	440	+91.4
Phule G-97110	275	385	+40
Vijay	300	275	-8.33
Phule G-96006	240	530	+120.8

Range	215-300	230-530
Mean	253	368
S.E.±	12	31

content in the leaves of chickpea upon water stress. Similar decrease in soluble proteins in roots and shoots of chickpea in response to water stress have been reported (Gorbanli *et al.*, 1998). The results obtained in present study are contradictory to these reports. The cultivars such as Phule G-96006 a well-known drought tolerant type, however, showed a marked increase in both proline as well as soluble proteins. These results indicated that the sources for proline synthesis is different than proteins, might be carbohydrates. This however needs further confirmation.

4.4 Effect of Water Stress on Reducing Sugars and Total Sugars

Table 4 contains the data on changes in leaf reducing sugars while data on changes in total sugar in response to drought in chickpea cultivars in summarized in Table 5. The leaf reducing sugar content were found to range from 43 to 79 mg/100 g in normal plants while it varied from 44 to 185 mg/100 g in stressed plants showing an average increase in reducing sugars due to water stress. Similarly, the total sugar exhibited a range of 188 to 325 mg/100 g in normal plants while 187.5 to 625.6 mg/100 g in stress plants. The mean reducing sugar content was found to increase from 63 to 119 mg/100 g while the mean total sugar was found to raise from 243 to 384 mg/100 g upon stress. The increase in reducing sugars was found to range from 13.5 to 266 per cent while the increase in total sugar was found to vary from 9.3 to 232.4 per cent. The cultivars known to be drought tolerant exhibited the highest increase in both reducing and total sugar due to water stress (Fig 7). These results clearly indicated a positive relationship with the drought tolerant character in chickpea. Among the cultivar studied the cvs. Phule G-9222-2 and BG-362 recorded the

Table 4. Effect of water stress on the content of reducing sugars in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants (mg/100g)	Stressed plants (mg/100g)	Increase(+)/ Decrease(-), %
Phule G-5	52	44	-15.3
BG-391	79	54	-31.6
Phule G-9222-2	50	180	+260
BG-256	74	84	+13.5
BG-362	43	135	+213.9
Phule G-97110	58	115	+98.2
Vijay	75	153	+104
Phule G-96006	70	185	+164

Range	43-79	44-185
Mean	63	119
S.E.±	5	18

Table 5. Effect of water stress on the content of total sugars in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants (mg/100g)	Stressed plants (mg/100g)	Increase(+)/ Decrease(-), %
Phule G-5	275.5	187.5	-31.9
BG-391	325.0	200.0	-38.4
Phule G-9222-2	237.5	362.5	+52.6
BG-256	188.0	625.0	+232.4
BG-362	287.5	450.0	+56.5
Phule G-97110	198.8	412.5	+10.74
Vijay	240.0	262.5	+9.3
Phule G-96006	187.5	575.0	+206.6

Range	188-325	187.5-625.0
Mean	243	384
S.E.±	16	54

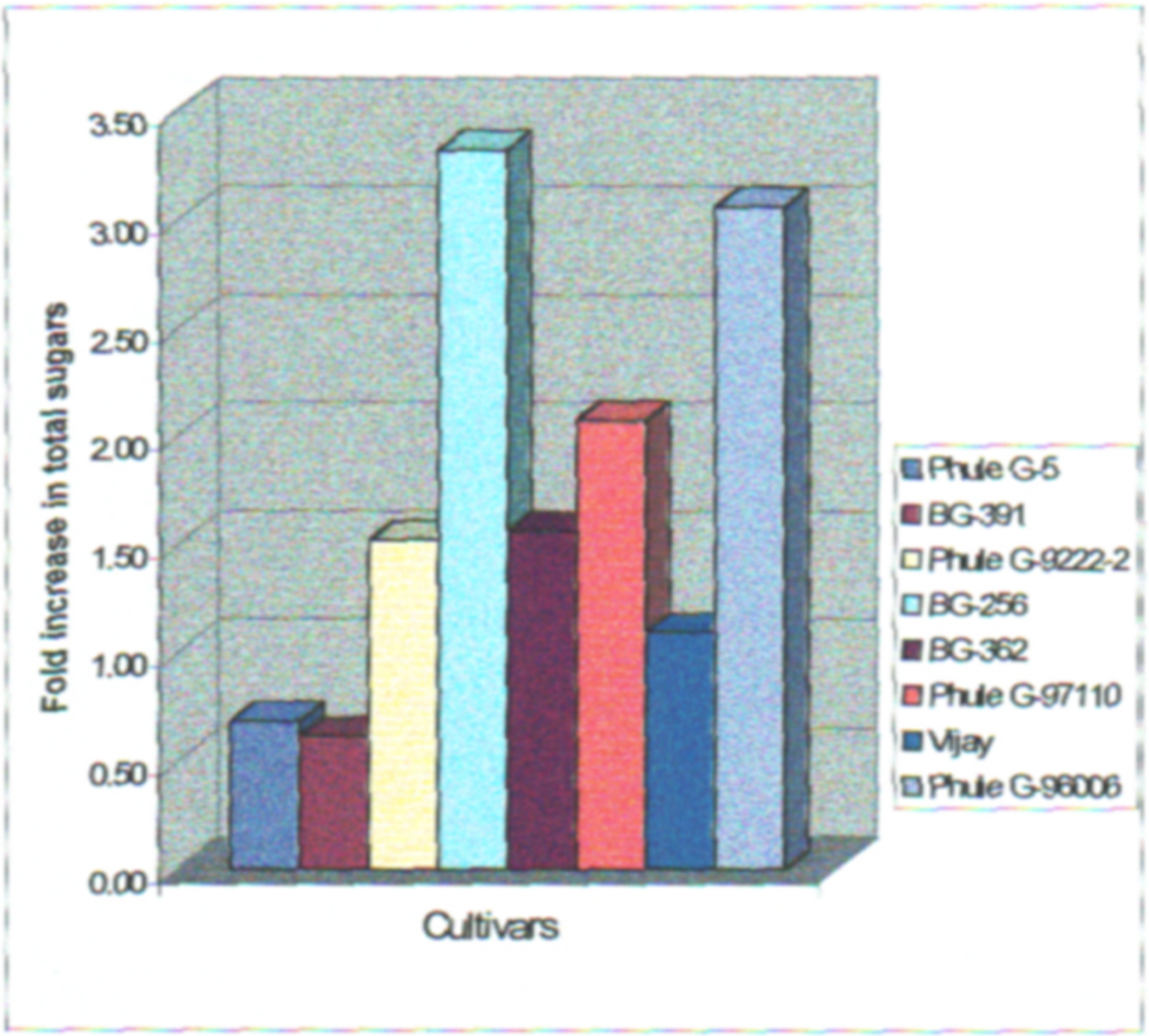

Fig 7. Increase in total sugars (folds) in different chickpea cultivars in response to water stress.

highest accumulation of total sugar upon water stress (Fig 7). Among the sugars, non-reducing sugars are predominant in leaves over reducing sugars. Thus, accumulation of total or non-reducing sugars may be good additional criteria for evaluating drought tolerant character in chickpea cultivars.

An increase in content of sugars in response to water stress in chickpea has been reported by several investigators (Vaaidia *et al.*, 1961; Singh and Rai, 1983; Koundal and Chopra, 1989). The results obtained in present study are in agreement with these reports. An increase in the content of sugars has been attributed to the hydrolysis of starch by enhanced amylase activity (Vaaidia *et al.*, 1961). The reason for an increase in non-reducing sugars in the leaf upon water stress remains to be elucidated. Identification of a source for this will be useful in explaining the drought tolerant mechanism in chickpea. Nevertheless the contents of the total and non-reducing sugars seem to be a good additional criteria in addition to proline content, for this purpose.

4.5 Effect of Water Stress on Total Polyphenols

The results on changes in total phenolics in chickpea plants as influenced by water stress are presented in Table 6. The total phenolic range from 0.8 to 2.8 mg/100 g while it was found to increase by 1.5 to 5.1 mg/100 g after the stress. The mean phenolic content of 1.7 mg/100 g was found to increase to 3.0 mg/100 g. The increase in phenolic content was about 5 to 322 per cent or 1 to 4.2 folds in different cultivars (Fig 8). The results further indicated that an increase in the total phenolics was observed in all the cultivars in response to drought. This indicated that an increase in phenolics due to

Table 6. Effect of water stress on the content of total phenolics in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants (mg/100g)	Stressed plants (mg/100g)	Increase(+)/ Decrease(-), %
Phule G-5	0.8	1.5	+88
BG-391	2.2	3.2	+45.4
Phule G-9222-2	1.2	2.7	+125
BG-256	1.3	3.3	+154
BG-362	0.9	3.8	+322
Phule G-97110	2.8	2.8	0.00
Vijay	2.0	2.1	+5
Phule G-96006	2.4	5.1	+113

Range	0.8-2.8	1.5-5.1
Mean	1.7	3.0
S.E.±	0.2	0.3

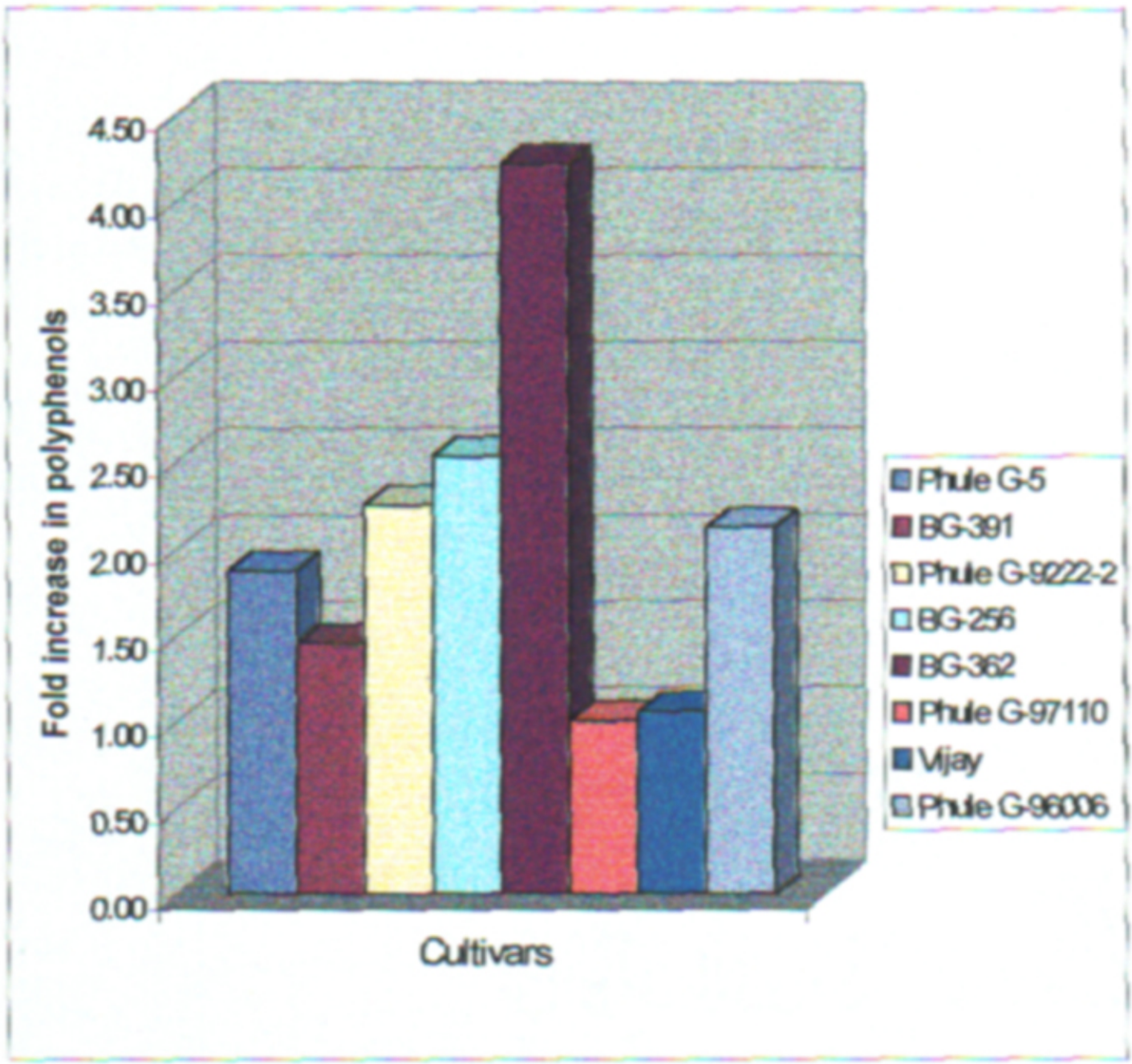

Fig 8. Increase in polyphenols (folds) in different chickpea cultivars in response to water stress.

stress seem to be ubiquitous phenomenon.

The changes in total phenolics content as influenced by water stress have been reported. Del (1972) observed an increase in phenolic contents due to water stress. It has been postulated that the increase in total phenolics is achieved by reducing the rate of growth to built up a level of lignin precursor which are then metabolically diverted to various phenolics. A 1.5 to 2.0 folds increase in total phenolics has also been reported due to water stress in chickpea (Sinha, 1979). The results obtained in present study are in line with these reports. However, the mode of increased the synthesis of phenolics and its physiological function in relation to osmotic regulation during water stress conditions needs to be explained. It is quite likely that it has similar effect as that of proline.

4.6 Effect of Water Stress on Chlorophyll

The changes due to water stress in chlorophyll a and chlorophyll b are presented in Tables 7 and Table 8, respectively. The chlorophyll a content was found to range from 10.0 to 13.3 mg/g of fresh leaves in normal plants of eight chickpea cultivars. The application of water stress caused a marked decrease in chlorophyll a content. It ranged from 6.5 to 10.5 mg/g in water stressed plants. The decrease in chlorophyll a content in the cultivar studied was found to vary from 17 to 49 per cent. The nature of decrease in chlorophyll a content did not exhibit any relationship with cultivars showing differential drought tolerance behavior.

Similar results were also obtained with respect to changes in chlorophyll b content in these cultivars. The chlorophyll b was found to range from 15.7 to 21.3 mg/g in normal plants while that it

Table 7. Effect of water stress on the content of chlorophyll a in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants (mg/g tissue)	Stressed plants (mg/g tissue)	Increase(+)/ Decrease(-), %
Phule G-5	10.0	7.7	-23
BG-391	11.2	8.6	-23
Phule G-9222-2	13.1	8.2	-37
BG-256	10.3	7.8	-25
BG-362	10.4	7.9	-24
Phule G-97110	12.5	10.5	-17
Vijay	12.8	6.5	-49
Phule G-96006	13.3	10.4	-22

Range	10.0-13.3	6.5-10.5
Mean	11.7	8.4
S.E.±	0.45	0.45

Table 8. Effect of water stress on the content of chlorophyll b in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control Plants (mg/g tissue)	Stressed plants (mg/g tissue)	Increase(+)/ Decrease(-), %
Phule G-5	16.5	15.0	-9
BG-391	17.9	13.2	-26
Phule G-9222-2	20.8	11.9	-43
BG-256	16.4	10.1	-38
BG-362	15.7	14.2	-10
Phule G-97110	18.5	15.7	-15
Vijay	20.0	9.1	-54
Phule G-96006	21.3	16.0	-25

Range	15.7-21.3	9.1-16.0
Mean	18.4	13.1
S.E.±	0.70	0.85

by 9.1 to 16.0 mg/g in water stressed plants. A decrease in chlorophyll b content was by 9 to 54 per cent in different cultivars. A definite relationship with respect to drought tolerant character and nature of loss in chlorophyll b was difficult to establish. The drought tolerant cultivars such as Phule G-96006, Vijay and BG-256 exhibited a large decrease in chlorophyll b. A decrease in chlorophyll a and chlorophyll b due to water stress has been reported in many crop plants. (Krishna *et al.,* 1989, Sharma *et al.,* 1990).

The chlorophyll stability index of eight cultivars of chickpea under normal moisture condition were investigated Table 9. The chlorophyll stability index range from 0.0 to 0.50 with a mean of 0.14. The cultivars known to be drought tolerant viz; Phule G-96006, BG-256 and Vijay exhibited a negligible or nil difference in the chlorophyll content before and after heating of the leaves at 56 ^{0}C for 30 min before the extraction. The drought susceptible cultivars such as Phule G-5, or Phule G-97110 showed a significant difference (0.22 and 0.50) in this respect cultivars. These observations indicated that chlorophyll stability index may be a useful criteria in evaluating a drought tolerant character in chickpea.

The chlorophyll is an important cell constituent in green plants. It is involved in biosynthesis of compounds through photosynthesis.

The biosynthesis of sugars, starch, amino acids etc will be influenced by the functionality of the chlorophyll which may affect the yields of the plants. The chlorophyll functionality is dependent on the turgidity of the leaf. The drought condition causing water deficits in the cells and tissues might affect the functional organization of the chlorophyll protein complexes which ultimately may result in decreased photosynthetic activity. The results obtained have indicated

Table 9. The chlorophyll stability index (CSI) in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	CSI
Phule G-5	0.22
BG-391	0.13
Phule G-9222-2	0.03
BG-256	0.00
BG-362	0.12
Phule G-97110	0.50
Vijay	0.09
Phule G-96006	0.00

Range	0.00-0.50
Mean	0.14
S.E.±	0.05

a significant decrease in the contents of chlorophyll a and chlorophyll b due to drought. Hence chlorophyll stability index of the normal plants (without water stress) as estimated by calculating the differences between chlorophyll extracted from the leaves before and after heat treatment will be the good criteria for evaluating drought tolerant character in chickpea. The cultivars known to be drought tolerant have shown a good chlorophyll stability index in present study. The reports on chlorophyll stability index in chickpea are however not available.

4.7 Effect of Water Stress on Phosphorous and Potassium.

The data obtained on the phosphorous content in the leaves of chickpea cultivars at normal and water stress condition are presented in Table 10. The phosphorous content in the leaves was found to range between 0.62 to 0.72 per cent with a mean of 0.67 per cent. When the plants were subjected to stress, the phosphorous content was found to increase in most of the varieties. The leaf phosphorus content in the stressed plant varied between 0.51 to 0.96 per cent with a mean of 0.71 per cent. An increase in the leaf phosphorous content was found to be more pronounced in drought susceptible cultivars as compared to tolerant one.

The changes in the contents in the potassium as influenced by drought are summarized in Table 11. The leaf potassium content exhibited a range of 2.87 to 6.00 with a mean of 4.14 per cent in different cultivars at normal conditions. The leaf potassium content was found to decrease markedly in drought susceptible cultivars while it was found slightly increased in drought tolerant cultivars. A well-known drought tolerant cultivar Phule G-96006 showed a 6.7 per cent

Table 10. Effect of water stress on the content of phosphorus in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants %	Stressed plants %	Increase(+)/ Decrease(-), %
Phule G-5	0.62	0.75	+21.0
BG-391	0.69	0.90	+30.4
Phule G-9222-2	0.66	0.56	-12.1
BG-256	0.67	0.51	-23.8
BG-362	0.72	0.79	+9.7
Phule G-97110	0.72	0.81	+12.5
Vijay	0.67	0.71	+5.9
Phule G-96006	0.65	0.67	+3.0

Range	0.62-0.72	0.51-0.96
Mean	0.67	0.71
S.E.±	0.03	0.04

Table 11. Effect of water stress on the content of potassium in leaves of chickpea cultivars (fresh wt. basis).

Cultivars	Control plants %	Stressed plants %	Increase(+)/ Decrease(-), %
Phule G-5	5.93	3.25	-45.1
BG-391	6.00	3.68	-38.6
Phule G-9222-2	3.18	2.88	-9.7
BG-256	3.75	2.87	-23.4
BG-362	4.31	5.37	+24.5
Phule G-97110	3.43	4.00	+16.6
Vijay	2.87	4.30	+50.0
Phule G-96006	3.68	3.93	+6.7

Range	2.87-6.00	2.87-5.37
Mean	4.14	3.78
S.E.±	0.39	0.27

increase while irrigated drought susceptible cultivar Phule G-5 exhibited a decrease of over 45 per cent in potassium content in response to drought. The results therefore indicated that maintenance of a level or marginal increase in potassium content in leaves in response to drought may be a good alternative or additional parameter evaluate drought tolerant character.

The reports on changes in leaf minerals in response to drought in chickpea are meager. Singh and Singh, (1999) studied the effect of salinity stress to chickpea genotypes and reported the higher or increase in potassium content in the tolerant genotypes. The results obtained in present study are in agreement with this report.

4.8 Correlation

A several fold increase in leaf proline content in response to a temporary water stress to the growing crop plants including chickpea in now well established. A drought tolerance is a physiological adaptation by the plant with the environment. The increase in proline content in the cells has been implicated in maintenance of osmotic conditions in the cells to protect the vital biochemical constituents during leaf water deficits. Hence it is important to investigate such biochemical constituents that are maintained at a stable level or increased in concentration in response to drought condition. The changes proline contents upon water stress exhibited a positive correlation with the accumulation of reducing sugars, total sugars and polyphenols while a negative correlation with accumulation of soluble proteins at 10 per cent level of probability Table 12. A higher level of probability was use to assets the significant of correlation because only eight varieties were included in the present study. These

Table 12. Correlation coefficients of proline content with some biochemical constituents.

Sr. No.	Factors	Correlation coefficient*	
		Normal plants	Stressed plants
1.	Proline x free amino acid	+ 0.0003	+ 0.4451
2.	Proline x soluble protein	- 0.0489	- 0.6855 *
3.	Proline x reducing sugars	- 0.1037	+ 0.6249 *
4.	Proline x total sugars	+ 0.5816	+ 0.6841 *
5.	Proline x total polyphenols	- 0.2685	+ 0.6729 *
6.	Proline x chlorophyll a	- 0.0362	+ 0.1511
7.	Proline x chlorophyll b	+ 0.0102	+ 0.1662
8.	Proline x chlorophyll stability index	- 0.1060	-
9.	Proline x phosphorus	- 0.0684	- 0.1184
10.	Proline x potassium	+ 0.3427	+ 0.4500

* Significant at 10 %

observation indicate the possibility of using additional/alternative parameter such as total sugars, polyphenols and soluble proteins as criteria for evaluation of drought tolerance in chickpea. However, a large number of cultivars need to be analyse to confirm these observation. The present studies conform the role of proline in imparting drought tolerance and also provide additional biochemical parameters. In this context, the increase in total and reducing sugar, phenolics, chlorophyll stability index and potassium may be good additional indices in evaluating the drought tolerant character of chickpea cultivars. Based on these indices, the cv. Phule G-96006, Vijay, Phule G-97110, BG-362 and BG-256 are found to be drought tolerant cultivars while others are susceptible.

Chapter Opener Page

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSIONS

Chickpea is one of the important food legumes grown in India as rainfed crops in rabi season on residual soil moisture, although an irrigated cultivation of chickpea is also taken to a less extent. The drought is one of the major environmental factors, which affect the productivity of chickpea to a great extent. Hence, attempts are being made to develop the drought tolerant cultivars in chickpea. The development of such cultivar through a traditional breeding procedure requires a significant time. Hence a biochemical markers have been identified which are positively associated with a drought tolerant character in crop plants. An increased accumulation of proline in the leaves in response to the drought has been reported in many field crops and seedlings of the forest trees. There is however a need to identify additional biochemical markers, if any, associated with drought tolerance.

Keeping in view the accumulation of proline, the promising chickpea cultivars with differential field reaction to the drought were grown under green house conditions. An artificial water stress was applied at 40 days of plant growth. The leaf samples of normal and stressed plants of each cultivar were analyzed for various biochemical constituents such as proline, free amino acids, soluble proteins, total and reducing sugars, total polyphenols, chlorophyll and minerals by following with standard procedures. The results obtained are briefly summarized in this chapter.

1. The mean proline content of 76 mg was found to increase to 1000 mg/100 g due to water stress in different chickpea cultivars. The

proline content was found to increase by over 1742 - 5043 % or 15 to 50 folds in different cultivars that are known to be drought tolerant. The cultivar Phule G-96006, a national check for drought tolerant chickpea has recorded the highest increase in proline accumulation upon water stress. This confirmed the role of proline accumulation for drought tolerance in chickpea. Based on data of proline accumulation, the cultivars Phule G-96006, Vijay, Phule G-97110, BG-256, BG-362 was found be drought tolerant cultivars.

2. The mean contents of FAA in normal plants was found to increase from 117 to 120 mg/100 g in stressed plants. There was a marginal increase in FAA in some cultivars while a slight decrease in others. These observations indicated that FAA content of leaves is not significantly affected due to water stress in chickpea.
3. The mean soluble protein content of 255 mg was found to increase to 368 mg/ 100 g upon water stress. Although the soluble proteins were found to increase in 6 out of 8 cultivars studied, such increase in soluble proteins was not of much significant order. An increase in both prolines upon water stress in drought tolerant cultivars (Phule G-96006) indicated that the sources for proline accumulation are different than protein hydrolysis during stress.
4. The mean reducing sugar content of 63 mg was found to rise to 119 mg/100 g upon stress. Similarly a mean total sugar content of 243 mg was found to increase to 384 mg/100 g upon water stress. There was a significant increase in both reducing and non-reducing sugars in 6 out of 8 cultivars studied. The drought

tolerant cultivar Phule G-96006 and BG-256 were found to record the highest increase in total sugars upon water stress. These observations indicated a positive relationship of total sugars, with a drought tolerant character in chickpea. Among the sugars, non-reducing are predominant in leaves over reducing sugars. Thus, accumulation of total or non-reducing sugars may be good additional criteria for evaluating drought tolerant character in chickpea cultivars.

5. The mean polyphenol content of 1.7 mg was found to increase to 3.0 mg/100 g upon water stress. All the cultivars studied exhibited an increase in total phenolics upon water stressed. This indicated that an increase in phenolics due to water stress seem to be a general phenomena. The increase in phenolics is not conspicuous in any variety. Hence, it is difficult to correlate it to drought tolerance.
6. The mean chlorophyll a content of 11.7 mg was found to decrease to 8.4 mg/100 g upon water stress. Similarly, a mean content of chlorophyll b was found to decrease from 18.4 to 13.1 mg/100 g upon water stress in different chickpea cultivars studied. A definite trend with respect to loss of chlorophyll could not be observed among the cultivars due to water stress. The cultivars such as Phule G-96006, BG-256 or Vijay exhibited higher chlorophyll stability index than that of drought susceptible cultivars. However, a definite relationship could not be established. Results therefore did not show any association of drought tolerance with changes in leaf chlorophyll.
7. The changes in leaf phosphorus content due to water stress were not of significant order. Although the mean potassium content of

4.14 per cent was found to decrease to 3.78 per cent in 8 cultivars, there was marked increase in cultivars that accumulated a significant amount of proline upon water stress. These results indicate that potassium content has a positive association with the drought tolerant character in chickpea. Based on these results, the cvs. Phule G-96006, Vijay, Phule G-97110, BG-362 and BG-256 were found to be drought tolerant cultivars.

8. Keeping in a view the proline accumulation as a standard parameter for drought tolerance, the correlation of proline content with some other biochemical constituents both under normal and stress condition were calculated. All the correlation calculated for normal plants were nonsignificant and the 'r' values were quite low. In stressed plants, were non significantly correlation with reducing and total sugars and polyphenol accumulation while significantly negative correlation with soluble proline at 10 per cent level of probability. These results indicated that the accumulation of total sugars or polyphenols as well as extent of loss of soluble proteins could be used as a additional or alternative parameters to evaluate drought tolerant character in new cultivar in chickpea. However, further investigations on a large number of chickpea cultivars are suggested to confirm these observations.

Chapter Opener Page

LITERATURE CITED

6. LITERATURE CITED

Agarawal, P. and Bhattacharya, L. 1980. Proximate composition of seeds of improved varieties of bengal gram (*Cicer arietinum L.*). Seed Res. 5 : 5-7.

AOAC, 1990. "Official Methods of Analysis " of Association of Official Aanlytical Chemists, Washington. D.C., 15th Ed.

Aman, P. 1979. Carbohydrates in raw and germinated seeds from mung bean and chickpea. J. Sci. Food agric. 30 : 869-72.

Angela, S., Fernando, F. and Migual, H. 1987. Chemical composition and nutritional value of Mexican varieties of chickpea. Qual. Plant. Pl. Food Hum. Nutr. 37 : 299-350.

Anonymous, 1999. Epitome of Agriculture in Maharashtra State, Part II. Department of Agriculture, M.S.

Arnon, D.I. 1949. Copper enzymes in isolate of chloroplast. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24 : 1-55.

Bates, L.S., Waldren, R.P. and Teare,L.D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39 : 205-207.

*Bradford,K.J. and Hsiao,T.C. 1982. Physiological responses to moderate water stress. pp. 264-324 in Physiological Plant Ecology II, Water Regulations and Carbon Assimilation, Springes-verlag, Berlin (eds) O.L. Lange, P.S. Nobel, C.B. Osmond, H. Ziegler.

Chapman, H.D. and Pratt, P.R. 1961. Mehtods of Analysis for Soils, Plants and Water. Univ. of Calif., Berley. pp. 169-170.

Chandra, S. and Arora, S.K. 1968. An estimation of protein, ascorbic acid and mineral matter content in some indigenous and exotic varieties of gram. Current Sci. 37 : 237-240.

Chandrasekhariah, A.M., Radder, G.D., Palled, Y.B. and Munirajappa, R. 1986. Response of gram to irrigation and phosphorous. Mysore J. Agri. Sci. 20 : 255-61.

Chaudhari, R.S. 2001. Studies on nutritional composition and trypsin inhibitor activity in parents and their promising hybrid composites in chickpea. M.Sc.(Agri.) Thesis. Mahatma Phule Krishi Vidyapeeth, Rahuri. M.S. India.

Chavan, J.K., Kachare, D.P., Deshmukh, R.B. and Kadam, S.S. 1993. Grain yield, dhal milling and cooking qualities of chickpea cultivars grown under rainfed and irrigated conditions. J. Maharashtra Agri. Univ., 18 : 281-283.

Chavan, J.K., Kadam, S.S. and Salunkhe, D.K. 1986. Biochemistry and technology of chickpea (*cicer arietinum L.*). seeds. CRC. Crit. Rev. Food Sci.. Nutr. 25 : 207-123.

Chen, D., Kessler, B. and Monselise, S.P. 1964 Studies on water regime and nitrogen metabolism of citrus seedlings grown under water stress. Plant physiol., 39 : 379-386.

Davies, D.R., Berry, G.J., Heath, M.C. and Dawkins, T.C.K. 1985. Pea (*Pisum sativum L.*) in " Grain legume crops " Collins, London, UK. (eds). Summerfield, R.J. and Roberts, E.H.

Day, W. and Legg, B.J. 1983. Water relations and irrigation response in the Faba Bean (*Vicia faba L.*) Butterworths, London, UK. (eds) Hebblethwaite, P.D.

*Del, M.R. 1972. On the variability of chlorogenic acid concentration. Oeclolgia. 9 : 289-300.

FAO, 1970., Amino Acid content of Foods, Food and Agriculture Organization, Rome.

FAO, 1998. Production Yearbook, FAO, Rome.

Farah, S.M., Arar, A. and Miller, D.E. 1988. Water requirements and irrigation management of pea, lentils, faba bean and chickpea crops in World crops: Cool Season Food Legumes. Dordrecht, The Netherlands Kluwer. (eds) Summerfield R.J.

Fernandez, M.L. and Berry, J.W. 1988. Nutritional evaluation of chickpea and germinated chickpea flours. J. Food Sci. 38 : 127-134.

*French, B.K. and Legg, B.J. 1979. Rothamsted irrigation, 1964-1976. J. Agric. Sci. (Combridge) 92 : 15-37.

Frechilla, S., Gonzalz, E.M., Royuela, M., Minchn, F.R., Aparicio-Tejo-P.M., Arrese-Igor, C. 2000. Source of Nitrogen nutrition (N-fixation or nitrate assimilation) in a major factor involved in pea response to moderate water stress. J. Pl. Physiol. 157 : 609-617.

Gorbanli, M., Hidari, R., Nojavan, M. and Farboundnia, T. 1998. The effect of water stress on the variation of soluble protein and amino acids in two different cultivars of chickpea. Iranian. J. Agril. Sci., 29 : 67-77.

Gupta, V., Yadav, V.K. and Yadav, N. 1997. Role of benzyladenine and GA in alleviating water stress effect in gram. Indian J. Agril. Sci., 29 : 381-387.

Gruda, N. and Schnitzler, W.H. 2000. The effect of water supply to seedlings cultivated in different substrates and raising systems on the bio-morphological and plant physiological

parameters of Lettuce. J. applied Botany, 74 : 240-247.

Hasio ,T.C. 1973. Plant responses to water stress. Ann. Rev. Plant Physiol., 24 : 519.

Hussain, M.M., Gallagher, J.N., Hill, G.D., Othman, H. and Reid, J.B. 1983. The nonexistence of moisture sensitive phases in *Vicia faba* L. grown under irrigation in cantesbury. Proc. Agron. Society (New Zealand)., 13 : 87-94.

Ivanov, V.P. 1959. Effect of foliar nutrition and soil moisture on the growth and development of maize. Frziol. Rast., 6 : 368.

Jackson, M.L. 1975. Soil Chemical Analysis Prentice Hall of India Pvt. Ltd., New Delhi.

Jambunathan, R. and Singh, U., 1978. Studies on desi and Kabuli chickpea (*Cicer arietinum L.*) cultivars. I. Chemical composition, in Proc. Int. Workshop on Chickpea, ICRISAT, Patancheru, Hyderabad, India, pp. 61.

Jambunathan, R. and Singh, U., 1981. Studies on desi and Kabuli chickea (*Cicer arietinum L.*) cultivars. III. Mineral and trace element composition, J. Agric. Food Chem., 29 : 1091-1094.

Jaya, R.V. and Venkataraman, L.V., 1979. Germinated legumes and their influence on liver, sernum cholesterol levels in rats : influence of different components of chickpea and green gram on tissue cholesterol levels in rats, Nutr. Rep. Intl, 20 : 283-285.

Jirali, D.I., Panchal, Y.C., Jangoudar, B.S. and Patil, B.C. 1989. Studies on physiological and biochemical characters in chickpea. India J. Physiol. 32 : 240-243.

Krishna ,C.V. and Rajeswarrao, G. 1989. Influences of foliar application of calcium on growth performance, chlorophyll and protein content in groundnut under stress. Narendradeva. J. Agril. Res. 4 : 7-11.

*Koundal, K.R. and Chopra, R.K. 1989. Effect of water deficit on various physiological and biochemical activities in root nodules of chickpea. Biochemie usd phygiologie der pflanzen. 185 : 69-74.

Lamptey, M.S. and Walker, B.L. 1976. A possible essential role of dietary linolenic acid in the development of the young rats, Nutr. Rep. Int ., 20 : 283-285.

Lineback, D.R. and Ke, C.H. 1975. Starches and low molecular weight carbohydrates from chickpea and horse gram flours, Cereal Chem., 52 : 334-336.

Loomis, W. and Shull,C.A. 1937. Methods in plant physiology, Mcgrow Hill, New York.

Lawry, O.H., Rosenbrough, H.J., Furr, A.L. and Randall, R.J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193 : 256-275.

Malik, C.P., Gupta, K. and Sharma, S. 1986. Effect of water stress on germination and seedling metabolism of gram (*Cicer arietinum* L.). Acta agronomica Hungarica, 35 : 11-16.

Mehrotra, S.C. and Bhist, A. 1990. Concentration of free amino acid in susceptible and tolerant cultivars of chickpea under iron deficiency stress. Indian J. Environ. Biology, 28 : 893 –894.

Millerd, A. 1975. Biochemistry of legume seed proteins, Annu. Rev. Plant physiol. 26 : 53-55.

Milner, M. 1972. Nutritional Improvement of Food Legumes by Breeding, Proc. Symp. PAG, Food and Agriculture Organization, Rome.

Mukane, M.A., Desai, B.B., Naik, R.M. and Chavan, U.D. 1996. Biochemical marker for water stress in pigeonpea genotypes. J. Maharastra agric. Univ., 21 : 140-141.

Munoj, F.J. Dopico, B. and Labrader, E. 1998. A cDNA encoding proline rich prolein from *Cicer arientinum* changes in expression during development and abiotic stress. Physiologia Plantarum. 102 : 582-590.

Murthy, K.S. and Urs, M.K. 1985. Effect of Bengal gram (*cicer arietinum*) proteins and lipids on serum and liver cholesterol levels in rats, J. food Sci. Technol., 22 : 54-56.

Nelson, N. 1944. A photometric adoption of the Somogyi method for determination of glucose. J. Biochem . 153 : 375-380.

*Pak, N. and Barja, I. 1974. Composition, content of toxic substances, protein quality and protein value of green peas, chickpeas and lentils grown in Chile, Cienciae Invest. Agraria, 1 : 105-106.

Panse, U.G. and Sukhatme, P.V. 1967. Statisti cal method for agricultural workers, I.C.A.R., New Delhi. 145-147.

Rackis, J.J. 1975. Oligosaccharides of food legumes, alpha galactosidase activity and the flatus problem. pp., 207 in "Physiological effects of Food Carbohydrates" (Eds), Jeanes, A. and Hodge, J. American Chemical Society, Washington.

*Randeva, V. and Ilieva, A. 1998. Relationship between the free proline content and the productivity of spring forage pea

cultivar under drought stress. Rasteniev "dni Nauki." 35 : 528-532.

Rao, K. and Subramanian, N. 1970. Essential amino acid composition of commonly used Indian pulses by paper chromatography. J. Food Sci. Technol. 7 : 31-32.

Rosen,R. 1957. A modified ninhydrin colorimetric analysis for amino acids, Arch. Biochem. Biophys. 67 : 10-15.

Salter, P.J. and Drew, D.H. 1965. Root growth as a factor in the response of *Pisum sativum* to irrigation. Nature, 206 : 1063-64.

Salunkhe ,D.K. 1982. Legumes in human nutrition : current status and future research needs. Curr. Sci. 51 : 387-394.

Salunkhe, D.K., Jadhav, S.J., Kadam, S.S. and Chavan, J.K. 1982. Chemical, biochemical and biological significance of polyphenols in cereals and legumes, Crit. Rev. Food Sci. Nutr. 17 : 277-180.

Samman, N., Maldonado, S., Alfano, M.E., Farfan, H. and Guteierrez, J. 1999. Composition of different bean varieties of north and western Argentina (Region No. A), J. Agrc. Food chem., 47 : 2685-2689.

Sharma, K.P. and Goswami, A.K. 1971. Chemical evaluation of some high yielding varieties of Bengal gram (*Cicer arietinum L.*). Indian J. Agric. Res., 5 : 109-112.

Sharma, B.K., Gupta, R.K. and Laxmichand, A.M. 1990. Influence of drought stress on photosynthesis and photorespiration in callus tissue of maize. Photosynthetica, 24 : 143-146.

Sharma, S., Kumar, R. and Malik, C.P. 1986. Salt stress induced

changes in protein degradation and proteloytic activity in germinating chickpea seeds. Legume Reasearch, 9 : 91-96.

Sheoran ,I.S., Luthra, Y.P. Kuhad, M.S. and Singh R. 1981. Effect of water stress on some enzymes of nitrogen metabolism in pigeonpea. Phytochemistry, 20 : 2675-2677.

Singh, U., Jambunathan, R. and Raju, S.M. 1981. Studies on desi and Kabuli chickpea (*Cicer arietinum L.*) cultivars. II. Seed protein fractions and amino acid composition. J. Food Sci. Techol. 18 : 86-88.

Singh, U. and Jambunathan, R. 1981. Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum L.)* pigeonpea (*Cajanus cajan L.*) , Qual. Plant. Plant Foods Hum. Nutr., 31 : 893-895.

Singh, R.A. and Kumar, P. 1992. Effect of water stress and hardening on metabolic changes in chickpea (*Cicer arietinum L.)* . Indian J. Plant Physiol., 35 : 252-257.

Singh, G. and Rai, V.K. 1983. Responce of two *Cicer arietinum* cultivars with differential sensitivities to water stress : Soluble sugars and drought resistance. Indian J. Ecology, 10 : 264-269.

Singh, A.K. and Singh, R.A. 1999. Effect of salinity on sodium, potassium and proline content of chickpea genotypes. Indian J. Plant Physiol. 4 : 111-113.

Sinha, R.K. 1979. Effect of Water Stress on Nitrogen Fixation in Cowpea (*Vigna sinensis savi*). M. Sc. Thesis, Haryana Agril. University, Hisar.

Sivakumar, V., Ramchandran, K., Ravichandran, V. and Vangamud,

M. 1998. Effect of drought hardening on proline content of tree seedlings. Ann. Plant Physiol. 12 : 82-84.

Steawart, C.R. , Clayton, J.M. and Thompson, J.F. 1966. Changes in amino acid content of excised leaves during incubation. II. Role of sugar in the accumulation of proline in wilted leaves. Plant Physiol. 41 : 1585-1590.

Steawart, C.R. 1972. The effect of wilting on proline metabolism in excised bean leaves in the dark. Plant Physiol. 51 : 508-932.

Swain, T. and Hills, W.E. (1959). Phenolic Constituents of *Prunus domestica* quantitative analysis of phenolic constituents. J. Sci. Food Agri., 10 : 63-65.

Tiwari, S.R., Sharma, R.D. and Ram, N. 1977. Mineral contents of some high yielding varities of Bengal gram (*Cicer arietinum L.*), J. Agric. Food Chem. 825 : 420-425.

Vaadia, V., Raney, F.C. and Naga, R.M. 1961. Plant water deficits and physiological processes. Ann. Rev. Plant. Physiol. 13 : 265-292.

Wadleigh, C.H. and Ayers, A.D. 1945. Growth and biochemical composition of bean plants as conditioned by soil moisture tension and salt concentration. Plant Physiol. 20 : 106-132.

Wilson, D.R., Jamieson, P.D., Jermyn, W.A. and Hanson, R. 1985. Models of growth and water use of field pea (*Pisum sativum L.*). In the pea crop London, UK : Butterworths, London, UK (Eds) Hebblethwaite, P.D., Heath, M.C. and Dawkins, T.C.K.

Woodhame, D.H. and Kozlowske, T.T. 1954. Effect of soil moisture stress on carbohydrate development and growth in plants. American J. Bot. 41 : 316-320.

Yadav, R.S. and Khare, R.B. 1995. Studies in proline accumulation in relation to seed yield in chickpea., Indian J. Pulses Res., 8 : 218-220.

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

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VITA

7. VITA

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of

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