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MY
BELOVED BROTHER

SHANTANU

CHARACTERIZATION OF CHICKPEA CULTIVARS (DESI AND KABULI) THROUGH MORPHOLOGICAL, CHEMICAL AND ELECTROPHORETIC TESTS

By

MISS. SHEETAL DINESH SAWALE

(Reg. No. 0135)

A Thesis Submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI – 413 722, DIST. AHMEDNAGAR, MAHARASHTRA, INDIA

in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

AGRICULTURAL BOTANY

(SEED TECHNOLOGY)

DEPARTMENT OF AGRICULTURAL BOTANY

POST GRADUATE INSTITUTE,

MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI – 413 722,
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Approved by

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2003

CANDIDATE'S DECLARATION

I hereby declare that
the thesis submitted for the
degree of Master of Science
or any part there of has not been
previously submitted by me or other person
to any other University or Institute
for a degree or diploma

Place: Rahuri

Dated: 27 / 6 / 2003

(MISS. SHEETAL D. SAWALE)

Prof. S. N. Mate,
Seed Research Officer,
Seed Technology Research Unit (NSP),
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Rahuri - 413 722, Dist. Ahmednagar
Maharashtra State (India).

CERTIFICATE

This is to certify that the dissertation entitled **"Characterization of Chickpea Cultivars (Desi and Kabuli) Through Morphological, Chemical and Electrophoritic Tests"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State, India in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL BOTANY**, embodies the results of piece of *bona fide* research work carried out by **MISS. SHEETAL DINESH SAWALE** under my guidance and supervision and that no part of this dissertation has been submitted to any other University for degree or diploma or publication in other form.

All the assistance and help received during the course of the investigation and sources of references has been duly acknowledged.

Place : M.P.K.V., Rahuri
Dated: 27 /06 /2003

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Place : M.P.K.V., Rahuri
Dated: 30 /06 /2003

(Dr. D.M. Sawant)

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Place : M.P.K.V., Rahuri.

Dated : 27/ 6/ 2003

(SHEETAL D. SAWALE)

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LIST OF ABBREVIATIONS

APS	: ammonium per sulphate
cm.	: Centimeter (s)
^0C	: Degree celsius
DAS	: Days after sowing
DDT	: Diphenyl dichloro triphenyl ethane
2,4 – D	: 2,4- Dichloro phenoxy acetic acid
et al.	: et alli (and others)
Fig.	: figure
g	: Gram
hr.	: Hour
i.e.	: id est (that is)
IAA	: Indol acetic acid
mA	: Milli Ampere
No.	: Number
NaOH	: sodium hydroxide
NSP	: national seed project
pH	: negative logarithm of hydrogen ion Concentration
ppm	: parts per million
%	: percent
Rm	: relative mobility
SDS-PAGE	: sodium dodocyl sulphate- polyacrylamide

	gel electrophoresis
TCA	: trichloro acetic acid
TEMED	: N,N,N,N',- Tetramethyl ethylene diamine
µg	: microgram
µl	: microlitre
Viz.	: videlicet (namely)
<	: less than
>	: more than

ABSTRACT

"CHARACTERIZATION OF CHICKPEA CULTIVARS (DESI AND KABULI) THROUGH MORPHOLOGICAL, CHEMICAL AND ELECTROPHORETIC TESTS"

by

MISS. SHEETAL DINESH SAWALE

A candidate for the degree of

MASTER OF SCIENCE (AGRICULTURE)

Research Guide	:	**Prof. S. N. Mate**
Department	:	**Agricultural Botany**
Major Discipline	:	**Seed Technology**

The present investigation "Characterization of chickpea cultivars (desi and kabuli) through morphological, chemical and electrophoritic tests" was carried out at Seed Technology Research Unit (NSP), Mahatma Phule Krishi Vidyapeeth, Rahuri 413722, Dist. Ahmednagar (Maharashtra), during 2001-03.

The experimental material consisted of twelve chickpea cultivars having 100 % genetic purity were obtained from Pulses Breeder, M.P.K.V., Rahuri. The observations were recorded on seedling, plant and seed morphological characters and also for chemical and electrophoritic tests.

The morphological characteristics exhibited by different cultivars studied indicated that although some of the cultivars have common morphological features in respect of one or few characters, they can be differentiated from each other on the basis of other characters.

Cultivars were studied for thirteen plant morphological characters namely growth habit (erect, semispreading and spreading), branching habit (less medium, profuse), stem colour (green and purple), foliage colour (green and dark green), leaflet size (small, medium and large) leaflet shape (oval and ovate) leaflet margin (medium and high leaf serration), flower colour (white, pink, dark pink), days to flower (early and medium), plant height (dwarf, medium and tall), pods per plant (less, medium and high), number of seeds per pod (single and double), pod size at maturity (small, medium and bold).

The chickpea cultivars can be characterized on the basis seedling characters. In all two seedling characters were studied for twelve cultivars namely pigmentation on seedling and colouration of leaflet at seedling stage. The cultivars PG-12, Vijay, Virat, PG-92307, PG-95311, PG-95421 and KAK-2 had green pigmentation on seedling while other showed purple pigmentation. The colouration of leaflet in cultivars PG-5, Vishal, PG-92926, PG-96005 and PG-96006 was purple while remaining cultivars showed green colouration of leaflets.

Further the chickpea cultivars can be characterized on the basis of seed size (small, medium and bold), seed surface texture (smooth and wrinkled) and seed colour (white, brown and dark brown).

According to the seed coat colour reaction, twelve cultivars were classified into two groups in NaOH test. Such as dark orange

red colour reaction (PG-5, PG-12, Vijay, Vishal, PG-92926, PG-96005 and PG-96006) and no colour reaction (Virat, PG-92307, PG-95311, PG-95421, KAK-2).

The results on electrophoretic banding pattern of seed storage protein revealed a total of 24 bands (Fig. 1) in material studied, out of which only band No. 15, RM 0.66 was commonly observed in seven cultivars (Vijay, PG-96005, Virat, PG-92307, PG-95311, PG-95421 and KAK) and it was absent in remaining five cultivars. The number of bands ranged between 23 to 24. All the bands, except band 15, were common in all cultivars but with differing intensities. The desi type and kabuli type cultivars can be differentiated from each other by presence or absence of band-15 corresponding to Rm value 0.66 because in all desi types (except Vijay and PG-96005) band corresponding to Rm value 0.66 was absent.

From the present study, it can be concluded that some of the morphological features of the chickpea cultivars alongwith NaOH test could be exploited for characterizing the cultivars and distinguishing them in different groups. However, electrophoresis gives more precise results for characterizing the chickpea cultivars being stable and not influenced by the environment.

INTRODUCTION

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important crop among the food legumes, contributing about 14% of the total world production of pulses and occupying about 15% of world area under pulses. The states of Uttar Pradesh, Rajasthan, Haryana, Madhya Pradesh and Maharashtra contributes about 80% to the total chickpea production in India. (Dahiya et al. 1995).

Chickpea is well adopted to warm, semiarid climate and in India it grows well in winter season.In India, it occupies 7.26 M.ha. area having a productivity of 855 Kg/ha. Chickpea is the major pulse crop of the country and it occupies 32% of the total production of pulses in the country. (Anonymous, 1996).

Vavilov(1926)considered India and Middle-Eastern countries as the probable center of origin for Chickpea. It belongs to the genus *Cicer* and tribe *vicieae*, suborder Papilionaceae of the order Leguminaceae.

1.1 Importance of characterization :-

Seed is the basic input in agriculture. Therefore, production of good seed is of prime importance in the seed production program. But, during the seed production cycle, the varietal purity is affected by a number of factors, such as cross pollination, mechanical mixtures, genetic shifts and selective influences of diseases etc. The extent of varietal deterioration

from initial breeders stock to the certified seed largely depends on care and precautions taken during the seed multiplication. Therefore, during the field inspection rouging of off types is done. In order to carry out rouging of off types in the seed plot, diagnostic characteristics of each variety are useful to distinguish varieties from one another. The crop varieties could be identified or distinguished to a reasonable degree of accuracy on the basis of clear and consistent differences in one or more essential characters.

1.2 Need for varietal identification :-

A number of varieties of Chickpea have been developed in the country and are being released and notified for a long time. The maintenance of genetic purity of released varieties is of at most importance in the seed production program. Therefore, documentation of distinguishing characters of varieties is essential to carryout scientific seed production. Unfortunately such complete information on distinguishing characters of different varieties is not available at one place. Therefore, it was considered essential to record the necessary detailed information on stable diagnostic characteristics of Chickpea cultivars. These distinguishing characteristics could be morphological, chemical or biochemical.

Intensive crop improvement programs have resulted in the development of alarge number of varieties in all important crop species. Variety identification has therefore attained critical importance in the national and international seed programs. Different cultivars are commonly identified on the basis of

taxonomic differences of seed, seedling and plant.

Distinguishing the varieties on the basis of morphology is not always possible, though it is undoubtedly, one of the most commonly used criterion. Thus, there is a need to develop alternative tests which can distinguish varieties on the basis of stable biochemical properties of seed or seedlings. Of the techniques available analysis of seed or seedling proteins and isoenzymes using elecrtophoresis techniques are most widely used because of their reliability, rapidity and cost effectiveness.

1.3 Plant Variety Protection :-

The Intellectual Property Right is the another reason for need of documentation of diagnostic characters of varieties. For protecting the property rights of original breeders of the varieties, it has become very essential that the concerned breeder or the organization should have detailed characteristics of the variety. Thus, the Intellectual Property Rights will give returns in terms of monitory benefits for which the breeder has invested money, time, energy, skill and knowledge. Based on these facts the Government of India has passed the Plant Variety Protection (PVP) and Farmers Right (FR) Bill with the adoption of effective system for varietal identification. The necessity of which encouraged development of system for documentation of diagnostic characters which will prove the identity if that variety. With the introduction of IPR (PBR) at Global level, it has become necessary to register, characterize and prepare documentation of varieties in seed production chain. In India, the characterization of varieties was previously

based on the UPOV (Union for Protection Of Variety) guidelines. But now a days a National system has been developed for characterization of varieties, called as a DUS (Distinctness, Uniformity and Stability) System. At present, the documentation of diagnostic characters of all the varieties from all crop species is being done as per national guidelines of DUS System.

The present study is concentrated on the documentation of most stable key morphological, chemical and biochemical diagnostic characters of seed, seedling and plant of twelve Chickpea cultivars with the help of coloured photographs.

At present, there is no compilation depicting the key diagnostic characters of important Chickpea cultivars. This study is an attempt in this direction, where efforts have been made to provide information on stable diagnostic characters of those released and notified varieties which are commonly cultivated, with following objectives-

i. To characterize chickpea cultivars by means of morphological characters.
ii. To identify chemical reaction on seed and seedling.
iii. To know the banding pattern of chickpea cultivars using elecrtophoresis.

The requirements of the seed producing agencies, seed certification agencies and seedman had been the main consideration while listing the stable diagnostic characters at different growth stages of the crop.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The literature relevant to the present investigation entitled "Characterization of Chickpea cultivars (desi & kabuli) through morphological, chemical and electrophoretic tests" has been reviewed in the following subheadings.

2.1 Morphological studies.
2.2 Chemical studies.
2.3 Electrophoresis.

2.1 Morphological studies :-

Attempts were made earlier to classify varieties by studying morphological characters in different crops. The morphological characters viz., seedling characters (pigmentation on seedling, colouration of leaflet at seedling stage), plant characters i.e. growth habit, branching pattern, stem colour, foliage colour, leaf size, leaf shape, leaf margin, flower colour, days to flower, plant height, pods per plant, number of seeds per pod, pod size at maturity etc. and seed characters (seed size, seed surface texture and seed colour) have effectively used by many workers to specify varieties of different crops at generic level in general and at species level in particular. The available literature pertaining to Chickpea and other pulse crops on similar aspects has been reviewed.

Joshi (1972) observed a wide range of variability in most of the yield contributing characters i.e. number of pods, number of seeds,

number of branches, 100- seed weight, days to flowering and maturity in a collection of twenty varieties of gram.

Singh and Tuwafe (1980) evaluated over 3000 kabuli germplasm for studying the variability for seed size and seeds per pod. The 100- seed weight of randomly picked seeds from each germplasm was recorded to the nearest gram.

Kumar et al. (1981) studied three hundred thirty Chickpea lines for variability. Data were recorded on three random plants in each line for days to flowering, plant height (cm.), plant spread (cm.), number of pod bearing branches, primary branches and pods per plant, pod length, seeds per pod, weight of 100 seeds (g), biological and grain yield per plant (g) and harvest index.

Adhikari and Pandey (1982) estimated genetic variability in thirty six genetically diverse lines of Chickpea for different characters viz., seed yield, number of pods per plant, foliage colour, seed weight, secondary branches per plant.

Srivastava and Gupta (1982) conducted an experiment with fortynine genotypes of Chickpea for studying genetic divergence. The number of days to flowering and maturity, number of primary branches per plant, pods per plant, plant height (cm.), biological and grain yield (g) per plant, harvest index (%), weight of 100 seeds were recorded on five competitive random plants from each genotype.

Dumbre et al. (1984) studied genetic diversity in gram and found that seed size, yield per plant and duration were the most important characters contributing towards genetic divergence.

Dumbre and Deshmukh (1984) evaluated seventeen Chickpea cultivars for genetic divergence. Days to 50% flowering maturity,

number of branches and pods per plant, plant height and plant spread, seed size and yield per plant were found to be different in each cultivar.

Kamble et al.(1984) observed the genetic variability for the components such as pods per plant, 100- seed weight, days to maturity, seed yield, etc. in five diverse Chickpea lines.

Srivastva et al.(1984) evaluated sixteen Chickpea genotypes for genetic divergence. They found the variations for the characters viz., days to flowering, days to maturity, plant height (cm.), plant yield (g), 100- seed weight (g).

Khorgade et al. (1985) conducted an experiment for studying the genetic variability in thirty two genetically diverse genotypes of Chickpea. They observed the variations in the characters such as time to 50% flowering, plant height, number of branches per plant, number of pods per plant, number of seeds per pod, 100- seed mass and yield per plant.

Payne (1987) showed variety testing in the test consist of two approaches, one being an examination of seed morphology and other field testing. Major disadvantage relying on the differences in the seed is that the seed morphological characters of many new varieties have same physical characteristics.

Chakrabarty and Agrawal(1989) studied morphological characteristics of seedlings of sixteen blackgram cultivars and seedlings were grouped in various diagnostic characteristics as stem pigmentation, hairiness, leaflet shape, hypocotyl length and radical length.

Govil and Kumar (1989) conducted an experiment to study

variations in genetic parameters in Chickpea. They observed variations for days to flowering, pods per plant,seeds per pod, etc.

Gurinder Singh et al. (1989) analyzed components of seed yield in Chickpea. The observations were recorded for grain yield per plant, pods per plant, 100-grain weight, harvest index, primary and secondary branches per plant, plant height. They found that these components mainly contribute for seed yield.

Agrawal and Pawar (1990) distinguished thirteen important Soybean varieties on the basis of morphological characteristics of seeds namely seed size, seed coat colour, hilum colour, etc. Out of thirteen cultivars, twelve were yellow seeded and one was black seeded. The twelve yellow seeded varieties could be classified into hilum colours as black, yellow and brown and all seeds were grouped on the basis of size as bold, medium and small.

Sharma et al.(1990) conducted an experiment on seventy genotypes of Chickpea for studying genetic variability. The variability was recorded for number of primary branches per plant, secondary branches per plant, days to flowering, days to maturity, plant height, 100- seed weight, seeds per pod, pods per plant, seed yield per plant, etc.

Arora (1991) estimated genetic variability for the characters such as pods per plant, 100- seed mass, seed yield per plant, plant height, canopy spread, length of pod bearing branch, primary and secondary branches per plant, seeds per pod, harvest index, days to flowering, days to maturity, etc.

Chaudhary et al.(1991) evaluated sixty Chickpea cultivars to study the yield attributes such as seed size, plant height, pods per

plant, seed yield per plant, biological yield.They observed variability in these attributes and concluded that all these attributes were responsible for higher yield.

Misra (1991) conducted an experiment with a set of eighteen Chickpea varieties for studying stability of heritability, genetic advance and character association. The observations were recorded for time to 50% flowering, time to maturity, plant height, branches per plant, pods per plant, seeds per plant, 100- seed mass, seed yield, etc.

Pundir et al. (1991) studied some physio- morphic and yield traits of twenty five Chickpea germplasm and recorded variations for characters viz., flowering days, pods per plant, seeds per pod, 100-seed weight (g), seed yield (Kg/ha).

Sandhu and Gumber (1991) carried out an investigation to estimate genetic divergence among fifty-nine strains of Chickpea. During the investigation, divergence was observed for primary branches per plant, secondary branches per plant, plant height (cm), pods per plant, seeds per plant, 100- seed mass (g), seed yield per plant (g), harvest index (%), etc.

Lokendra Kumar and Arora (1992) evaluated forty genotypes of Chickpea collected from different parts of India for multivariate analysis. They observed the variations for days to initial flowering, days to 50% flowering, days to 100%flowering, days to maturity, reproductive period, plant height (cm), canopy spread (cm), length of pod bearing branch, primary and secondary branches per plant, first pod forming node, pods per plant, seeds per pod, seeds per plant, 100- seed weight (g), biological yield per plant, harvest index (%) and

seed yield per plant.

Arora and Kumar (1994) during their experiment for studying path coefficient analysis, observed variations for plant spread, pods per plant, plant height, 100- seed weight, biological yield per plant, etc in forty genotypes of Chickpea.

Jahagirdar et al. (1994) studied genetic variability in Chickpea for the characters viz., number of pods per plant, days to 50% flowering, plant height, number of primary and secondary branches per plant, 100- grain weight.

Dahiya et al. (1995) worked on the identification of Chickpea cultivars namely, Annigeri-1, BG- 256, BG- 267, C- 235, Gaurav, H-208, Haryana Chana- 1(HC- 1), K- 850, L- 550, PG- 5 and PBG- 1, based on field parameters. They characterized these varieties according to foliage colour, flower colour, pod size,seed colour, seed size and seed surface, etc.

Singh et al. (1998) evaluated Chickpea (*Cicer arietinum* L.) germplasm comprising 140 diverse lines of both exotic and indigenous types for yield and five yield components. Of the 140 lines eleven were of kabuli type, one was black seeded and rest were of desi type. Considerable variations were observed for the characters viz., days to flower, plant height (cm), pods per plant, seeds per pod, 100- seed weight (g), yield per plant (g).

Jain et al.(2000) conducted an experiment on fifteen varieties of mungbean to develop a system of varietal identification in mungbean based on characters at seed, seedling and plant level. They also conducted chemical tests to verify the varieties at seed level.

Among all the characters at seed level, seed luster, hilum

shape, seed coat colour and seed shape were found most stable, uniform and distinguishing. Among quick, rapid and repeatable biochemical tests, Peroxidase activity and KOH test can be used to verify the varieties.

Also pigmentation on stem, leaf, growth habit, days to flowering and plant height, etc, characters were also studied for their distinguishness, uniformity and stability.

R. Sankarpandian (2000) studied eighteen predominant cultivars of pulses which are popular and grown in more acreage in Tamilnadu. Among those pulses, four each in red gram and blackgram varieties, six greengram varieties and four cowpea varieties are cultivated in the state. He observed distinguishing characters viz., stem colour, petiole colour, hairiness, pod colour and grain colour exhibited in green gram cultivars and varied leaf shape, stem colour, pod shape and colour and seed colour exhibited on different cowpea varieties.

Suryawanshi et al. (2000) characterized seven cultivars of Soybean viz., PK- 1029, MACS- 450, MACS- 124, MAUS-1, MAUS-2, DS- 186 and JS -335 cultivated in Maharashtra based on different morphological characters. Among the different morphological characters, pigmentation on seedling, foliage colour, leaf surface, stem pubescence, flower colour, pod hairiness, pod colour at maturity, seed coat colour and seed shape were observed most stable diagnostic characters under Rahuri condition. These characters are much helpful for identifying the genotype at flowering and maturity stage during field inspections.

Yadav and Srivastavs (2000) characterized the varieties both desi and kabuli types of Chickpea based on UPOV guidelines, because in the present scenario of Intellectual Property Rights, the characterization of plant variety is given the top priority in our country. While characterizing the varieties, it was realized that some characters were lacking which invariably showed complete expression and also few statistical majors of quantitative traits which posed a problem while classifying the experimental material. Attempts were therefore made to supplement these information and document them with the help of coloured photographs. DUS characteristics of Chickpea varieties were, thus, finaly studied, based on seed colour, seed shape, seed size, seedling pigmentation, growth habit, branching habit, stem pigmentation, leaflet size, foliage colour, plant height, pods per plant, etc. These informations need to be utilised while characterizing varieties of Chickpea.

2.2 Chemical studies :-

Among various chemical tests, Phenol test, Modified Phenol test, Peroxidase test, NaOH test and 2, 4- D test have reported effective to discriminate varieties of different crops. Extensive studies on these tests have been made by many workers. Their reports have been revealed as follows.

Wall (1965) has given a standard phenol colour reaction test method for testing wheat seeds for cultivar purity.

Buttery et al. (1968) studied the peroxidase activities in seeds of Soybean cultivars. They separated Soybean varieties into two main groups on the basis of higher or lower (positive) and no (negative) activities of peroxidase.

Banerjee and Chandra (1977) studied the modified phenol test for variety identification of wheat and used $CuSO_4$, thiourea and Na_2CO_3. They grouped varieties on the basis of colour of seed developed.

Clancy et al. (1982) conducted phenol test on seven winter wheat cultivars to determine at what stage of seed development this method can be used for varietal identification. They reported that the phenol test could be used for cultivar identification once the seed reached the hard dough stage and chlorophyll content has declined to 0.2 µg/mg dried chaff.

Wagner and McDonald (1982) used various laboratory tests namely hilum colour, hypocotyl colour, seed coat peroxidase and electrophoresis of B- amylase and urease in unimbibed seeds 0f thirty-six Soybean cultivars.

Panwar and Chandgiram (1988) studied eleven varieties of

wheat for identification by phenol and modified phenol tests. Their results indicated that phenol test formed primary groups while modified phenol test formed secondary groups. It has been suggested that phenol test may be supplemented by other laboratory techniques such as PAGE (Polyacrylamide Gel Electrophoresis) for effective discrimination of wheat varieties.

Chakrabarty and Agrawal (1990) studied growth response to added chemicals in blackgram varieties. The effect of growth hormones (IAA & GA_3), weedicide (2, 4- D & Lasso) and DDT on seedling growth was studied. Application of 2,4- D decreased the hypocotyl length. The sixteen blackgram cultivars were grouped as short hypocotyl length, medium hypocotyl length and long hypocotyl length.

The study of hypocotyl length and its response to various chemicals like growth hormones, herbicides and insecticides indicated that seedling growth and its response could easily be determined in laboratory on a routine basis and can be used as an important diagnostic trait for distinguishing blackgram varieties.

Dahiya et al. (1995) characterized Chickpea cultivars based on some laboratory tests. They used chemical tests for identifying the varieties, viz., response to 2,4- D, peroxidase activity and UV fluorescent test. They categorized the varieties for 2,4-D test as highly sensitive, sensitive and tolerent, while all the varieties showed negative reaction to seed coat peroxidase test.

Muthuraj et al. (1999) screened twenty nine Soybean cultivars for peroxidase test. Seeds of fourteen Soybean cultivars showed a positive and the rest fifteen cultivars a negative reaction with respect

to peroxidase test.

Sambsiva Rao et al. (2000) characterized thiryseven genotypes of groundnut by utilizing the biochemical tools. This study characterized and compared the total soluble seed proteins from extracts of individual seed by PAGE.

Seedling characteristics for their reaction to NaOH, KOH, GA_3 and 2,4- D were studied. It was possible to differentiate between all the cultivars into different groups like light brown and dark brown and also low, moderate and high response to coleoptile length.

Sivakumar et al. (2000) studied seventeen cultivars of Cluster bean (*Cymopsis tetragonoloba*) for variation of peroxidase activity in seed coat as well as cotyledon. Seed coat response was grouped into three (negative, dark brown and dark reddish brown) whereas in case of cotyledon response, four groups (negative, light reddish brown, red and dark red) were made.

Anonymous (2001). Simple laboratory tests such as Phenol (standard & modified), NaOH, $FeSO_4$, KOHand KOH- bleach tests were found very effective both for characterization and identification of varieties.

Using such techniques i.e. NaOH test, $FeSO_4$ test, Phenol (CuSO4) test and Phenol (Na2CO3) test, ten Chickpea varieties were classified including Vijay, Vishal and PG- 5.

2.3 Electrophoresis :-

Attempts were made earlier to classify varieties by electrophoretic studies in different crops by many workers. Their reports have been reviewed as follows.

Larsen (1967) used electrophoretic technique in many chemotaxonomic studies to find the relationship between different cultivars.

McKee (1973) gave emphasis on the use of chemistry in characterizing plant varieties and pointed out that the application of chemical and biochemical techniques to plant taxonomy at the species level is becoming of increasingly important. By definition, each variety of a cultivated species should differ from other varieties in one or more characteristics. Thus, if, varieties are distinct, there should be corresponding chemical differences. This fact plus the limited quantity of tissue available in an individual seed or seedling, suggest the use of electrophoretic techniques to help to identify varieties, perhaps, utilizing 'Fingerprint' techniques.

Konarev et al. (1981) reported electrophoretic methods useful for cultivar identification and testing cultivar purity and serological methods for species identification and determination of the species components and mixtures.

Blogg (1982) used starch gel electrophoresis for analysis of dry seed, cotyledons, unifoliate seedling, leaves and trifoliate leaves in seven Soybean cultivars. They obtained upto five distinct zymograms per enzyme for seven genotypes. Only four of the nine enzymes were useful in differentiating the seven genotypes.

Kapse and Nerkar (1985) identified Cotton cultivars using

polyacrylamide gel electrophoresis (PAGE) of seed proteins. Four intraspecies (*Gossypium hirsutum x Gossypium barbadense)* hybrids, the parents of hybrids and two varieties of the *Gossypium hirsutum* and *Gossypium arboreum* species were examined. The cultivars could be identified from electrophoregram of soluble proteins of single seed..Thus, the technique can serve as a supplement, if not a substitute to field tests in the genetic purity of Cotton cultivars.

McDonald and Drake (1990) evaluated a rapid electrophoretic system for varietal identification and testing. They reported that an increased number of new varietal release and an anticipated impact of biotechnology on varietal development will exelrate the ability of seed analyst to differentiate varieties by traditional seed technology approaches. However, problems associated with cost, number of seed samples per run and standardization of results have hindered its ready acceptance by those conducting seed quality tests.

Singh et al. (1992) analyzed the total salt soluble fractions in the non- denaturing PAGE System for distribution of protein profile along with an insoluble fraction (G1). A total of seven bands were recorded based on visual observations as major and minor bands. Total nine varieties were studied.

Cooke (1993) reported that the gel electrophoresis of seeds or vegetative proteins and enzymes is well documented and increasingly widely used techniques for the identification of varieties of agricultural and horticultural crops. Some aspects of uses of electrophoresis and practical application in quality control, distinctness testing and certification procedure have been reviewed briefly by him.

Cooke (1999) explained some of the modern method that are being applied to cultivar verification / identification namely the use of computerized image analysis system, the use of gel electrophoresis method to analyze protein and enzymes and the various DNA profiling techniques.

Anonymous (1999) electrophoretic techniques based on seed / seedling proteins or isozyme banding patterns were standardized. at IARA,New Delhi in different crops to identify genotypes. Following electrophoresis methods were found useful to identify cultivars.

Chickpea and Soybean- SDS- PAGE of soluble proteins.

Pigeonpea-SDS- PAGE of globulins in gradient gels.

Cotton-SDS-PAGE of soluble proteins and globulins.

Sunflower-SDS- PAGE of soluble proteins and Esterase isoenzymes.

Raghvirendra Sing et al. (2000) subjected twenty three Chickpea varities including both desi and kabuli namely,Annigiri,Avrodhi,c-235,CSG-8962,Dahod Yellow, DCP-92-3, Gaurav, GCP-101, GCP-102, H-86-18, Ujjain-21, Vijay, Vishal, HK-89-131, L-550, Pragati, Pusa-327, Pusa-1003 to SDS-polycridamide gel electrophoresis of total soluble seed proteins for variety identification. A total of 55 bands were observed. Desi varieties have more protein bands in comparison to five kabuli varieties. Two kabuli varieties L- 550 and Pusa- 267 can be distinguished clearly from the others as having least number of bands (18), moreover all the kabuli varieties have three bands of high molecular weight, number- 1,2 and 3 (Rm value 0.10, 0.11 and 0.12 respectively) which are absent in desi Chickpea varieties. Thus , this technique serves as an important tool to differentiate between desi and kabuli Chickpea varieties.

Gurpreet Singh et al. (2000) planned the varietal identification within different legume crops viz., Moong(Phaseolus mungo) Chickpea(Cicer arietinum), Urdbean (Vigna mungo) and Lentil (Lens esculanta) keeping in view,the importance of electrophoretic technique . A unique banding pattern having 12 - 21 bands from six varities of moong seeds was obtained using SDS - PAGE at 15% gel concentration. Band number 11 was present only in local varieties and absent in all other varieties. Band number 3 was absent in Ps varieties and present in SML varieties . Work on varietal identification of other crops is in progress.

Anonymous (2001) electrophoresis and other simple laboratory techniques were standardized for characterization and identification of Chickpea , Soybean , Rice, Cotton, Pigeonpea, Maize and Sorghum varieties. SDS - PAGE profiles of total soluble seed protein, salt soluble and alcohol soluble protein fractions of ten Chickpea , eighteen Soybean , thirty-one Rice , twenty-three Cotton, twelve Pigeonpea and twelve Sorghum varieties were performed.

MATERIAL AND METHODS

3. MATERIAL AND METHODS

The present investigation entitled "Characterization of Chickpea cultivars (desi & kabuli) through morphological, chemical and electrophoretic tests" was carried out at Seed Technology Research Unit (NSP), Mahatma Phule Krishi Vidyapeeth, Rahuri – 413722, Dist.- Ahmednagar, Maharashtra, during Rabi 2001- 02 and 2002- 03.

The details of the seed material used and methods adopted for characterizing different cultivars in the present studies are given under various subheadings in this chapter.

3.1 Experimental material :-

The genetically pure seeds of the following twelve Chickpea cultivars (desi & kabuli) were obtained from Pulses Breeder, Pulses Improvement Scheme, M. P. K. V., Rahuri.

Sr. No.	Name if cultivar	Pedigree
	Desi type	
1	PG - 5	B –11 x N – 31
2	PG – 12	GW – 517 x Ceylon - 2
3	Vijay	P – 1270 x Annigeri
4	Vishal	K – 850 x ICC - 80074
5	PG – 92926	ICCC – 42 x ICC -12237
6	PG – 96005	ICCC – 42 x ICCV – 10
7	PG – 96006	ICCC – 42 x ICCV – 10

	Kabuli type	
8	Virat	(ICC–7676xICCC–32)x[(ICCC-49x FLIP-82-1C)xICCV-3
9	PG - 92307	(ICCV-2 x Surutato 77) x ICC -7344
10	PG - 95311	(ICCC-32xICCL-80004)x[(ICCC-49x FLIP-81-86)xICCV-3
11	PG - 95421	(ICCC-32xL-144)x[(ICCC-49xFLIP-82-1C)x[ICCV-3]
12	KAK - 2	(ICCV-2xSurutato-77)xICC-7344

Pure seeds of each cultivars were examined for seed, seedling and plant morphological characters. The chemical tests i.e. 2,4 – D test, peroxidase test, NaOH test, phenol test and modified phenol test and electrophoretic test i.e. separation of seed storage proteins by SDS–PAGE method of total soluble seed proteins were also performed.

3.2 Field Experiment Method :-

Field experiments were conducted during Rabi 2001- 02 and 2002- 03.

Details of experiment :-

i. No. of rows :- two rows of 4.95 x 5 m^2 for each genotype.

ii. No. of replications :- two

iii. Spacing :- 45 x 15 cm.

iv. Season :- Rabi-2001and Rabi-2002.

The genetically and physically pure seeds of twelve Chickpea genotypes were sown in two rows each at Seed Technology

Research Unit farm, M. P. K. V., Rahuri.

The recommended dose of fertilizers was applied at the time of sowing. One irrigation was given after sowing and subsequent irrigations were given as and when required. The weeding and interculturing operations were done from time to time and experimental plot was kept clean throughout the period.

To study the stable diagnostic characteristics, the observations were noted at seedling, flowering and maturity stages on five randomly selected plants of each genotype.

3.2.1 Morphological characters :-

Observations were recorded for seedling, plant and seed characters and categorized into different groups as given in table.

Table- Characters studied for their variations :-

Sr. No.	Characters	Plate No.	Category	Stage
1	Pigmentation on seedling	1	a. Green b. Purple	Seedling
2	Colouration of leaflet	2	a. Green b. Purple	Seedling
3	Growth habit	3	a. Erect b. Semispreading c. Spreading	50% flowering
4	Branching habit	4	a. Less(4-5 branches) b. Medium(6-9branches) c. Profuse (>9 branches)	50% flowering
5	Stem colour	5	a. Green b. Purple	50% flowering
6	Foliage colour	6	a. Green b. Dark green	50% flowering

7	Leaflet size	7	a. Small b. Medium c. Large	50% flowering
8	Leaflet shape	8	a. Oval b. Ovate	50% flowering
9	Leaflet margin	9	a. Medium b. High	50% flowering
10	Flower colour	10	a. White b. Pink c. Dark pink	50% flowering
11	Days to flower	-	a. Late (>60 days) b. Medium (50-60 days) c. Early (40-50 days)	Initiation of flowering
12	Plant height	11	a. Dwarf (<50cm.) b. Medium (50-60cm.) c. Tall (>60cm.)	Maturity
13	Pods per plant	-	a. Less (<70 pods) b. Medium (70-100 pods) c. High (>100 pods)	Harvesting
14	No. of seeds per pod	12	a. Single b. Double	Harvesting
15	Pod size at maturity	13	a. Small b. Medium c. Bold	Maturity
16	Seed size (100-seed wt.)	14	a. Small(<20g/100seed) b. Medium(20-30g/100seed) c. Bold (>30g/100seed)	Ripe seed
17	Seed surface texture	15	a. Smooth b. Wrinkled	Ripe seed
18	Seed colour	16	a. White b. Brown c. Dark brown	Ripe seed

3.2.3 Chemical studies :-

1. Phenol test:-

Twenty seeds of each genotype in four replication were soaked in water for 16 hrs. at room temperature (25-30 ^{0}C) . The soaked seeds were placed in petridish ,lined with filter paper soaked with 1% phenol solution . The petridishes were kept under laboratory condition at 30± 1 ^{0}C . The final reaction was observed after four hours and genotypes were classified into following colour reaction groups –

i. No colour reaction.
ii. Light brown.
iii. Brown.

2. Modified phenol test :-

In phenol test, it is difficult to identify individual cultivars within a colour group. This limitation could be avoided considerably by modified phenol test using critical concentration of chemicals such as copper sulphate and sodium carbonate.

Modified phenol test with copper sulphate :-

Twenty seeds of each variety in four replication were soaked in copper sulphate in concentration of 0.04% for 16 hrs. at 20 ± 1 ^{0}C temperature. The soaked seeds were placed in petridishes , lined with 1% phenol solution. The petridishes were kept under laboratory condition at 30 ± 1 ^{0}C. The final reaction was observed after 4 hrs. and genotypes were classified into three colour reaction groups mentioned as in phenol test.

Modified phenol test with sodium carbonate :-

In this test seeds were soaked in sodium carbonate of 0.06% concentration and similar procedure is carried out as mentioned under modified phenol test with copper sulphate.

3. Peroxidase activity test :-

The seed coat was crushed and put in test tubes, then about ten drops of 0.5% guaiacol was added. After ten minutes one drop of 0.1% Hydrogen Peroxide was added. One minute after adding the H_2O_2, the seed coat was observed for peroxidase activity. All the genotypes were grouped according to the intensity of colour reaction into two categories as moderate and high.

4. NaOH test :-

The NaOH reaction for seed coat colour was observed with 10-15 seeds of each genotype in two replications. The seeds were soaked in 5% NaOH solution at room temperature (25-30 ^{0}C). The change in colour of seed coat was observed after four hours. The cultivars were classified into two colour reaction groups as dark orange red colour and no colour.

5. 2,4- D test :-

Fifty randomly selected seeds (10 seeds each in five replications) were placed in two layers of moistened germination towels. These rolled towels were then placed in vertical position in a seed germinator at 25 ^{0}C. At the end of seventh day, rolled towels were taken out from the germinator and hypocotyl length was measured in mm. on five randomly selected seedlings. The varieties were classified into short, medium and long respectively for hypocotyl length. For studying the effect of weedicides, the seedlings were

raised in similar manner except that the germination towels were moistened with different solutions prepared in distilled water. The hypocotyl growth response to weedicides was determined on the basis of percent increase or decrease in hypocotyl length over that of control.

The varieties were classified into highly affected, moderately affected and least affected for 2,4- D solution of 5 ppm concentration.

3.2.3 Electrophoresis :-

Sodium Dodecyl Sulphate Polyacylamide Gel Electrophoresis (SDS-PAGE) on individual seed from each genotype was conducted. The detailed methodology is described below.

Material :-

The genetically pure seed of each variety was used for electrophoresis study.

Apparatus and Equipments :-

i. The vertical slab gel electrophoresis apparatus of ATTO make.
ii. Rubber gasket
iii. Spacer (1mm)
iv. Comb (13 wells)
v. Automatic power supply

Chemicals / Reagents :-

The following chemicals / reagents (analytical grades) were used for preparation of different solutions.

i. Acrylamide (specially purified for electrophoresis)
ii. Bis- acrylamide (specially purified for

electrophoresis)

iii. Tris buffer

iv. Glacial acetic acid

v. Glycine

vi. Ammonium Per Sulphate (APS)

vii. N.N.N.N'.Tetramethylene diamine (TEMED)

viii. Ethanol

ix. Hydrocloric acid

x. Bromophenol blue dye

xi. Sucrose

xii. Commassic brilliant blue salt

Methodology :-

I. Single seed of each genotype was extracted in 0.3-0.5 ml of working protein extraction solution and kept at room temperature for overnight.

ii. The samples were kept in boiling water bath for ten minutes.

iii. The samples were centrifused at 12,000 rpm for 15 minutes in refrigerated centrifuse.

iv. Clear supernatant of protein extract was used for loading on gel.

Gel preparation :-

A. Separating gel (10%)

i. Tris – HCl (pH 8.8) – 12 ml

ii. Distilled water – 7.4 ml

iii. 30% Acrylamide – 20 ml

iv. 10% SDS – 0.4 ml

v. 5% APS (freshly prepared) – 0.6 - 0.8 ml

vi. TEMED – 40µl

B. Staking gel :-

i. Tris – HCl (pH 6.8) – 1.5 ml

ii. Distilled water – 6 ml

iii. 30% Acrylamide – 2ml

iv. 5% APS (freshly prepared) – 0.6 – 0.8 ml

v. 10% SDS – 0.10 ml

vi. TEMED – 40µl

Sample loading :-

10uml of clear supernatant of protein extract solution per well was loaded.

Electrophoresis :-

Voltage :- 220 Volts

Current :-

i. Initially 1.5 mA per well till the tracking dye reach at the interface of separating gel.

ii. the current was subsequently increased to 2 mA per well till the dye reached to the end of resolving gel.

Staining :-

After complete running, gels were stained in 2.5% Commassic Brilliant Blue (R-250) overnight, the gels were destained several times with destaining solution.

Staining solution :-

i. Commassic Brilliant Blue – 2.5 g

ii. Glacial acetic acid -100 ml

iii. Methanol –500 ml

Make final volume of one liter with distilled water.

Destaining solution :-

Destaining of gel should be done with the help of following chemicals –

i. Glacial acetic acid – 70 ml

ii. Methanol - 250ml

Make final volume of one liter with distilled water.

Interpretation :-

i. Presence or absence of specific bands.

ii. Intensity of bands- a. Dense (D)
b. Medium (M)
c. Light (L)
d. Weak (W)

$$\text{Rm value} = \frac{\text{Distance traveled by protein bands.}}{\text{Distance traveled by tracking dye.}}$$

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

The pure seeds of twelve Chickpea (*Cicer arietinum* L.) cultivars were analyzed visually for their morphological features of seedling, plant and seed. The chemical test viz., phenol colour reaction, modified phenol test, peroxidase test, NaOH test and 2,4- D test and electrophoresis of seed protein for banding pattern was carried out separately. The results obtained from these studies are presented below.

4.1 Morphological tests :-

The results of morphological studies of Chickpea cultivars were classified on the basis of seedling, plant and seed characteristics.

4.1.1 Seedling morphology :-

The seedling characteristics of Chickpea cultivars were studied in field and all the twelve cultivars were classified on the basis of pigmentation on seedling and colouration if leaflet at seedling stage as under.

a. Pigmentation on seedling :-

On the basis of pigmentation on seedling at seedling stage (10 DAS), the seedlings were observed and all twelve cultivars were grouped into two categories as green (PG-12, Vijay, Virat, PG-92307, PG-95311, PG-95421, KAK-2) and purple(PG-5, Vishal, PG-92926, PG-96005, PG-96006).

b. Colouration of leaflet at seedling stage :-

On the basis if colouration of leaflet at seedling stage, the cultivars were classified into two classes as green(PG-12, Vijay,

Plate No. 1

Pigmentation on seedling

Green

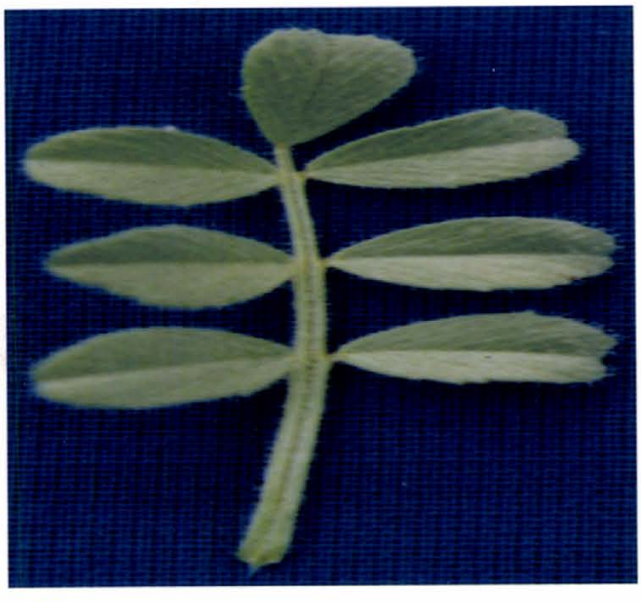
(PG-12,Vijay, Virat,
PG-92307,PG-95311,
PG-95421,KAK-2)

Purple

(PG-5,Vishal,PG-92926,
PG-96005,PG-96006)

Plate No.2.

Colouration of leaflet at Seedling stage



Green

(PG- 12, Vijay, Virat,
PG-92307,PG-95311,
PG-95421,KAK-2)

Purple

(PG- 5, Vishal,
PG--92926,
PG-96005, PG-96006)

Virat, PG-92307, PG-95311, PG-95421, KAK-2) and purple(PG-5, Vishal, PG-92926, PG-96005, PG-96006).

4.1.2 Plant morphology

The plant characteristics of Chickpea were studied under field conditions during 2001- 2003 and all the twelve cultivars were classified on the basis of plant morphology as follows.

a. Growth habit :-

On the basis of growth habit at 50% flowering, the plants were observed and all the twelve genotypes were categorized into three classes as spreading (PG-12, Vijay, and KAK-2), semispreading (PG-92926, Virat, PG -92307, PG-95311,PG-95421) and erect (PG-5, Vishal, PG-96005,PG-96006).

b. Branching habit :-

On the basis of number of primary and secondary branches, observed at 50% flowering, the twelve cultivars were grouped into three classes as less (PG-12), medium (PG-5, Vijay, Vishal, PG-92926, PG-96005, PG-96006, Virat, PG-95421, KAK-2) and profuse(PG-92307, PG-95311) branching habit.

c. Stem colour :-

On the basis of stem colour at 50% flowering, all the cultivars were grouped into two groups as green (PG-5, Virat, PG-92307, PG-95421, KAK-2)and purple (PG-12, Vijay, Vishal, PG-92926, PG-96005, PG-96006).

d.Foliage colour :-

On the basis of foliage colour, at 50% flowering stage, the cultivars were classified into two classes as green (PG-12, Vijay, PG-

Plate No. 3

Growth habit

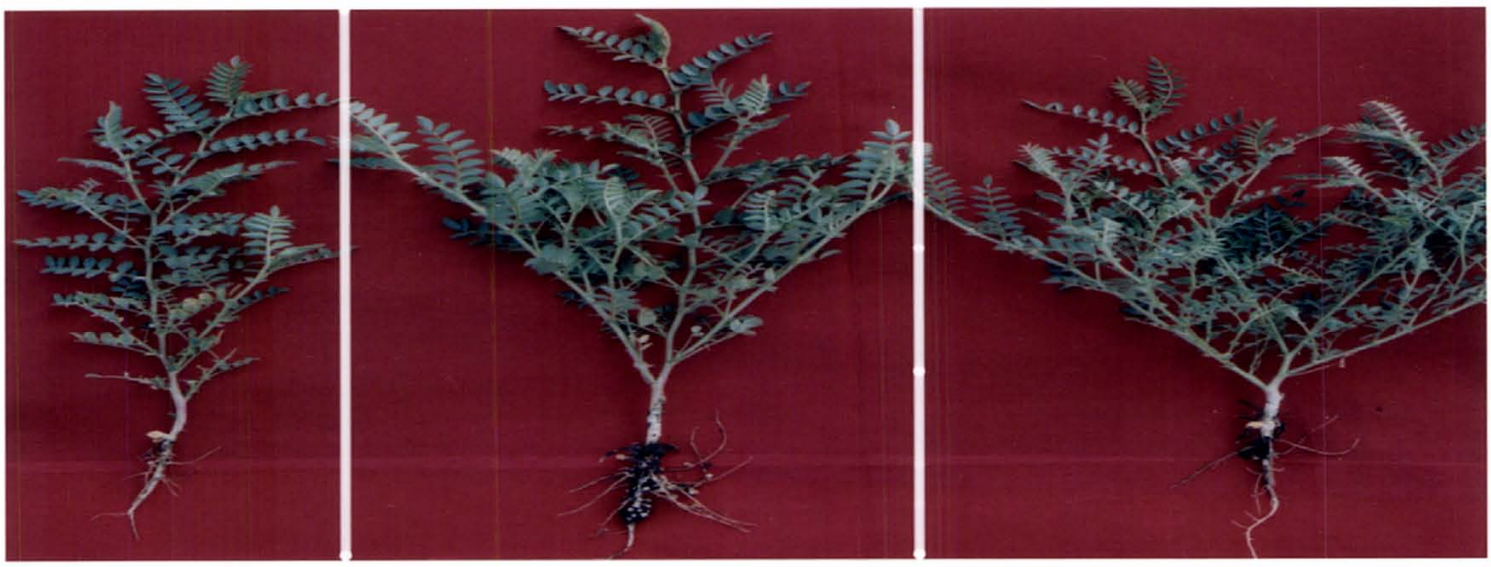
Erect
(PG-5, Vishal, PG-96005, PG-96006)

Semi spreading
(PG-92926, Virat, PG-92307, PG-95311, PG-95421)

Spreading
(PG-12, Vijay, KAK-2)

Plate No.4

Branching habit



Less
(PG-12)

Medium
(PG-5, Vijay, Vishal, PG-92926, PG-96005, PG-96006, Virat, PG-95421, KAK-2)

Profuse
(PG-92307, PG-95311)

Plate No. 5

Stem Colour

Green

(PG-5, Virat,
PG-92307,PG-95311,
PG-95421,KAK-2)

Purple

(PG-12,Vijay,Vishal,
PG-92926,
PG-96005,PG-96006)

Plate No.6

Foliage Colour

Green

(PG- 12, Vijay, Virat,
PG-96006,PG-92307,
PG-95311,PG-95421,KAK-2)

Dark Greeen

(PG- 5, Vishal,
PG--92926,PG-96005)

96006, Virat, PG-92307, PG-95311, PG-95421, KAK-2) and dark green(PG-5, Vishal, PG-92926, PG-96005).

d. Leaflet size :-

On the basis of leaflet size, twelve Chickpea genotypes were grouped into three classes as small (PG-12, Vijay), medium (PG-5, Vishal, PG-92926, PG-96005, PG-96006, Virat, PG-95311) and large(PG-92307, PG-95421, KAK-2).

e. Leaflet shape :-

On the basis of leaflet shape, all the cultivars were categorized under two classes as oval and ovate. All the twelve cultivars were having oval leaf shape.

f. Leaflet margin :-

On the basis of leaflet margin, at 50% flowering stage, the twelve genotypes of Chickpea were classified into two classes as medium (PG-5, PG-12, Vijay, Vishal, PG-92926, PG-96005, PG-96006, PG-92307, PG-95311, PG-95421, KAK-2) and high (Virat) leaf serration.

g. Flower colour :-

On the basis of flower colour, at initiation of flowering, all the Chickpea genotypes were grouped into three classes as white (Virat, PG-92307, PG-95311, PG-95421, KAK-2), pink (PG-5, Vishal) and dark pink (PG-12, Vijay, PG-92926, PG-96005, PG-96006).

h. Days to flower :-

On the basis of days to 50% flowering all the genotypes were grouped into two groups as medium (PG-5, Virat, PG-95311) and early (PG-12, Vijay, Vishal, PG-92926, PG-96005, PG-96006, PG-92307, PG-95421, KAK-2).

Plate No. 7

Leaflet size

Small
(PG-12,Vijay)

Medium
(PG-5, Vishal, PG-92926,Virat, PG-96005,PG-96006, PG-95311)

Large
(PG-92307, PG-95421, KAK-2)

Plate No.8

Leaflet shape

Oval
(PG-5,PG- 12, Vijay, Vishal, PG-92926,PG-96005, PG-95311, Virat,PG-96006, PG-92307,PG-95421,KAK-2

Ovate

Plate No. 9

Leaflet margin

Medium

(PG-5,PG-12,Vijay, Vishal,PG-92307,PG-92926, PG-95311,PG-96006, PG-96005,PG-95421,KAK-2)

High

(Virat)

Plate No.10

Flower Colour

White

(Virat, PG-92307, PG-95311,PG-95421, KAK-2)

Pink

(PG-5, Vishal)

Dark Pink

(PG-12, Vijay, PG-92926,PG-96005, PG-96006)

i. Plant height :-

On the basis of plant height, at maturity, the twelve Chickpea genotypes were grouped into three groups as dwarf (PG-12, Vijay, PG-92926), medium (PG-5, Vishal, PG-96005, PG-96006, Virat, PG-92307, KAK-2) and tall (PG-95311, PG-95421).

j. Number of pods per plant :-

On the basis of number of pods recorded at harvesting, the cultivars were classified as less pods (PG-5, PG-12, Vishal, PG-92926, Virat), medium pods (Vijay, PG-96005, PG-92307, KAK-2) and high pods (PG-96006, PG-95311, PG-95421).

k. Number of seeds per pod :-

On the basis of number of seeds per pod, at harvesting stage, the twelve cultivars were grouped into two classes as single seeded (PG-5, PG-12, Vijay, Vishal, PG-92926, Virat, PG-92307, PG-95311, PG-95421) and double seeded (PG-96005, PG-96006, KAK-2).

l. Pod size at maturity :-

On the basis of pod size at maturity, all the cultivars were classified as small (PG-12, Vijay), medium (PG-5, Vishal, PG-92926, PG-96005) and bold (PG-96006, Virat, PG-92307, PG-95311, PG-95421, KAK-2).

4.1.3 Seed morphology :-

On the basis of seed characteristics, the twelve Chickpea genotypes were classified as under.

a. Seed size (weight of 100- seeds in gram) :-

On the basis of seed size, at full ripe stage, all the cultivars were categorized into three classes as small (PG-12, Vijay, PG-

Plate No. 11

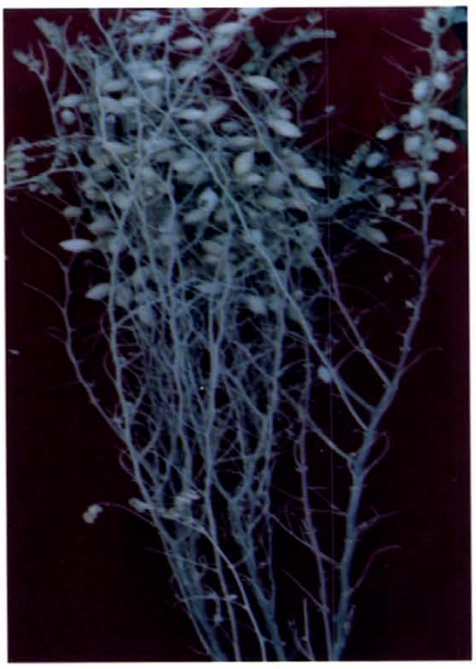
Plant height

Dwarf

(PG-12, Vijay, PG-92926)

Medium

(PG-5, Vishal, PG-96005, PG-96006, Virat, PG-92307, KAK-2)



Tall

(PG-95311, PG-95421)

Plate No.12

Number of Seeds / Pod

Singal

(PG-5, PG-12, Vijay, Vishal, PG-92926, Virat, PG-92307, PG-95311, PG-95421)

Double

(PG-96005, PG-96006, KAK-2)

Plate No. 13

Pod Size at maturity

Small
(PG-12, Vijay)

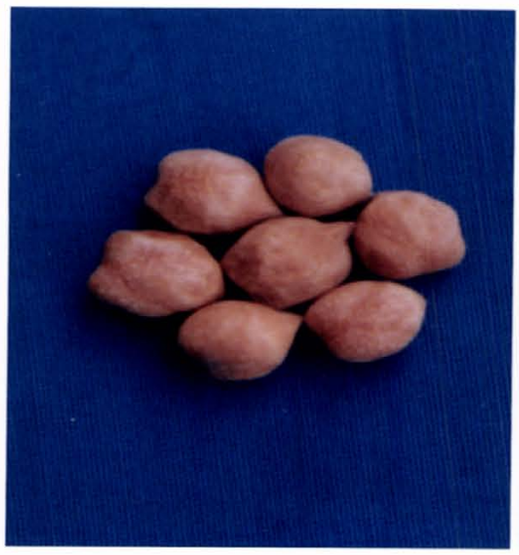
Medium
(PG-5, Vishal, PG-92926, PG-96005)

Bold
(PG-96006, Virat, PG-92307, PG-95311, PG-95421, KAK-2)

Plate No. 14

Seed Size

Small
(PG-12, Vijay, PG-96006)

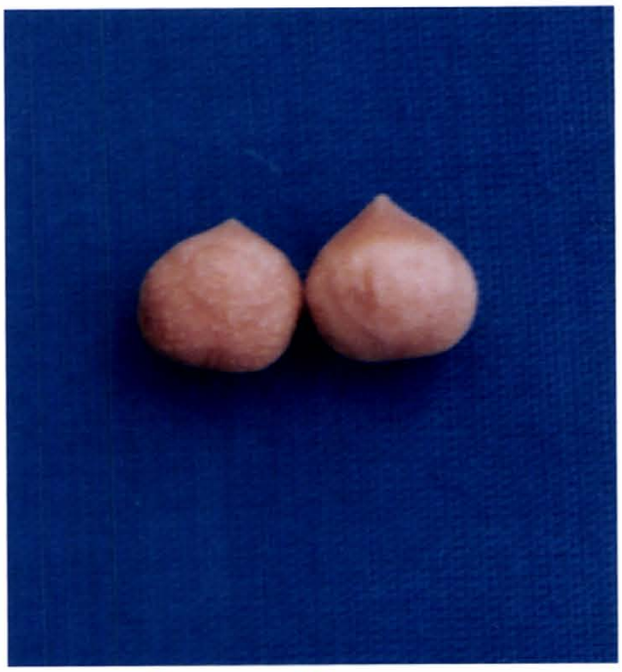


Medium
(PG-5, Vishal, PG-92926, PG-96005, PG-95421)

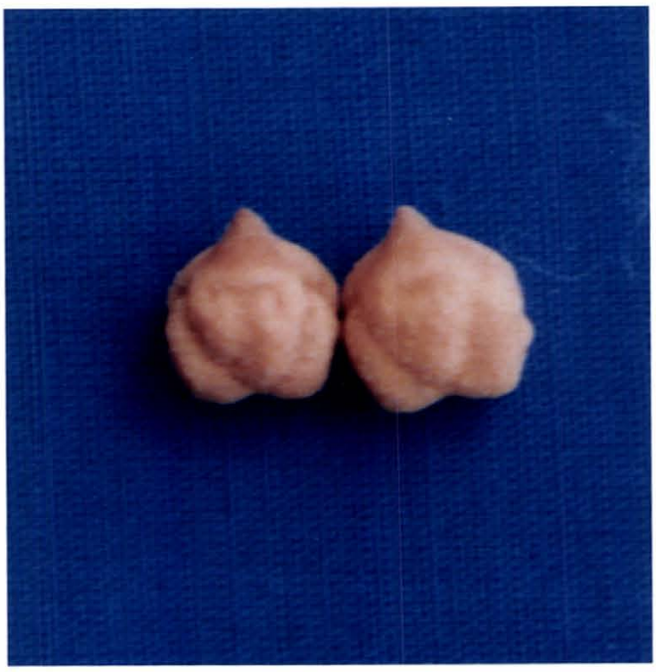
Bold
(Virat, PG-92307, PG-95311, KAK-2)

Plate No. 15

Seed surface texture



Smooth
(PG-5,PG-92307)



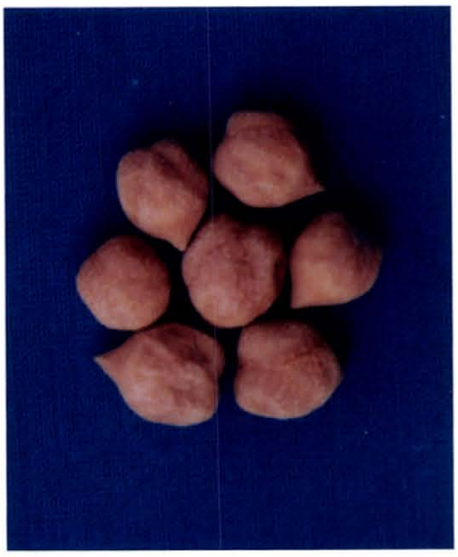
Wrinkled
(PG-12,Vijay,Vishal,
PG-92926,PG-96005,
PG-96006, Virat,
PG--95311,PG-95421,KAK-2)

Plate No.16

Seed Colour

White
(Virat,PG-92307,
PG-95311,
PG-95421,
KAK-2)

Brown
(PG-96006,
PG-96005,Vishal,
PG-92926, Vijay,
PG-12)



Dark brown
(PG-5)

96006), medium (PG-5, Vishal, PG-92926, PG-96005, PG-95421) and bold (Virat, PG-92307, PG-95311, KAK-2).

b. Seed surface texture :-

On the basis of seed surface texture, all the genotypes were classified into two classes as smooth (PG-5, PG-92307) and wrinkled (PG-12, Vijay, Vishal, PG-92926, PG-96005, PG-96006, Virat, PG-95311, PG-95421, KAK-2).

c. Seed colour :-

On the basis of seed colour, all the twelve cultivars under study were classified into three classes as white (Virat, PG-92307, PG-95311, PG-95421, KAK-2), brown (PG-12, Vijay, Vishal, PG-92926, PG-96005, PG-96006) and dark brown (PG-5).

PG - 5

Pedigree :- B- 11 x N- 31

Year of release :- 1982

Area of adoption :- Central zone and South zone

Distinguishing characters :- (Plate No.-19)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Purple
2.	Colouration of leaflet at seedling stage	Purple
3.	Growth habit	Erect
4.	Branching habit	Medium
5.	Stem colour	Green
6.	Foliage colour	Dark green
7.	Leaflet size	Medium
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	Pink
11.	Days to flower	Medium (53days)
12.	Plant height	Medium (50.14cm.)
13.	Pods per plant	Less (48pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Medium
16.	Seed size (100- seed weight)	Medium
17.	Seed surface texture	Smooth
18.	Seed colour	Dark brown

PG- 12

Pedigree :- GW- 517 x Ceylon- 2

Year of release :- 1985

Area of adoption :- Maharashtra State

Distinguishing characters :- (Plate No.-20)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Green
2.	Colouration of leaflet at seedling stage	Green
3.	Growth habit	Spreading
4.	Branching habit	Low
5.	Stem colour	Purple
6.	Foliage colour	Green
7.	Leaflet size	Small
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	Pink
11.	Days to flower	Early (47days)
12.	Plant height	Dwarf (43.72 cm.)
13.	Pods per plant	Less (70 pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Small
16.	Seed size (100- seed weight)	Small
17.	Seed surface texture	Wrinkled
18.	Seed colour	Brown

Plate No. 19

PG-5

Plate No. 20

PG-12

Plate No. 21

Vijay

Plate No. 22

Vishal

Vijay

Pedigree :- P- 1270 x Annigeri

Year of release :- 1993

Area of adoption :- Central zone

Distinguishing characters :- (Plate No.-21)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Green
2.	Colouration of leaflet at seedling stage	Green
3.	Growth habit	Spreading
4.	Branching habit	Medium
5.	Stem colour	Purple
6.	Foliage colour	Green
7.	Leaflet size	Small
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	Dark pink
11.	Days to flower	Early (45 days)
12.	Plant height	Dwarf (48.5 cm.)
13.	Pods per plant	Medium (70 pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Small
16.	Seed size (100- seed weight)	Small
17.	Seed surface texture	Wrinkled
18.	Seed colour	Brown

Vishal

Pedigree :- K- 850 x ICC- 80074

Year of release :- 1995

Area of adoption :- Maharashtra State

Distinguishing characters :- (Plate No.-22)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Purple
2.	Colouration of leaflet at seedling stage	Purple
3.	Growth habit	Erect
4.	Branching habit	Medium
5.	Stem colour	Purple
6.	Foliage colour	Dark green
7.	Leaflet size	Medium
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	Pink
11.	Days to flower	Early (45 days)
12.	Plant height	Medium (55.02 cm.)
13.	Pods per plant	Less (57 pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Medium
16.	Seed size (100- seed weight)	Medium
17.	Seed surface texture	Wrinkled
18.	Seed colour	Brown

PG- 92926

Pedigree :- ICCC- 42 x ICC- 12237

Year of release :- Promising variety

Distinguishing characters :- (Plate No.-23)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Purple
2.	Colouration of leaflet at seedling stage	Purple
3.	Growth habit	Semispreading
4.	Branching habit	Medium
5.	Stem colour	Purple
6.	Foliage colour	Dark green
7.	Leaflet size	Medium
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	Dark pink
11.	Days to flower	Early (45 days)
12.	Plant height	Dwarf (49.16 cm.)
13.	Pods per plant	Less (57 pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Medium
16.	Seed size (100- seed weight)	Medium
17.	Seed surface texture	Wrinkled
18.	Seed colour	Brown

PG- 96005

Pedigree :- ICCC- 42 x ICCV- 10

Year of release :- Promising variety

Distinguishing characters :- (Plate No.-24)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Purple
2.	Colouration of leaflet at seedling stage	Purple
3.	Growth habit	Erect
4.	Branching habit	Medium
5.	Stem colour	Purple
6.	Foliage colour	Dark green
7.	Leaflet size	Medium
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	Dark pink
11.	Days to flower	Early (47 days)
12.	Plant height	Medium (58.08)
13.	Pods per plant	Medium (88 pods)
14.	Number of seeds per pod	Double
15.	Pod size at maturity	Medium
16.	Seed size (100- seed weight)	Medium
17.	Seed surface texture	Wrinkled
18.	Seed colour	Brown

PG- 96006

Pedigree :- ICCC- 42 x ICCV- 10

Year of release :- Promising variety

Distinguishing characters :- (Plate No.-25)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Purple
2.	Colouration of leaflet at seedling stage	Purple
3.	Growth habit	Erect
4.	Branching habit	Medium
5.	Stem colour	Purple
6.	Foliage colour	Green
7.	Leaflet size	Medium
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	Dark pink
11.	Days to flower	Early (47 days)
12.	Plant height	Medium (59.68 cm.)
13.	Pods per plant	High (117 pods)
14.	Number of seeds per pod	Double
15.	Pod size at maturity	Bold
16.	Seed size (100- seed weight)	Small
17.	Seed surface texture	Wrinkled
18.	Seed colour	Brown

Virat

Pedigree :- (ICC-7676xICCC-32)x[(ICCC-49xFLIP-82-1C)xICCV-3

Year of release :- 2000

Area of adoption :- Maharashtra State

Distinguishing characters :- (Plate No.-26)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Green
2.	Colouration of leaflet at seedling stage	Green
3.	Growth habit	Semispreading
4.	Branching habit	Medium
5.	Stem colour	Green
6.	Foliage colour	Green
7.	Leaflet size	Medium
8.	Leaflet shape	Oval
9.	Leaflet margin	High
10.	Flower colour	White
11.	Days to flower	Medium (54 days)
12.	Plant height	Medium (58.64 cm.)
13.	Pods per plant	Less 59 pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Bold
16.	Seed size (100- seed weight)	Bold
17.	Seed surface texture	Wrinkled
18.	Seed colour	White

Plate No.23

PG-92926

Plate No. 24

PG-96005

Plate No. 25

PG-96006

Plate No. 26

Virat

PG- 92307

Pedigree :- (ICCV-2xSurutato-77)x ICC-7344

Year of release :- Promising variety

Distinguishing characters :- (Plate No.-27)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Green
2.	Colouration of leaflet at seedling stage	Green
3.	Growth habit	Semispreading
4.	Branching habit	Profuse
5.	Stem colour	Green
6.	Foliage colour	Green
7.	Leaflet size	Large
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	White
11.	Days to flower	Early (44 days)
12.	Plant height	Medium (57.22 cm.)
13.	Pods per plant	Medium (94 pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Bold
16.	Seed size (100- seed weight)	Bold
17.	Seed surface texture	Smooth
18.	Seed colour	White

PG- 95311

Pedigree :- (ICCC-32xICCL-80004)x[(ICCC-49 xFLIP-82-86)xICCV-3

Year of release :- 2002

Area of adoption :- South zone

Distinguishing characters :- (Plate No.-28)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Green
2.	Colouration of leaflet at seedling stage	Green
3.	Growth habit	Semispreading
4.	Branching habit	Profuse
5.	Stem colour	Green
6.	Foliage colour	Green
7.	Leaflet size	Medium
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	White
11.	Days to flower	Medium (54 days)
12.	Plant height	Tall (61.80 cm.)
13.	Pods per plant	High (124 pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Bold
16.	Seed size (100- seed weight)	Bold
17.	Seed surface texture	Wrinkled
18.	Seed colour	White

Plate No. 27
PG-92307

Plate No. 28
PG-95311

Plate No. 29
PG-95421

Plate No.30
KAK-2

PG- 95421

Pedigree :- (ICCC-32xL-144)x[(ICCC-49xFLIP - 82- 1C)xICCV-3

Year of release :- Promising variety

Distinguishing characters :- (Plate No.-29)

Sr. No.	Characters	Category
1.	*Pigmentation on* seedling	Green
2.	Colouration of leaflet at seedling stage	Green
3.	Growth habit	Semispreading
4.	Branching habit	Medium
5.	Stem colour	Green
6.	Foliage colour	Green
7.	Leaflet size	Large
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	White
11.	Days to flower	Early (44 days)
12.	Plant height	Tall (66.50 cm.)
13.	Pods per plant	High (136 pods)
14.	Number of seeds per pod	Single
15.	Pod size at maturity	Bold
16.	Seed size (100- seed weight)	Medium
17.	Seed surface texture	Wrinkled
18.	Seed colour	White

KAK - 2

Pedigree :- (ICCV-2xSurutato-77)xICC-7344

Year of release :- 1999

Distinguishing characters :- (Plate No.-30)

Sr. No.	Characters	Category
1.	Pigmentation on seedling	Green
2.	Colouration of leaflet at seedling stage	Green
3.	Growth habit	Spreading
4.	Branching habit	Medium
5.	Stem colour	Green
6.	Foliage colour	Green
7.	Leaflet size	Large
8.	Leaflet shape	Oval
9.	Leaflet margin	Medium
10.	Flower colour	White
11.	Days to flower	Early (45 days)
12.	Plant height	Medium (57.50 cm.)
13.	Pods per plant	Medium (79 pods)
14.	Number of seeds per pod	Double
15.	Pod size at maturity	Bold
16.	Seed size (100- seed weight)	Bold
17.	Seed surface texture	Wrinkled
18.	Seed colour	White

4.2 chemical tests :-

4.2.1 Phenol test :-

The phenol test was carried out according to standard procedure. However, no colour reaction was noticed in any of the Chickpea cultivars studied.

4.2.2 Modified Phenol test :-

The modified phenol tests using $CuSO_4$ and Na_2CO_3, were performed according to standard procedure. However, all the cultivars showed no colour reaction in both of the cases.

4.2.3 Peroxidase test :-

The standard peroxidase test was carried out. But all the Chickpea cultivars under study showed negative peroxidase activity.

4.2.4 NaOH test :-

The standard NaOH test for seed coat reaction was carried out. according to this test, the cultivars were grouped into two colour reaction groups as dark orange red (PG-5, PG-12, Vijay, Vishal, PG-92926, PG-96005, PG-96006) and no colour [negative] (Virat, PG-92307, PG-95311, PG-95421, KAK-2).

4.2.5 2,4 – D test :-

The 2,4 – D test was carried out according to standard procedure. However, all the twelve Chickpea cultivars showed only radical initiation (highly affected).

Table – Results of chemical tests :-

Cultivars	Phenol test	Modified phenol test	Peroxidase test	NaOH test	2,4 – D test
PG-5	Negative	Negative	Negative	Dark orange red colour	Highly affected
PG-12	Negative	Negative	Negative	Dark orange red colour	Highly affected
Vijay	Negative	Negative	Negative	Dark orange red colour	Highly affected
Vishal	Negative	Negative	Negative	Dark orange red colour	Highly affected
PG-92926	Negative	Negative	Negative	Dark orange red colour	Highly affected
PG-96005	Negative	Negative	Negative	Dark orange red colour	Highly affected
PG-96006	Negative	Negative	Negative	Dark orange red colour	Highly affected
Virat	Negative	Negative	Negative	No colour reaction	Highly affected
PG-92307	Negative	Negative	Negative	No colour reaction	Highly affected
PG-95311	Negative	Negative	Negative	No colour reaction	Highly affected
PG-95421	Negative	Negative	Negative	No colour reaction	Highly affected
KAK-2	Negative	Negative	Negative	No colour reaction	Highly affected

Plate No. 17

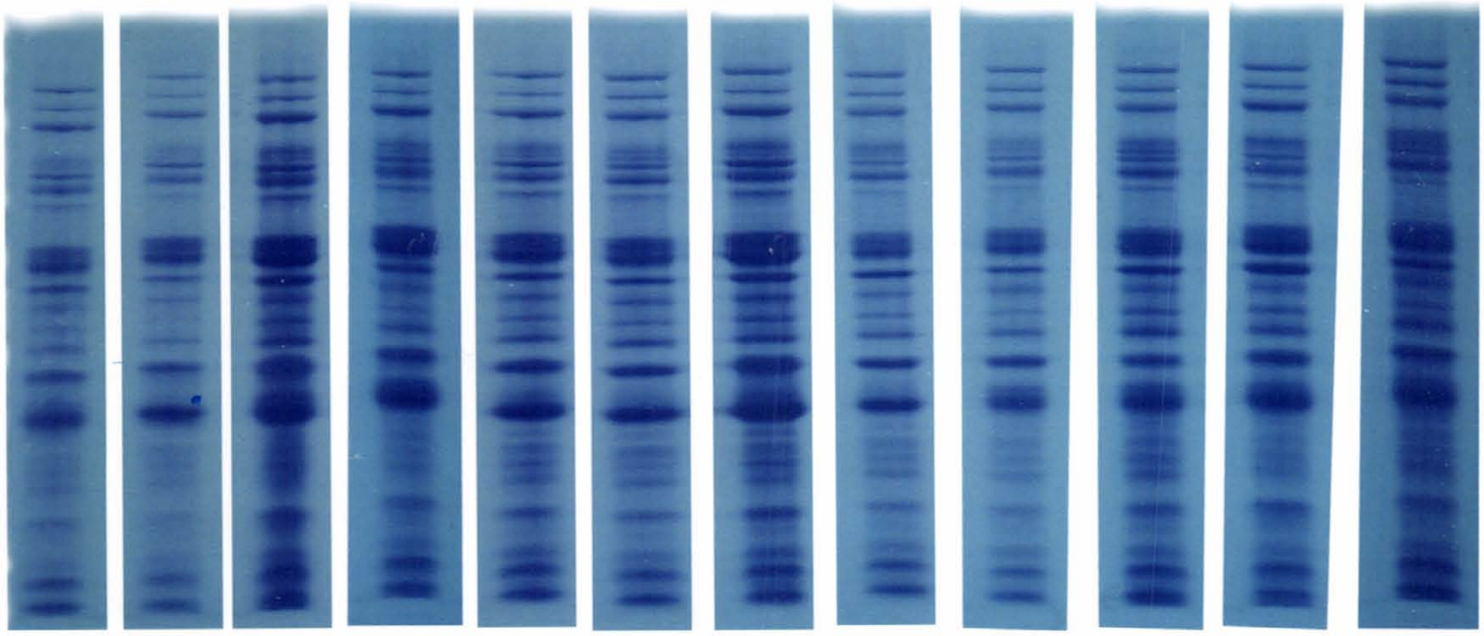
NaOH Test

Dark orange red colour
(PG-5,PG-12,Vijay PG-92926,Vishal, PG-95005,PG-95006)

No colour
(Virat, PG-92307, PG-95311,PG-95421, KAK-2)

Plate No.18

Electrophoretic banding pattern



4.3 Electrophoresis test :-

The classification of banding pattern and number of bands and their intensities in each cultivar are presented in table no. 2 and 3. The results revealed that the electrophoretic patterns of Chickpea cultivars under study were unique and varietal differences were revealed by the presence or absence of particular band and differences in the intensity of bands in the electrophoregram.

It can be observed from the electrophoregram that all together twenty-four bands were recognized in the gel material of the seed of different cultivars under study. In the electrophoregram of all the cultivars, band no. 15 with Rm value 0.66 was common only in seven cultivars. However, it was medium in five cultivars and light in remaining two cultivars. Except band no.- 15, all the bands were common in all the genotypes, but with different intensities.

The qualitative and quantitative variations were observed in the banding pattern of seed protein of Chickpea cultivars. the overall banding pattern showed a variation mostly in the intensity of the bands of the cultivars. There was very less variation in the number of bands among the cultivars. the number of bands in each cultivar ranged from 23 to 24. (plate no.-18).

According to the banding pattern, cultivar PG-5 was having total twenty three bands, including one dense, four medium, ten light and eight weak bands.

In PG-12, there were total twenty three bands, including one den three medium seven light and twelve weak bands.

There were twenty-four bands observed in Vijay, including eleven dense and thirteen medium bands. Band no.-15 was present in Vijay. There was not a single light or weak band.

In Vishal, there were twenty-three total bands, out of which, five were dense, six were medium and twelve were light.

Cultivars PG-92926 was having total twenty three bands, including three dense, six medium nine light and five weak bands.

In PG-96005, there were twenty-four bands, including eleven dense, eight medium and five light bands. Band no.-15 was present in this cultivar with light intensity.

In PG-96006, twenty-three bands were observed, out of which three were dense, four were medium, eleven were light and five were weak bands.

There were twenty-four bands observed in Virat, including only one dense, four medium, twelve light and seven weak bands. In this cultivar band no.-15 was present with light intensity.

In PG-92307, there were total twenty-four bands with differing intensities. Seven were dense, eleven were medium and six were light. Band no.-15 was having medium intensity.

Cultivar PG-95311 was having twenty-four bands with differing intensities. There were six dense, eleven medium and seven light bands.

There were twenty-four bands in PG-95421, including six dense, ten medium and eight light bands.

In KAK-2, there were also twenty four bands with differing intensities. Nine were dense, ten were medium and five were light bands.

Thus, all the Chickpea cultivars under study could be identified on the basis of different intensities of the bands. All the kabuli cultivars were having twenty four bands while all the desi cultivars were having twenty three bands except two cultivars i.e. Vijay and PG-96005.

Table :- Classification of Chickpea cultivars according to number of bands and their intensities.

Sr. No.	Cultivars	No. of bands	Dense	Medium	Light	Weak
1.	PG-5	23	1	4	10	8
2.	PG-12	23	1	3	7	12
3.	Vijay	24	11	13	-	-
4.	Vishal	23	5	6	12	-
5.	PG-92926	23	3	6	9	5
6.	PG-96005	24	11	8	5	-
7.	PG-96006	23	3	4	11	5
8.	Virat	24	1	4	12	7
9.	PG-92307	24	7	11	6	-
10.	PG-95311	24	6	11	7	-
11.	PG-95421	24	6	10	8	-
12.	KAK-2	24	9	10	5	-

Table :- Banding pattern of twelve Chickpea cultivars.

Band No.	Rm value	1	2	3	4	5	6	7	8	9	10	11	12
1.	0.16	++	++	+++	++	++	+++	++	++	++	+++	+++	+++
2.	0.18	++	++	+++	++	++	+++	++	++	+++	+++	+++	+++
3.	0.22	++	++	++++	+++	+++	++++	++	++	+++	++++	++++	++++
4.	0.26	+	+	+++	++	++	+++	++	++	+++	+++	+++	+++
5.	0.29	++	++	++++	+++	++	+++	++	++	+++	+++	+++	+++
6.	0.32	++	++	++++	+++	+++	++++	++	++	+++	++	++	++++
7.	0.35	+	+	+++	++	+	+++	+	+	++	++	++	++
8.	0.41	+++	+++	++++	++++	+++	++++	+++	+++	++++	++++	++++	++++
9.	0.46	+++	+++	++++	++++	+++	++++	+++	+++	++++	++++	++++	++++
10.	0.48	+++	++	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++
11.	0.51	++	+	+++	++	++	+++	++	++	+++	+++	+++	+++
12.	0.54	++	+	+++	++	++	+++	++	+	+++	+++	++	+++
13.	0.58	++	+	+++	++	++	+++	++	++	+++	+++	+++	+++
14.	0.62	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
15.	0.66	-	-	+++	-	-	++	-	++	+++	+++	+++	+++
16.	0.68	++++	+	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++
17.	0.73	+	+	+++	++	+	++	+	+	++	++	++	++
18.	0.76	+	+	+++	++	+	++	+	+	++	++	++	++
19.	0.77	+	+	+++	++	+	++	+	+	++	++	++	++
20.	0.79	+	+	+++	++	+	++	+	+	++	++	++	++
21.	0.84	+	+	++++	+++	++	+++	++	++	+++	+++	+++	+++
22.	0.91	+	+	+++	++	++	+++	++	++	+++	++	++	+++
23.	0.94	++	+	++++	+++	+++	++++	+++	++	++++	+++	+++	++++
24.	0.97	++	++	++++	+++	+++	++++	+++	++	++++	+++	+++	++++

1.PG-5
2.PG-12
3.Vijay
4.Vishal
5.PG-92926
6.PG-96005
7.PG-96006
8.Virat
9.PG-92307
10.PG-95311
11.PG95421
12.KAK-2

(++++ - Dense , +++ - Medium
++ - Light , + - Weak)

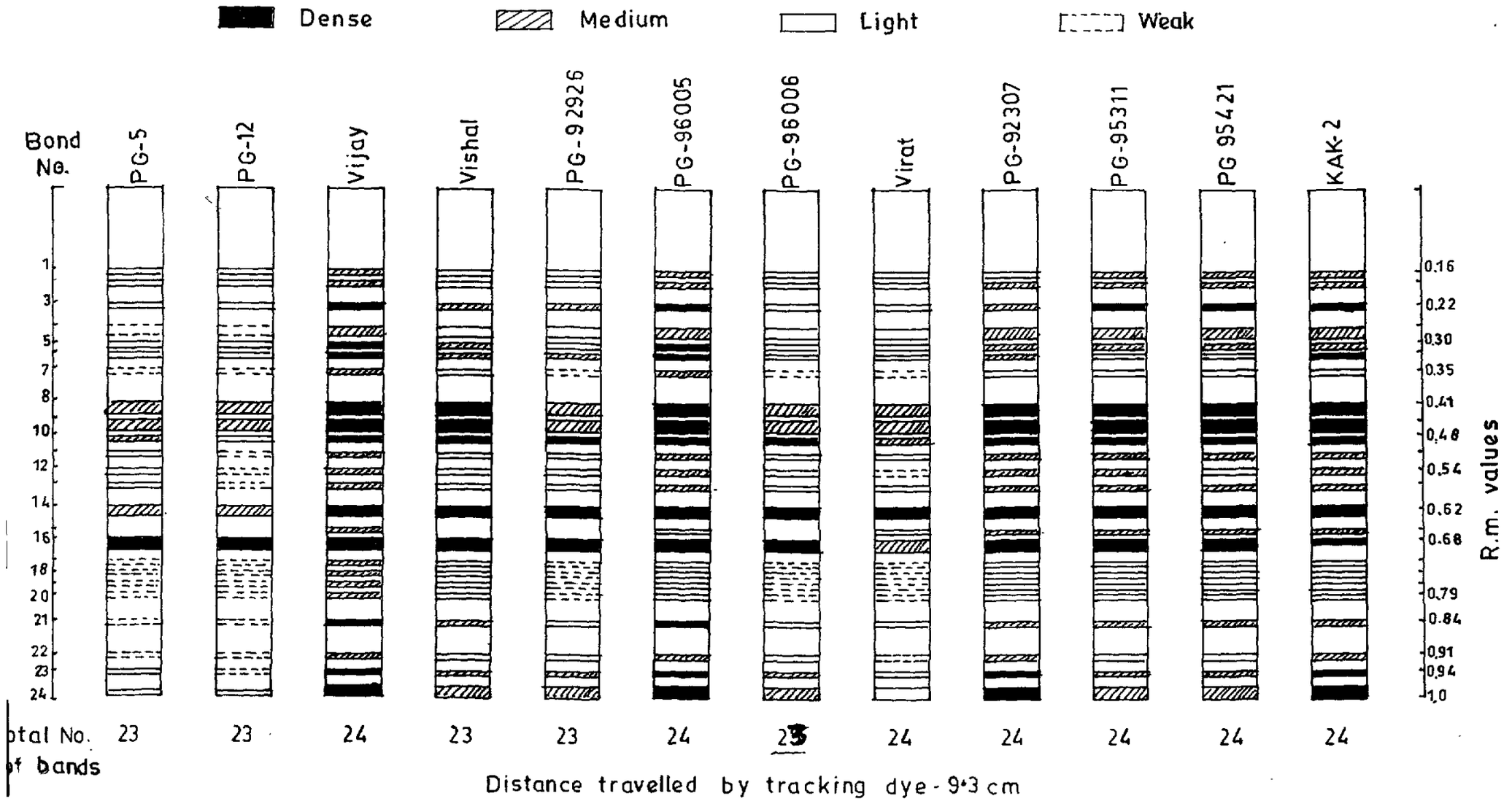


Fig.:- Elecrtophoregram of chickpea cultivars :

DISCUSSION

5. DISCUSSION

The genetic purity is one of the most important characteristics of good quality seed. In seed production program careful attention is paid at every stage of production to maintain genetic purity of cultivars. The characterization of cultivars has therefore attained a critical importance in the national and international seed program. Different cultivars are commonly characterized on the basis of morphological differences of seed, seedling and plant. But now a days many chemical and biochemical tests have been developed for this purpose.

A new crop variety should be

i. Clearly distinguishable (D) by one or more characteristics from any other variety.
ii. Sufficiently uniform (U).
iii. Stable (S) in its essential characteristics.

In characterization of cultivars, it is important to decide the criterion which is used to find distinctness among the cultivars. While selecting the criterion, consideration should be given to the factors such as cost convenience and time availability. Morphological characteristics have traditionally been used to describe and to examine the varietal distinctness.

In the past 20 to 30 years, there were fewer varieties available compared to number of varieties being grown today. In many cases, the cultivars grown in same geographic area shows different seed

characteristics mainly due to G + E interaction, thus causing difficulties in identification.

Characterization of cultivars at seed or seedling stage saves space and efforts by screening large population for certain desired characters by finding genetic markers which have important implications in plant breeding. A major disadvantage of use of morphological characters to characterize the cultivars is that, many new cultivars have seeds with similar characteristics. Alternately field testing is advocated to characterize among the cultivars by employing standard check for comparing test samples. Although this method is simple and reliable, it usually takes an entire growing season to complete the test and obtain the results. In addition, this test has to be performed outside the laboratory and adequate field space must be available.

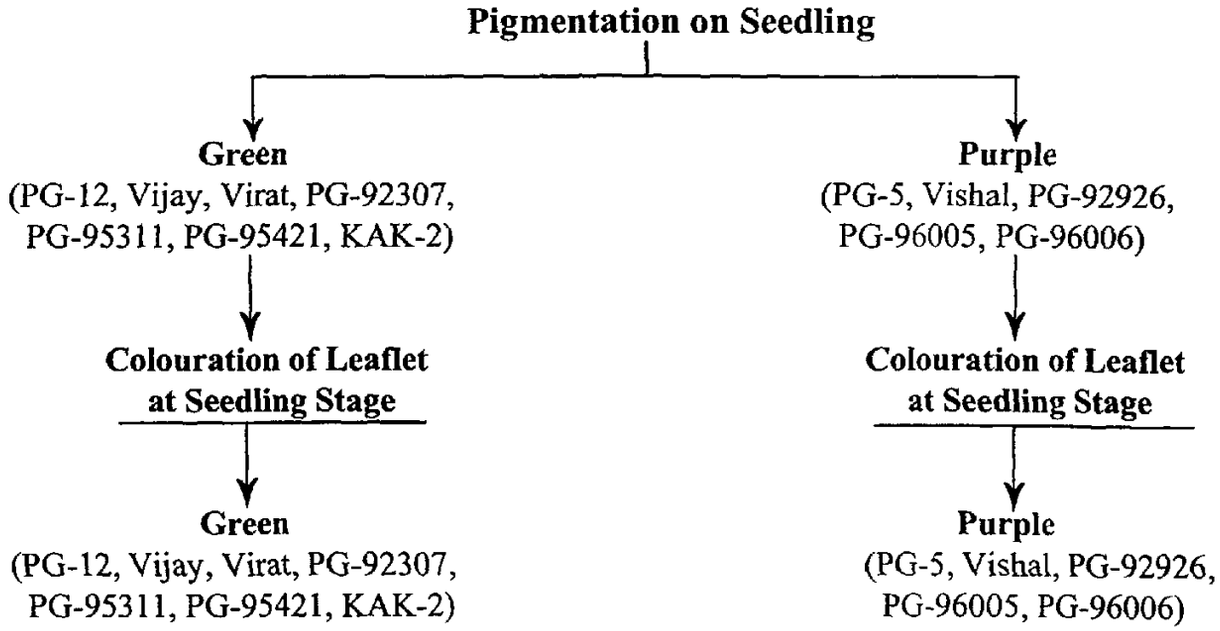
Field and laboratory tests for the experiment "characterization of Chickpea cultivars (desi & kabuli) through morphological, chemical and electrophoretic tests" was conducted during the year 2001-2003. The results reported in previous chapter are discussed here as under.

5.1 Morphological tests :-

5.1.1 Seedling morphology :-

Two seedling characteristics namely pigmentation on seedling and colouration of leaflet at seedling stage were studied in all twelve cultivars of Chickpea. It was revealed that both of these characters were common in all the cultivars i.e. the cultivars with green pigmentation on seedling had green colouration of leaflet. (flow chart –1).

FLOW CHART - 1 Seedling Morphology



The cultivars PG-12, Vijay, Virat PG-92307, PG-95311, PG-95421 and KAK-2 had green pigmentation on seedling while others showed purple pigmentation.

Chakrabarty and Agrawal (1989) also identified blackgram varieties on the basis of seedling pigmentation (dark purple and green).

The colouration of leaflet in cultivars PG-5, Vishal, PG-92926, PG-96005 and PG-96006 was purple while remaining cultivars showed green colouration of leaflet.

These seedling characteristics, thus, could be used in distinguishing Chickpea cultivars. The results obtained were in accordance with Anonymous (1988).

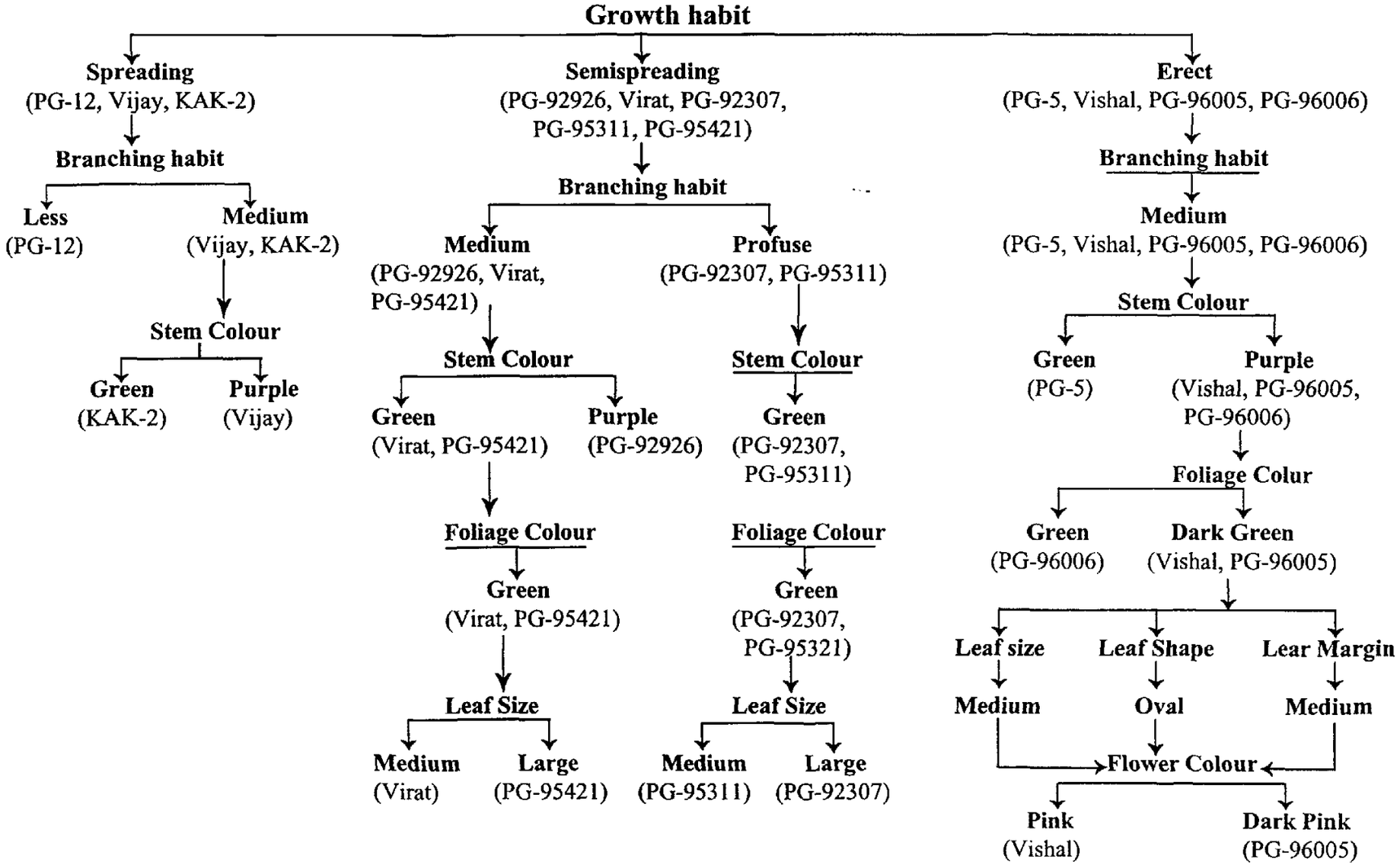
5.1.2 Plant morphology :-

The plant characteristics exhibited by different cultivars indicated that although some of the cultivars had common morphological features in respect of one few characters, they can be distinguished from each other on the basis of other characters. (flow chart –2).

On the basis of growth habit, all the cultivars were grouped into three categories viz., spreading (PG-12,Vijay, KAK-2), semispreading (PG-92926, Virat, PG-92307, PG-95311, PG-95421) and erect (PG-5, Vishal, PG-96005, PG-96006).

On the basis of branching habit, three categories were made i.e. less (PG-12), medium (PG-5, Vijay, Vishal, PG-92926, PG-96005, PG-96006, Virat, PG-95421, KAK-2) and profuse (PG-92307 & PG-95311).

FLOW CHART - 2 Plant Morphology



Stem colour varied from green in PG-5, Virat, PG-92307, PG-95311, PG-95421 and KAK-2 to purple in other remaining cultivars.

Foliage colour also varied from green in PG-12, Vi jay, PG-96006, Virat, PG-92307, PG-95311, PG-95421 and KAK-2 to dark green in other cultivars.

On the basis of leaflet size, the cultivars were grouped into three categories namely small (PG-12, Vijay), medium (PG-5, Vishal, PG-92926, PG-96005, PG-96006, Virat and PG-95311), large (PG-92307, PG-95421 and KAK-2).

All the twelve cultivars have showed oval leaflet shape.

The cultivar Virat showed high serration of leaflet margin while other showed medium leaf serration.

For differentiation of cultivars, flower colour was observed and they were grouped into three different groups viz., white (Virat, PG-92307, PG-95311, PG-95421 & KAK-2), pink (PG-5 & Vishal) and dark pink (PG-12, Vijay, PG-92926, PG-96005, PG-96006).

On the basis of days to flower, all the genotypes were grouped into two categories viz., early (PG-12, Vijay, Vishal, PG-92926, PG-96005, PG-96006,PG-92307, PG-95421, KAK-2) and medium (PG-5,Viray,PG-95311).

On the basis of plant height at the time of harvesting, the cultivars were differentiated into three groups i.e. tall (PG-95311, PG-95421), medium (PG-5, Vishal, PG-96005, PG-96006, Virat, PG-92307, KAK-2), dwarf (PG-12, Vijay, PG-92926).

Pods per plant were high in PG-96006, PG-95311, PG-95421, medium in Vijay, PG-96005, PG-92307, KAK-2 while less in PG-5, PG-12, Vishal, PG-92926 and Virat.

Number of seeds per pod varied from single in PG-5, PG-12, Vijay, Vishal, PG-92926, Virat, PG-92307, PG-95311, PG-95421 to double in PG-96005, PG-96006 and KAK-2.

On the basis of pod size at maturity, all the cultivars were grouped into three categories viz., bold (PG-96006, Virat, PG-92307, PG-95311, PG-95421, KAK-2), medium (PG-5, Vishal, PG-92926, PG-96005), small (PG-12, Vijay). Flow chart –2)

The plant morphological characters thus, could be used in *distinguishing chickpea cultivars*. The results obtained were in conformity with the findings of Anonymous (1988), Chakrabarty and Agrawal (1989) and Yadav and Srivastava (2000).

5.1.3 Seed morphology :-

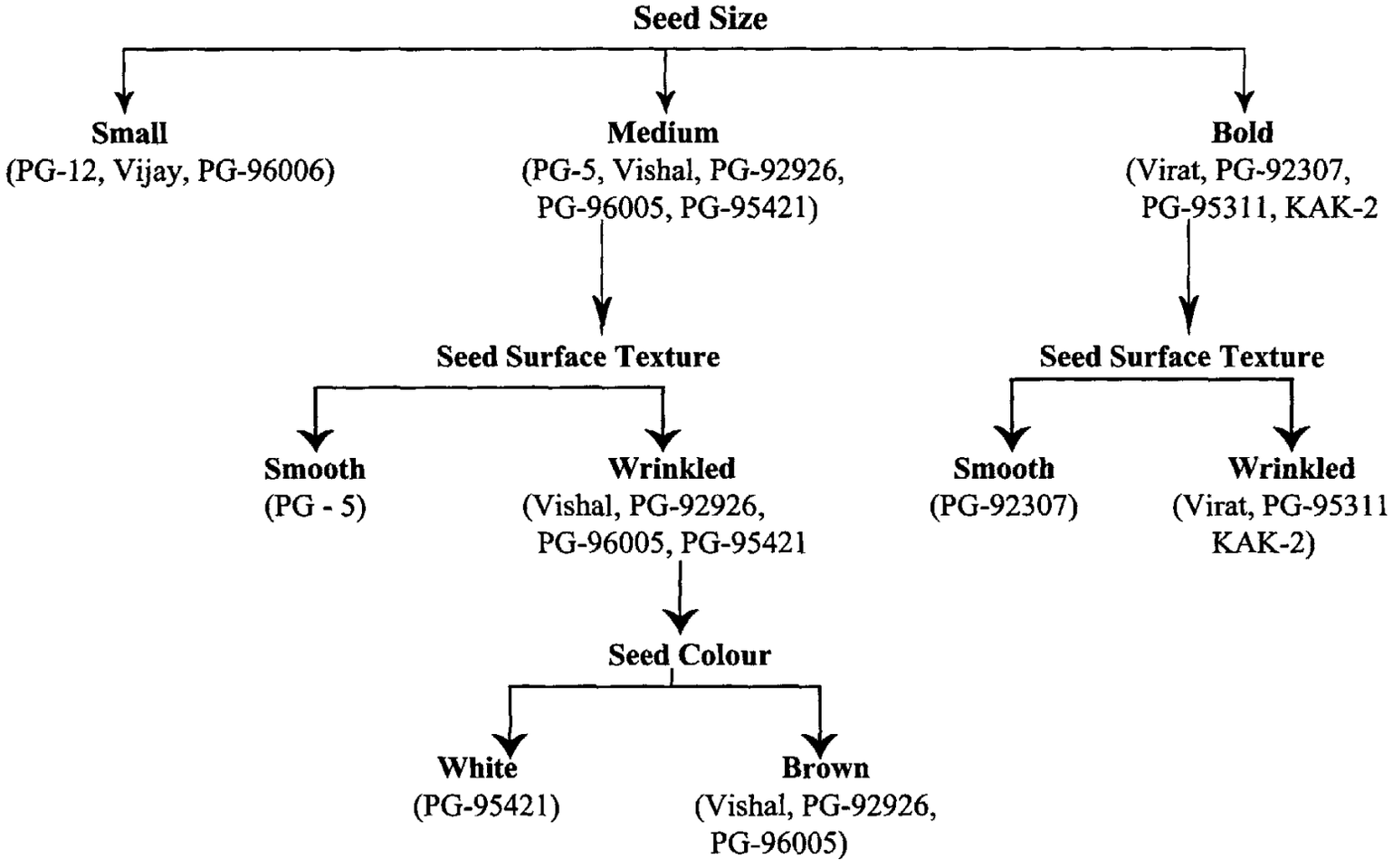
The seed characteristics exhibited by different cultivars studied, indicated that although some of the cultivars have common morphological features in respect of one or two characters, they can be differentiated from each other on the basis of other characters. (Flow chart-3).

The classification of Chickpea cultivars described in flow chart-3 revealed that the cultivars PG-12, Vijay, PG-96006 had small seed size, while PG-5, Vishal, PG-92926, PG-96005, PG-95421 had *medium* seed size and remaining Virat, PG-92307, PG-95311 and KAK-2 were bold in size.

The cultivars PG-5 and PG-92307 were recorded for smooth seed surface texture while remaining all had wrinkled seed surface texture.

Three types of seed colour s were found in the cultivars studied viz., white (Virat, PG-92307, PG-95311, PG-95421, KAK-2), brown

FLOW CHART - 3 Seed Morphology



(PG-96005, PG-96005, PG92926, Vishal, Vijay, PG-12 and dark brown (PG-5).

The results were in accordance with Anonymous (1988), in chickpea, Chakrabarty and Agrawal (1989) in blackgram, Dahiya et.al. (1995) in chickpea and Yadav and Srivastava (2000)in chickpea.

5.2 Chemical tests :-

5.2.1 Phenol test :-

Phenol test has been studied by a very large number of workers in wheat varieties. It was observed to be very simple, quick and accurate method for classification of wheat varieties into different groups. However, this test was not found suitable for Chickpea cultivars because the colour reaction could not be noticed in any Chickpea cultivar.

5.2.2 Modified Phenol test :-

The modified phenol test has been used by many of the workers for variety testing in wheat crop. The modified phenol tests using $CuSO_4$ and Na_2CO_3 were performed by the workers. But this test was not found suitable for Chickpea cultivars because the colour reaction could not be noticed in any Chickpea cultivar.

The results of phenol test using $CuSO_4$ were in accordance with Anonymous (2001) for cultivara PG-5, Vijay and Vishal. However, the results obtained for phenol test using Na_2CO_3 were in contrast.

5.2.3 Peroxidase test :-

The peroxidase test had been used for variety testing extensively in Soybean. It has been used as a qualitative test i.e. peroxidase activity is present or absent by visual observation, but not

quantitatively. This test is very simple. But not useful for the identification of Chickpea cultivars because not a single cultivar of Chickpea had shown a positive peroxidase activity.

The results were in accordance with Dahiya et al. (1995)in chickpea.

5.2.4 NaOH test :-

The NaOH test had been studied by the workers for varietal identification of different crops. This test had been carried out as per the method described in Chapter- 3. All the kabuli cultivars had shown negative colour reaction while all the desi type cultivars had shown dark orange red colour reaction of seed coat.

Thus, this test could be used for identifying desi and kabuli chickpea cultivars. The results were in accordance with Anonymous (2001). However, colour observed for PG-5, Vijay and Vishal was dark brown.

5.2.5 2,4- D test :-

The 2,4- D test was used to characterize Chickpea, Blackgram, Groundnut. According to this test, varieties could be catagorized into different groups as per the hypocotyl length or according to their sensitivity. However, this test was not useful for the twelve Chickpea cultivars because they had shown only radical initiation (highly affected).

The results obtained were in conformity with Dahiya et al.(1995).

5.3 Electrophoresis test :-

SDS-PAGE electrophoregram of 12 chickpea cultivars containing 23-24 bands (Table 2) were distinctly different. The

maximum number of bands (24) were observed in all kabuli type chickpea cultivars and also in two desi cultivars i.e. Vijay and PG-96005. Remaining all desi cultivars contained 23 bands. This indicates polymorphism in protein pattern among the genotypes under study.

The results obtained are in contrary with Raghvirendra Singh et al. (2000), where they reported more bands in desi cultivars in comparison to kabuli cultivars.

All the bands, except band no. 15, corresponding to Rm 0.66, were common in twelve cultivars. Those are common, the cultivars can be differentiated on the basis of differing intensities of these bands. The common bands may serve as reference for comparison and presence / absence of specific bands can be used for genotype differentiation from gene pool. Variation in band intensity observed may be attributed to large variations in the amount of various prolamiens present in protein extract. Similar trends were observed in the protein banding pattern of sorghum genotypes by Manjre et al. (2002).

Similarity index :-

		1	2	3	4	5	6	7	8	9	10	11	12
PG-5	1	-	100	95.83	100	100	95.83	100	95.83	95.83	95.83	95.83	95.83
PG-12	2		-	95.83	100	100	95.83	100	95.83	95.83	95.83	95.83	95.83
Vijay	3			-	95.83	95.83	100	95.83	100	100	100	100	100
Vishal	4				-	100	95.83	100	95.83	95.83	95.83	95.83	95.83
PG-92926	5					-	95.83	100	95.83	95.83	95.83	95.83	95.83
PG-96005	6						-	95.83	100	100	100	100	100
PG-96006	7							-	95.83	95.83	95.83	95.83	95.83
Virat	8								-	100	100	100	100
PG-92307	9									-	100	100	100
PG-95311	10										-	100	100
PG-95421	11											-	100
KAK-2	12												-

The similarity index was worked out in order to evaluate degree of closeness among different cultivars under study and their evolutionary relationship. The results presented in the above table indicated that the similarity index values were in the range of 95.83 and 100. The high similarity values suggest genetic closeness which might be either due to common source or accumulation of similar genes from different parents during the development of genotypes. Thus, these similarly index values based on soluble protein polymorphism may not be used to establish evolutionary relationship among the chickpea cultivars.

From the observation, it appears that chickpea cultivars under study were identified by the presence or absence of specific band (s) and also the intensities of bands could be used as genetic markers. The electrophoretic banding patterns of twelve chickpea cultivars were unique and qualitative and quantitative variations were observed in the banding pattern of chickpea cultivars. Seed protein

that showed genetic variation may be used as probes to mark genotypes. Also the desi type and kabuli type chickpea cultivars showed different banding patterns which could be used for identification of desi type and kabuli type chickpea.

SUMMARY AND CONCLUSIONS

6. SUMMARY AND CONCLUSIONS

6.1 Summary :-

The present investigations were undertaken to characterise the chickpea cultivars by means of morphological, chemical, and electrophoretic tests.

The investigation were carried out using twelve chickpea cultivars. The observations were recorded for their seedling, plant and seed morphological characters and analysed chemically for their response to phenol, modified phenol, NaOH, 2, 4-D and peroxidase tests. The bonding patterns of chickpea seed storage proteins were studied through sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) at 10 % gel concentration.

The important findings of the present investigations have been summarized below.

1. All the twelve chickpea cultivars were studied for two seedling morphological characters namely pigmentation on seedlings and colouration of leaflet at seedling stage. The cultivars PG-12, Vijay, Virat, PG-92307, PG-95311, PG-95421 and KAK-2 were recorded for green pigmentation while remaining had shown purple pigmentation.

2. Further, the cultivars were studied for thirteen plant morphological characters, namely growth habit, branching habit, stem colour, foliage colour, leaflet size, leaflet shape,

leaflet margin, flower colour, days to flower, plant height, pods per plant, number of seeds per pod, pod size at maturity.

On the basis of growth habit, all the cultivars were grouped as errect (PG-5, Vishal,PG-96005, PG-96006) semispreading (PG-92926, Virat, PG-92307, PG-95311, PG-95421) and spreading (PG-12, Vijay, KAK-2).

Branching habit i.e. number of primary and secondary branches were found medium in majority of cultivars except in PG-92307 and PG-95311 (Profuse) and PG-12 (less) cultivars PG-5, Virat, PG-92307, PG-95311, PG-95421 and KAK-2 were found to be having green stem colour, while other have purple.

Four cultivars i.e. PG-5, Vishal, PG-92926 and PG-96005 had dark green foliage colour while remaining eight cultivars were observed to be green.

Majority of the cultivars were having medium leaf size i.e. PG-5, Vishal, PG-92926, PG-96005, PG-96006, Virat and PG-95311 while in PG-12 and Vijay it was small and in PG-92307, PG-95421 and KAK-2 it was large.

The leaf shape was found to be oval in all the cultivars.

The leaf serration of the margin was high only in cultivar KAK-2 while remaining eleven cultivars showed medium leaf margin.

Flower colour was found to be white in all kabuli types culitvars while in desi type, PG-5 and Vishal showed pink colour and remaining cultivars showed dark pink colour.

Cultivars PG-5, Virat and PG-95311 were found medium for days to flower while remaining all were early for days to flower.

On the basis of plant height all the cultivars were grouped into three groups namely dwarf (PG-12, Vijay, PG-92926), medium (PG-5, Vishal, PG-96005, PG-96005, Virat, PG-92307, KAK-2) and tall (PG-95311 and PG-95421).

The cultivars PG-5, PG-12, Vishal, PG-92926 and Virat showed less number of pods per plant while in cultivars Vijay, PG-96005, PG-92307, KAK-2 the pods were medium and PG-96006, PG-95311, PG-95421 the number of pods was higher in comparison to others.

The number of seeds per pod was found to be double only in PG-96005, PG-96006 and KAK-2 and the remaining cultivars were having single seed per pod.

The cultivars PG-12 and Vijay were having small pod size while it was medium in PG-5, Vishal, PG-92926, PG-96005 and bold in PG-96006, Virat, PG-92307, PG-95311, PG-95421, KAK-2.

3. Seed morphological characters (i.e. seed size, seed surface texture and seed colour) were studied for varietal identification.

Some cultivars showed common characters and could be differentiated by the presence or absence of other characters.

The cultivars PG-12, Vijay, PG-96006 has small seed size, while PG-5, Vishal, PG-92926, PG-96005, PG-95421 have medium seed size and remaining cultivars were bold in size.

Two types of seed surface textures were observed i.e. smooth and wrinkled. Only the cultivars PG-5 and PG-92307 had shown smooth seed surface while remaining all had wrinkled seed surface.

Seed colour was observed to be white in all kabuli type culitvars while in desi type cultivars only PG-5 had dark brown colour and remaining cultivars had brown colour.

4. The chickpea cultivars did not show any response to phenol test, modified phenol test, peroxidase test and 2, 4-D test.

5. The twelve chickpea cultivars could be classified into two groups as per the response to NaOH test. All the desi type chickpea cultivars i.e. PG-5, PG-12, Vijay, Vishal, PG-92926, PG-96005 and PG-96006 had shown positive response to NaOH test. They showed dark orange red colour of seed coat. But in all the kabuli type cultivars, there was no any colour reaction, i.e. negative response.

6. The electrophoretic studies revealed that the number of bands in different cultivars did not vary much and a total of 24 bands were recognised (Fig. 1).

All the desi cultivars, except Vijay and PG-96005 had 23 bands. Band 15 corresponding to Rm. 0.66, was absent in these cultivars. Total 24 bands were present in Kabuli type chickpea cultivars.

Though all the cultivars were having the same number of bands, the cultivars could be differentiated on the basis of varying intensities of these bands.

7. Each cultivar exhibited a unique banding pattern.

8. The variation of band intensity observed may be attributed to variations in the amount of various proteins present in the extract.

9. The high similarity indices showed closeness among the genotypes.

6.2 Conclusion :-

The morphological characteristics exhibited by different chickpea cultivars, showed that although some of the varieties have common morphological features in respect of one or more characters, they can be differentiated from each other on the basis of other characters. Some of the characters such as flower colour, seed colour can be used to distinguish between desi type and Kabuli type chickpea cultivars.

The chemical tests such as phenol test, modified phenol test, peroxidase test and 2, 4-D test are not suitable for characterization of chickpea cultivars except NaOH test, which

is suitable for differentiating desi type and kabuli type chickpea cultivars.

Electrophoretic patterns of chickpea cultivars under study are unique. Varietal differences are revealed by the presence or absence of particular band in the electrophoregrams. The desi type and kabuli typecultivars can be differentiated easily from the banding pattern. Though the common bands are same in all the cultivars each band is having varying intensity for each cultivar

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7. LITERATURE CITED

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