

**VARIETAL IDENTIFICATION AND SEED
VIGOUR ASSESSMENT IN FODDER
SORGHUM [*Sorghum bicolor* (L.) Moench].**

BY

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In
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The logo of Chaudhary Charan Singh Haryana Agricultural University is a circular emblem. It features a central figure of a person holding a scythe, standing on a platform. Below the figure are a tractor and a cow. The emblem is surrounded by text in Hindi and English. The Hindi text at the top reads 'श्री चरन सिंह हरियाणा कृषि विश्वविद्यालय' and at the bottom '1970'. The English text at the top reads 'CHAUDHARY CHARAN SINGH HARYANA AGRICULTURAL UNIVERSITY' and at the bottom '1970'. The emblem is set against a background of a green field and a blue sky.

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CERTIFICATE – I

This is to certify that this thesis entitled “**Varietal identification and seed vigour assessment in fodder sorghum [*Sorghum bicolor* (L.) Moench].**” submitted for the degree of Master of Science in the subject of **Seed Science and Technology** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. Gend Dnyaneshwar Gajanan Adm. No. 2012A64M** under my supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

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PLACE: HISAR

GEND DNYANESHWAR GAJANAN

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CHAPTER – I

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the major crops of the world. It is known under variety of names such as, great millet and guinea corn in West Africa, Kafir corn in South Africa, dura in Sudan, mtama in Eastern Africa and jowar in India. It is originated in Africa and it is often-cross pollinated, diploid ($2n=2x=20$), C4 grass with a high photosynthetic efficiency. It belongs to the 'Poaceae' family and the tribe of 'Andropogoneae' (Harlan & de Wet, 1972). The largest diversity of cultivated and wild sorghum is in Africa (Doggett, 1988; De Wet and Harlan, 1971; De Wet, 1977). According to Vavilov, Indian subcontinent is considered to be secondary centre of origin of sorghum (Dorofeev, 1992). Sorghum can be grown in a wide range of ecological conditions and yield well under unfavourable conditions of drought stress and high temperatures. It is mainly grown for food and fodder purpose. It is preferred over other non-leguminous fodder due to its high yielding capacity, better quality and palatability and its utilization in various forms *i.e.*, green fodder, stover, silage and hay. Besides food and fodder, it is also used for preparation of alcoholic beverages, fibres, sugar and syrup.

In India, the Government enacted the legislation on Protection of Plant Varieties and Farmer's Rights Act -2001 (PVP & FRA 2001), that provides protection of new varieties. Novelty, Distinctness, Uniformity and Stability are the essential requirements for grant of protection to all the new varieties. The morphological study includes the study of seed, seedling and full grown plant could be taken into account for morphological characterization. Grow out test of the variety is conducted by growing the plant under field condition for determining the genuineness of the variety. Different cultivars could be identified based on above said characterization in the field conditions but it is laborious and time consuming. Varietal characterization using morphological characters possess several undesirable features like seasonal dependence, large space requirement, time consuming, tedious and environmental influence. To overcome this limitation, there is also a need for rapid and reliable method of varietal identification and genetic purity testing. The alternative way to speed up the testing procedure is to use bio-chemical test or electrophoresis in place of morphological markers. During the past 30 years, various techniques have been used for analysis of genetic variability at the molecular level in plants. As per DUS Guidelines, seed protein profiles (Ladizinsky and Hymowitz,

1979) are still powerful tools for determining genetic homology at the molecular level and for solving problems of varietal differentiation in systematic manner. Several investigators have emphasized the importance of protein and enzyme electrophoresis for the individuals and cultivars of different species. Protein and enzymes are the primary products of the genes and hence the most suited for genetic purity determination. According to Cook *et al.* (1983), electrophoresis is the proper method to resolve protein polymorphism. Polyacrylamide gel electrophoresis (PAGE) was introduced by Raymond and Weintraub (1959). A simple vertical slab gel electrophoresis was constructed by Beileski and Reid (1968). This technique commonly used in developing genetic fingerprint of crop varieties, inbred lines and hybrids through specific 'Banding pattern' that is based on the principle that any charged particle migrates towards the electrode the opposite sign under externally applied electric field. The rate of migration is affected by large number of factors such as strength of field, net charge, size and shape of molecules, ionic strength, viscosity, pH of buffer system, concentration of acrylamide and bis-acrylamide, running duration and temperature. So, it is essential to develop field and laboratory key characters to test the varietal purity of fodder sorghum.

Seed quality is determined by different factors including seed viability and vigour which imparts inherent capacity to grow under favorable and unfavorable conditions. Seeds of fodder sorghum are poor storer crop and it deteriorate sharply with passage of time under ambient conditions. Seed vigour is a concept describing several characteristics which includes the rate and uniformity of germination and growth, tolerance to environmental stress after sowing and retention of performance after storage. The seed vigour comprises those seed properties which determine the potential for rapid, uniform emergence and development of normal seedling under a wide range of field conditions. Vigour is the first component of seed quality, which is lost, followed by a loss of germination capacity and viability (Trawatha *et al.*, 1995). Now a days, the seed vigour as a quality attribute has gained significance which is a highly complex character influenced by many parameters.

Keeping in view the importance of fodder sorghum as one of the potential forage crops, it is essential to study the morphological characters in field and laboratory for key characters to test the varietal purity. The methods are required on routine basis to have an effective check on the seeds moving in commercial channels. So far, only scanty reports are available to identify fodder sorghum cultivars by using such methods. In the light of above facts, the present study on varietal identification and seed vigour assessment of fodder sorghum varieties was planned with the following main objectives,

1. To identify stable diagnostic characteristics of seed, seedling and plant morphology of fodder sorghum varieties.
2. To distinguish fodder sorghum varieties by Electrophoresis technique.
3. To assess the seed vigour potential of different fodder sorghum varieties.

Characterization and identification of cultivars is crucial to the variety improvement, registration and seed production programme. The term cultivar denotes as an assemblage of cultivated plants which is clearly distinguished by any character (morphological, physiological, biochemical or others) or which when produced (sexually or asexually) retains its distinguishing character. Practically a variety must show distinct, uniform and stable variation in the characters that are adopted for use in varietal characterization and identification. The subject is reviewed under following heads.

2.1. VARIETAL IDENTIFICATION

2.1.1 Morphological characters

Registration of new varieties is largely based on characterization of submitted genotypes through morphological observations or measurement on both seed and vegetative plant structures. Number of studies has been conducted in identifying stable morphological traits for variety characterization in crops are as follows:

Kolberg (1999) studied characterization of 124 accessions of sorghum landraces from Namibia using International plant Genetic Resources Institute (IPGRI) descriptors and observed considerable morphological variability. Some interesting trends were observed reflecting the use of this cereal in the country. From the set of characters observed, future collecting priorities and possible gaps in the existing collection were identified.

Nagaraja *et al.* (2000) studied the characterization of 23 varieties including five hybrids of sorghum along with their parental lines and four additional inbreds were described and characterized by seed and seedling morphology and electrophoresis of seed proteins and isoenzymes. On the basis of seed colour and shape in profile view, the hybrids and inbreds could be grouped into two categories while on the basis of germ size, they could be grouped into the three categories. The varieties could be divided into five groups based on the pattern of anthocyanin pigmentation in different parts of the seedling. Three varieties could be identified individually by their characteristics pigmentation pattern.

Selvaraju *et al.* (2000) observed varietal identification keys in breeder seeds of 19 sorghum varieties. The keys were made based on seed and seedling

morphological characters and chemical tests. The varieties were identified based on the variations in the morphological characters of seeds, such as length, breadth, length/breadth ratio and colour. They also reported that the coleoptiles colour of seedlings could also be combined with the seed characters for variety identification.

Pahuja *et al.* (2002) evaluated forage sorghum hybrids for yield and morphological traits.

Umakanth *et al.* (2002) studied genetic diversity of 18 exotic collection and 30 established sorghum genotypes from the national programme, with different geographical origins. The analysis of variance showed highly significant differences among genotypes for all the characters studied. Plant height made the greatest contribution to the total variability at (60%) followed by panicle length (12%) and days to flowering (11%).

Agarwal *et al.* (2002) studied multivariate classification of elite lines in 41 sorghum genotypes. The genotypes were grouped into five non overlapping clusters based on nonhierarchical euclidean cluster analysis. Cluster 1 had six genotypes, whereas cluster 2 had 12 genotypes.

Sangwan *et al.* (2005) studied varietal identification of morphological traits of 12 forage sorghum genotypes. They classified the forage sorghum genotype in different groups like non-tan type genotypes, white midribs, green midribs, rough margins, smooth margins and Stem thickness. The genotypes were classified into various groups based on inflorescence compactness and shape of panicle.

Thangavel *et al.* (2005) reported the varietal grouping of 12 sorghum (*Sorghum bicolor* L. Moench) cultivars including one hybrid and its parental lines by seed and seedling morphology. All 12 cultivars showed variation in the seed descriptors *viz.*, seed colour, seed size, seed shape, size of mark of germ, texture of endosperm, colour of vitreous albumin and seed lusture and seedling descriptors *viz.*, anthocyanin pigmentation on coleoptiles, dorsal side first leaf, sheath of first leaf and on leaf blade (at five leaf stage).

Elangovan (2006) characterized and evaluated 179 accessions of sorghum germplasm collected from different parts of the world. The accessions exhibited good variability in both qualitative and quantitative traits. In qualitative traits, maximum diversity was observed in earhead shape, earhead compactness, glume colour, seed size and seed colour. Days to 50% flowering (69-116 days), plant height (68-279 cm), earhead length (17-40 cm), stem fresh weight (40-1000 g per plant), stem dry weight (20-317 g per plant), grain yield (9-104 g per plant) and brix percentage (2-27%) were also highly variable.

Nabi *et al.* (2006) compared five advanced lines of sorghum including check (Hegari) for plant height, stem thickness, leaf area, number of leaves per plant, sweetness, crude protein, green fodder and dry matter yields. Cultivar JS 88 showed significant differences with higher number of leaves per plants (13.5), stem thickness (1.8 cm) and leaf area (512.5 cm²) as compared to check (10.6 average number of leaves/plant, 1.3 cm stem thickness and 445.5 cm² leaf areas). Similarly, JS 88 was superior in green fodder yield, dry matter and quality (sweetness and crude protein) to Hegari. On an average JS 88 produced 73.8 tons per hectare green fodder and 12.8 tons per hectare dry matter yield as compared to Hegari (59.6 t/ha green fodder and 8.4 t/ha dry matter yield). They also reported that early growth stages had high crude protein level than late stages. Reddy *et al.* (2006) characterized ICRISAT-bred sorghum hybrid parents of 269 designated lines and evaluated for specific traits and/or grain yield compared to known controls and assigned ICRISAT numbers.

Elangovan *et al.* (2007) characterized and evaluated 157 sorghum accessions. Wide range of variability was recorded in both qualitative and quantitative characters. Majority of the accessions showed tan type, dark green leaf colour, erect leaf orientation, and stay green types (non-senescence). However, variation was observed in seedling vigour, midrib colour, earhead shape, earhead compactness, glume colour, glume covering, seed size, seed colour, and races. The quantitative characters also showed wide variation in the evaluated sorghum germplasm. The characters of stem dry weight (70-665 g), stem fresh weight (110-1040 g), grain yield (3-75 g), brix (3-25%), and 100-seed weight (0.6-4.5 g) were highly variable based on coefficient of variability percentage.

Mujaju and Chakauya (2008) studied 47 sorghum germplasm from different areas. Genetic similarities were calculated and genetic relationships between accessions were analysed using principal component analysis and cluster analysis. The landraces were grouped into six clusters according to the geographical location of collection, suggesting environmental adaptation. Accessions with the same name had a tendency to group together, although some of the materials were found to be scattered in the dendrogram. Agro morphological traits were highly variable even for landraces with the same farmer-given name and source. The study suggested that optimization of on-farm conservation strategy for this germplasm should primarily focus on high diversity areas and perhaps recognize those traits envisaged to be of importance by farmers for varietal identification.

Reddy *et al.* (2008) studied the inheritance of midrib colour, awning, midrib type, glume type and plant colour in a set of sorghum cultivars. A total of eight segregating F₂ populations representing one cross segregating for midrib colour, six crosses for awning, one cross for both midrib type and glume type and three crosses for plant colour were used. Observations were recorded on the midrib colour (brown or white), awning (presence or absence), midrib type (pithy or juicy), open or closed glume (complete encapsulation of grain by glume) and tan or purple plant colour. Results showed that white and pithy midrib in leaves, presence of awns, open glume shape in panicles and purple plant colour in sorghum were under monogenic control with dominant expression in the parents used in the study.

Joshi *et al.* (2009) studied morphological characterization of 26 forage sorghum [*Sorghum bicolor* (L.) Moench] varieties which included 20 released and notified and six indigenous local varieties for DUS testing. These were characterized using 40 morphological descriptors adopted from the DUS guidelines of PVP & FR Authority and subsequently examined for their Distinctiveness, Uniformity and Stability. No intra-varietal variation was observed for any of the visual characteristics and expression of characters in different varieties remained same for the two consecutive years confirming the uniformity and stability of the varieties.

Reddy *et al.* (2009) studied genetic variability and divergence in 29 sorghum (*Sorghum bicolor*) cultivars for 39 agro-morphological traits used in DUS testing. Large variation among cultivars was found for the traits, time of panicle emergence (65- 87days), plant height (120-272cm), leaf length (57-87 cm), panicle length (22- 35cm) and 1000-grain weight (23-46 g).

Raghuvanshi *et al.* (2014) studied morphogenetic characterization based on DUS testing, to distinguish between seventeen varieties of forage sorghum, twenty six essential morphogenetic characteristics of seedling, plant (vegetative stage and at maturity) and matured seeds, as per the National Test Guideline for DUS test of Sorghum, were considered. 3 character wise elaborations of results for distinguishing forage sorghum varieties are done. It was possible to distinguish all the seventeen varieties included in the present study on the basis of grouping characteristics based on physiology of plant its morphology and seed colour.

2.1.2 Electrophoresis of seed proteins

Analysis of seeds and seedlings proteins or isoenzymes by electrophoresis and the subsequent use of the information provided for varietal identification is now well established and has been reviewed comprehensively (Cooke,1988, 1995; Smith

and Smith, 1992). There are many reports available in literature for varietal identification and characterization on the basis of SDS-PAGE of seed protein.

Parra *et al.* (1993) extracted protein from eight cultivars of maize with distilled water and analyzed them by SDS –PAGE. The banding pattern of protein varied among the cultivar enabling their identification.

Hiers *et al.* (1994) distinguished six *Fescue* grass cultivars by SDS–PAGE of proteins. Distinct banding patterns were obtained enabling the cultivars identification. Banding patterns were independent of locality and year of production of the seed.

Fourteen genotype of pearl millet, consisting of elite hybrids and their eight parental lines (three female line along with their maintainer and two restorer lines) were identified using PAGE of soluble protein (Kumar *et al.*, 1995). The banding pattern of soluble proteins by PAGE was quite different for each of the genotype and therefore it was possible to identify each genotype individually including the hybrid and parental lines. This indicates that electrophoresis is reliable technique for varietal identification in pearl millet.

International Seed Testing Association (ISTA) has adopted and recommended SDS-PAGE as standard reference method for identification of commercial seed lots of *Pisum and lolium* cultivars (ISTA, 1996). SDS-PAGE of seed glutenin has been recommended by UPOV (1994) for DUS testing of wheat varieties. Varietal characterization using electrophoresis of total seed protein is reported in sorghum (Chauhan, 2000), potato (tuber protein electrophoresis) by (Mishra *et al.*, 2005), bottle gourd (Upadhayay and Ram, 2006), rice (Nethra *et al.*, 2007).

Nunes A. *et al.* (2005) studied the quantitative and qualitative analysis of uncooked zein and kafirin fractions performed through sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and electrophoretic profiles. In his study, Kafirins and zeins present the same oligomer and monomer compositions with the exception of a 66 kDa oligomer that is only present in kafirins.

Akhare *et al.* (2007) studies the applicability of protein markers for identification of sorghum hybrids and its parents and to test the genetic purity for commercial samples.

Sharmila *et al.* (2013), in their studies they analyzed protein of six genotype (HC 260, M 35-1, CSV -15, CSV-17, CSV-20, CSV -22) of sorghum by using SDS –PAGE, protein estimation and phylogenetic relationship among them. The phylogenetic relationships among six genotypes were investigated based on

seed protein profile produce by SDS –PAGE. In their studies, they found 56 polypeptide bands were scored of which 30 were polymorphic and 26 were monomorphic.

2.2 SEED VIGOUR STUDIES

Seed viability and vigour are the most important attributes of the seed which, with the passage of time, lead to reduction in seed quality, performance and stand establishment (Christiansen and Rowland, 1981). This is the major economic concern in agriculture production (McDonald, 1999). Various seed viability and vigour tests have been used extensively in a number of crops to predict the planting value of seed lots in the field and the literature reported on this aspect have been reviewed here as under:

2.2.1 Standard germination (%)

Standard germination test is an accepted measure of seed quality and it provides information about emergence capacity of lots under favorable conditions (ISTA, 2003). This test, which is an excellent measure of viability and vigour, accurately predicts the field performance of soybean seeds when planted in ideal soil conditions that seldom exists (Tekrony, 1973). Significant and positive correlation between seedling establishment and standard germination was reported by Kavak *et al.* (2008) in pepper and Mor *et al.* (2009) in fennel crop.

Demir *et al.* (2005) observed that standard germination test unable to predict seedling emergence potential of aubergine. Williams *et.al.* (2007) reported loss in germination percentage from *Eucalyptus camaldulensis* seed with lose of vigour and increased ageing. Schuab *et al.* (2007) studied that germination test under water stress condition pointed out difference on physiological potential of different seed lots of soybean.

2.2.2 Vigour indices

A combination of standard germination test value with seedling length and dry matter production provides a broad evaluation of seed vigour. Vigour index offers the possibilities of categorizing seed lots into various classes of seed quality. Woodstock (1976) forwarded the seed dry weight as a parameter of vigour and approved by AOSA. Abdul Baki and Anderson (1973a, b) established vigour level in soybean by germinating the seeds in standard germination test for five days and then normal germination and length of hypocotyl were determined. Measuring plumule growth as a vigour indicator for cereals was the first suggested by Germ (1960). Similarly the conclusions were also drawn by Trigo *et al.* (2000) in rye, wheat, barley, and triticale.

Verma *et al.* (1999) reported that standard germination and seedling vigour indices were positively correlated with seedling dry weight. DHA test, protein content and seed yield. They also found that seedling vigour decreased with increased age of seeds in rapeseed and mustard. The dry weight of the seedling showed a positive response to the water and nutritional uptake (Choi *et al.*, 1997). Yadav and Dhankar, (2001) reported that vigour index-I and vigour index –II were positively and significantly correlated with standard germination, seedling length and seedling dry weight and negatively correlated with electrical conductivity in okra. Kumar (2002) observed that seed lot having higher vigour index means better seedling establishment under field condition than lower vigour index in cotton. Ajala (2003) reported that seedling vigour and electrical conductivity was the most sensitive indicators in the predication of field emergence of winged bean and pigeon pea. The vigour index –I and vigour index –II was decreased as the quality of seed become poor due to duration of storage period increased in maize (Basu *et.al.*,2004). Ilbi and Eser (2006) emphasized that seed vigour as an important seed quality parameter because standard germination test does not consistently predict the field performance of seed lot. Estimation of vigour not only provides indication of field emergence value but also give storage value of seed lot.

2.2.3 Accelerating ageing test (%)

The accelerating ageing test was first developed to measure the relatively storability of the seed (Delouche, 1965; Delouche *et al.*, 1967; Delouche and Baskin, 1973) and later on improved and evaluated as indicator of seed vigour in a wide range of species. Delouch and Baskin (1973) and AOSA (1983) observed that the germination percentage after accelerating ageing was correlated with vigour of lot and hence to the lot capacity to perform well under field condition. Subsequent studies have verified the accuracy of this test in predicting the life span of a number different species. Basra *et al.* (2003) found that vigour level, mean emergence time and quality parameters could be predicted by accelerating ageing test. Accelerating ageing test can be used to evaluate the physiological potential (vigour) of pumpkin and zucchini seeds (Dutra and Vieira, 2006). Mavi and Demir (2007) found that seedling emergence under stressful condition and longevity of watermelon seed could be predicted by accelerating ageing test. Rabiei *et al.* (2009) studied the effect of drought stress on seed vigour and germination of oat (*Avena sativa* L.) and that drought stress had significant effect on seed vigour, germination percentage, radical and stem dry weight in oat. Standard germination test is an accepted measure of seed quality and it provides information about emergence capacity of lots under favorable conditions (ISTA, 2003).

2.2.4 Electrical conductivity (mS/cm/g)

The electric conductivity test measures the amount of electrolytes which leach out from seed as they deteriorate (ISTA, 1999). This test has been used to measure the seed viability (Presley, 1958) and later on development into a vigour test for prediction of field emergence. However, the electrical conductivity test cannot be taken as a sole indicator of seed quality and deterioration as Abdul – Baki and Anderson (1973) showed that leaching of sugar from mechanical injured seed was higher than whole seed with equal viability. Electrical conductivity test exhibited good potential as a rapid and efficient method for discriminating soybean seed lots of different vigour level (Dias and Filho, 1996). Ilbi and Eser (2006) reported in onion that electrical conductivity test rapidly provide information about difference in quality status of seed sample and hence possibly their vigour. Wang *et al.* (2004) reported that among that different test the electrical conductivity (EC) test provide the best estimate of seed vigour for legume species purple vetch (*Vicia benghalensis*) and alfalfa (*Medicago sativa L.*). Gupta *et al.* (2005) reported that electrical conductivity increased after the seeds were subjected to accelerated ageing because of membrane deterioration and metabolic changes in the seed.

2.2.5 Dehydrogenase acidity test

The tetrazolium test based on enzyme activity is one of the most valuable techniques for analyzing seed viability. The measurement of the activity of specific enzymes was one of earliest biochemical techniques used to assess deterioration and predict the seed viability. The activity of dehydrogenase enzyme is directly correlated with seed vigour. According to Chhetri *et al.* (1993) when seeds of French bean, pea, lentil and millet were aged artificially, the dehydrogenase activity was higher in the chemically fortified seeds as compared with control. Verma *et al.* (1999) also observed such a relationship in rapeseed and mustard. They observed that as the age of the seed increased the dehydrogenase activity decreased significantly.

Contrary to this, Shenoy *et al.* (1990) reported that the conductance of seed leachates, dehydrogenase activity and germination following accelerating ageing did not show any significance association in rice. Verma *et al.* (2003) observed that the dehydrogenase activity was reduced as the ageing progressed and was found lowest after four year of storage on *Brassica Spp.*

2.2.6 Tetrazolium test

Tetrazolium test has gained wide acceptance not only as a rapid technique for estimating viability but also a power full tool for assessing vigour. On the basis of topographical pattern of seed staining, this test can be used to measure seed

viability (Moore, 1974). Lather *et al.* (1990) revealed that Tz test was the most useful and quick test for assessing the field emergence potential and viability of cotton seeds. Narwal (1995) observed that Tz test is a reliable indicator of seed quality and field performance in okra. Perez and Arguello (1997) reported that application of Tz test to groundnut seeds showed that the test was an accurate method for evaluating the quality of seed lots. Deswal and Chand (1997) observed a significant correlation between Tz test, standard germination test and field emergence in rice bean. The Tz test permits the development of clear and clean coloration and is adequate for the identification of critical areas related to germination and also provided consistent data for the assessment of viability and vigour of tomato seeds (Santos *et al.*, 2007).

2.2.7 Seed weight

Seed weight is also considered to be one of the parameters which influence performance of various crop seeds. Seed vigour in lettuce varied according to seed weight (Smith *et al.*, 1973). The hypocotyl and shoot length, vigour index and DNA production were also significantly correlated with seed weight (Paul and Ramaswamy 1979). Shahi *et al.* (1986) reported that wide range of viability for 100 –seed weight in lentil which was positively associated with permeability and germination percentage. The speed of germination index, seedling dry weight and 100- seed weight had significant positive correlation with speed of emergence index and seedling establishment (Kumar *et al.* 1989).

2.2.8 Seedling establishment and other indices

Field emergence index has been given different names such as emergence rate index (Allan *et al.* 1962), germination rate (Maguire 1962), Speed of germination (Lawrence, 1963) and germination or peak value (Djavanshir and Pourbeik 1976). The speed germination has also been shown to be positive correlation with seed vigour in wheat, maize and barley (Germ, 1960), barley (Cobb and Jones, 1966) and Corn (Gill and Delouche 1973). Mathews and Hosseini (2006) reported that measurement of mean emergence time in oat as a vigour assessment tool of different seed lots of maize. Contrary to this, Hampton *et al.* (2009) reported that standard germination of all 26 lots of *Brassica Spp* was not related to field emergence but electrical conductivity was more strongly related to field emergence.

CHAPTER – III

MATERIAL AND METHODS

The present investigation entitled “Varietal identification and vigour assessment in fodder sorghum [*Sorghum bicolor* (L.) Moench]” comprised of twelve fodder sorghum varieties viz., HC-136, HC-171, HC-308, HJ-513, HJ-541, Pant Chari-7, GFS-5, Pant Chari-3, PC-5, SSV-84, CSV-15 and UP Chari-2 were used in present study. The seeds of different varieties were collected from Forage Section, Department of Genetics and Plant Breeding CCS Haryana Agricultural University, Hisar during 2013. The details of materials and techniques followed for recording different observations during the course of this investigation have been described in this chapter and are given below.

3.1 EXPERIMENT SITE AND LOCATION

The field experiment was conducted in Research Area of Forage Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during the year 2012-2013.

3.2 EXPERIMENTAL DESIGN AND LAYOUT PLAN

The seeds of all fodder sorghum varieties were sown in Randomized Block Design (RBD) with three replication during July 2013. Five rows per plot were sown and the row length was 6 m and row to row and plant to plant spacing was 60 and 15 centimeter, respectively. The seed was sown in shallow furrows. The non-experimental rows were also maintained to avoid border effects. Recommended agronomic package and practices were followed to grow a good and healthy crop.

3.3 RECORDING OF EXPERIMENTAL DATA

A total of five competitive plants (excluding border plants) from each plot were randomly selected and tagged. These tagged plants were used for recording the data pertaining to morphological characters for varietal identification.

A. VARIETAL IDENTIFICATION IN FODDER SORGHUM VARIETIES

3.4 MORPHOLOGICAL CHARACTERS

FIELD STUDIES

i. Leaf sheath anthocyanin coloration

Anthocyanin colour of leaf sheath was observed at 5th leaf stage on the basis of colour present on the leaf sheath from five randomly selected plants in each replication and grouped as yellow green and grayed purple.

ii. Leaf midrib colour

Leaf midrib colour of 5th leaf was observed from five randomly selected plants in each replication and grouped as white, yellow green, grayed yellow and grayed purple.

iii. Flag leaf yellow colouration of midrib

The observation was recorded from five randomly selected plants in each replication on the basis of presence of yellow colour on the midrib of flag leaf and varieties were grouped as present/absent.

iv. Lemma arista formation

The observation was recorded from five randomly selected panicles of each replication and varieties were grouped on the basis of presence and absence of formation of lemma arista.

v. Stigma anthocyanin colouration

The anthocyanin colouration of stigma was observed at end of flowering from five selected panicles (upper portion) in each replication with the help of stereo microscope. The varieties were grouped on the basis of presence and absence of colour on stigma.

vi. Flower with pedicel: length of flower

The observation was recorded at the time of flowering from the five randomly selected panicles in each replication. The varieties were grouped as very short/ short/ medium/ long/ very long.

vii. Anther colour of dry anther

Anther colour was recorded at the time of flowering from five randomly selected panicles in each replication with the help of magnifying lens. The varieties were grouped as yellow orange, orange, orange red and grayed orange.

viii. Glume colour

The colour of glume was recorded at the time of physiological maturity of grain from five randomly selected panicles in each replication with the help of magnifying lens and varieties were grouped as green white, yellow white, grayed yellow, grayed orange, grayed red and grayed purple.

ix. Stem diameter (cm)

The observation was recorded from lower one third height of plant at the time of physiological maturity from five randomly selected plants in each replication. The varieties were grouped as small (<2 cm)/ medium (2-4 cm)/ large (>4cm).

x. Leaf width of blade (cm)

Leaf width of blade was recorded at the time of physiological maturity in third leaf from top including flag leaf from five selected plants in each replication. Varieties were grouped as short (<4.1 cm)/medium (4.1-6.0 cm)/long (6.1-8.0cm)/very long (>8.0 cm).

xi. Panicle length without peduncle (cm)

The observation was recorded at the time of physiological maturity from five randomly selected panicles in each replication. The varieties were grouped as very short (<11cm)/short (11-20 cm)/medium (21-30cm)/long (31-40 cm)/very long (>40 cm).

xii. Panicle length of branches (cm)

Middle third branches from base of selected panicle from five randomly selected panicle in each replication was recorded at the time of physiological maturity and varieties were grouped as short (5.1cm)/medium (5.1-10cm)/long (10.1-15cm)/very long (>15cm).

xiii. Panicle shape

The observation was recorded on visual basis at the time of physiological maturity from five randomly selected panicles in each replication and varieties were grouped as panicle broader in upper part/symmetric/panicle broader in lower part/pyramidal.

xiv. Glume length

Glume length was recorded at the time of physiological maturity from five panicles in each replication. The varieties were grouped as very short (25% of grain covered)/short (50% of grain covered)/medium (75% grain covered)/long (100% grain covered)/very long (longer than the grain).

xv. Caryopsis colour

The colour of caryopsis was observed visually after threshing and varieties were grouped as white, grayed white, yellow white, yellow orange and grayed orange.

xvi. Grain shape in profile view

Grain shape in profile view was recorded visually after threshing and varieties were grouped as narrow elliptic/elliptic/circular.

xvii. Grain texture of endosperm

The grain texture of endosperm was recorded after cutting the grain in longitudinal section and analysed visually. The varieties were grouped as fully vitreous (100% corneous)/ 3/4 vitreous (75% corneous)/ half vitreous (50% corneous)/ 3/4 farinaceous (25% corneous)/ fully farinaceous (0% corneous).

xviii. Grain luster

The observation was recorded visually after threshing and varieties were grouped as non-lustrous/lustrous.

LABORATORY STUDIES**i. Seed size**

The observation was recorded visually after threshing and varieties were grouped as very small/small/medium/large/very large.

ii. Seed shape

Grain shape was recorded visually after threshing and varieties were grouped as narrow elliptic/elliptic/circular.

iii. Seed colour

The colour of caryopsis was observed visually after threshing and varieties were grouped as white, grayed white, yellow white, yellow orange and grayed orange.

iv. 1000 seed weight (g)

Grains were counted after removal of glume of the healthy seed manually and mean weight of three replication was taken and varieties were grouped as very low (<16g) / low (16-25g) / medium (26-35g) / high (36-45g) / very high (>45g).

v. Seedling pigmentation

The observation was recorded at 7-8 days after sowing from five plants selected randomly in each replication and varieties were grouped on the basis of colour present on the coleoptiles as yellow green and grayed purple.

ELECTROPHORESIS OF TOTAL SOLUBLE SEED PROTEIN (SDS – PAGE)

The protein profile for fodder sorghum seed was obtained by electrophoresis technique (SDS-PAGE) following the procedure described by Dadlani & Varier (1993) with some modification.

I. Preparation of reagents**a. Separating (resolving) gel buffer (1.5 M Tris-HCl), pH 8.8**

Tris (18.15 g) was dissolved in 50 ml of distilled water and pH was adjusted to 8.8 by adding concentrated 4N HCl drop by drop. Final volume was made to 100 ml by adding distilled water and stored in refrigerator.

b. Stacking gel buffer (0.5 M Tris-HCl) pH 6.8

Tris (6.05 g) was dissolved in 50 ml of distilled water and pH was adjusted to 6.8 by adding concentrated 4N HCl drop by drop. Final volume was made to 100 ml by adding distilled water.

c. 30% acrylamide – bisacrylamide solution for resolving gel

Acrylamide (73.05 g) and bisacrylamide (1.95 g) were dissolved in distilled water and final volume was made to 250 ml with distilled water.

d. 30 % Acrylamide – bisacrylamide solution for stacking gel

Acrylamide (29.61g) and bisacrylamide (0.39 g) were dissolved in distilled water and final volume was made to 100 ml with distilled water.

e. SDS stock solution (10 % w/v)

SDS (10 g) was dissolved in distilled water with constant stirring and gentle heating. The final volume was made to 100 ml with distilled water and stored at room temperature.

f. Ammonium per sulphate (10 % w/v)

Ammonium persulphate (0.1 g) was dissolved in 1 ml distilled water to make 10 % solution. It was always prepared fresh for use.

g. Running (Tank) Buffer (SDS –Tris glycine, pH 8.3)

Tris (9.075 g), Glycine (43.2 g) and SDS (3 g) were dissolved in distilled water and the volume was made to 3 liter. This buffer can normally be used for 2-3 subsequent runs.

h. Defatting solvent mixture (2:1:1 chloroform : Methanol : Acetone v/v/v, respectively)

Chloroform (200 ml), Methanol (100 ml) and acetone (100 ml) were added to prepare the defatting solvent mixture.

i. Protein extraction buffer (2X sample buffer)

1M Tris HCl (pH 6.8)	2.5 ml
Sodium dodecyl sulphate (SDS)	0.4 g
β- mercaptoethanol	1.0 ml
20 % glycerol	2.0 ml
1 % bromophenol blue	0.4 ml
Final volume (by adding distilled water)	10.0 ml

j. Fixing solution (15 % TCA)

Trichloro acetic acid (150 g) was dissolved in distilled water and final volume was made to one liter.

k. Staining solution

To 100 ml of 15 % TCA solution, 15 ml of 1% CBBR (dissolve 1.0 g of coomassie Brilliant Blue dye in 100 ml methanol) was added to prepare staining solution.

I. Destaining solution

Methanol, acetic acid and water were mixed in ratio of 50:70:880 (v/v/v), respectively to prepare destaining solution.

II. Preparation of gel

Gel plates were cleaned, dried and assembled in the gel cassette. A gel thickness of 1.0 mm was used.

a. Separating gel :

The following solutions were mixed to prepare one slab of gel.

Tris buffer pH 8.8	6.0 ml
Water	5.7 ml
30 % resolving acrylamide	8.9 ml
10 % Ammonium per sulphate (APS)	0.1 ml
10 % Sodium dodecyl sulphate (SDS)	0.2 ml

N,N,N',N'- tetramethylene diamine (TEMED) (0.02 ml) was added just before pouring the gel mixture.

b. **Stacking gel** : After polymerization of the main gel stacking gel mixture was added over it carefully.

Tris buffer pH 6.8	1.50 ml
Water	6.0 ml
30 % stacking gel acrylamide	2.00 ml
10 % Ammonium per sulphate (APS)	0.20 ml
10 % Sodium dodecyl sulphate (SDS)	0.10 ml (For one slab only)

N,N,N',N'- tetramethylene diamine (TEMED) (0.04 ml) was added just before pouring the solution.

III. Preparation of protein samples

Five sound pure seed were randomly selected from each variety and ground into fine powder with the help of pestle and mortar. The powder transferred to test tube and 10 ml of defatting solvent mixture added. The test tubes were covered with aluminum foil and allowed for defatting. After 3 h., solvent mixture decanted and the process was repeated three to four times so that the oil content could be removed completely. The seed powder was then dried by leaving the test tubes open at room temperature.

IV. Protein extraction (Total Tris soluble protein)

One hundred milligram of defatted seed powder was taken in eppendorf tube and 0.5 ml of 2x sample buffer was added to it. The contents were thoroughly mixed and kept for overnight in refrigerator. The tubes were taken out and content were mixed properly and then it was subjected to centrifugation at 10,000 rpm for 10

minutes. The supernatants were taken into separate glass tubes. These samples were then boiled in water bath for 10 minute, cooled and finally it was used as protein source for electrophoresis.

V. Electrophoresis

The electrophoresis was carried out using the apparatus of M/S Atto, Japan. The plates were thoroughly washed with water followed by ethanol and then air dried. The plates were fitted in gel casting assembly and tygon tube was used to make it air tight. After mixing all components of separating gel, the solution was poured immediately in between the glass slabs. A layer of water was then gently poured using a syringe and after polymerization of gen, water was removed using filter paper stripe. Thereafter, the stacking gel was mixed and immediately poured over the separating gel. The comb was inserted with care so that on air bubble could be trapped. The tygon tube was removed after polymerization of gel and the gel assembly was fitted into the electrophoresis apparatus. Running or Tank buffer was poured in upper and lower reservoirs of the electrophoresis apparatus. The comb was removed and wells were washed properly and carefully. In each well 25 μ l sample was loaded. Electrophoresis was carried out first at 1.5 mA per well till the sample migrated to the resolving gel and later at 2 mA per well till the tracking dye reached the bottom of the gel.

VI. Fixing and staining

Cassette was removed from the unit and the gel was taken out gently. It was placed in a tray and incubated for 15 minutes in 15% TCA solution and thereafter, staining solution was added to the tray after removing fixing solution and incubated for 8 hrs. The staining solution was decanted after expiry of time and gel was rinsed with water. Destaining was done for 1-2 days with destaining solution to clear the gel background. After proper destaining, gels were photographed and stored in 7% acetic acid for longer period.

VII. Evaluation and documentation

The varieties were separated into different groups according to number of bands (presence or absence) and intensities of some specific bands. The relative mobility (R_m) value of each band was calculated as follows

$$\text{Relative mobility (R}_m\text{)} = \frac{\text{Distance migrated by the protien band from the origin (cm)}}{\text{Distance migrated by the tracking dye (cm)}}$$

VIII. Similarity index

Similarity index value was calculated based on proportion on common bands between two lines by using the following formula (Nei and Li, 1979).

$$F = \frac{2M_{xy}}{M_x + M_y}$$

Where F is the similarity index, M_x is the number of bands common in variety x, M_y is the number of bands in variety y and M_{xy} is the number of bands common to both x and y. $F \times 100$ gives the percent similarity between two variety, thus $F = 1.0$ would mean that the patterns in the varieties are identical.

IX. Dendrogram analysis

Analysis was done with the help of computer by using D-UPGMA software.

B. SEED VIGOUR ASSESSMENT IN FODDER SORGHUM VARIETIES

All fodder sorghum varieties used for varietal identification, were further used for seed quality assessment and the following observations were recorded in the laboratory and field conditions.

Laboratory parameters

The study was conducted in the laboratories of the Department of Seed Science and Technology during 2013-2014 and following observations were recorded.

i. Standard Germination test (%)

One hundred seeds with three replication each of all the fodder sorghum varieties were tested in the laboratory according to the Rules of International Seed Testing Association (ISTA), 2011. The final count of germination was recorded on 8th day and the number of normal seedlings were counted and expressed as percent germination.

ii. Seedling length (cm)

Ten normal seedlings in three replication of each variety were selected randomly during germination test and measured in centimeter and average seedling length was calculated.

iii. Seedling dry weight (mg)

Ten normal seedlings selected at randomly during germination testing were taken for measuring dry-weight (mg). These seedlings were placed in paper bags and then transferred into hot air oven. The average weights of ten dry seedlings were taken and seedling dry weight expressed in milligrams.

iv. Vigour Indices

Seedling vigor indices were calculated according to the formulae suggested by Abdul-Baki and Anderson, (1973).

Vigour Index-I = Standard germination (%) x seedling length (cm)

Vigour Index-II = Standard germination (%) x seedling dry weight (mg)

v. Electrical Conductivity test (mS/cm/100 seeds)

Electrical conductivity of the seed leachates was measured to know the status of membrane permeability. Hundred normal and undamaged seeds taken randomly (replicated thrice) from each seed lot were soaked in 100 ml beakers each containing 75ml of distilled water. The seeds were immersed completely in water and beakers were covered with the aluminium foil. Thereafter, these samples were kept in the germinator at 25°C for 24h. The electrical conductivity of seed leachates was measured by Conductivity Meter and was expressed in mS/cm/100seeds.

vi. Accelerated Ageing test (%)

Sufficient number of seeds from each variety were taken and put on in a single layer on wire mesh tray fitted in plastic boxes having 20 ml of distilled water in bottom. The boxes were placed in accelerating ageing chamber after closing their lids. The seeds were aged at 40 ± 1 °C temperature and about 100 % RH for 72 h. and then tested for germination in three replications of 100 seeds each according to Rules of International Seed Testing Association (ISTA), 2011. The number of normal seedlings was counted and expressed in percentage.

vii. Tetrazolium Test (%)

Viability test (Moore, 1974) based on three replications of 50 seeds were conducted by soaking seeds in 50 ml water for 16 h. at 25° C to activate dehydrogenase enzymes. After the pre-conditioning, soaked seed subjected to longitudinal cut and the prepared seeds were stained in 0.5 % tetrazolium solution (2,3,5-triphenyl tetrazolium chloride) for 4 h. at 30°C in petriplates. Thereafter, solution was poured off and seeds were rinsed in water and examined under magnifications. The number of seeds stained red were considered as viable seeds and expressed in per cent.

Dehydrogenase activity test (OD)

As suggested by Kittock and Law (1968), the representative seed samples of each fodder sorghum variety were grounded to pass through 20-mesh screen. 200 mg fine grounded material was soaked in 5 ml of freshly prepared 0.5% TTC solution having pH 7.0. After that the mixture was incubated at a temperature of 38°C for 4 h. Then it was centrifuged at 10,000 rpm for 3 minutes and the supernatant was poured off. The formazan was extracted with 10 ml acetone for 16 h. at room temperature.

It was again centrifuged for 3 minutes at 10,000 rpm. Then acetone solution containing formazan was transferred to the cuvette. The absorbance reading of the solution (OD) was taken at 520 nm wave length using Systronics Spectrophotometer-169.

Field parameters

One hundred seeds of all the twelve fodder sorghum varieties in three replication each were sown in a Randomized Block Design (RBD) in the Research farm, Department of Seed Science and Technology, CCS Haryana Agriculture University, Hisar and the following observations were recorded.

I. Field Emergence Index

The number of seedlings emerged were counted on each day up to seedling establishment. The field emergence index (speed of emergence) was calculated as described by Maguire (1962).

Field Emergence Index (FEI) =

$$\frac{\text{No. of seedlings emerged}}{\text{Day of first count}} + \dots + \frac{\text{No. of seedlings emerged}}{\text{Day of final count}}$$

II. Mean Emergence Time (days)

The mean emergence time (MET) was calculated for each treatment combination using the formula cited by Ellis and Roberts (1980).

$$\text{MET} = \frac{\sum nt}{\sum n}$$

Where, n- number of seeds newly germinated at time 't'

t - Days from sowing.

$\sum n$ - Final emergence of seedlings.

III. Seedling Establishment (%)

The seedling establishment was determined by counting the total number of seedlings when the emergence was completed or when there was no further addition in the total emergence.

Statistical Analysis

The mean value of observations recorded on different parameters was subjected to statistical and graphical analysis. The factorials CRD (Completely Randomized Design) for laboratory parameters and RBD (Randomized Block Design) for field parameters were used for analysis of variance (Panse and Sukhatme, 1967).

ANOVA for RBD

Source of variation	Degree of freedom (Df)	Sum of squares (SS)	Mean squares (MS)	F-value
Replications	(r-1)	SSr	MSr	MSr/EMS
Treatment combinations	(t-1)	SSt	MSt	MSt/EMS
Error	(r-1)(t-1)	SSe	MSe(EMS)	
Total	(rt-1)	Total SS		

Where,

- r : Number of replications
t : Number of treatment combinations
SSt : Sum of squares due to treatments combination
SSe : Error sum of squares
MSt : Mean squares due to treatments
EMS : Error mean squares

The standard error of differences (SEd), Standard error of means (SEm), Critical difference (CD) and coefficient of variation (CV) were calculated as follows:

$$SE(m) = \sqrt{\frac{EMS}{r}}$$

$$SE(d) = \sqrt{\frac{2 \times EMS}{r}}$$

CD (5%): SEd x t value at error d.f.

$$CV (\%) = \frac{\sqrt{EMS}}{\bar{X}} \times 100$$

Where, r = number of replicates; \bar{X} = overall mean (grand total /n)

The correlation coefficients among various laboratory parameters were estimated as per standard formulae as given below:

$$r = \frac{\text{Cov}(x,y)}{\sigma_x \cdot \sigma_y}$$

Where,

r	=	Correlation coefficient
Cov(x,y)	=	Covariance between characters x and y
σ_x	=	Standard deviation of character x
σ_y	=	Standard deviation of character y

In the present investigation, twelve varieties of fodder sorghum [*Sorghum bicolor* (L.) Moench] were evaluated at Research Area of Forage Section, Department of Genetics and Plant Breeding CCS Haryana Agricultural University, Hisar. Various morphological characters were studied to characterize all these varieties. The observations were recorded and results obtained for various characters are mentioned below.

4.1 Classification on the basis of Morphological characteristics Field Study

Fodder sorghum varieties were grouped into different classes on the basis of observations recorded as per the guidelines of Protection of Plant Varieties & Farmers Right Authority for morphological characters in the field at different stages of plant growth.

4.1.1 Leaf sheath anthocyanin colouration

On the basis of leaf sheath anthocyanin colouration, varieties were classified into two categories. Six varieties (PC-3, PC-5, GFS-5, PC-7, HJ 513 and HJ 541) were yellow green and remaining 6 varieties (HC-136, HC-171, HC-308, SSV-84, CSV-15 and UP Chari-2) were grayed purple in colour. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.1.

4.1.2 Leaf mid rib colour

On the basis of leaf mid rib colour of 5th fully developed leaf, varieties were classified into two groups. Only two varieties (PC 7 and HJ 513) had white and remaining 10 varieties (GFS-5, HJ-541, HC-308, HC-136, HC-171, SSV-84, CSV-15, UP Chari-2, PC-3 and PC-5) showed yellow green leaf mid rib colour. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.2.

4.1.3 Flag leaf yellow colouration of mid rib

On the basis of flag leaf yellow colouration of mid rib, varieties were classified into two categories *viz.*, absence and presence of yellow colouration. Two varieties (CSV 15 and HJ 513) had white mid rib and remaining 10 varieties (HC - 171, HC-136, HJ-541, HC-308, SSV-84, PC-3, UP Chari-2, PC-5, GFS-5 and PC-7) recorded yellow green colour. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.3.

Table 1: Diagnostic plant characters for varietal identification in different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Character (s)	Variety (s)											
	PC 7	PC 3	GFS 5	PC 5	SSV 84	CSV 15	HC 136	HC 171	HC 308	HJ 513	HJ 541	UP Chari 2
Leaf Sheath Anthocyanin colouration	Yellow green	Yellow green	Yellow green	Yellow green	Grayed purple	Grayed purple	Grayed purple	Grayed purple	Grayed purple	Yellow green	Yellow green	Grayed purple
Leaf midrib colour (5 th fully developed leaf)	White	Yellow green	Yellow green	Yellow green	Yellow green	Yellow green	Yellow green	Yellow green	Yellow green	White	Yellow green	Yellow green
Flag leaf yellow colouration of midrib	Present	Present	Present	Present	Present	Absent	Present	Present	Present	Absent	Present	Present
Lemma arista formation	Present	Present	Absent	Present	Absent	Absent	Present	Present	Present	Present	Present	Present
Stigma anthocyanin colouration	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent	Present	Present	Present	Absent
Flower with pedicel : length of flower	Medium	Medium	Long	Medium	Long	Long	Medium	Very long	Very long	Long	Long	Very long
Anther colour of dry anther	Grayed orange	Grayed orange	Grayed orange	Grayed orange	Yellow orange	Grayed orange	Grayed orange	Yellow orange	orange	Grayed orange	Yellow orange	Yellow orange
Glume colour	Grayed yellow	Yellow white	Yellow white	Grayed yellow	Grayed red	Grayed red	Grayed orange	Yellow white	Green white	Green white	Grayed orange	Yellow white
Glume length	Short	Very short	Medium	Short	Short	Short	Very short	Medium	Very short	Long	Long	Very long
Stem diameter	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Small	Medium	Medium	Medium

Contd..

Character(s)	Variety(s)											
	PC 7	PC 3	GFS 5	PC 5	SSV 84	CSV 15	HC 136	HC 171	HC 308	HJ 513	HJ 541	UP Chari 2
Leaf Width of blade	long	Long	Medium	Medium	Very long	Very long	Long	Long	Long	Long	long	Very long
Panicle length of branches	Medium	Medium	Medium	Medium	Medium	Short	Short	Short	Medium	Medium	Medium	long
Panicle length without peduncle	Medium	Medium	long	Medium	long	Medium	Short	Short	Medium	Medium	Medium	Medium
Panicle shape	Symmetrical	Panicle broader in upper part	Pyramidal	Symmetrical	Symmetrical	Symmetrical	Symmetrical	Panicle broader in upper part	Symmetrical	Panicle broader in lower part	Panicle broader in lower part	Symmetrical
Caryopsis colour after threshing	Yellow white	Yellow white	Yellow white	White	White	White	Yellow white	Yellow white	Yellow white	Yellow white	Yellow white	Yellow white
Grain shape in profile view	Elliptical	Circular	Circular	Circular	Circular	Circular	Circular	Elliptical	Circular	Circular	Elliptical	Circular
Grain texture of endosperm	$\frac{3}{4}$ vitreous	$\frac{3}{4}$ vitreous	$\frac{1}{2}$ vitreous	$\frac{3}{4}$ vitreous	$\frac{1}{2}$ vitreous	$\frac{1}{2}$ vitreous	Vitreous	$\frac{3}{4}$ farinaceous	$\frac{3}{4}$ farinaceous	$\frac{1}{2}$ vitreous	$\frac{3}{4}$ farinaceous	$\frac{1}{2}$ vitreous
Grain luster	lusterous	Non Lusterous	Lusterous	Lusterous	Lusterous	Lusterous	Lusterous	Non Lusterous	Lusterous	Lusterous	lusterous	Non lusterous
Seed size	Large	Large	Large	Medium	Medium	Medium	Very large	Medium	Medium	Very large	Medium	Large

Fig. 1 Flow chart based on morphological characters for different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

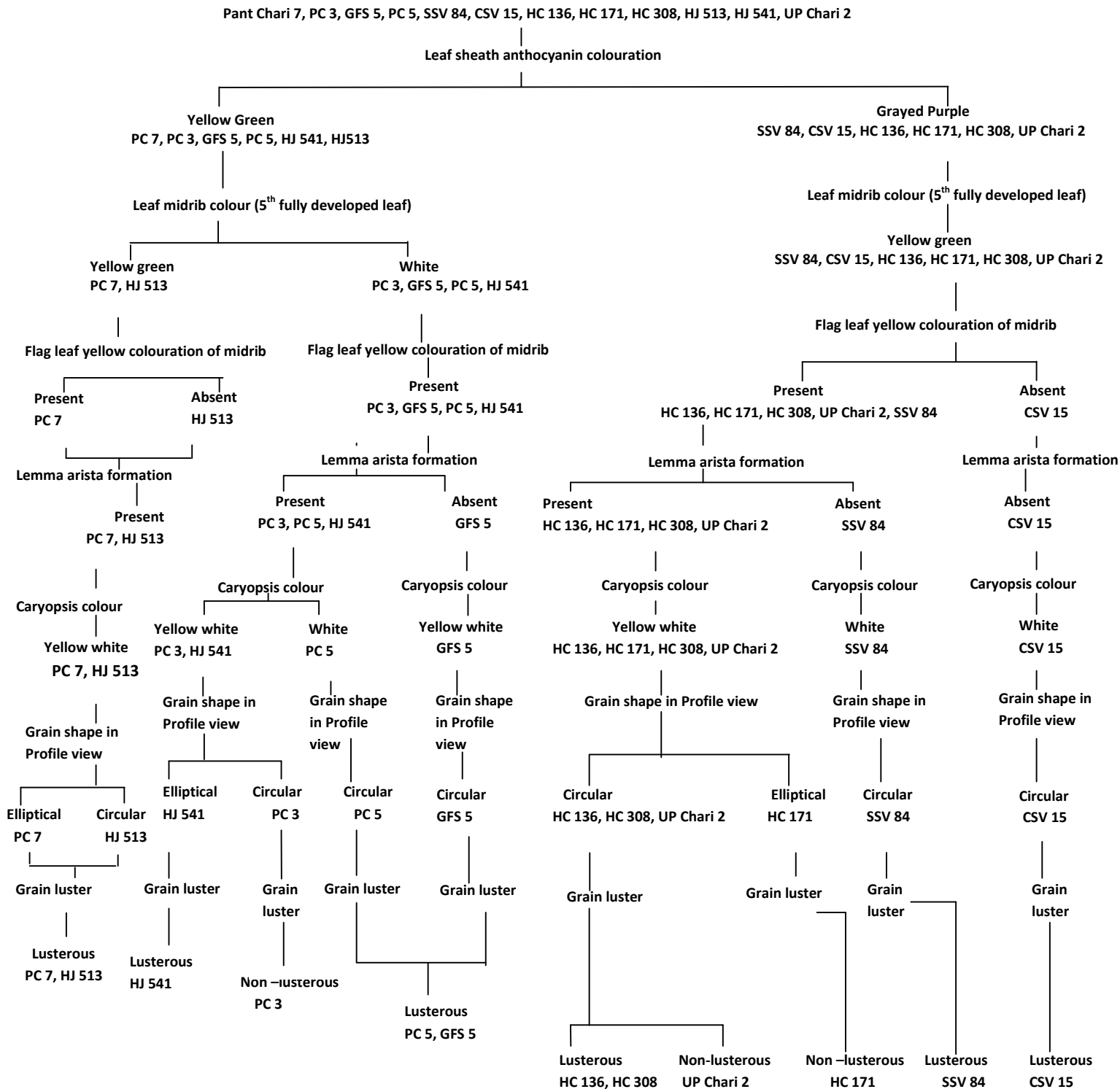


PLATE 4.1: Leaf sheath anthocyanin colouration



Grayed purple

HC-136, HC-171, HC-308, SSV-84,
CSV-15, UP Chari-2

Yellow green

PC-3, PC-5, GFS-5, PC-7,
HJ 513, HJ 541

PLATE 4.2: Leaf mid rib colour (5th fully developed leaf)



White

HJ 513, PC-7



Yellow green

GFS-5, HJ-541, HC-308, HC-136, HC-171, SSV-84,
CSV-15, UP Chari-2, PC-3, PC-5,

PLATE 4.3 : Flag leaf yellow colouration of mid rib



Present

HC-171, HC-136, HJ-541, HC-308,
SSV-84, PC-3, UP Chari-2, PC-5,
GFS-5, PC-7



Absent

HJ 513, CSV-15

PLATE 4.4 : Lemma arista formation



Absent
SSV-84, GFS-5, CSV-15,



Present
HJ-513, HC-171, HC-136, HJ-541, HC-308, UP Chari-2, PC-7, PC-3, PC-5

PLATE 4.5 : Stigma anthocyanin colouration



Present
HJ-513, HJ-541, HC-308, SSV-84



Absent
HC-171, HC-136, CSV-15, UP Chari-2, PC-3, PC-5, PC-7, GFS-5

PLATE 4.6: Flower with pedicel: length of flower



Medium
HC-136, PC-5, PC-3, PC-7



Long
CSV-15, HJ-513, SSV-84, HJ-541, GFS-5



Very long
UP Chari-2, HC-171, HC-308

4.1.4 4.1.4 Lemma arista formation

Varieties were also classified on the basis of lemma arista formation and most of the varieties (HJ-513, HC-171, HC-136, HJ-541, HC-308, UP Chari-2, PC-7, PC-3 and PC-5) had lemma arista formation except 3 varieties (SSV-84, GFS-5 and CSV-15). The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.4.

4.1.5 Stigma anthocyanin colouration

On the basis of stigma anthocyanin colouration, varieties were classified into two categories *viz.*, absence and presence of stigma anthocyanin colouration. Eight varieties (HC-171, HC-136, CSV-15, UP Chari-2, PC-3, PC-5, PC-7 and GFS-5) had non stigma anthocyanin colour and remaining 4 varieties (HJ-513, HJ-541, HC-308 and SSV-84) had stigma anthocyanin colour. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.5.

4.1.6 Flower with pedicel: length of flower

On the basis of flower with pedicel length of flower, varieties were grouped into three categories *viz.*, medium, long and very long. Four varieties (HC-136, PC-5, PC-3 and PC-7) recorded medium, five varieties (CSV-15, HJ-513, SSV-84, HJ-541 and GFS-5) recorded with long pedicel and remaining 3 varieties (UP Chari-2, HC-171 and HC-308) were with very long pedicel length. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.6.

4.1.7 Anther colour of dry anther

On the basis of anther colour of dry anther, varieties were categories into three group's *viz.*, yellow orange, orange and grayed orange. Four varieties (HC-171, HJ-541, SSV-84 and UP Chari-2) found with yellow orange, seven varieties (HJ-513, CSV-15, PC-3, PC-5, GFS-5, HC-136 and PC-7) were grayed orange and only HC 308 found with orange anther colour of dry anther. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.7.

4.1.8 Glume colour

All the varieties were classified into five categories *viz.* green white, yellow white, grayed yellow, grayed orange and grayed red on the basis of glume colour as shown in Table 1 and Plate 4.8. Varieties HC 308 and HJ 513 had with green white; GFS-5, UP Chari-2, PC-3 and HC 171 had yellow white; two varieties (PC-5 and PC-7) had grayed yellow; two varieties (HC- 136, HJ-541) showed grayed orange and SSV-84 and CSV-15 had grayed red glume colour.

4.1.9 Glume length

Varieties classified on the basis of glume length have been presented in Table 1 and various groups have been shown in Plate 4.9. Four varieties (HC 308, HC 136, PC-3 and UP Chari-2) were very short; five (SSV-84, CSV-15, HJ 541, PC-5 and PC-7) were short; two (HC 171 and GFS-5) were medium and HJ 513 was found with long glume length.

4.1.10 Stem diameter

On the basis of stem diameter, varieties were classified into two group's *viz.*, small and medium. Only HC 308 was found small (<2cm) and remaining eleven (HJ-541, HC-171, HJ-513, HC-136, GFS-5, PC-7, CSV-15, UP Chari-2, PC-3, PC-5 and SSV-84) recorded with medium stem diameter (2-4 cm). The classification of varieties on the basis of stem diameter has been presented in Table 1 and various groups have been shown in Plate 4.10.

4.1.11 Leaf width of blade

On the basis of leaf width of blade, varieties were grouped into three category *viz.*, medium (4.1-6.0 cm), long (6.0-8.0 cm) and very long (>8.0 cm). Two varieties (PC-5 and GFS-5) were medium, seven varieties (PC-3, PC-7, HC-171, HJ-541, HJ-513, HC-136 and HC-308) were long and three varieties (UP Chari-2, CSV-15 and SSV-84) recorded very long leaf width of blade. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.11.

4.1.12 Panicle length without peduncle

On the basis of panicle length without peduncle, varieties were classified into four categories *viz.*, short, medium, long and very long. Two varieties (HC-136 and HC-171) had short (11-20 cm); eight varieties (PC-7, CSV-15, UP Chari-2, PC-3, PC-5, HC-308, HJ-513 and HJ-541) had medium (21-30 cm); two varieties (GFS-5 and SSV-84) recorded long (31-40 cm) panicle length. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.12.

4.1.13 Panicle length of branches

Classification of varieties on the basis of panicle length of branches has been presented in Table 1 and various groups have been shown in Plate 4.13. Varieties were classified into four categories *viz.*, short, medium, long and very long. Varieties HC-171, CSV-15 and HC 136 showed short (< 5.1 cm); eight varieties (HC-308, HJ-513, HJ-541, SSV-84, GFS-5, PC-7, PC-3 and PC-5) had medium (5.1-10 cm); only UP Chari 2 had long (10.1-15cm) panicle length of branches.

PLATE 4.7 : Anther colour of dry anther



Orange
HC-308



Grayed orange
HJ-513, CSV-15, PC-3,
PC-5, GFS-5, HC-136,
PC-7

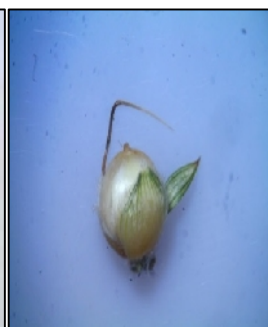


Yellow orange
HC-171, HJ-541, SSV-
84, UP Chari-2,

PLATE 4.8: Glume colour



Yellow white
GFS-5, HC-
171, UP Chari-
2, PC-3



Grayed yellow
PC-5, PC-7



Grayed orange
HC- 136, HJ-541



Grayed Red
SSV-84, CSV-15



PLATE 4.9 : Glume length



Very short (25% of grain covered)
HC 308, HC 136, PC-3,
UP Chari-2



Short (50% of grain covered)
SSV-84, CSV-15,
HJ 541, PC-5, PC-7



Medium (75% of grain covered)
HC 171, GFS-5



Long (100% of grain covered)
HJ 513

PLATE 4.10: Stem diameter



Small (<2 cm)
HC 308



Medium (2-4 cm)
HJ-541, HC-171, HJ-513, HC-136, GFS-5, PC-7,
CSV-15, UP Chari-2, PC-3, PC-5, SSV-84

PLATE 4.11: Leaf width of blade



Medium (4.1-6.0 cm)
PC-5, GFS-5



Long (6.0-8.0cm)
PC-3, PC-7, HC-171, HJ-541, HJ-
513, HC- 136, HC-308



Very long (> 8.0 cm)
UP Chari-2, CSV-15,
SSV-84.

PLATE 4.12 : Panicle length without peduncle



Short (11-20 cm)
HC-136, HC-171



Medium (21-30cm)
PC-7, CSV-15, UP Chari-2,
PC-3, PC-5, HC-308, HJ-513,



Long (31-40 cm)
GFS-5, SSV-84

4.1.14 Panicle shape

Varieties were classified on the basis of panicle shape has been presented in Table 1 and the various groups have been shown in Plate 4.14. Seven varieties (HC-308, SSV-84, CSV-15, PC-5, PC-7, UP Chari-2 and HC-136) had symmetrical; two varieties (HJ-513 and HJ-541) had panicle broader in lower part, two varieties (HC-171 and PC-3) showed panicle broader in upper part and GFS 5 had pyramidal in their panicle shape.

4.1.15 Caryopsis colour

On the basis of caryopsis colour all the varieties were classified into two categories *viz.*, white and yellow white. Three varieties (SSV-84, CSV-15, and PC-5) showed white; nine varieties (HC-308, HJ-513, HC-136, HC-171, HJ-541, UP Chari-2, PC-3, GFS 5 and PC-7) showed yellow white. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.15.

4.1.16 Grain shape in profile view

All the varieties were also classified on the basis of grain shape in profile view which has been presented in Table 1 and various groups have been shown in Plate 4.16. Nine varieties (HC-136, HC-308, SSV-84, CSV-15, UP Chari-2, PC-3, PC-5, GFS-5 and HJ-513) were circular; three (HJ-541, HC-171, PC-7) were found elliptic in their grain shape.

4.1.17 Grain texture of endosperm

On the basis of grain texture of endosperm, varieties were classified into four categories *viz.*, vitreous, $\frac{3}{4}$ vitreous, $\frac{1}{2}$ vitreous, and $\frac{3}{4}$ farinaceous. Variety HC-136 was 100% vitreous; three varieties (PC-3, PC-5 and PC-7) were observed with $\frac{3}{4}$ vitreous; five varieties (SSV-84, GFS-5, CSV-15, UP Chari-2 and HJ-513) were $\frac{1}{2}$ vitreous and three varieties (HJ-541, HC-171 and HC-308) were $\frac{3}{4}$ farinaceous. The classification of varieties has presented in Table 1 and various groups have been shown in Plate 4.17.

4.1.18 Grain luster

On the basis of grain luster, varieties were categories in two groups *viz.*, lusterous and non lusterous. Nine varieties (HJ-541, HC-136, HJ-513, SSV-84, CSV-15, GFS-5, HC-308, PC-5 and PC- 7) showed lusterous grain while remaining three varieties (HC-171, UP Chari-2 and PC-3) had non lusterous grain. The classification of varieties has been presented in Table 1 and various groups have been shown in Plate 4.18.

Laboratory studies

i. Seed Size

Varieties were also classified on the basis of grain size and the results are presented in Table 1, six varieties (HC-308, HC-171, HJ-541, CSV-15, SSV-84 and PC-5) were found medium in size, four varieties (GFS-5, UP Chari-2, PC-3 and PC-7) showed large and HC 136 and HJ 513 were very large seed size.

ii. Seed shape

Varieties were also classified on the basis of grain shape and the results are showed in Table 1. Nine varieties (HC-136, HC-308, SSV-84, CSV-15, UP Chari-2, PC-3, PC-5, GFS-5 and HJ-513) showed circular; three varieties (HJ-541, HC-171 and PC-7) were found elliptic in their grain shape.

iii. Seed colour

On the basis of Seed colour, all the varieties were classified into two categories i.e white and yellow white. Data showed that where three varieties (SSV-84, CSV-15, and PC-5) had white while nine varieties (HC-308, HJ-513, HC-136, GFS 5, HC-171, HJ-541, UP Chari-2, PC-3 and PC-7) recorded yellow white. (Table 1)

iv. 1000-Seed Weight

On the basis of 1000 seed weight, all the varieties were classified into three groups (Table 6) viz., low (16-25 g), Medium (26-35 g) and high (36-40 g). On the basis of above classification, three varieties (GFS-5, HC-308 and SSV-84) were found low, eight varieties (PC-7, PC-3, PC-5, UP Chari-2, CSV-15, HC-171, HC-513 and HC-541) recorded medium while HC-136 showed high seed weight.

v. Seedling pigmentation

On the basis of seedling pigmentation, different varieties of fodder sorghum were classified in two categories i.e yellow green and grayed purple. Nine varieties (HJ 513, HJ 541, CSV-15, UP chari-2, PC-3, PC-5, GFS-5, SSV-84 and PC-7) were found yellow green while HC 308, HC 136 and HC 171 recorded grayed purple colour seedling pigmentation.

4.2 Electrophoresis of seed protein

All 12 fodder sorghum varieties were subjected to SDS-PAGE analysis for total seed proteins (plate 14.19). A wide quantitative variation was noticed in terms of relative mobility (R_m) value, electrophoretic mobility of protein bands, intensity of bands and also in banding pattern of different sorghum varieties. Polymorphism in protein banding of all varieties were noticed and it was observed that they varied in intensity and thickness also.

PLATE 4.13 : Panicle length of branches



Short (<5.1 cm)
HC- 136, HC- 171,
CSV-15

Medium (5.1-10 cm)
HC- 308, HJ- 513, HJ- 541, SSV-
84, GFS-5, PC-7, PC-3, PC-5

Long (10.1-15 cm)
UP Chari-2

PLATE 4.14 : Panicle shape



Symmetrical
HC- 308, SSV-84,
CSV-15, PC-5, PC-7,
UP Chari-2, HC-136

**Panicle broader in
lower part**
HJ -513, HJ-541

Pyramidal
GFS-5

**Panicle broader in
upper part**
HC-171, PC-3

PLATE 4.15 : Caryopsis colour



White
SSV-84,
CSV-15,
GFS-5, PC-5



Yellow white
HC-308, HJ-513,
HC-136, HC-171,
HJ-541, UP Chari-
2, PC-3, PC-7

PLATE 4.16: Grain shape in profile view



Circular

HC-136, HC-308, SSV-84, CSV-15,
UP Chari-2, PC-3, PC-5, GFS-5, HJ-
513



Elliptic

HJ-541, HC-171, PC-7

PLATE 4.17: Grain texture of endosperm



Vitreous
HC136



½ Vitreous
SSV-84, GFS-5,
CSV-15, UP Chari-
2, HJ-513,



¾ Vitreous
PC-3, PC-5, PC-7



¾ Farinaceous
HJ-541, HC-171,
HC-308,

PLATE 4.18 : Grain luster



Lusterous

HJ-541, HC-136, HJ-513,
SSV-84, CSV-15, GFS-5,
HC-308, PC-5, PC-7



Non lusterous

HC-171, UP Chari-2, PC-3

The SDS- PAGE analyses of the total soluble proteins of twelve varieties revealed a maximum seventeen bands were recorded in total soluble seed protein electrophoresis profile. A wide quantitative variation (having different Rm values) was observed in pattern of protein bands, their electrophoretic mobility, position and intensity. The standard protein marker bands [2 (Rm 0.24), 6 (Rm 0.40), 9 (Rm 0.53), 11 (Rm 0.64), 13(Rm 0.68), 16 (Rm 0.88) and 17 (Rm 0.91)] were observed invariably common in almost all the varieties (Table 2). The maximum number of bands were observed in varieties HC-136 (Dense-1, Medium-8, light-5 and weak-3), HJ-513 (Dense-3, medium-3, light-7 and weak 4), PC-5 (Dense-3, Medium-2, light-11, weak-1) and lowest were observed in variety PC-3 (Medium-2, light-6, weak-3). All the varieties were differentiated by using the presence/absence of specific combination of bands or single band. In this way, all the twelve varieties were individually identified. (Table 3)

The analysis was carried out along with the standard molecular weight marker having range 14.4 to 116.0 kDa. The bands between 45 to 66.2 kDa molecular weight were more thick and intense as compared to higher and lower molecular weight bands. This was a clear indication that these molecular size protein molecules constituted majority of total soluble seed protein in sorghum varieties.

The similarity index was calculated based on the protein of all fodder sorghum varieties. Similarity index based on the relative mobility of protein band indicated the association among different varieties. The similarity index was calculated in all combination and their values obtained are present in Table 5. The similarity index value ranges from 0.143 to 1.000. The variety PC-3 showed less value with other varieties showing dissimilarity with other remaining varieties.

Unweighted paired group method with arithmetic mean (UPGMA) was used to construct dendrogram for all the twelve sorghum varieties. The dendrogram showed two major clusters at coefficient of 0.7774 as the varieties PC- 3 and PC 7 in first cluster and rest varieties in second cluster. The second cluster was further divided into two sub-clusters. The complete dendrogram is shown in Fig.2.

4.3 Seed vigour assessment studies

The seed vigour is an important aspect of the seed quality which varies even in the seed lot having same germination percentage. The loss of seed viability in storage is preceded by a wide range of symptoms which collectively contribute to the loss of seed vigour. The loss of seed vigour usually reflected in the lack of uniformity and decreased field emergence. Generally, the germination standard

Table 2: Number of bands, their position and Rm values of seed protein in different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Band number	Position (cm)	Rm value
1	1.89	0.18
2	2.52	0.24
3	2.94	0.28
4	3.26	0.31
5	3.36	0.32
6	4.20	0.40
7	4.73	0.45
8	5.04	0.48
9	5.57	0.53
10	5.99	0.57
11	6.72	0.64
12	6.93	0.66
13	7.14	0.68
14	7.56	0.72
15	8.51	0.81
16	9.24	0.88
17	9.56	0.91

Table 3: Seed Protein bands present or absent in different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Band No.	HC 136	HC 171	HC 308	HJ 513	HJ 541	PC 3	PC 5	PC 7	UP Chari 2	SSV 84	CSV 15	GFS 5
1	+	+	++	+	+	-	+	-	+	+	-	+
2	++	+++	+++	+++	+++	+	+++	+++	++	+++	+++	+++
3	+	++	++	+	+	+	+++	++	++	+++	+	++
4	+++	++	+++	++	++	-	++	+	++	++	++	+
5	+++	++++	-	+	++	+++	++++	+++	++++	++++	++++	++
6	+	++	++++	++++	++++	++	++	++	+++	+	-	++
7	++++	++++	++	++	+++	++	++	++	+++	+++	+++	+++
8	+++	++++	++	++	++	+++	++++	++	+++	+++	+++	+
9	++	++	+++	++++	++++	-	++++	++	+++	+++	+++	+++
10	++	+++	++++	++++	++++	+	++	++	++	-	-	+++
11	++	++	++	+++	++	-	++	-	+	++	+	-
12	+++	+++	+++	+++	+++	++	++	++	+	-	-	+
13	+++	+++	++	++	-	++	++	+	-	-	-	-
14	+++	+++	+++	++	++	++	++	++	++	++	++	++
15	+++	+++	++	++	++	++	++	-	++	++	++	++
16	+++	-	++	++	++	-	++	-	++	++	++	++
17	++	++	++	+	++	-	++	++	-	++	++	+

++++ Dense; +++ Medium; ++ Light; + weak; - Band absent

Table 4: Band map based on seed protein analysis different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Varieties / Protein marker Band	1	2	3	4	5	6	7
HC 136	1	1	1	1	1	1	1
HC 171	1	1	1	1	1	0	1
HC 308	1	1	1	1	1	1	1
HJ 513	1	1	1	1	1	1	1
HJ 541	1	1	1	1	0	1	1
PC 3	1	1	0	0	1	0	0
PC 5	1	1	1	1	1	1	1
PC 7	1	1	1	0	1	0	0
UP Chari 2	1	1	1	1	0	1	1
SSV 84	1	1	1	1	0	1	1
CSV 15	1	0	1	1	0	1	1
GFS 5	1	1	1	0	0	1	1

Table 5: Similarity index based on seed protein profile analysis in different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Sr.no	Varieties	1	2	3	4	5	6	7	8	9	10	11	12
1.	HC 136	1	0.857	1.000	1.000	0.857	0.429	1.000	0.571	0.857	0.857	0.714	0.714
2.	HC 171		1	0.857	0.857	0.714	0.500	0.857	0.667	0.714	0.714	0.571	0.571
3.	HC 308			1	1.000	0.857	0.429	1.000	0.571	0.857	0.857	0.714	0.714
4.	HJ 513				1	0.857	0.429	1.000	0.571	0.857	0.857	0.714	0.714
5.	HJ 541					1	0.286	0.857	0.429	1.000	1.000	0.833	0.833
6.	PC 3						1	0.429	0.750	0.286	0.286	0.143	0.333
7.	PC 5							1	0.571	0.857	0.857	0.714	0.714
8.	PC 7								1	0.429	0.429	0.286	0.500
9.	UP Chari 2									1	1.000	0.833	0.833
10.	SSV 84										1	0.833	0.833
11.	CSV 15											1	0.667
12.	GFS 5												1

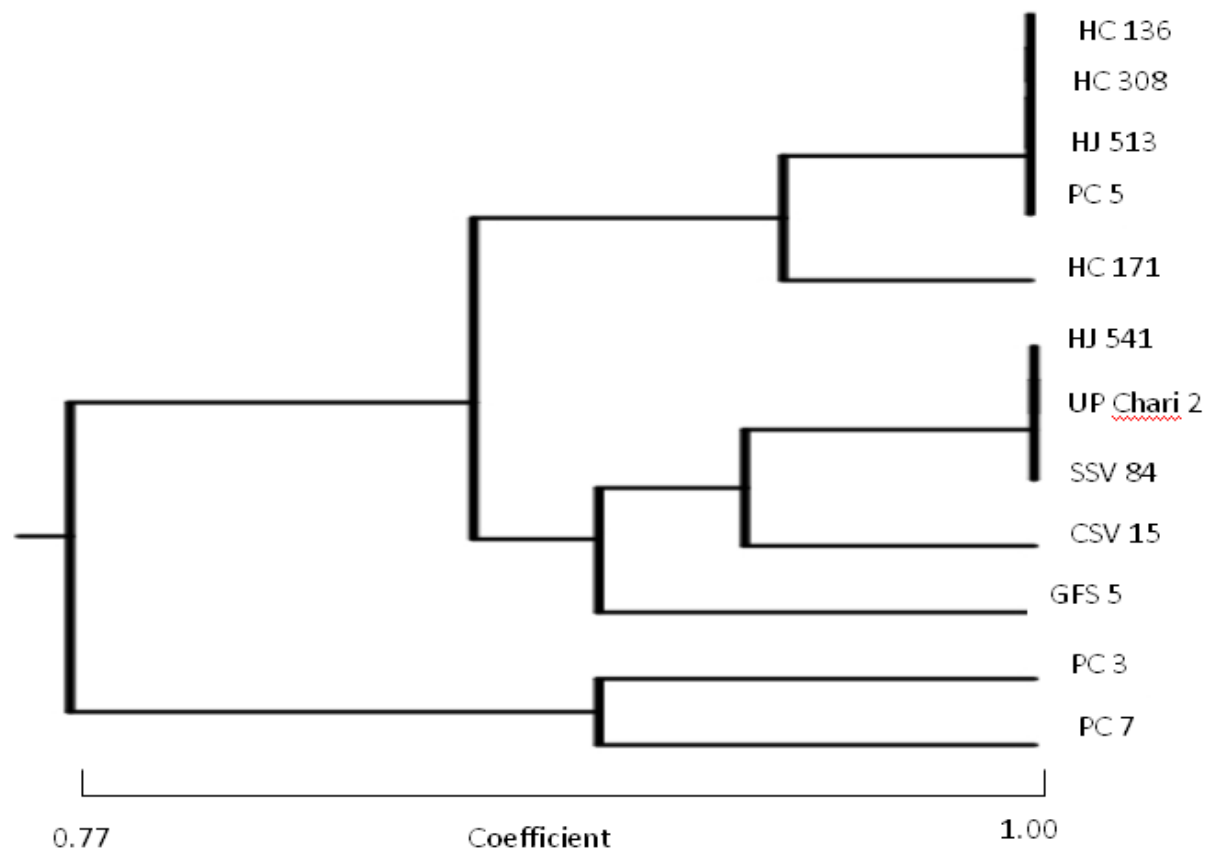
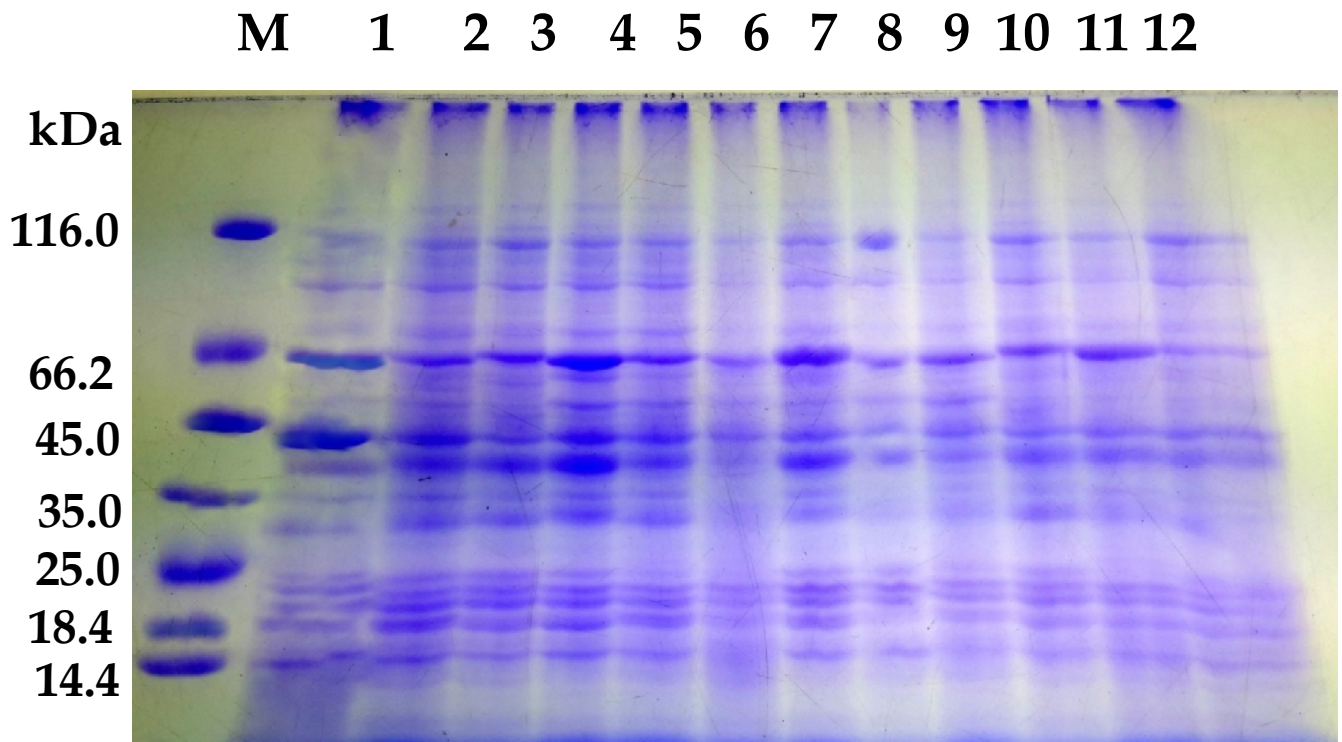


Fig 2: Dendrogram based on protein profile analysis of different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Plate 4.19: Electrophoretic pattern of total soluble proteins in different varieties of fodder sorghum [*Sorghum bicolor* (L.) Moench].

{M -Marker, 1- HC 136, 2- HC 171, 3-HC 308, 4- HJ 513, 5-HJ541, 6-PC 3, 7-PC 5, 8-PC 7, 9-UP Chari 2, 10-SSV 84, 11-CSV 15, 12-GFS 5 .}



alone does not give the actual estimate of field performance of any seed lot. So, there is need to have some reliable parameters for evaluation of the seed quality in sorghum. In order to assess the seed quality of different varieties of sorghum in terms of field performance, the seeds were subjected to number of physiological and biochemical tests which can be used reliably to predict crop establishment under the field conditions.

In the present investigation, all the varieties were subjected to different vigour and viability tests viz., standard germination, seedling vigour index-I, seedling vigour index -II, accelerating ageing test, electrical conductivity test, dehydrogenase activity test, tetrazolium test, test weight, seedling establishment, field emergence index and mean germination time. The results obtained during the course of studies have been presented in the following heads.

i. Standard germination (%)

The result obtained for standard germination test of all sorghum varieties are presented in Table 6. The variety HJ 513 and HJ 541 recorded maximum germination (80 %) followed by CSV 15 and SSV 84 (79 %), whereas the variety HC 308 showed minimum germination (74.33 %). The standard germination ranged from 74.33 - 80.00, in the all varieties the variety PC 7, GFS-5, Pant Chari-3, PC-5, UP Chari-2, HC-171 and HC-136 were found at par value with each other.

ii. Vigour Index-I

Vigour index-I was calculated by multiplying the standard germination percentage with seedling length (cm) and the results obtained for vigour index-I of all fodder sorghum varieties are presented in Table 6. The variety PC 7 was recorded maximum value (3083.40) followed by HJ 513 (3023.47) while the variety HC-136 showed minimum value (2345.30). The vigour index-I ranged from 2345.30 – 3083.40. All sorghum varieties were significantly superior over HC-136.

iii. Vigour Index-II

Similarly, vigour index-II was calculated by multiplying standard germination percentage with seedling dry weight (mg) and result are presented in Table 6. The data revealed that the variety HC-136 showed maximum vigour index-II (14775.00) whereas PC 3 had minimum (9992.33). Vigour index -II ranged from 9992.3-14775.00 and the all fodder sorghum varieties were significantly superior over PC 3 except HJ-513 and HJ-541 which showed value at par.

iv. Accelerated ageing test (%)

All sorghum varieties were subjected to stress condition i.e $40 \pm 1^{\circ}$ C and 100 % relative humidity for 72 h. The seed lot which gave maximum germination even after going under the stress was regarded as vigourous seed and the results are

Table 6 : Seed viability and vigour parameters in different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Varieties	SG(%)	SL (cm)	SDW (mg)	V.I-I	V.I-II	AAT (%)	EC (mS/cm/ 100 Seed)	Tz test (%)	DHA	TW (g)	FEI	MET	SE (%)
Pant Chari 7	76.667 (61.106)	40.200	139.667	3,083.40	10,709.00	43.667 (41.344)	0.113	86.000 (68.033)	0.083	29.290	12.917	5.047	60.667 (51.199)
GFS 5	75.000 (59.979)	33.233	145.333	2,493.17	10,900.67	39.667 (39.019)	0.100	74.000 (59.330)	0.076	23.667	13.213	4.647	59.333 (50.411)
Pant Chari 3	75.333 (60.210)	34.833	132.667	2,625.17	9,992.333	45.667 (42.496)	0.077	79.333 (62.962)	0.084	27.100	11.657	5.137	57.667 (49.403)
PC 5	75.000 (59.979)	33.900	141.667	2,543.07	10,626.67	49.333 (44.600)	0.100	79.333 (62.945)	0.071	34.610	12.183	4.800	53.333 (46.893)
HC- 308	74.333 (59.555)	36.367	150.333	2,704.90	11,176.33	52.667 (46.511)	0.087	73.000 (58.672)	0.097	24.433	12.640	4.803	56.000 (48.427)
SSV- 84	79.000 (62.704)	32.100	136.333	2,537.97	10,769.67	39.000 (38.626)	0.087	85.667 (67.762)	0.088	24.400	16.097	4.600	67.667 (55.331)
UP chari -2	76.000 (60.645)	36.767	142.667	2,793.43	10,843.67	47.667 (43.645)	0.083	76.667 (61.112)	0.098	29.100	13.103	4.900	58.000 (49.604)
CSV -15	79.000 (62.716)	31.133	179.333	2,456.93	14,151.33	54.667 (47.659)	0.083	77.000 (61.331)	0.081	32.000	14.027	4.990	61.000 (51.366)
HC 171	76.000 (60.645)	33.867	142.333	2,574.60	10,817.00	45.333 (42.305)	0.090	77.333 (61.546)	0.089	27.667	12.953	5.000	58.000 (49.588)
HC- 136	75.000 (59.979)	31.267	197.000	2,345.30	14,775.00	55.667 (48.23)	0.167	81.333 (64.388)	0.158	37.800	14.070	4.950	64.000 (53.124)
HJ -513	80.000 (63.414)	37.800	147.667	3,023.47	11,812.00	54.667 (47.659)	0.073	71.333 (57.610)	0.089	29.767	15.467	4.597	67.667 (55.348)
HJ-541	80.000 (63.442)	36.633	145.667	2,935.37	11,654.33	47.667 (43.645)	0.103	76.000 (60.65)	0.092	35.000	13.417	4.793	59.333 (50.381)
SE(m)	0.957	1.008	2.675	97.98	218.55	0.739	0.006	1.072	0.001	0.669	0.497	0.118	2.293
C.D. at 5 %	2.811	2.959	7.855	287.68	641.71	2.170	0.016	3.146	0.003	1.963	1.468	0.349	6.768

SG- Standard germination , SL-Seedling length , SDW-Seedling dry weight, V.I-I – vigour index-I, V.I-II – vigour index –II, AAT- Accelerating ageing test, EC- Electrical conductivity, Tz- Tetrazolium test, DHA – Dehydrogenase activity test, TW- Test weight, FEI- Field emergence index, MET – Mean emergence time, SE – Seedling establishment.

presented in Table 6. The variety HC 136 recorded maximum value (55.67 %) followed by HJ 513 (54.66 %) and CSV 15 (54.66 %) whereas SSV 84 and GFS 5 recorded minimum value (39.00 and 39.67 % respectively). The value of accelerated ageing test ranged from 39.00% to 55.67 % in all the varieties. The variety SSV -84 and GFS-5 were found significantly superior over all varieties except UP Chari-2 and HJ-541 at par value.

v. Electrical conductivity (mS/cm/100 Seeds)

The amount of seed leachates dissolved in the water was measured in all fodder sorghum varieties after 24 h. of imbibition and the result are presented in Table 6. The variety HC 136 recorded maximum value (0.167) while variety HJ-513 showed minimum value (0.073). The electrical conductivity ranged from 0.073 to 0.167 in all the fodder sorghum varieties. All fodder sorghum varieties were found significantly superior over variety HJ-513 except Pant Chari-3 show at par value.

vi. Tetrazolium test (%)

The tetrazolium test results recorded for all fodder sorghum varieties are presented in Table 6. The variety PC 7 recorded maximum value (86 %) followed by SSV 84 (85.66 %) and the variety HJ-513 recorded minimum value (71.33 %). The value of tetrazolium test ranged from 71.33 - 86.00 in all the varieties. All the varieties were found significantly superior over HJ-513 except HC-308 which showed at par value.

vii. Dehydrogenase activity test (OD)

This test was referred as biochemical test and the enzymatic activity of all fodder sorghum varieties was measured. More activity of the dehydrogenase would result into more vigorous seed and vice-versa. The highest value for Dehydrogenase activity was recorded with HC-136 (0.158) and lowest PC-5 (0.071). The values of dehydrogenase activity test ranged from 0.071 - 0.158 in all the varieties. (Table 6)

viii. Test weight (g)

The weight of 1000 seed of all fodder sorghum varieties was recorded and the results are presented in Table 6. The highest test weight was recorded for HC-136 (37.80 g) and lowest for GFS-5 (23.67 g). The test weight ranged from 23.67 – 37.80, all sorghum varieties were found significantly superior over GFS-5 for test weight.

ix. Field emergence index

The field emergence index for all fodder sorghum varieties was recorded and presented in Table 6. The highest value was recorded in SSV-84 (16.10) followed by HJ 513 (15.47) while variety PC 3 showed minimum value (11.66). The field

emergence index ranged from 11.66 – 16.10 in all the varieties. All varieties were found significantly superior over PC 3.

x. Mean emergence time (days)

The emergence time was calculated for all fodder sorghum varieties and the results are presented in Table 6. The variety PC 3 recorded highest value (5.13) followed by PC 7 (5.00) and HC 171 (5.00), while SSV 84 and HJ 513 recorded lowest value (4.60). The Mean emergence time ranged from 4.60-5.13 in all the varieties. All sorghum varieties were found significantly superior over SSV-84 and HJ-513 except HJ-541 and GFS-5 which were found at par.

xi. Seedling establishment (%)

The results of seedling establishment are presented in Table 6 and the variety SSV 84 and HJ 513 recorded highest seedling establishment (67.67 % each) followed by HC 136 (64.00%) while, the variety PC 5 recorded lowest value (53.33%). The seedling establishment ranged from 53.33 -67.67 in all the varieties. All sorghum varieties were found significantly superior over PC-5 except Pant Chari-3, HC-308, UP Chari -2.

Relationship among various vigour parameter and Seedling establishment

Correlation coefficient analysis was employed to find out the association among various seed vigour parameters and presented in Table 7. It was found that most of the vigour parameters are correlated significantly.

The standard germination showed positive and significant association with seedling length (0.989**), seedling dry weight (0.980**), vigour index -I (0.980**), vigour index-II (0.715**), accelerated ageing test (0.715**), electrical conductivity (0.726**), field emergence index (0.501) and seedling establishment (0.458). Similarly vigour index-I and II also show the positive and significantly correlation with accelerated ageing test, electrical conductivity, tetrazolium test, dehydrogenase activity test, test weight, field emergence index, mean germination time and seedling establishment. The tetrazolium test showed positive and significant correlation with field emergence index (0.938**), mean germination time (0.969**) and seedling establishment (0.974**). Dehydrogenase activity test also showed positive and significant association with all vigour parameter except standard germination percentage.

The seedling establishment also showed the significantly and positive correlation with vigour index –II (0.740**), accelerated ageing test (0.740**), electrical conductivity (0.764**), tetrazolium test (0.974**), dehydrogenase activity test (0.962**), test weight (0.947**), field emergence index (0.970**) and mean emergence time (0.998**).

Table 7: Correlation coefficient (r) among different viability and vigour parameters in different varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench).

Vigour parameters	SG	SL	SDW	V.I-I	V.I-II	AAT	EC	Tz	DHA	TW	FEI	MGT	SE
SG	1												
SL	0.989**	1											
SDW	0.980**	0.993**	1										
V.I-I	0.980**	0.992**	0.999**	1									
V.I-II	0.715**	0.738**	0.792**	0.791**	1								
AAT	0.715**	0.733**	0.778**	0.777**	0.991**	1							
EC	0.726**	0.740**	0.781**	0.781**	0.984**	0.997**	1						
Tz	0.493	0.536	0.572	0.577*	0.800**	0.812**	0.833**	1					
DHA	0.537	0.581*	0.636*	0.641*	0.856**	0.841**	0.853**	0.974**	1				
TW	0.512	0.563	0.621*	0.624*	0.843**	0.821**	0.829**	0.961**	0.996**	1			
FEI	0.501	0.549	0.608*	0.615*	0.806**	0.779**	0.791**	0.938**	0.979**	0.976**	1		
MGT	0.454	0.502	0.545	0.552	0.746**	0.741**	0.763**	0.969**	0.966**	0.954**	0.979**	1	
SE	0.458	0.503	0.543	0.551	0.740**	0.740**	0.764**	0.974**	0.962**	0.947**	0.970**	0.998**	1

* Significant at 5% (p= 0.05), ** Significant at 1% (p= 0.01).

The ability to identify plant varieties quickly as well as precisely is a basic requirement for running seed trade smoothly and efficiently. Further, the description of varieties along with the assessment of varietal identity and purity are important for seed certification as well as quality seed production. Now, the farmers are aware of worthiness of quality seed and they always insist on purchasing a product of known identity and quality. Thus, there is considerable need for reliable method of varietal identification in the present scenario of agriculture in the world.

The basic objectives of varietal identification are to test the occurrence of traits that helps in identifying a particular variety (Flenner and Smith, 1983). Characterization of the cultivars developed on the ideotype concept to establish their distinctiveness is of crucial importance to fulfil the conditions of Distinctness, Uniformity and Stability (DUS) as laid down by the International Union for protection of plant Varieties (UPOV, 1994) and seed act (1966). The need for a detailed examination of diagnostic characteristics thus becomes imperative in new PVP-opting countries to maintain identity of released and notified varieties and their parental lines. Sharma (1991) has indicated that no system of variety registration exists in India apart from rather vague variety release proposals provided by the breeders and there is a need for establishing a DUS testing system. Such work additionally assists in protecting morphologically and often agronomically, similar but distinct varieties when a PVP system is established *de novo* in a country. The requirement also finds support from the work of Singhal & Prakash (1992) who have identified a high degree of resemblance in morphology in recently developed wheat varieties in India. Virk & Witcombe (1998) have also stated that the selection strategy employed in the All India Coordinated Crop Improvement Programs, the system of multi-location testing of new varieties, concentrates on selection for yield with emphasis in selection on one important adaptive trait i.e. flowering time, towards which there is strong stabilizing selection.

The interest is being shown by the government of India to develop procedure that will help in identification of varietal purity in both field and at seed level. The Government of India, therefore, enacted “The Protection of Plant Varieties and Farmers’ Right Act (PPV&FRA 2001)” in 2001 and the regulations for implementing the Act were formulated in 2003. Varietal descriptions provided by

the concerned breeders are generally inadequate to characterize a variety or varietal mixture. Therefore, there is a great need to develop keys to identify varietal purity. Protection of Plant Variety and Farmers' Right Authority (2007) published guidelines for DUS testing in sorghum. For varietal identification of twelve fodder sorghum varieties by morphological characters in field were applied in present study based on DUS guidelines published by PPV&FRA authority.

5.1 Morphological characteristics

5.1.1 Seedling stage

A morphological characteristic of sorghum seedling anthocyanin colouration of coleoptiles was examined at seedling stage for their characterization. Based on the variation observed on this character, it was possible to distinguish all varieties broadly into two categories: yellow green (nine varieties) and grayed purple (three varieties). Similarly, the results were reported by Nagaraja *et al.* (2000) characterized twenty three varieties including five hybrids of sorghum on the basis of seed colour and seedling morphology and Selvaraju *et al.* (2000) characterized 19 sorghum genotypes on the basis of seedling anthocyanin colouration of coleoptiles.

5.1.2 5th leaf stage

At 5th leaf stage of varieties were characterized on the basis of two characters; leaf sheath anthocyanin colouration and leaf midrib colour of 5th fully developed leaf. All varieties exhibited similar colouration pattern as was observed in case of seedling anthocyanin colouration of coleoptiles i.e. yellow green (six varieties) and grayed purple (six varieties). On the basis of leaf midrib colour the genotypes were categorized into two groups: white (two varieties) and yellow green (ten varieties). Sangwan *et al.* (2005) and Elangovan *et al.* (2007) characterized 12 and 157 sorghum genotypes on the basis of midrib colour (white and green), respectively.

5.1.3 Panicle emergence stage

Forage sorghum varieties were characterized on the basis of flag leaf yellow colouration of midrib at panicle emergence stage. Two fodder sorghum varieties exhibited yellow colouration of midrib while ten fodder sorghum varieties were without yellow colouration in midrib of flag leaf. Similarly, the result were reported by Raghuvanshi *et al.* (2014) studied morphogenetic characterization based on DUS testing, to distinguish between seventeen varieties of forage sorghum, twenty six essential morphogenetic characteristics of seedling, plant (vegetative stage and at maturity) and matured seeds. It was possible to distinguish all the seventeen varieties included in the present study on the basis of grouping characteristics based on physiology of plant, its morphology and seed colour. Likewise Elangovan (2006)

characterized 179 accessions of sorghum on the basis of days to 50% flowering along with other morphological characters.

5.1.4 Flowering stage

Forage sorghum varieties were classified into different groups based on lemma arista formation, stigma anthocyanin colouration, colour of dry anther and flower with pedicle length of flower. Based on colour of dry anther genotypes were classified into three categories *viz.*, yellow orange (four varieties), orange (one variety) and grayed orange (Seven varieties) while, for flower with pedicle: length of flower, varieties were also divided into three groups i.e medium (four varieties), long (five varieties) and very long (three varieties). However, on the basis of stigma anthocyanin colouration, varieties were divided into two groups absent (eight varieties) and present (four varieties). On the basis of lemma arista formation sorghum varieties were divided into two categories *viz.*, present (9 varieties) and absent (3 varieties). Kolberg (1999) characterized 124 accessions of sorghum using IPGRI descriptors. Reddy *et al.* (2006), Reddy *et al.* (2009) and Joshi *et al.* (2009) characterized 269, 26 and 29 sorghum genotypes using DUS guidelines for sorghum, respectively.

5.1.5 Physiological maturity

Characters like, glume colour, stem diameter, leaf width of blade, panicle length without peduncle, panicle length of branches, panicle shape, glume length were recorded for characterization of varieties at physiological maturity. On the basis of glume colour varieties were categorized into five groups *viz.*, green white (two varieties), yellow white (four varieties), grayed yellow (two varieties), grayed orange (two varieties), grayed purple (two varieties). Genotypes could be divided into four groups *viz.*, very short (four varieties), short (five varieties), medium (two varieties) and long (one varieties) on the basis of glume length. On the basis of leaf width of blade, varieties could be divided into three categories *viz.*, medium (two varieties), long (seven varieties) and very long (three varieties). On the basis of panicle length without peduncle, varieties were categorized into three groups namely, short (two varieties), medium (eight varieties), long (two varieties) whereas, varieties were characterized into three groups *viz.*, short (three varieties), medium (eight varieties), long (one variety) on the basis of panicle length of branches. In case of panicle shape, varieties were categorized into three groups symmetrical (seven varieties), panicle broader in lower part (two varieties), and panicle broader in upper part (two varieties) and pyramidal (one varieties). However, on the basis of stem diameter varieties are categorized into two group small (<2 cm, one varieties) and medium (2-4 cm, eleven varieties). Pahuja *et al.*

(2002) evaluated 18 sorghum hybrids on the basis of plant height. Umakanth *et al.* (2002) studied diversity of 48 sorghum genotypes on the basis of plant height and panicle length. Sangwan *et al.* (2005) studied identification of 12 sorghum genotypes on the basis of panicle compactness, shape of panicle and plant height. Elangovan *et al.* (2006) characterized 179 accessions on the basis of earhead compactness, earhead shape, glume colour, earhead length and plant height. Nabi *et al.* (2006) compared 5 advance lines of sorghum on the basis of stem thickness, plant height and leaf area. Reddy *et al.* (2009) characterized 29 sorghum genotypes on the basis of plant height and panicle length.

5.1.6 Seed characters

After threshing, the varieties were classified on the basis of caryopsis colour, grain shape in profile view, grain texture of endosperm, grain luster, seed size and 1000 seed weight. Varieties were characterized into two groups *viz.*, white (three varieties), and yellow white (nine varieties) on the basis of caryopsis colour. However, on the basis of grain textures of endosperm, varieties were divided into four groups namely, vitreous (one variety), $\frac{3}{4}$ vitreous (three varieties), $\frac{1}{2}$ vitreous (five varieties) and $\frac{3}{4}$ farinaceous (three varieties). On the basis of grain shape in profile view, genotypes were also divided into two group's *viz.*, circular (nine varieties) and elliptical (three varieties). Varieties could be divided into two groups namely, lustrous (nine varieties) and non lustrous (three varieties) on the basis of grain luster. On the basis of seed size, varieties are divided into three group *viz.*, Medium (five varieties), large (four varieties) and very large (two varieties), whereas, low (16-25 g, three varieties), medium (26-35 g, eight varieties) and high (36-45 g, one variety) on the basis of 1000 seed weight. Nagaraja *et al.* (2000) characterized 23 sorghum genotypes on the basis of seed colour and seed shape in profile view into two categories while size of mark of germ into three categories. Selvaraju *et al.* (2000) classified 19 sorghum varieties on the basis of seeds colour. Thangavel *et al.* (2005) classified 12 sorghum cultivars on the basis of seed colour, seed size, seed shape, size of mark of germ, texture of endosperm, colour of vitreous albumen and seed luster. Elangovan (2006) characterized 179 sorghum accessions on the basis of seed size and seed colour. Reddy *et al.* (2009) classified 29 sorghum genotypes on the basis of 1000-grain weight and reported wide range of variation in these genotypes.

5.2 Electrophoretic technique

The term electrophoretic technique refers to the migration of a charged particle under the influence of an electric field. The rate of movement depends primarily on two factors i.e. (a) difference in size of molecules: particles with

smaller molecular weight migrate fast than those with higher weight and (b) difference in charge: molecules with the higher charge migrate faster than those with a lower charge. This technique, by employing the above principle, separates the protein molecules on acrylamide gel into different bands which is used for identification of varieties.

The banding pattern of twelve fodder sorghum varieties revealed that a total of seven bands were present in each variety as present in standard protein marker but the total number bands varied in all varieties. A wide quantitative variation was noticed in terms of relative mobility (R_m) value, electrophoretic mobility of protein bands, intensity of bands and also in banding pattern of different fodder sorghum varieties.

The seven standard protein marker bands numbers *viz.*, 2 (R_m 0.24), 6 (R_m 0.40), 9 (R_m 0.53), 11 (R_m 0.64), 13 (R_m 0.68), 16 (R_m 0.88) and 17 (0.91) were observed invariably common in almost all the varieties. The maximum number of bands were observed in varieties HC-136 (Dense-1, Medium-8, light-5 and weak-3) followed by HJ-513 (Dense-3, medium-3, light-7 and weak 4), PC-5 (Dense-3, Medium-2, light-11, weak-1) and lowest number of bands was observed in variety PC-3 (Medium-2, light-6, weak-3). All the varieties were differentiated by using the presence/absence of specific combination of bands or single band.

In this way, all the twelve varieties were individually identified. Based on the presence or absence of bands (Table 3), similarity index was calculated for all the fodder sorghum varieties (Table 5). The similarity index of twelve varieties revealed the closeness among the different varieties that may be either due to common parentage or accumulation of similar genes from different parents in the development of varieties as well as germplasm lines. Based on the dendrogram analysis, the dendrogram showed two major clusters at coefficient of 0.7774, two varieties in first cluster and rest varieties in second cluster (Fig. 2). The second cluster was further divided in to two clusters. Similarly, SDS-PAGE was used by different workers for varietal identification different in crops such as varietal characterization using electrophoresis of total seed protein is reported in sorghum (Chauhan, 2000), potato (tuber protein electrophoresis) by (Mishra *et al.*,2005), bottle gourd (Upadhyay and Ram, 2006), rice (Nethra *et al.*,2007). Khare *et al.*, (2007), studied the applicability of protein markers for identification of sorghum hybrids and its parents and to test the genetic purity for commercial samples. Sharmila *et al.*,(2013), in their studies they analysed protein of six genotype (HC 260, M 35-1, CSV -15, CSV-17, CSV-20, CSV -22) of sorghum by using SDS – PAGE, protein estimation and phylogenetic relationship among them.

5.3 Vigour studies

Seed quality deterioration during storage is well known phenomenon however; the extent of loss is governed by a number of intrinsic and extrinsic factors. Intrinsic factors include all such variation in seed metabolism which occurs due to the differences in environmental and edaphic conditions during the plant growth, particularly during development and growth of seed. The extrinsic factors include relative humidity, temperature and oxygen availability in storage. Due to these factors, the rate and extent of decline in seed quality with respect to viability and vigour varies considerably among different cultivars of same species and different seed lots of the same varieties.

In the present study, different seed samples of twelve fodder sorghum varieties were evaluated with a view to have substantial information on their vigour parameters. The mean sum of squares due to varieties was highly significant for all the parameter which indicated presence of substantial amount of variability among the varieties. Perusal of data presented in Table 1 revealed that fodder sorghum varieties showed variation in respect of different vigour parameters. The maximum germination per cent was recorded with HJ-513 and HJ-541 (80 %) and minimum in HC 308 (74.33 %). The varieties viz., GFS-5, PC-3, PC-5, UP Chari-2, HC-171, HC-136 were at par with HC-308. The maximum value for vigour index-I and vigour index-II was recorded in PC-7 and HC-136 respectively. After the accelerated ageing test variety HC 136 recorded maximum value (55.66 %) and varieties GFS-5 and SSV-84 shows minimum values (39 %). The varieties HC-136, HJ-541, CSV-15, and HC-308 were found at par with HC-136. The maximum value of electrical conductivity was observed in HC-136 and minimum in HJ-513. It showed that variety HJ-513 had recorded high vigour potential than other varieties. In case of tetrazolium test, the variety Pant Chari-7 showed the maximum value (86.00%) and minimum in HJ-513 (71.33%). The varieties SSV-84 HC-136 were found at par with Pant Chari-7 varieties. The maximum value for dehydrogenase activity was recorded in HC-136 (0.158) and minimum in PC-5 (0.071) and all varieties were found significantly superior over PC-5.

The maximum test weight was recorded for HC-136 (37.80 g) while minimum GFS-5 (23.66 g). All varieties were found significantly superior over GFS-5 except HC-308, SSV-84 were at par with GFS-5. The maximum value of field emergence index was recorded in HJ 513 (15.46) and minimum in Pant Chari-3 (11.65). The maximum Value of mean emergence time was recorded Pant Chari-3 (5.137) and minimum in SSV-84 (4.6). The maximum seedling establishment per cent was recorded in SSV-84 (67.66 %) and HJ-513 (67.66%) and minimum value

was recorded in PC-5 (53.33%). Overall maximum vigour potential was shown by HC-136, HJ-513, SSV-84 and HJ-541 varieties, which showed superior for almost all vigour parameters. Similarly, results were reported by Chauhan, (2003) who also found that fenugreek varieties subjected to vigour test showed variation for different vigour parameters and that could be correlated for the evaluation of field performance of the varieties.

The correlation study among various vigour parameters such as accelerated ageing test, standard germination, vigour indices, test weight, and seedling establishment was found informative in quality assessment of the seed lots of fodder sorghum varieties.

Standard germination, vigour indices, accelerated ageing test and tetrazolium test, were found positively correlated with seedling establishment (Table 7). It is well known and widely accepted that a seed lot with high vigour and viability will also give the better performance in field conditions. The accelerated ageing test provides the information in respect to the storability and capacity of seeds to tolerate stress. In the present study, a gradual reduction in normal seedling was observed which declined faster with the advancement of stress period when seeds were subjected to accelerated ageing test. Further, the accelerated ageing test was significantly positively associated with all remained vigour and viability parameters. The dehydrogenase activity test showed the significantly positive association with all vigour parameter whereas, the test weight was significantly positively associated with vigour indices, accelerated ageing test field emergence index and seedling establishment. That it shows that higher test weight of seed lot which had ultimately high vigour. Similarly, results were recorded by Kavak *et al.* (2008) found that highest correlation among controlled deterioration, germination and field emergence. Mor *et al.* (2009) reported that field emergence index and seedling establishment were significantly and positively correlated with standard germination, speed of germination, seedling length, seedling vigour index, accelerated ageing test, dehydrogenase activity, respiration rate and test weight (g) whereas significantly and negatively correlation were observed with EC and seed density (g/cc).

CHAPTER – VI

SUMMARY AND CONCLUSION

The present investigation entitled, “Varietal identification and seed vigour assessment in fodder sorghum. [*Sorghum bicolor* (L.) Moench].” was conducted during 2012-14. The field work was carried out at Research Area of Forage Section, Department of Genetics and Plant Breeding, whereas laboratory work at Department of Seed Science and Technology, CCS Haryana Agricultural, University, Hisar. The result obtained during the course of investigation are summarized and concluded as under.

In the present investigation twelve different varieties of fodder sorghum were used for varietal identification based on the morphological and electrophoresis technique. All the varieties showed differences for various morphological characters which provided basis for their grouping into different categories. Banding pattern of total soluble seed protein showed variation among the varieties. The various vigour parameters were also studied to assess seed vigour potential of different fodder sorghum varieties. The results obtained during the course of investigation are summarized as below.

- The study revealed that morphological characters viz., leaf sheath anthocyanin colouration, flag leaf mid rib colouration, anther colour after dry, lemma arista formation panicle shape, caryopsis colour after threshing, grain shape in profile view, grain luster and test weight were the most important diagnostic characters for varietal identification in fodder sorghum varieties.
- Test weight (1000-seed) also varied with fodder sorghum varieties and grouped were made accordingly into low (16-25 g), medium (26-35 g) and high (36-40 g) among the different varieties of fodder sorghum classified in which three varieties (GFS-5, HC-308 and SSV-84) are low, eight varieties (PC-7, PC-3, PC-5, UP Chari-2, CSV-15, HC-171, HC-513 and HC-541) are medium and HC-136 showed high test weight.
- The morphological characters like glume length, flower with pedicel length could not differentiated fodder sorghum varieties as better than other character among the varieties.
- The banding pattern of total soluble protein showed considerable variation among the twelve fodder sorghum varieties. Seven protein marker bands

were present in all varieties but the intensity and thickness varies from variety to variety.

- The analysis of total soluble protein banding pattern in all the fodder sorghum varieties were carried out along with the standard protein molecular weight marker having 14.4 to 116.0 kDa. The bands between 45 to 66.2 kDa were more thick and intense as compared to higher and lower molecular weight bands. This was a clear indication that these molecular size protein molecules constituted majority of total soluble seed protein in sorghum varieties.
- Unweighted paired group method with arithmetic mean (UPGMA) was used to construct dendrogram for all the twelve sorghum varieties. The dendrogram showed two major clusters at coefficient of 0.7774, which is shown in Fig. 2.
- The maximum vigour potential was found in HJ-513, HJ-541, SSV-84, HC-136 and CSV-15 which showed superiority almost for all vigour parameters among all the varieties.
- The standard germination accelerated ageing test, tetrazolium test and electrical conductivity that were found most suitable vigour parameters for prediction of seedling establishment in field conditions. All vigour parameters showed positive association with the seedling establishment.

From the above results, it is concluded that morphological characters can be supported with the bio chemical technique to differentiate the fodder sorghum varieties. The observed morphological data recorded during course of investigation were found most valuable varietal improvement programme. The banding pattern of total soluble protein by SDS-PAGE differentiated all the varieties from one another and it the most reliable method for varietal identification.

Further the results revealed that the present study appeared to be quite sufficient in differentiating and characterization of fodder sorghum varieties. The information generated from the study can also be used further in the improvement of fodder sorghum varieties through various breeding programme and also to distinguish the available germplasm of fodder sorghum varieties to claim for registration.

REFERENCES

- Abdul –Baki, A. A. and Anderson, J.D. (1973b). Relationship between decarboxylation of glutamic acid and vigour in soybean seeds. *Crop Science*. **13**, 227-232.
- Abdul –Baki, A.A. and Anderson, J.D. (1973). Physiological and biochemical deterioration of seed Biology (Ed by T. T. Koziowski), pp.283-315. Academic Press, New York.
- Abdul –Baki, A.A. and Anderson, J.D. (1973a). Vigour determination in soybean seed by multiple criteria. *Crop Science*, **13**, 630 -633.
- Agarwal, M., Singh, R., and Shukla, A. (2002). Multivariate classification of elite lines in sorghum. *Forage Res.* **27**(4): 243-246.
- Ajala, M. O. (2003). Influence of seed quality attribute on field emergence of pigeonpea (*Cajanus cajan* L.,) *Tropical Agriculture*. **80** (2), 118-122.
- Akhare, A.A., Sakhare, S.B., Kulwal, P.L., Dhumale, D.B and Kharkar, A. (2007). Protein electrophoresis for identification of hybrids and their parents in *Sorghum bicolor* (L). Moench. *Asian J. of Bio Sci.* Vol. 3 No. 1: (176-179).
- Allan, R.E., Vogel, O.A. Russel and Peterson, C.J. (Jr.) (1962). Seedling emergence rate of late sown wheat and its association with plant height and coleoptile length. *Agronomy Journal*. **54**, 347-350.
- AOSA (1983). Seed vigour testing handbook. *Assoc.Seed Analt. Publ. AOSA Handbook*, **32**.
- Basra, S.M.A., Ahmad, N., Khan, M.M., Iqbal, N. and Cheema M.A (2003). Assessment of cotton seed deterioration during accelerated aging. *Seed Science & Technology*, **31**, 531- 540.
- Basu, S., Sharma, S.P and Dadhani, M. (2004). Storability studies on maize (*Zea mays* L.) parental line seeds under natural and accelerated aging conditions. *Seed Science & Technology*. **32**, 239-245.
- Beileski, R.L. and Reid, M.S. (1968). A simple apparatus for varietal flat sheet polyacrylamide gel electrophoresis. *Annal. Biochem.*, **22**, 347-351.
- Chauhan, P. (2000). Varietal identification in forage sorghum. M.Sc. Thesis. Deptt. Of Seed Science and Technology, CCSHAU, Hisar.
- Chhetri, D.R., Rai, A.S. and Bhattacharjee, A. (1993). Chemical manipulation of seed longevity for four crops in an unfavorable storage environment. *Seed Science & Technology*, **21**, 31- 44.
- Choi, W. Y., Kwon, Y.W., Soh, C. H., Choi, W.Y., Kwon, Y.W and Sou, C.H. (1997). Physiology responses of water stress at seedling stage in barley. *Korean Journal of crop Science*, **42**(2), 189- 195.

- Christiansen, H.R. and Rowland, R. (1981). Cotton physiology seed and germination. *In: Proceeding of beltwise cotton production research conference* (Ed by J.M. Brown, 4-8 Jan, New Orleans, L.A. Publ. Natl.Cotton. Counc., Memphis, T.N.)
- Cobb, R.D. and Jones, L.G. (1966). Development of sensitive laboratory growth tests to measure seed deterioration. *Proceedings of Association of Official Seed Analysis*, **56**, 52-60.
- Cook, R.J., Cliff, E.M. and Draper, S.R. (1983) Barley cultivar identification of hordein electrophoretograms. *J. Natl. Int. Agric. Bot.*, **16**, 197-206.
- Cooke, R.J. (1988). Electrophoresis in plant testing and breeding. *Advances in Electrophoresis*, **2**, 171-261.
- Cooke, R.J. (1995). Varietal identification of crop plants. New diagnostics in crop science (Ed by I.H. Skeritt and R. Appies), pp. 33-83. CAB International.
- Dadlani, M and Varier, A. (1993). Electrophoresis for varietal identification. *Technical Bulletin*. Division of Seed Science and Technology, IARI, New Delhi.
- De Wet, J.M.J. (1977) Domestication of African cereals. *Afr. Econ. Hist.*, **3**, 15.
- De Wet, J.M.J. and Harlan, J.R. (1971). The origin and domestication of *Sorghum bicolor*. *Econ. Bot*, **25**, 128-135.
- Delouche, J.C. (1965). An accelerated ageing technique for predicting relative storability of crimson clover and tall fescue seed lots. *Agronomy Abstract*, **40**, 165-171.
- Delouche, J.C. and Baskin, C.C (1973). Accelerated ageing techniques for predicting the relative storability of seed lots. *Seed Science & Technology*, **1**, 427-452.
- Delouche, J.C., Rusting, T.T and Baskin, C.C (1967). Predicting the relatively storability of seed lots. *Rep. American Seed Research Foundation*, pp.22. Miss state Uni. State College.
- Demir, I., Ermis, S., Okcu, G. and Matthews, S. (2005). Vigour tests for predicting seedling emergence of aubergine (*Solanum melongena* L.) seed lots. *Seed Science & Technology*, **33**, 481-484.
- Deswal, D.P. and Chand, U. 1997. Standardization of the tetrazolium test for viability estimation in rice bean (*Vigna umbellata* (Thunb.) Ohwi and Ohashi) seeds. *Seed Science & Technology*. **25** (3), 409-417.
- Dias, D. C .F. S and marcos –Filho, J (1996). Electric conductivity test for evaluation I soybean seeds. *Seed Research*, **24**, 1-10.
- Djavanshir, K. and Pourbeik, H. (1976). Germination value a new formula. *Silva Genetica*, **25**, 79-82.
- Dogget, H. (1988). Sorghum. Wiley, New York.

- Dorofeev, V.F. (1992). Origin and geography of cultivated plants. Cambridge University Press, Cambridge, U.K. 332.
- Dutra, S. and Vieira, R.D. (2006). Accelerated ageing test to evaluate seed vigour in pumpkin and zucchini seeds. *Seed Science & Technology*, **34**, 209-254.
- Elangovan, M. (2006). Characterization and evaluation of exotic sorghum germplasm collections. *International J. Agril. Sci.* **2**(1): 231-234.
- Elangovan, M.; Prabhakar.; Reddy, D.C.S. (2007). Characterization and evaluation of sorghum [*Sorghum bicolor* (L.) Moench] germplasm from Karnataka, India. *Karnataka J. Agril. Sci.* **20**(4): 840-842.
- Ellis, R.H. and Roberts, E.H. (1980). Towards a rational basis for testing seed quality. Seed production (Ed by P.D. Hebblethwaite), pp. 605-635, Butterworth, London.
- Flenner, B and Smith, S (1983). Pioneer corn description system, *Pioneer Technical Bulletin*. pp. 1-60.
- Germ, H. (1960). Methodology of the vigour test for wheat, maize, and barley in rolled paper. *Proceeding of International Seed Testing Association*, **25**, 515- 518.
- Gill, N.S. and Delouche, J.C. (1973). Deterioration of seed corn during storage. *Proceedings of Association of official Seed Analysts*, **63**, 92-96.
- Gupta, v., arya, L., Pandya, c. and Kak, A. (2005). Effect of accelerated ageing on seed vigour in pearl millet (*Pennisetum glaucum*) hybrids and their parents. *Indian Journal of Agricultural Sciences*. **75** (6): 346-347.
- Hampton, J.G., Leeks, C.R .F and McKenzie, B.A. (2009). Conductivity as a vigour test for *Brassica species* . *Seed Science & Technology*, **37**, 214-221.
- Harlan ,J.R & de wet.T.M.J. (1972). A simplified classification of sorghum. *Crop science* **12**, 172.
- Hiers, R.E; Villiers, O.T. de; Bosman, M and de Villiers, O.T., (1994). Identification of tall *Fescue* grass cultivars by sodium dodecyl sulphate and acid polyacrylamide gel electrophoresis. *South Africa J.Plant Soil.* **11**(1); 26-29.
- Ibbi, H. and Eser, B. (2006). The potential of vigour tests to identify difference in the extent of ageing in onion seeds. *Seeds Science & Technology*, **34**, 713-718.
- ISTA. (1996). International rules for seed testing .Verification of species and cultivars. *Seed Science & Technology*, **24** (Suppl.), 253-270.
- ISTA. (1999). International rules for seed testing. *Seed Science and Technology*, **27**, 50-52.

- ISTA. (2003). International seed rules for testing. (Edi. 2003), *International Seed Testing Association*. Zurich, Switzerland.
- ISTA. (2011). International Rules for Seed Testing. Chapter 5: The Germination Test. *International Seed Testing Association*, Baserdorf, Swizerland.
- Joshi, D.C.; Shrotria, P.K.; Singh, R.; Chawla, H.S. (2009). Morphological characterization of forage sorghum [*Sorghum bicolor* (L.) Moench] varieties for DUS testing. *Indian J. Genetics Plant Breeding*. **69**(4): 383-393.
- Kavak, S., Iibi, H. and Eser, B. (2008). Controlled deterioration test determine vigour and predicts field emergence in pepper seed lots. *Seed Science & Technology*, **36**,456-462.
- Kittock, D. L. and Law, A. G.(1968). Relationship of seedling vigour to respiration and tetrazolium chloride reduction by germinating wheat seeds. *Agronomy Journal*, **60**, 286-288.
- Kolberg, H. (1999). Morphological variability in sorghum (*Sorghum bicolor* (L.) Moench subsp. bicolor) accession from Namibia. *Plant Genetic Resources Newsletter*. **117**: 51-54.
- Kumar, A., (2002). Pre- sowing treatments for improving seed quality in cotton. (*Gossypium spp.*). M.Sc. Thesis, CCS HAU, Hisar.
- Kumar, A; Choudhary, R.k.; Kapoor, R. L and Dahiya, O.S., (1995). Identification of pearl millet hybrids and their parental lines using seedling characters, Chemical tests and gel electrophoresis. *Seed Sci. Technology.*, 23(1):21-32.
- Kumar, R., Tyagi, C. S and Ram, C. (1989). Evaluation of vigour on mungbean seeds: Relationship of speed of germination, dry matter per seedling, 100- seed weight and vigour index of field performance. *Seed and Farms* (July - August), pp. 33-37.
- Ladizinsky, G. and Hymowitz, T. (1979). Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. Appl. Genet.*, 54: 145-151.
- Lather, B.P.S., Deswal D.P and Punia R.C. (1990). The relationship of Tz test to field establishment of cotton cultivars. *Proceedings of Seed Science & Technology*. (National seminar), pp. 113-117. December 14-16, held at University of Mysore.
- Lawerence, T. (1963). A comparison of methods of evaluating Russian wild ryegrass for seedling vigour. *Canadian Journal of plant science*, **48**, 307-312.
- Maguire, J.D. (1962). Speed of germination – Aid in selection and evolution for seedling emergence and vigour. *Crop Science*, **2**, 176-177.
- Mathews, S. and Hosseini, M.K. (2006). Mean germination time as indicator of emergence performance in soil of seed lots of maize. *International Seed Testing Association*. **17**: 647-660.

- Mavi, K. and Demir, I. (2007). Controlled deterioration and acceleration ageing tests to predict seedling emergence of watermelon under stressful conditions and seed longevity. *Seed Science & Technology*, **35**, 445- 459.
- McDonald, M.B (1999). Seed deterioration: physiology, repair and assessment. *Seed science & Technology*, **27**, 177-200.
- Mishra, A.C., Singh, N.P and Ram, H. H. (2005). Banding pattern of protein subunits with tuber development in *Solanum tuberosum*. L. *Indian Journal of plant physiology*, **10** (1), 100 -102.
- Moore, R.P. (1974). Tetrazolium stain for assessing seed quality. *Seeds Ecology* (Ed. By W. Heydecker), pp. 347-366. The Pennsylvania state university, Univ. park. P.A.
- Mor, V.S., Verma, S.S. and Verma, U. (2009). Studies on seed viability and vigour in various order umbels of fennel (*Foeniculum vulgare*). *Seed Science & Technology*, **37**, 747-757.
- Mujaju, C.; Chakauya, E. (2008). Morphological variation of sorghum landrace accessions on-farm in semi-arid areas of Zimbabwe. *International J. Bot.* **4**(4): 376-382.
- Nabi, C.G.; Muhammad, R.; Ghulam, A. (2006). Comparison of some advanced lines of {*sorghum bicolor* (L.) Monech} for green fodder/dry matter yields and morpho-economic parameters. *J. Agril. Res. Lahore.* **44**(3): 191-196.
- Nagaraja, G.S. Varier, A. Singh, U. Sharma, S.P. (2000). Characterization of inbreds and hybrids of sorghum {*Sorghum bicolor* (L.) Moench} by seed and seedling morphology and electrophoresis of seed proteins and isoenzymes. *Plant Varieties and Seeds.* **13**(1): 17-29.
- Narwal, A.L., (1995). Studies in seed viability in okra (*Abelmoschus esculentus* L. Moench) Ph.D Thesis, CCS HAU ,Hisar.
- Nei, M. and Li, W. (1979). Mathematical method for studying genetic variations in terms of restriction endonuclease. *Proceeding of National Academy of Science, USA.* **76**, 5269- 5273.
- Nethra, n., Prasad,S. Rajendra, Vishwanath, K.,Dhanraj, K.N. and Gowda, Rame (2007). Identification of rice hybrids and their parental lines based on seed, seedling characters, chemical tests and gel electrophoresis of total soluble seed protein. *Seed Science & Technology*, **35**, 176-186.
- Nunes, A. Isabel Correia, Antnio Barros, and Ivonne Delgadillo., (2005). Characterization of kafirin and zein oligomers by preparative sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. *J.Agric. Food Chem.*, **53**(3), pp. 639-643.
- Pahuja, S.K.; Grewal, R.P.S.; Singh, N.; Singh, P.; Jindal, Y.; Pundir, S.R. (2002). Evaluation of forage sorghum hybrids for yield and morphological traits. *International Sorghum and Millets Newsletter.* **43**: 42-45.

- Panse, V.G. and Sukhatme, P.V. (1967). Statistical methods for agricultural workers. pp. 381. ICAR, New Delhi.
- Parra, P; Ortiz de Bertorelli, L. and De Bertorelli, L. O., (1993). Development of an electrophoretic method for identification of maize cultivars. *Revista de la Facultad de Agronomia, Central de Venezuela*, 19 (3): 213-216.
- Paul, S.R., and Ramaswamy, K.R. (1979). Relationship between seed size and se quality attribute in cowpea (*Vigna sinensis* L.). *Seed Research*, 7, 63-70.
- Perez, M.A. and Arguello, J.A. (1997). Determination of vigour of Groundnut (*Arachis hypogaea* L.) seeds kept under different conditions using the tetrazolium test. *Agriculture Scientia*. 14, 19-24.
- Presley, J. T. (1958). Relation to protoplant permeability to cotton seed viability and predicts position to seedling disease. *Plant Dis.Rept* .42, 852.
- Rabiei, E.; Khodambashi, M.; Pirbalouti, A.G. and Shahraki, A.D. 2009. Effect of drought stress on seed traits on oat (*Avena sativa*). *Research on Crops*. 10(1): 12-15.
- Raghuvanshi, S., Swamy, D., Mahadevakumar, S., Singh, O. P. (2014). DUS descriptor of seventeen forage cultivars of sorghum [*Sorghum bicolor* (L.) Moench]. *Global Journal of Bio- Science and Biotechnology*, 3(1) 2014: 100-108.
- Raymond, L. and Weintraub, A. (1959) In: G.J. Brewer and C.F. Singh (eds.). Introduction to isozyme technique. New York Academic Press. 1-20.
- Reddy, R.N.; Mohan, S.M.; Madhusudhana, R.; Umakanth, A.V.; Satish, K.; Srinivas, G. (2008). Inheritance of morphological characters in sorghum. *J. SAT Agril. Res*. 6: 1-3.
- Reddy, B.V.S.; Sharma, H.C.; Thakur, R.P.; Ramesh, S. (2006). Special issue: Characterization of ICRISAT- bred Sorghum hybrid parents (set I). *International Sorghum and Millets Newsletter*. 47: 138.
- Reddy, D.C.S.; Audilakshmi, S.; Seetharama, N. (2009). Genetic variability and divergence for DUS testing traits in sorghum (*Sorghum bicolor*). *Indian J. Agril. Sci*. 79(4): 286-290.
- Sangwan, V.P.; Chauhan, P.; Ram, C. (2005). Varietal identification of forage sorghum through morphological characters. *Seed Res*. 33(1): 117-118.
- Santos, M.A.O., Novembre, A.D.L.C. and Marcos –filho, J. (2007). Tetrazolium test to assess viability and vigour of tomatillo seeds. *Seed Science & Technology*, 35, 213-223.
- Schuab, S.R.P., Baccini, A.L., Scapim, C.A., Franca –neto, J.B., Meschede, D.K and Avila, D.K. (2007). Germination test under water stress condition to evaluate soybean seed vigour. *Seed Science & Technology*, 35, 187 -199.

- Seed Act (1966). Government of India, Manager Publication, G.O.I., New Delhi.
- Selvaraju, P., Sivasubramaniam, K. (2000). Identification keys for sorghum varieties. *Madras Agril. J.* **87**: 10-12.
- Shahi, J.P., Singh, J., Agrawal, I. and Lal, M.S. (1986). Studies on variability for seed size, permeability of seed coat to water and germination in lentil. *LENS Newsletter*, **13** (2), 14-15.
- Sharma, S.P. (1991). Plant variety testing system in UK -lessons for India. *Seed Research*. **19**: 101-105.
- Sharmila, P., Sarada Mani, N and Ratan Kumar, P.K. (2013). Genetic variability among genotypes of sorghum based on protein profile of seed storage proteins. *International Journal of Advanced Biotechnology and Research*. Vol.4, pp. 280-285.
- Shenoy, V.V., Dadlani, M and Sheshu, D. (1990). Association of laboratory assessed parameter with field emergence in rice. The non-ionic acid stress as assed vigour test. *Seed Research*, **18** (1), 60-69.
- Singhal, N.C.; and Prakash, S. (1992). The characterization and identification of wheat cultivars integrated approach. In: Proceedings of the Indo- British Workshop on Plant Breeders' Rights, seed Certification and Storage. Division of Seed Science and Technology, *Indian Agril Res. Institute, New Delhi*.
- Smith, J.S.C. and Smith, O.S. (1992). Finger printing of crops varieties. *Advances in Agronomy*, **47**, 85-140.
- Smith, O.E., Welch, N.C. and McCoy, O.D. (1973). Studies on lettuce seed quality II. Relationship of seed vigour to emergence, seedling weight and yield. *Journal of the American Society for Horticulture Scirnce*, **28**, 552-555.
- Tekrony, D.M. (1973). The soybean seed field emergence complex. *Proceedings third soybean seed conference, American Seed trade Association*, **3**, 22-28.
- Thangavel, P.; Bhatati, A.; Natarajan, N.; Evera, T. (2005). Varietal grouping in sorghum by seed and seedling morphology and response to chemical testing. *Karnataka J. Agril. Sci.* **18**(3): 664-672.
- Trawath, S.E., Tekrony, D.M and Hidedrand. D.F., (1995). Relationship of soybean seed quality to fatty acid and C₆-aldehyde levels during storage. *Crop Science*, **35**: 141-142.
- Trigo, L.F.N., Nedel, J.L.,Peske. S.T. and M.F.O.O. (2000). Seed and seedling characteristics associated with variation in early vigour of triticale at post emergence. *Revista Cientifica Riral*, **5**(1), 105-114.
- Umakanth, A.V.; Madhusudhana, R.; Swarnlata, K.; Rana, B.S. (2002). Genetic diversity studies in sorghum. *International Sorghum and Millets Newsletter*. **43**: 31-33.

- Upadhyay, Mgha and Ram, H.H (2006). Characterization of bottle gourd germplasm on SDS-PAGE of seed protein. *Vegetable Science*, **33** (1), 18-20.
- UPOV (1994). Guideline for the conduct of test for Distinctness, Uniformity and Stability: wheat (*Triticum aestivum* L.). International Union for Protection of New Varieties of plants. pp. 37-43. Geneva.
- Verma S.S., Tomar, R.P.S. and Verma, U. (1999). Study on seed quality parameters in rapeseed and mustard under ambient condition. *Indian Journal of Agriculture Science*, **39** (12), 840-842.
- Verma S.S., Verma, U. and Tomar, R.P.S. (2003). Studies on seed quality parameter in deterioration seeds in brassica (*Brassica campestris*). *Seed Science & Technology*, **31**, 389-396.
- Virk, D.S.; Witcombe, J.R. (1998). An analysis of varietal testing 1. Introduction. In: (Eds John R. Witcombe, Daljit S. Virk & John Farrington), *Seeds of Choice- Making the Most of New Varieties for Small Farmers*. 3-6.
- Wang, Y.R., Yu, L., Nan, Z.B and Liu, Y.L. (2004). Vigour test used to rank seed lot quality and predict field emergence in four forage species. *Crop Science*, **44** (2), 535-541.
- Williams, E.R., Gunn, B.V.W., Solomon, D.J. and Doran, J.C. (2007). Reliability of germination testing procedure and germination performance of stored *Eucalyptus camaldulensis* seed of different ages. *Seed Science & Technology*, **35**, 1-8.
- Wood Stock, L.W. (1976). Progress report on the seed vigour testing handbook. *Association of Official Seed Analysts, Newsletter*, **50**, 1-78.
- Yadav, S.K and Dhankhar, B.S (2001). Correlation studies between various fields parameters and seed quality traits in okra cv., Versha Uphar, *Seed Research*, **29**(1), 84-88.

APPENDIX I

Table showing observation recorded

	Parameters	States	Stage of observation
1.	Leaf sheath anthocyanin colouration	yellow green grayed purple	5 th leaf
2.	Leaf mid rib colour (5 th fully developed leaf)	white yellow green grayed yellow grayed purple	5 th leaf
3.	Flag leaf yellow colouration of midrib	absent present	panicle emergence
4.	Lemma arista formation	absent present	flowering
5.	Stigma anthocyanin colouration	absent present	upper portion of the panicle at the end of flowering
6.	Flower with pedicel: length of flower	very short short medium long very long	flowering
7.	Anther colour of dry anther	yellow orange orange orange red grayed orange	end of flowering
8.	Glume colour	green white yellow white grayed yellow grayed orange grayed red grayed purple	physiological maturity of grain
9.	Stem diameter (at lower one third height of plant)	small (<2 cm) medium (2 – 4 cm) large (> 4 cm)	physiological maturity
10.	Leaf width of blade (the third leaf from top including flag leaf)	narrow (< 4.1 cm) medium (4.1 – 6.0 cm) broad (6.1 – 8.0 cm) very broad (> 8.0 cm)	physiological maturity
11.	Panicle length without peduncle	very short (<11 cm) short (11 – 20 cm) medium (21 – 30 cm) long (31 – 40 cm) very long (> 40 cm)	physiological maturity
12.	Panicle length of branches (middle third of panicle)	short (<5.1 cm) medium (5.1-10 cm) long (10.1-15 cm) very long (>15 cm)	physiological maturity
13.	Panicle shape	reversed pyramid panicle broader in upper part symmetric panicle broader in lower part pyramidal	physiological maturity

	Parameters	States	Stage of observation
14.	Glume length	very short (25% of grain covered) short (50% of grain covered) medium (75% of grain covered) long (100% of grain covered) very long (longer than the grain)	physiological maturity
15.	Caryopsis colour after threshing	white RHS 155 grayed white RHS 156 yellow white RHS 158 yellow orange RHS 14-20 grayed orange RHS 200	after threshing
16.	Grain weight of 1000 grains	very low (< 16 g) low (16-25 g) medium (26-35 g) high (36-45 g) very high (> 45 g)	after threshing
17.	Grain shape in profile view	narrow elliptic elliptic circular	after threshing
18.	Grain size of mark of germ	very small small medium large very large	after threshing
19.	Grain texture of endosperm (in longitudinal section)	fully vitreous (100% corneous) $\frac{3}{4}$ vitreous (75% corneous) half vitreous (50% corneous) $\frac{3}{4}$ farinaceous (25% corneous) fully farinaceous (0% corneous)	after threshing
20.	Grain lustre	non-lustrous lustrous	after threshing

ABSTRACT

Title of thesis	:	“Varietal identification and seed vigour assessment in fodder sorghum [<i>Sorghum bicolor</i> (L.) Moench].”
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Year of award of degree	:	2014
Major subject	:	Seed Science and Technology
Total number of pages in the thesis	:	52+viii
Number of words in the abstract	:	303 Approx.

Key words: Varietal identification, SDS-PAGE, vigour, morphological characters.

The present investigation comprised of 12 varieties of fodder sorghum (*Sorghum bicolor* (L.) Moench) was undertaken for varietal identification based on morphological and SDS-PAGE and to assess their vigour potential. The results revealed that morphological character like leaf sheath anthocyanin colouration, leaf midrib colour, flag leaf yellow colouration of midrib, lemma arista formation, anther colour of dry anther, glume colour, glume length, panicle length without peduncle, panicle shape, caryopsis colour and grain luster were found important diagnostic characters for categorizing these varieties. Seed characters such as seed size, seed shape and test weight clearly differentiated the varieties into different groups. Total soluble seed protein analysis using SDS-PAGE revealed that maximum total number of bands were 17. A wide quantitative variation (having different R_m values) was observed in pattern of protein bands, their electrophoretic mobility, position and intensity. The standard protein marker bands [2 (R_m 0.24), 6 (R_m 0.40), 9 (R_m 0.53), 11 (R_m 0.64), 13 (R_m 0.68), 16 (R_m 0.88) and 17 (R_m 0.91)] were observed invariably common in almost all the varieties. All the varieties were differentiated by using the presence/absence of specific combination of bands or single band. The similarity was calculated in all the combination and the similarity index values ranged from 0.143 to 1.00 and the variety PC 3 gave less value with the comparison of other varieties showed dissimilarity with other remaining varieties. The dendrogram showed two major clusters at coefficient of 0.7774, the varieties PC- 3 and PC 7 in first cluster and rest varieties in second cluster. The maximum vigour potential was shown by HJ-513, HJ-541, SSV-84, HC-136 and CSV-15 varieties which showed superiorly form the all other variety. The standard germination, accelerated ageing test, tetrazolium test and electrical conductivity were found the most suitable vigour parameters for prediction of seedling establishment in field conditions.

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