I J T A
© Serials Publications
National Academy of Agricultural Science (NAAS)
Rating : 3. 03

Chemical, Biochemical and Seed Composition Assays for Characterization of Pigeonpea [*Cajanus Cajan* (L.) Millsp.] Genotypes

*Mahammadrafik Nadaf, *N.M. Shakuntala, R.V. Beladadi, S.N. Vasudevan and Y.S. Amaresh*

Abstract: *Raichur is coming under North-Eastern dry zone (Zone 2) of Karnataka state, wherein among the major pulse crops, pigeonpea is grown in maximum cultivable area during kharif, hence it is regarded as pulse bowl of South India. This region is blessed with suitable agro-climatic conditions for quality seed production of varieties of pulses. Ten important pigeonpea genotypes well suitable which are adjusted to this region were identified by knowing their response to different chemical (Phenol, Modified phenol with Copper sulphate, $FeSO_4$, NaOH, KOH and Peroxidise) tests. All the genotypes were identified by using key based chemical tests. Phenol, Modified phenol with Copper sulphate, $FeSO_4$ and KOH tests were able to distinguish all the genotypes. In $FeSO_4$ test, the genotypes did not show any reaction which was considered as absent. Chemical tests could be used as a powerful tool to identify every genotype in a short period of time. The highest electrical conductivity was recorded in Maruti (0.66 dSm^{-1}) and lowest in PT-221, Asha (0.62 dSm^{-1}) where as highest Dehydrogenase enzyme activity was observed in BSMR-736 (0.78) and lowest in Maruti (0.63). Among ten genotypes, significantly highest seed protein content was noticed in Maruti (22.50%) followed by ICPL-87 (21.60%), TS-3 (21.50%), and remaining six genotypes had low protein content.*

Keywords: *Pigeonpea, Chemical test, Varietal identification,dehydrogenase, protein content.*

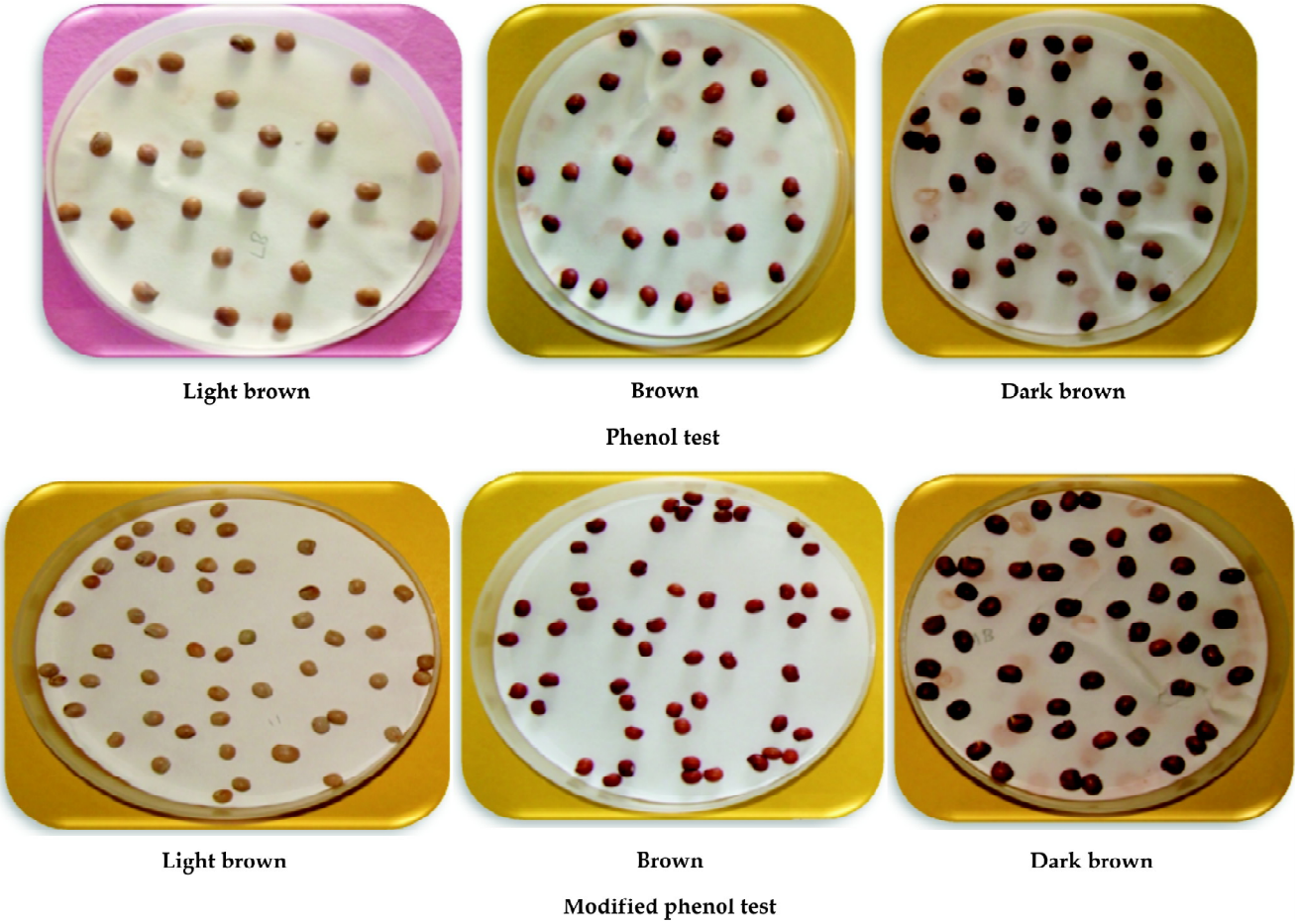
INTRODUCTION

Pigeonpea is the major pulse grown in India and valuable multi-purpose grain legume crop which is extensively grown in arid and semiarid tropics. In India, it is one of the very important grain legumes and occupies second position in area and production next to chickpea.

India has the distinction of being world's largest producer of pulses, with the production of 14.69 million tonnes annually from an area of 28.31 million hectares (Anon., 2011). It is grown on an area of 4.04 million hectares with an annual production of 2.65 million tonnes with a productivity of 656 kg ha^{-1}. Its area, production and productivity trends in India for the last five decades showed that there was about 2 per cent increase in area per year but the yield levels were stagnated around 600-700 kg ha^{-1} (Anon., 2012).

To sustain its high production and productivity, a number of high yielding varieties and hybrids have been developed and notified in the recent past, out of which many varieties and hybrids are now in seed production chain. The release of large number of rice hybrids has increased the task as well as the responsibilities of seed technologists in order to ensure the quality of seed. Seed technologists must be well equipped to identify different varieties and hybrids, both at field and at seed level. Varietal descriptions given by the breeders most often relate to field characters and not sufficient to identify genotypes or seed lot adequately. The alternative way to speed up the

* Department of Seed Science and Technology, University of Agricultural Sciences, College of Agriculture, Raichur – 584 104, India.



testing procedures is to use chemical tests in place of morphological markers. These chemical tests are very quick, easy to do, reproducible and can be undertaken throughout the year under controlled conditions.

MATERIAL AND METHODS

The experiments were carried out in the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur The seed material of most important different pigeonpea genotypes *viz*, BSMR-736, ICPL-87, TS-3R, Maruti, Asha, WRP-1, GS-1, PT-221, TS-3 and GC-11-39 were obtained from pigeonpea breeder, AICRP on pulses, Agricultural Research Station, Gulbarga for the present investigation.

The experiments consist of 10 treatments and three replication with Completely Randomised Design (CRD).

Different Chemical Tests

Phenol test

The standard phenol test for varietal purity testing as suggested by Walls (1965) was followed. Four replications of 100 seeds each were soaked in distilled water for 24 hours. The seeds were then placed in petri dishes containing filter paper moistened with 5 ml of 1% phenol solution and kept at room temperature (28°C) for 24 hours. After that, the seeds were examined and grouped into different colour classes as, no colour change, light brown, brown and dark brown (Figure 1).

Modified phenol test

As described by Banerjee and Chandra (1977), the procedure is similar to the standard phenol test except that, the seeds were soaked in a solution of 0.5% $CuSO_4$ instead of soaking the seeds in distilled water. The seeds were examined and grouped into five distinct groups namely, no colour change, light brown, brown, dark brown and black (Figure 1).

Ferrous sulphate ($FeSO_4$) test

The ferrous sulphate test as described by Kirankumar Reddy(2004) was followed. Four replications of 100 seeds were soaked in one per cent ferrous sulphate solution and kept in an incubator for two hours and the black colour uniformly making them undistinguishable from each other.

Potassium hydroxide (KOH) test

The test procedure as suggested by Mckee (1973), the seeds (1 g) of genotypes were washed in distilled water and then soaked in 10 ml of 6% potassium hydroxide solution for one hour in test tube at an ambient temperature. The solution was decanted and used for visual observation. Based on the change in colour of the solution, the genotypes were grouped as, light yellow, light brown, brown and dark brown (Figure 2).

Sodium hydroxide (NaOH) test

Four replications of fifty seeds each were soaked in 3% NaOH solution for 3 hours and thereafter the change in colour of the solution was observed (Agrawal, 1987). Based on intensity of colour reaction, the genotypes were classified into three groups *viz.,* no colour change, light yellow and wine red (Figure 3).

Peroxidase test

It was studied as per the procedure given by Buttery and Buzzell (1968). Ten seed coats were removed and placed separately in the test tube, with three replications for all the varieties and added 10 drops of 0.5 per cent Guaiacol solution into test tube, after ten minutes one drop of 0.1 per cent solution of hydrogen peroxide (H_2O_2) was added and the reactions were noted exactly after sixty seconds. The colouration due to peroxidase activity was observed to group the varieties as, brown colour solution and colourless solution.

Biochemical Test

Electrical conductivity (dSm^{-1})

Five grams of seeds in three replications were soaked in acetone for half a minute and thoroughly washed in distilled water for three times. Then the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at 25°C ± 1°C for twelve hours. The seed leachate was collected and the volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant 1.0 and the mean values were expressed in desi Simons per meter (dSm^{-1}) (Jackson, 1973).

Dehydrogenase enzyme activity (OD Value)

The dehydrogenase enzyme activity was measured using the method followed by Shenoy *et al.* (1990). Representative seeds (25) from each genotypes were taken and preconditioned by soaking in water for overnight at room temperature. Seeds were taken at random and the embryos were excised. The embryos were steeped in 0.25 per cent solution of 2, 3, 5-triphenyl tetrazolium chloride solution and kept in dark for two hours at 40°C for staining. The stained seeds were thoroughly washed with water and then soaked in five ml of 2 methoxy ethanol (methyl cellosolve) and kept overnight for extracting the red colour formazan. The intensity of red colour was measured using ELICO UV-VI Spectrophotometer (model SL-159) using blue filter (470 nm) and methyl cellosolve as the blank. The OD value obtained was reported as dehydrogenase enzyme activity.

Alpha amylase activity (mm)

The α-amylase activity was analyzed as per the method suggested by Simpson and Naylor (1962). Two gram of agar shreds and one gram of potato starch was mixed together in water to form paste and the volume was made up to 100 ml with distilled water. The homogenous solution of agar-starch mixture after boiling was poured into sterilized Petri-dishes and allowed to settle in the from of gel after cooling. The pre-soaked (for 8 hours) and half cut seeds (with their half endosperm and embryo portion intact) were placed in the petri-dishes in such a way that the endospermic part remained in contact with agar-starch gel. The petri-dishes were closed and kept in dark at 30°C. After 24 hours the Petri-dishes were uniformly smeared with potassium iodide solution (0.44 g of iodine crystal + 20.008 g potassium

Table 1
Grouping of genotypes based on chemical tests

Genotypes	*Phenol test*	*Modified phenol test*	*$FeSO_4$ test*	*KOH test*	*NaOH test*	*Peroxidase test*
BSMR-736	Medium brown	Light Brown	Black	Light yellow	Dark red	Present
ICPL-87	Dark brown	Dark brown	Black	Light Yellow	Dark red	Present
TS-3R	Medium Brown	Dark brown	Black	Yellow	Dark red	Present
Maruti	Medium brown	Brown	Black	Light brown	Dark red	Present
Asha	Dark brown	Light brown	Black	Light brown	Yellow	Present
WRP-1	Light brown	Dark brown	Black	Yellow	Yellow	Absent
GS-1	Light Brown	Light brown	Black	Yellow	Yellow	Absent
PT-221	Light brown	Brown	Black	Yellow	Dark red	Absent
TS-3	Light Brown	Dark brown	Black	Light brown	Yellow	Absent
GC-11-39	Dark brown	Light brown	Black	Yellow	Yellow	Present

Phenol test : Medium brown, Light brown, Dark brown
Modified phenol : Light brown, Dark brown, Brown
KOH test : Light yellow, Light brown, Yellow
NaOH test : Yellow, Dark red
$FeSO_4$ test : Black
Peroxidase test :
Present : Reddish brown Colour solution
Absent : Colourless solution

iodide in 500 ml distilled water) and excess solution was drained off after few minutes. The diameter of halo (clear) zone formed around the seed was measured in mm and reported as α-amylase activity.

Seed Composition Estimation

Protein content (%)

The five gram seeds of different genotypes were weighed and powdered separately to assess protein content in the sample. Total nitrogen in powdered sample was determined by employing the Kjeldahl's method described by Jackson (1973). Percentage of total protein was obtained by multiplying the per cent N by 6.25 factor.

Starch (%)

100 mg of powdered seed sample of different genotypes was taken separately into the centrifuge tube, then 5-10 ml of 80 per cent ethanol was added and the tube was placed in a water bath at 80-85 °C for 5-10 min. Further centrifuge it for 10 min at 3000 rpm, decant and save the supernatant, ethanol extraction were repeated thrice and save the supernatant every time. To the residues, add 3 ml of distilled water followed by 6.5 ml of 52 per cent perchloric acid. Stir the content for 5 min and then occasionally for the next 15 min. Add little water and centrifuge at 3000 rpm for 5 min. Decant the supernatant into a 100 ml volumetric flask. Repeat the extraction with perchloric acid thrice, increasing the time from 15 to 25 and finally to 30 min and save the supernatant. Make up the volume of the pooled supernatants to 100 ml with distilled water. Neutralize the supernatant, make further dilutions, if necessary and estimate the glucose content of the hydrolysate by any one of the method for the estimation of reducing sugars.

Oil content (%)

The oil content of each genotype was determined with the help of NMR (Nuclear Magnetic resonance spectrometer) and the genotypes were grouped as low, and high oil content types.

RESULTS AND DISCUSSION

Varietal identification by morphological characters is laborious, time consuming, tedious, cumbersome and costly affair. A number of chemical tests have been developed for varietal identification such as

Table 2
Grouping of pigeonpea genotypes based on biochemical test

Genotypes	*Electrical conductivity (dSm^{-1})*	*Group*	*Dehydrogenase enzyme activity (OD Value)*	*Group*	*α-amylase activity (mm)*	*Group*
BSMR-736	0.59	Low	0.78	High	10.37	Low
ICPL-87	0.64	High	0.67	High	11.93	High
TS-3R	0.65	High	0.65	High	10.47	Low
Maruti	0.66	High	0.63	Low	10.43	Low
Asha	0.62	Low	0.69	High	10.33	Low
WRP-1	0.65	High	0.66	High	12.27	High
GS-1	0.60	Low	0.76	High	13.03	High
PT-221	0.62	Low	0.68	High	10.97	Low
TS-3	0.59	Low	0.78	High	10.80	Low
GC-11-39	0.65	High	0.64	Low	10.77	Low
MEAN	0.63		0.69		11.14	
S.Em±	0.001		0.001		0.110	
CD (1%)	0.004		0.005		0.441	

Electrical conductivity	*Dehydrogenase enzyme activity*	*α-amylase activity*
High : > 0.63 dSm^{-1}	High : > 0.65 (OD Value)	High : > 11 mm
Low : < 0.63 dSm^{-1}	Low : < 0.65 (OD Value)	Low : < 11mm

Phenol test, Modified phenol test, Peroxidase test, Potassium hydroxide test, Sodium hydroxide, Ferrous sulphate test. These chemical tests are very quick, easy and reproducible (Ashwani Kumar *et al.*, 1995). Very often these tests provide supportive evidence for morphological evaluation of seeds (Vanderburg and Vanzwol, 1991) and varietal identification keys were prepared (Figure 4 and 5).

The genotypes were grouped on the basis of the reaction of seeds to the various chemical tests (Table 1).

On the Basis of seed colouration with phenol, the pigeonpea genotypes were grouped into three categories (light brown, brown and dark brown), four genotypes *viz.*, (WRP-1, GS-1, PT-221 and TS-3) showed light brown, three genotypes *viz.*, (BSMR-736, TS-3R and Maruti) showed medium brown and three genotypes *viz.*, (ICPL-87, Asha and GC-11-39) showed dark brown. Seed colouration with phenol is one of the important qualitative characteristic which is not affected by environmental condition. The result of phenol test is usually distinct and easily interpreted. Walls (1965) reported that the phenol colour reaction depends on the quality and quantity of oxidase enzymes present in seeds (Figure 1).

While on the basis of modified phenol test with $CuSO_4$, the genotypes were classified into four groups (no colour change, light brown, brown and dark brown). Were four genotypes *viz.*, (GS-1, TS-3, WRP-1 and PT-221) showed light brown, three genotypes *viz.*, (BSMR-736, GC-11-39 and Maruti) showed brown and three genotypes *viz.*, (ICPL-87, TS-3R and Asha) showed dark brown colour reaction. Banerjee and Chandra (1977) reported Cu^{++} ions act as catalysts which increased the enzymatic process thereby enhancing the resolution of phenol colour reaction (Figure 1).

The peroxidise test, the genotypes were grouped into (Reddish brown and colourless solution). Six genotypes *viz.*, (BSMR-736, ICPL-87, TS-3R, Maruti, Asha and GC-11-39) showed reddish brown colour solution, four genotypes *viz.*, (WRP-1, GS-1, PT-221 and TS-3) showed colourless solution (Figure 2).

Table 3
Grouping of pigeonpea genotypes based on seed composition

Genotypes	*Protein content (%)*	*Groups*	*Starch content (%)*	*Groups*	*Oil content (%)*	*Groups*
BSMR-736	22.10	High	52.00	High	1.40	Low
ICPL-87	21.60	High	49.80	Low	1.30	Low
TS-3R	20.75	Low	51.50	High	1.50	High
Maruti	22.50	High	49.75	Low	1.40	Low
Asha	21.20	High	51.75	High	1.50	High
WRP-1	20.80	Low	50.25	Low	1.40	Low
GS-1	21.30	High	51.10	High	1.30	Low
PT-221	21.10	High	50.50	Low	1.60	High
TS-3	21.50	High	49.40	Low	1.50	High
GC-11-39	20.75	Low	51.20	High	1.40	Low
MEAN	21.36		50.73		1.43	
S.Em±	0.277		0.223		0.058	
CD (1%)	0.115		0.899		0.232	

Protein
High : > 21%
Low : < 21%

Starch
High : > 51%
Medium : 51%
Low : < 51%

Oil content
High : > 1.5%
Low : < 1.5%

The sodium hydroxide test, the genotypes were grouped into (Dark red and Yellow). Five genotypes *viz*., (ASHA, WRP-1, GS-1, TS-3 and GC-11-39) showed yellow and remaining five genotypes *viz*., (BSMR-736, ICPL-87, TS-3R, MARUTI and PT-221) showed dark red. The difference in colour reaction of seeds seems to be due to differences in genetic back ground concerning the enzyme system (Chakrabarthy and Agrawal, 1990). In our study with respect to ferrous sulphate test did not vary with the ferrous sulphate test is absent (Figure 2).

The potassium hydroxide test is useful in determining the varietal difference based on the chemical reaction. On the basis of colour reaction with KOH solution, the genotypes were categorised into yellow, light yellow and light brown. Were two genotypes *viz*., (BSMR-736 and ICPL-87) showed light yellow, three genotypes *viz*., (Maruti, Asha and TS-3) showed light brown and five genotypes *viz*., (TS-3R, WRP-1, GS-1 and PT-221) showed yellow colour solution. Varied colour reaction may be due to the chemical composition of seed or selective action of enzymes present which may be governed genetically (Figure 3).

The chemical assays will serve as a useful tool in grouping genotypes which are crop specific based on genetic makeup and enzymatic activity. Among the various chemical assays the pigeonpea crop responded well for phenol, modified phenol with $CuSO_4$, peroxidase, KOH and NaOH tests.Table 1. Grouping of genotypes based on chemical tests

Biochemical Analysis

The results pertaining to electrical conductivity are presented in Table 2.

The electrical conductivity was ranged from 0.59 dSm^{-1} to 0.66 dSm^{-1} with mean 0.63 dSm^{-1}. The genotypes were grouped into two groups having low (< 0.63 dSm^{-1}) and high (> 0.63 dSm^{-1}) on content.

Among ten genotypes, five genotypes had low electrical conductivity *viz*., BSMR-736 (0.59 dSm^{-1}), TS-3 (0.59 dSm^{-1}), GS-1 (0.60 dSm^{-1}), PT-221 (0.62 dSm^{-1}), Asha (0.62 dSm^{-1}) and five genotypes had high electrical conductivity *viz*., ICPL-87 (0.64 dSm^{-1}), TS-3R (0.65 dSm^{-1}), WRP-1 (0.65 dSm^{-1}), GC-11-39 (0.65 dSm^{-1}) and Maruti (0.66 dSm^{-1}).

The results pertaining to dehydrogenase enzyme activity presented in the Table 2. The Dehydrogenase enzyme activity was ranged from 0.63-0.78 with mean 0.69. The genotypes were grouped into two groups having low (< 0.65) and high (> 0.65).

Among ten genotypes, eight genotypes had high dehydrogenase enzyme activity *viz*., BSMR-736 (0.78), TS-3 (0.78), GS-1 (0.76), Asha (0.69), PT-221 (0.68), ICPL-87 (0.67), WRP-1 (0.66), TS-3R (0.65) and two genotypes had low dehydrogenase enzyme activity *viz*., Maruti (0.63) and GC-11-39 (0.64).

The results regarding α-amylase activity are presented in the (Table 2). The α-amylase activity was ranged from 10.33 mm to 13.03 mm with mean 11.14 mm. The genotypes were grouped into two groups having low (< 11) and high (> 11).

Among ten genotypes, three genotypes had high α-amylase activity namely GS-1 (13.03 mm), WRP-1 (12.27 mm), ICPL-87 (11.93 mm) and another seven genotypes had low α-amylase activity namely PT-221 (10.97 mm), TS-3 (10.80 mm), GC-11-39 (10.77 mm), TS-3R (10.47 mm), Maruti (10.43 mm), BSMR-736 (10.37 mm) and Asha (10.33 mm).

Seed Composition

The protein content varied significantly among the pigeonpea genotypes (Table 3). The mean protein content of the genotypes was 21.36 per cent. The protein content of the genotypes ranged from 20.75% (TS-3R) to 22.50% (Maruti). Based on percentage of protein content, genotypes were grouped into two groups having low (< 21.5%) and high (> 21.5%).

Among ten genotypes, significant high seed protein content was noticed in Maruti (22.50%) followed by ICPL-87 (21.60%), TS-3 (21.50%), and six genotypes had low protein content *viz*., GS-1 (21.30%), Asha (21.20%), PT-221(21.10%), WRP-1 (20.80%), GC-11-39 (20.75%) and TS-3R (20.75%).

The data pertaining to starch content (%) in pigeonpea genotypes is presented in Table 2. The mean starch content of the genotypes was 50.73% per cent. The starch content of genotypes ranged from 49.40% (TS-3) to 52.00% (BSMR-736). Based on percentage of starch content, genotypes were grouped into two groups having low (< 51%) and high (> 51%) on content.

Among ten genotypes, five genotypes showed high seed starch *viz*., BSMR-736 (52.00) followed by Asha (51.75%), TS-3R (51.50%), ICPL-87 (51.20%), Maruti (51.10%) and five genotypes had low seed starch *viz*., PT-221 (50.50%), WRP-1 (50.25%), GC-11-39 (49.80%), GS-1 (49.75%) and TS-3 (49.40%).

The results regarding oil content (%) of pigeonpea genotypes is presented in Table 3. Oil content varied among the genotypes. The mean oil content was 1.43 per cent. The oil content of genotypes ranged from 1.30% (ICPL-87 and GS-1) to 1.60% (PT-221). Based on percentage of oil content, genotypes were grouped into two groups having low (< 1.5%) and high (> 1.5%) on content.

Among ten genotypes, four genotypes had high seed oil content *viz*., PT-221 (1.60%), TS-3R (1.50%), Asha (1.50%), TS-3 (1.50%) and six genotypes had low oil content *viz*., BSMR-736 (1.40%), Maruti (1.40%), WRP-1 (1.40%), GC-11-39 (1.40%), ICPL-87 (1.30%) and GS-1 (1.30%).

Grouping Based on Biochemical Analysis

Biochemical assays like electrical conductivity, dehydrogenase enzyme activity and α-amylase activity will be helpful in grouping of genotypes enhancing them for further seed quality studies. A number of water soluble compounds such as electrolytes, sugars, aminoacids and organic acids are released in the water. The electrical conductivity of seed leachate indicates the membrane integrity and quality of seed and it was negatively correlated with seed quality.

The highest electrical conductivity was recorded in Maruti (0.66 dSm^{-1}) and lowest in PT-221, Asha (0.62 dSm^{-1}), with the mean of 0.63 dSm^{-1}. Based on this, the genotypes were grouped as low electrical conductivity (<0.63 dSm^{-1}) with five genotypes and high electrical conductivity (> 0.63 dSm^{-1}) with five genotypes. Higher seed leachate due to controlled deterioration at higher moisture content was noticed as evident from electrical conductivity test. This might due to increase in the membrane damage when controlled deterioration was done at higher moisture contents.

The highest Dehydrogenase enzyme activity was observed in BSMR-736 (0.78) and lowest in Maruti (0.63), with the mean of 0.69. Based on this, the genotypes were grouped as low dehydrogenase enzyme activity (< 0.65) with two genotypes and high dehydrogenase enzyme activity (> 0.65) with eight genotypes. Dehydrogenase enzyme which exists in mitochondria and necessary for respiratory process indicate the level of seed viability and vigour (Anon., 2012).

The dehydrogenase enzyme activity is a good stable metabolic marker to estimate the degree of vigour in seeds (Saxena *et al*., 1987) and have positive association with vigour and viability of seeds (Rurdrapal and Basu, 1982 and Kharluki, 1983).

The highest α-amylase activity was recorded in GS-1 (13.03 mm) and lowest in Asha (10.33 mm), with the mean of 11.14 mm. Based on this, the genotypes were grouped as low α-amylase activity (< 11 mm) with seven genotypes and high α-amylase activity (> 11 mm) with three genotypes in red clover (Dejan, 2009).

Grouping Based on Seed Composition

The highest protein content was noticed in BSMR-736 (22.10%) ICPL-87 (21.60%) and lowest in GC-11-39 (20.75%), with the mean of 21.36%. Based on this the genotypes were grouped as low protein (< 21%) with three genotypes, high protein (> 21) with seven genotypes.

The highest starch content was observed BSMR-736 (52.00%) and lowest in PT-221 (49.40%), with the mean of 50.73%. Based on this, the genotypes were grouped as low starch (< 51%) with seven genotypes and high starch (> 51%) with three genotypes.

The highest oil content was noticed in PT-221 (1.60%) and lowest in GS-1 (1.30%), with the mean of 1.43%. Based on this, the genotypes were grouped as low oil content (1.5%) with six genotypes and high oil content (>1.5%) with four genotypes. Similar results were earlier reported by Lingaraj (2009) and sebetha *et al*. (2010) in cowpea. Luan and Han (1990) in groundnut, Shadakshri *et al*. (1995) in sesamum. The variation in oil content might be a genetic factor. Weiss (1971) reported that two to seven polygenes were involved in the inheritance of oil content.

SUMMARY AND CONCLUSION

The genotypes were grouped based on the variation in protein content in to low (< 21%) with (TS-3R, WRP-1 and GC-11-39) three genotypes and high (> 21%) with (BSMR-736, ICPL-87, Maruti, Asha, GS-1, PT-221 and TS-3) seven genotypes. Based on variation in the starch content, the genotypes were (ICPL-87, Maruti, WRP-1, PT-221 and TS-3) grouped into low (<51%) with five genotypes and high (>51%) with five genotypes (BSMR-736, TS-3R, Asha, GS-1 and GC-11-39). Based on variation in the oil content, the genotypes were grouped into low (<1.5%) with (BSMR-736, ICPL-87, Maruti, WRP-1, GC-11-39 and GS-1) six genotypes and high (>1.5%) with (TS-3R, Asha, PT-221 and TS-3) four genotypes.

Based on electrical conductivity the genotypes were grouped into, low (< 0.63 $dSm^{-1)}$ with (BSMR-736, Asha, GS-1, PT-221 and TS-3) five genotypes and high (> 0.63 dSm^{-1}) with (ICPL-87, TS-3R, Maruti, WRP-1 and GC-11-39) five genotypes. Based on dehydrogenase enzyme activity the genotypes were grouped into, low (< 0.65 OD Value) with (Maruti and GC-11-39) two genotypes and high (>0.65 OD Value) with (BSMR-736, ICPL-87, TS-3R, Asha, WRP-1, GS-1, PT-221 and TS-3) eight genotypes. Based on α-amylase activity the genotypes were (BSMR-736, TS-3R, Maruti, Asha, WRP-1, GC-11-39, PT-221 and TS-3) grouped into low (< 11 mm) with seven genotypes and high (> 11 mm) with (ICPL-87, WRP-1 and GS-1) three genotypes.

References

Agrawal, P.K., (1987), Cultivar purity test In. *Seed Sci. and Technol.*, South Asian Publ, New Delhi, p.160.

Anonymous, (2011), Pulses production up by 30 per cent. *The Hindu*, 30, March, Vol. 134(76): 3.

Anonymous, (2012), Knowledge based N management for increasing both grain yield and N-use efficiency in intensive irrigated rice system in eastern India. In: Annual reports for 2011-12, Central Rice Research Institute, Cuttack, p.75.

Ashwani Kumar, Chowdhary, R.K., Kapoor, R.L. and Dahiya, O.S., (1995), Identification of pearl millet hybrids and their parental lines using seeds and seedling characters, chemical tests and gel electrophoresis. *Seed Sci. and Technol.,* 23: 21-32.

Banerjee, S.K. and Chandra, S., (1977), Modification phenol test for the varietal identification of wheat seed. *Seed Sci. and Technol.,* 5: 53-60.

Buttery, B.R. and Buzzell, R.I., (1968), Peroxidase activity in seeds of soybean varieties. Crop Sci., 8: 722-724.

Chakrabarthy, S.K. and Agrawal, R.L., (1990), Identification of blackgram varieties-III: Utilization of seedling growth response to added chemical. *Seed Res*., 18(1): 24-31.

Jackson, M. L., 1973, *Soil Chemical Analysis.* Prentice Hall of India Pvt. Ltd., New Delhi.

Kharlukhi, L., (1983), Physiological and biochemical changes in seeds during storage under controlled condition. *Ph.D. Thesis*, IARI, New Delhi.

Kirankumar Reddy, C., (2004), Studies on laboratory techniques for identification of cotton (*Gossypium* spp.) genotypes. *M. Sc. (Agri.) Thesis*, Acharya N. G. Ranga Agric. Univ., Hyderabad (India).

Lingaraj, C., Halemani, (2009), Assessment of genetic diversity in cowpea (*Vigna unguiculata* L. Walp.) germplasm. *M.Sc. (Agri) Thesis,* Univ. of Agric. Sci., Dharwad, Karnataka (India).

Luan, W.Q. and Han, S.P., (1990), Preliminary analysis of nutritive value of groundnut germplasm resources in shangadong province. *Crop Genetic Res.,* 2: 22-25.

Mckee, (1973), Chemicals and biochemical techniques for varietal identification. *Seed Sci. and Technol.,* 1: 181-199

Rudrapal, A.B. and Basu, R.N., (1982), Lipid peroxidation and membrane damage in deterioration of wheat and mustard seed. *Indian J. Expt. Biol*. 20: 465-470.

Saxena, V.P., Singh, G., Pakuraiah, T. and Pandey, N., (1987), Seed deterioration studies in some vegetable seeds. *Acta. Hortic*., 215: 39-44.

Sebetha, E.T., Ayodele, V.I., Kutu, F.R. and Mariga, I.K., (2010), Yields and protein content of two cowpea varieties grown under different production practices in Limpopo province, South Africa. *Afr. J. Biotechnol.,* 9(5): 628-634.

Shadakshari, Y.G., Virupakshappa, K. and Shivashankar, G., (1995), Genetic variability studies in germplasm collection of sesamum (*Sesamum indicum* L.). *Mysore J. Agric. Sci*, 29: 133-137.

Shenoy, V.V., Dadlani, M. and Seshu, D.V., (1990), Association of laboratory assessed parameters with field emergence in rice. The non-anoic acid stress as a seed vigour test. *Seed Res*., 18(1): 60-69.

Simpson, G.M., and Naylor, J.M., (1962), Dormancy studies in seeds of *Avena fatura* and a relationship between maltase, amylases and gibberellins. *Canadian J. of Botany,* 40: 1959-1673.

Vanderburg, N.J. and Vanzwol, R.A., (1991), Rapid identification techniques used in laboratories of the International Seed Testing Association: a survey. *Seed Sci. and Technol.,* 19: 687-700.

Walls, W.E., (1965), A standardized phenol method for testing wheat seed for varietal purity. *Association of Official Seed Analyst,* p. 28.

Weiss, E.A., (1971), Castor, sesame and safflower, *Leonard Hill Books*, London, 311-355.