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Faster Chemical Methods to Determine Genetic Purity in Pigeon Pea (*Cajanus cajan* (L.) Mill sp.)

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A B S T R A C T

The present investigation was carried out to find faster chemical methods to determine genetic purity in ten pigeon pea varieties. The faster methods are Ferrous Sulphate test, Sodium hydroxide test, peroxidase test, seedling growth response to GA_3, kinetin and 2-4D. Histogram using pixel luminance in ferrous sulphate test based on the grey scale produces values ranging from 17377 to 90616 which can be used to distinguish pigeon pea varieties. Similarly, sodium hydroxide test produced six coloured palette based on RGB values can distinguish varieties in pigeon pea. Also in peroxidase test, values for all ten pigeon pea varieties based on absorbance (0.252-2.372), peroxidase enzyme activity (0.791-7.442 IU/mg) and peroxidase enzyme volume (1.581-14.883 U/g) can be used for distinguishing varieties. Among the seedling growth response tests, in GA_3 test two varieties Asha (-2) and GC-11-39 (-6) were not responded and were recorded lowest values than the control. But remaining varieties can be distinguished as medium to high response types. Also in kinetin test two varieties not responded (GRG-811 and Asha) and all the other varieties can be distinguished based on this test. In case of 2-4D test all the varieties were recorded as susceptible. The co-efficient of variation for all the three tests based on the increase in coleoptile length over the control was ranged from 0.20 (Kinetin test) to 0.37 (GA_3 test).

Introduction

Pigeon pea is one of the strategic and vital dry land crops having significance in food grain sustainability and nutrition. The seed production and testing of this crop is of is crucial in achieving productivity and positive socio-economic impact. The determination of genetic purity is very crucial in seed inventory management and distribution. To ensure the genuineness of seed the genetic purity testing is vital in supplying seeds with "*surety on* *purity*". Since pigeon pea is often cross-pollinated (3-45 %), there is a possibility of genetic contamination during its seed production cycle (Saxena *et al.,* 1990). The seed produced need to be tested in field grow-out-test (GoT) for genetic purity before it is released for commercial cultivation thus confirming its genuineness and true-to-type characters. The conventional methods using field grow out test based on morphological characters take very long time to determine genetic purity which is dependent on occurrence of specific plant characters which is again depends on environmental conditions. Moreover, GOT is time-consuming which may take one full growing season for completion, tedious and highly vulnerable to workforce abuse and infrastructure used (Bora *et al.,* 2016). Also, this method is highly seasonal and depends on soil fertility for the expression of individual characters (Mc. Donald, 1995). Though conducting field GoT under controlled conditions like greenhouse or insect proof nets is possible but expensive.

Eleven local and popular rice varieties of Assam were characterised based on the grain characters such as grain colour, length, width, L/B ratio, grain type and reactions to chemical tests such as phenol, modified phenol, NaOH, KOH-resistance, peroxidise test and $FeSO_4$ tests (Bora *et al.,* 2008). Saharan (1991) classified 33 genotypes of rice varieties into four groups viz., brown spot, brown streaks, grey spot and grey streaks kernels by using 1.5 per cent ferrous sulphate solution. Ponnuswamy *et al.,* (2003) evaluated 22 cotton genotypes which have shown no response to one per cent ferrous sulphate solution soaked for two hours. Further, Kirankumar Reddy (2004) observed that all the 22 cotton genotype developed black colour uniformly making them undistinguishable from each other when soaked in one per cent ferrous sulphate solution and kept in an incubator at 35± 1° C and observed after two hours. Sambasivarao *et al.,* (2002) classified thirty-seven groundnut genotypes based on seedling response to 2,4-D as the low, moderate and high response.

Chakrabarthy and Agarwal (1989) grouped 16 blackgram varieties based on the colour reaction of seed coat peroxidase activity (low, moderate and high peroxidase activity). Agarwal and Pawar (1990) identified 13 soybean varieties by peroxidase activity of the seed coat. Peroxidase test conducted on 29 soybean cultivars, fourteen showed positive results and fifteen cultivars showed a negative reaction. However, only 11 cultivars were pure for peroxidase test and the off types ranged from 4-40 per cent. The reason for the presence of off-types in certain cultivars could be hidden variation, as no selection was made on the biochemical basis in the development of cultivars. Screening by this method is quicker and useful for single seed screening on large scale basis.

Nair *et al.,* (1989) showed that H-4 cotton hybrid had higher peroxidase activity as compared to its parents, but hybrid H-6 had reverse pattern. Ashwanikumar *et al.,* (1993) studied the usefulness of peroxidase activity discriminating 14 genotypes of pearl millet. Muthuraj *et al.,* (1999) observed that among 29 soybean cultivars, 14 cultivars showed positive results to peroxidase test and 15 cultivars showed negative results concerning peroxidase. Ponnuswamy *et al.,* (2003) reported that the cotton genotypes could be classified by peroxidase activity as low, moderate, high and very high. Ponnuswamy *et al.,* (2003) reported that seedling response to 2,4-D at 0.5 ppm on moistened filter paper was proved to be a futile exercise as it could not distinguish the cotton genotypes.

Biradarpatil *et al.,* (2006) classified 20 genotypes of safflower based on response to 2,4-D at 5ppm into three groups namely highly susceptible, susceptible and less susceptible. Similarly, in the case of Tomato, chemical tests are used to identify and characterize different cultivars. Though no single chemical test could distinguish all the varieties distinguishable chemical characters were used to develop the keys for the identification of each cultivar (Vishwanath *et al.,* 2013).

Materials and Methods

The present investigation was carried out during the year 2017-18 in the Department of Seed Science and Technology, College of Agriculture, Dharwad. To overcome the difficulties in morphological characterisation, which is labour intensive and risk to the crop grown due to environmental conditions, rapid methods like chemical tests can be used. The rapid chemical tests utilise specific treatments such as chemicals to reveal chemical differences among seeds or seedlings of different varieties. They require virtually no technical expertise and can be completed in a relatively short time. Also, these tests are inexpensive to conduct and need no sophisticated or expensive equipment. The results of these tests are usually distinct and easily interpreted. Since the individual seeds or seedlings are tested the percentage of varietal contamination or variants can be readily determined.

Varieties and seed source

The ten varieties of pigeon pea seeds grown during two seasons for morphological characterisation using DUS criteria are used for chemical tests. The varieties viz., Maruti, TS3R, GRG-811, Asha, BSMR-736, GRG-833, GC-11-39, WRP-1, ICPL-87 and Bannur local were used. These seeds are genetically pure or true to type as described by the breeder and are collected from plants marked for collection during morphological characterisation from the crop grown at ARS, Annigeri during 2017 and 2018 (two seasons). All the chemical tests are conducted in the Seed Testing Laboratory at Department of Seed Science and Technology, UAS, Dharwad.

Chemical tests

The seeds of pigeon pea varieties can be distinguished based on the physical and chemical tests done on seeds, seedlings and seed extract based on the colour changes on these. The tests carried out are summarised in Table 1.

Ferrous Sulphate ($FeSO_4$) test

Seeds were soaked in 1.5% solution of $FeSO_4$ for four hours in ambient conditions. Seeds and excess moisture was removed and evaluated for colour change as dark grey streaks, brown streaks, and brown spots on the seed coat. Since all the pigeon pea seeds were turned to grey colour, it was difficult to distinguish varieties. Hence, luminance meter was used to measure pixel luminance and plot a histogram based on the 8-bit gray scale. In a grey scale there are 255 different possible intensities and histogram will display 255 numbers showing the exact distribution of pixels among the grey colour samples. The gray scale measures pixel distribution from 0-255, 0 is black, 128 is grey and 255 is white (Figure 1).

Sodium hydroxide (NaOH) test

Hundred seeds in four replications were soaked in five per cent NaOH solution for one hour at room temperature. Changes in the colour of the seeds were observed after one hour. Based on the colour intensity of the seed, the genotypes were classified into three groups viz., orange, brown and straw types (Agrawal, 1987).

Peroxidase test (Modified)

The Peroxidase test was carried out as per the procedure given by Agarwal and Pawar (1990) with slight modification. Seeds were soaked in distilled water overnight and decanted seeds were soaked in ten ml of 0.5 per cent guaiacol solution for one hour. Then five ml of guaiacol solution was taken out and 0.5 per cent hydrogen peroxide solution was added. The change in colour of the solution was observed after twenty minutes.

Modification on the existing method

In the current method, based on a change in colour of the solution, the varieties were grouped as red, dark red and reddish orange. Since it is tough to classify varieties based on the colour change in solution, Peroxidase (POX) activity was assayed, an increase in optical density due to oxidation of guaiacol to tetra-guaiacol following Castillo *et al.*, (1994) with minor modifications at 470 nm absorbance using a reaction mixture containing 12 mM hydrogen peroxide and 96 mM guaiacol in phosphate buffer (pH 7.0). One gram of decorated seeds of all ten pigeon pea varieties along with one contaminant were homogenized in 15 ml of 100 mM Potassium phosphate buffer (pH 7.8) with a pinch of Poly Vinyl Pyrrolidone (PVP). The extract was centrifuged at 10000 rpm for 10 min at 4°C. The supernatant was collected and used as enzyme extract. Three ml reaction mixture containing one ml 100 mM phosphate buffer (pH 7.0); 0.5 ml each 96 mM guaiacol; 12 mm H_2O_2; 50 µl enzyme extract and 950 µl distilled water. Absorbance due to the formation of tetra-guaiacol was recorded at 470 nm and enzyme activity was calculated as per the extinction coefficient of its oxidation product, tetra–guaiacol E =26.6 nM/cm.

This will give enzyme activity expressed as µmoles/cm/min/g seed fresh weight and also provide an absorbance of solutions which is specific to varieties. These particular values of absorbance (no unit) can be used to distinguish varieties and are more useful than the conventional method where varieties are grouped as red, dark red and reddish orange.

For classification of varieties with a contaminant, specific values absorbance are taken and distinguished.

Seedling growth response to GA_3

The seeds of pigeon pea genotypes were surface sterilised by washing in distilled water.

Hundred seeds each in three replications were placed on two layers of blotter paper moistened with 100 ppm GA_3 solution and incubated at 25 ± 1^0C as per ISTA procedure (Anon., 1996).

The water soaked blotter papers were used as the control. On the seventh day, twenty-five seedlings were selected randomly and growth response was measured regarding per cent increase in coleoptile length over that of control using the following formula.

Per cent increase over Control =

$$\frac{\text{Coleoptile length in GA}_3\text{-Coleoptile length in control}}{\text{Coleoptile length in control}} \times 100$$

Mean of increased seedling length was determined and the genotypes were grouped into three categories according to Agarwal and Pawar (1990).

Category of per cent increase over control

A. Low response	: < 25
B. Medium response type	: 25 to 50
C. High response	: > 50

Seedling growth response to Kinetin

The increase in coleoptile length due to exogenous application of kinetin was measured. The seeds (100 x 4) were soaked in 50 ppm kinetin solutions for 24 hours and then germinated in rolled towels at 25 + 1°C as per ISTA (l996). Twenty five seedlings were selected randomly and growth response was measured at 14th day of germination in terms of per cent increase in shoot length and root length over control. The mean of increased seedling length was determined and the genotypes were grouped into five categories according to Agarwal and Pawar (l990) based per cent increase over control viz., Very low response (< 25%), Low response type (25 to 50%), Medium response type (50 to 75%), High response type (75 to 100%) and Very high response type (> 100%).

Seedling growth response to 2, 4-D

The reduction in coleoptile growth due to exogenous application of 2,4-D was measured. The seeds of pigeon pea genotypes were surface sterilized by washing in distilled water.

Hundred seeds each in three replications were placed on two layers of blotter paper moistened with 10 ppm 2,4-D solution and incubated at 25 ± 1^0C as per ISTA procedure (Anon., 1996). The water soaked blotter papers were used as the control. On the seventh day, twenty-five seedlings were selected randomly and growth response was measured regarding per cent decrease in coleoptile length over that of control using the following formula.

Per cent decrease over Control =

$$\frac{\text{Coleoptile length in control-Coleoptile length in 2,4-D}}{\text{Coleoptile length in control}} \times 100$$

Mean of decreased coleoptile length was determined and the genotypes were grouped into two categories according to Agarwal and Pawar (1990).

Category Per cent reduction over control

a. Susceptible : < 60 per cent
b. Highly susceptible : > 60 per cent

Results and Discussion

Ferrous Sulphate test ($FeSO_4$)

The results of Ferrous Sulphate test ($FeSO_4$) were in-conclusive on the visual observation. Since all the ten varieties failed to respond to this test and produced grey colour change, it is decided to conduct a pixel luminance test using luminance meter.

All the ten varieties responded differently in the pixel luminance histogram (Figure 2). The white varieties GRG-833 (17377) and WRP-1 (17914) were recorded lowest values. The highest values were recorded in varieties BSMR-736 (90616) and GRG-811 (81334). The other varieties like Maruti (50293), TS3R (45366), Asha (65852), GC-11-39 (57921), ICPL-87 (58802) and Bannur Local (47346) recorded mid range values on the grey scale. This histogram can be used for conducting genetic purity test using Ferrous Sulphate test ($FeSO_4$) and these values can be reference values for such test. These results were not in line with Saharan (1991) who classified 33 genotypes of rice varieties into four groups viz., brown spot, brown streaks, grey spot and grey streaks kernels by using 1.5 per cent ferrous sulphate solution without using luminance histogram. Also not in line with Ponnuswamy *et al.,* (2003) evaluated 22 cotton genotypes which have shown no response to one per cent ferrous sulphate solution soaked for two hours. The difference in the response of pigeon pea and other crops indicate that this test is also a crop specific but still using histogram pigeon pea varieties can be distinguished using this test.

Sodium hydroxide (NaOH) test

The varieties of pigeon pea under this investigation responded to this test and varieties were distinguished based on the change in the solution. But unlike in recommended crops like rice and sunflower, pigeon pea seeds not produced yellow or light yellow colour but produced brown colour. To distinguish pigeon pea varieties based on the brown colour change in solution k-means colour palette was created (Figure 3) based on the six dominant colours. Among the ten varieties, TS3R, Asha and GC-11-39 were observed as brown (#873E23), GRG-833 was observed as beige (#C3A690), WRP-1 was observed as light brown (#B79174), ICPL-87 and Bannur Local were observed as saddle brown (#945837), Maruti and BSMR-736 were observed as sandy brown (#A31B17) and GRG-811 was observed as dark brown (#431B17).

Peroxidise test (Modified)

The peroxidise test is done to quantify the peroxidise enzyme activity in soya bean. However this test can also be used for distinguishing the varieties of pigeon pea based on the absorbance, peroxidise enzyme activity (IU/mg) and peroxidise enzyme volume (U/g). The results of peroxidise test are furnished in Figure 4 and Table 2. The mean, range, standard deviation and co-efficient of variation for peroxidise test was analysed. The co-efficient of variation ranged was recorded as 0.46 for all the parameters.

Among the varieties, the very low (<0.5) absorbance was recorded in Maruti (0.252). The varieties TS3R (0.588) and random contaminant (0.804) were recorded low absorbance (0.5-1.0). Medium (1.0-1.5) absorbance was recorded in the varieties GRG-811 (1.32), Asha (1.374), GC-11-39 (1.256), WRP-1(1.169) and ICPL-87 (1.212). High (1.5-2.0) absorbance was recorded in BSMR-736 (1.615) and Bannur local (1.531). Very high (>2.0) absorbance was recorded in GRG-833 (2.372). The absorbance of the solution can be used as distinguishing character which is similar to earlier results in cotton (Kirankumar Reddy, 2004).

The peroxidise enzyme activity was recorded among the varieties as very low, low, medium, high and very high. Among the ten varieties, Maruti (0.791) and TS3R (1.845) were recorded as very low (< 2.0 IU/mg). The varieties, GC-11-39 (3.94), WRP-1 (3.667), ICPL-87 (3.802) and contaminant (2.522) were recorded as low (2.0-4.0 IU/mg). The varieties, GRG-811 (4.141), Asha (4.311) and Bannur local (4.803) were recorded as medium (4.0-5.0 IU/mg). The varieties, BSMR-736 (5.067) and GRG-833 (7.442) were recorded as high (5.0-6.0 IU/mg) and very high (>6.0 IU/mg) peroxidise enzyme activity respectively. The peroxidise enzyme volume also differs across all the varieties and classified as very low, low, medium, high and very high. Among the varieties, Maruti (1.581) recorded lowest peroxidise enzyme volume (<3.0 U/g). TS3R and Contaminant were recorded as low (3.0-6.0 U/g). The varieties, WRP-1(7.335), GC-11-39 (7.881) and ICPL-87 (7.605) were recorded as medium (6.0-8.0 U/g). The varieties, GRG-811, Asha and Bannur local were recorded as high (9.0-10.0 U/g). Whereas, the varieties BSMR-736 (10.133) and GRG-833 (14.883) were recorded very high (>10.0 U/g). Similar results were recorded by Chakrabarthy and Agarwal (1989) grouped 16 blackgram varieties based on the colour reaction of seed coat peroxidase activity (low, moderate and high peroxidase activity). These observations were in line with results of peroxidise test in soya bean (Agarwal and Pawar, 1990). Peroxidise enzyme activity in pigeon pea can be used for distinguishing varieties which is similar to the observations by Nair *et al.*, (1989) who recorded that H-4 cotton hybrid had higher peroxidase activity as compared to its parents, but hybrid H-6 had reverse pattern. Also results were in line with similar test in pearl millet (Ashwanikumar *et al.*, 1993), cotton genotypes (Ponnuswamy *et al.*, 2003).

Seedling growth response to GA_3

The percent increase in coleoptile length during seedling response to GA_3 was recorded as Low response (< 25), Medium response type (25 to 50) and High response (> 50). Among the ten varieties, two varieties Asha (-2) and GC-11-39 (-6) were not responded to GA_3 application and recorded less coleoptile length than the control (0.5 cm). The variety GRG-811 (50 percent) was recorded as medium response and all the remaining varieties like Maruti (110 percent), TS3R (138 percent), BSMR-736 (91 percent), GRG-833 (80 percent), WRP-1(170 percent) and Bannur local (57 percent) were recorded as high response type (Fig. 5–7).

Table.1 Chemical tests to distinguish pigeon pea varieties

Sl no	Physical / Chemical Tests	Seed	Seedling	Seed Coat Extract
1	Ferrous Sulphate test ($FeSO_4$)	√		
2	Sodium hydroxide (NaOH) test	√		
3	Peroxidase test (Modified)			√
4	Seedling growth response to GA_3		√	
5	Seedling growth response to Kinetin		√	
6	Seedling growth response to 2-4D		√	

Table.2 Peroxidise enzyme activity for distinguishing pigeon pea varieties

Sl. no	Varieties	Absorbance	Peroxidise Enzyme Activity (IU/mg)	Peroxidise Enzyme Activity (U/g)
1	Maruti	0.252	0.791	1.581
2	TS3R	0.588	1.845	3.689
3	GRG-811	1.320	4.141	8.282
4	Asha	1.374	4.311	8.621
5	BSMR-736	1.615	5.067	10.133
6	GRG-833	2.372	7.442	14.883
7	GC-11-39	1.256	3.940	7.881
8	WRP-1	1.169	3.667	7.335
9	ICPL-87	1.212	3.802	7.605
10	Bannur Local	1.531	4.803	9.606
11	Contaminant	0.804	2.522	5.045
	Mean	1.23	3.85	7.70
	Range	0.252-2.372	0.791-7.442	1.581-14.883
	S.D	0.56	1.75	3.51
	C.V (%)	0.46	0.46	0.46

Table.3 Chemical tests on coleoptile length for distinguishing pigeon pea varieties

Varieties	GA_3 Test		Kinetin Test		2-4D test	
	Mean Values	Per cent Increase over the control	Mean Values	Per cent Increase over the control	Mean Values	Per cent Decrease over the control
Maruti	1.1	110	0.6	28	0.5	2
TS3R	1.2	138	0.6	12	0.2	65
GRG-811	0.8	50	0.5	-10	0.4	21
Asha	0.5	-2	0.5	-4	0.3	31
BSMR-736	1.0	91	0.5	3	0.3	43
GRG-833	0.9	80	0.6	22	0.4	11
GC-11-39	0.5	-6	0.6	10	0.5	5
WRP-1	1.4	170	0.7	44	0.3	36
ICPL-87	0.6	14	0.6	26	0.5	8
Bannur Local	0.8	57	0.8	66	0.5	2
Control	0.5	0	0.5	0	0.5	0
Mean	0.8	70	0.6	20	0.4	22
Range	0.5-1.4	(-6)-170	0.5-0.8	(-10) -66	0.2-0.5	2- 65
S.D	0.30	59.10	0.12	22.91	0.10	20.90
C.V (%)	0.37	0.84	0.20	1.16	0.26	0.93

Table.4 Descriptors in chemical tests based on the chemical tests

Sl No	Variety	Recommended Test	Character response to specific test compared to all other varieties	Type of Sample (Seed / Seed Solution / Seedling)
1	Maruti	Peroxidise Test- Based on the Absorbance	Low absorbance	Seed Solution
2	TS3R	Seedling response to 2-4D	Less susceptibility	Seedling
3	GRG-811	Sodium hydroxide (NaOH) test	Dark brown seeds	Seed
4	Asha	Seedling growth response to GA_3	Low response	Seedling
5	BSMR-736	Ferrous Sulphate test ($FeSO_4$)- Based on histogram	High pixel luminance	Seed
6	GRG-833	Peroxidise Test- Based on the Absorbance	Highest absorbance	Seed Solution
7	GC-11-39	Seedling growth response to GA_3	Low response	Seedling
8	WRP-1	Sodium hydroxide (NaOH) test	Light brown seeds	Seed
9	ICPL-87	Sodium hydroxide (NaOH) test	Saddle brown seeds	Seed
10	Bannur Local	Seedling growth response to Kinetin	Medium response	Seedling

Fig.1 Grey scale for plotting histogram on pixel luminance

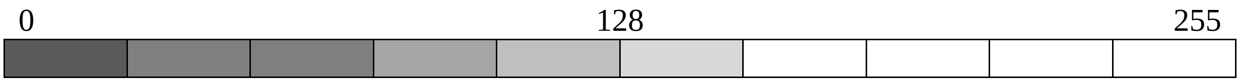


Fig.2 FeSO4 chemical test to distinguish pigeon pea varieties (histogram based on peak pixel luminance)

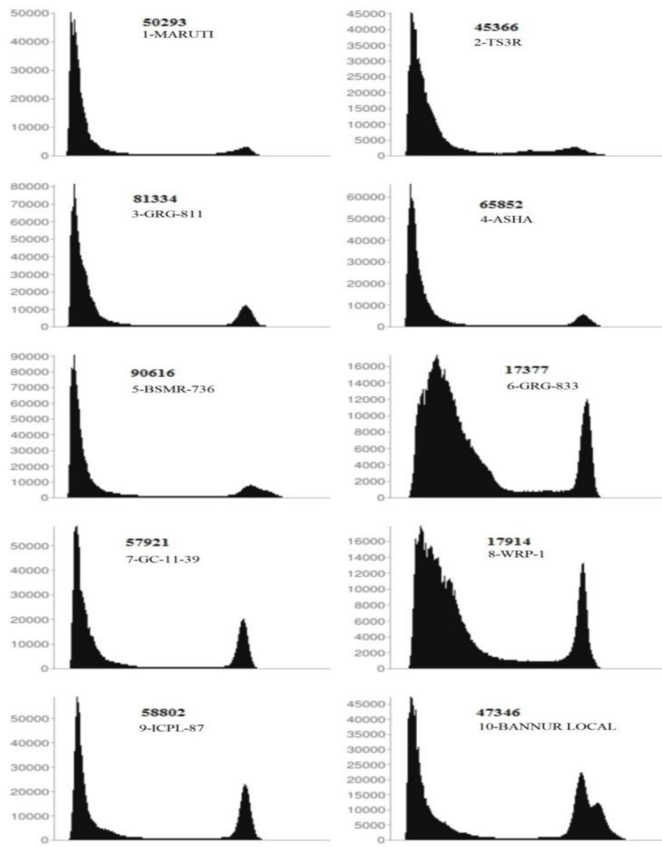


Fig.3 NaOH Chemical test for distinguishing varieties based on k-means colour palette (Six dominant colours)

Legend -

1: MARUTI	**2: TS3R**	**3: GRG-811**	**4: ASHA**	**5: BSMR-736**
6: GRG-833	**7: GC-11-39**	**8: WRP-1**	**9: ICPL-87**	**10: BANNUR LOCAL**

Fig.4 Peroxidise Chemical test for distinguishing varieties based on absorbance

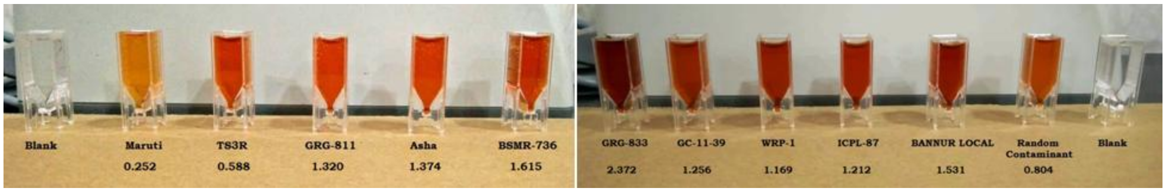
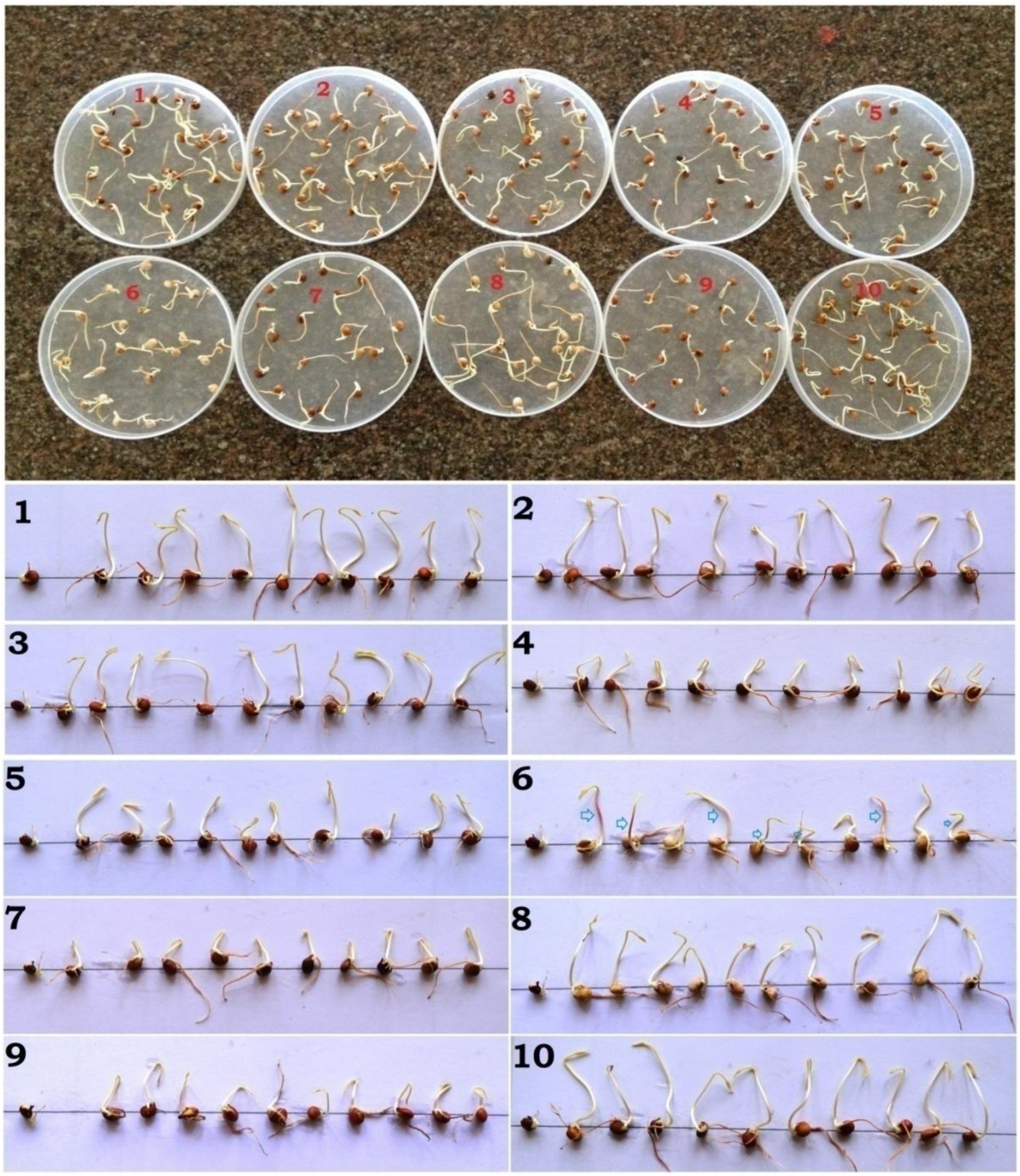


Fig.5 Response of seedlings to GA3 Chemical test



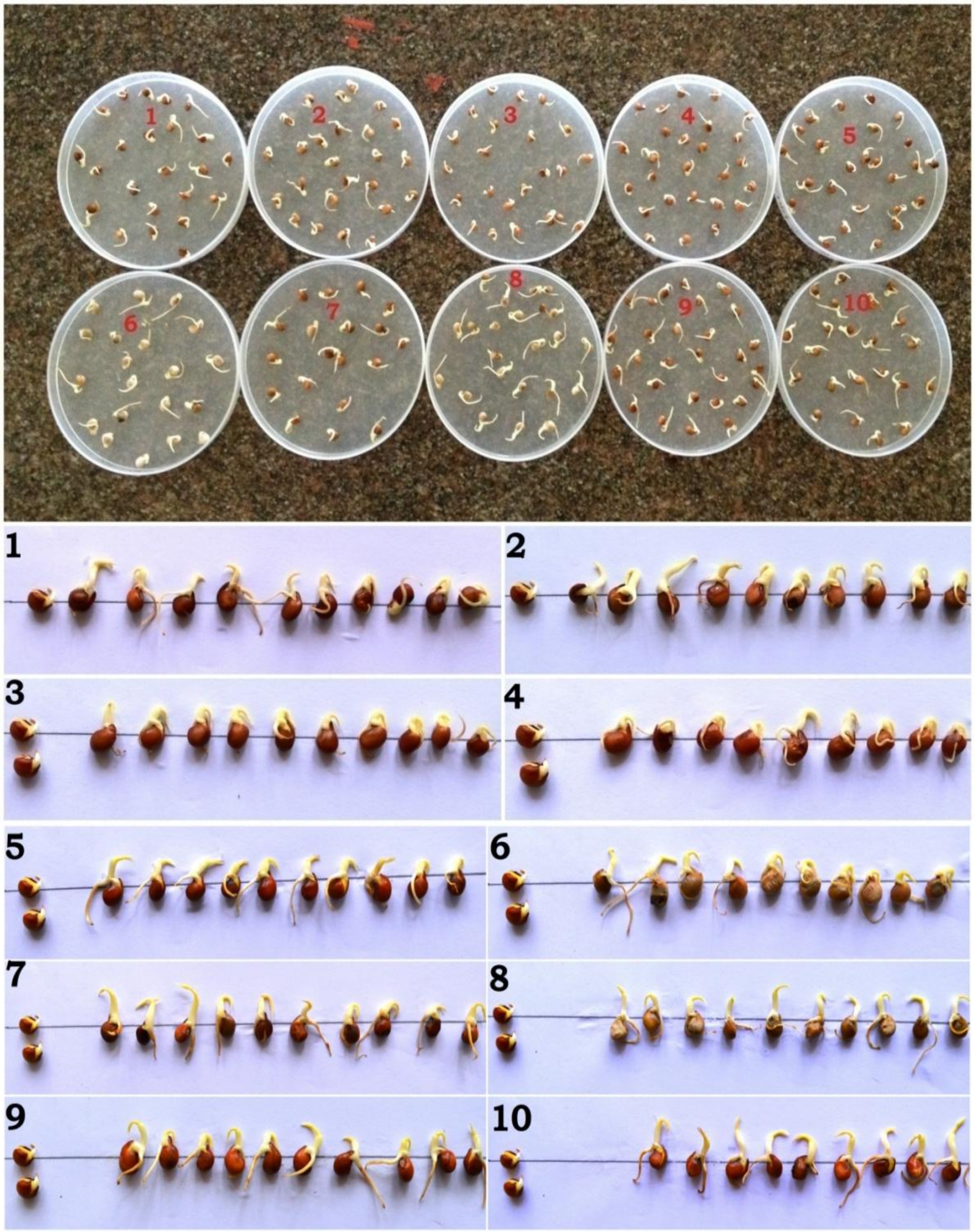
☆ **The seeds in the left side are the control (without GA_3 treatment)**

Legend -

1: MARUTI 2: TS3R 3: GRG-811 4: ASHA 5: BSMR-736

6: GRG-833 7: GC-11-39 8: WRP-1 9: ICPL-87 10: BANNUR LOCAL

Fig.6 Response of seedlings to Kinetin Chemical test

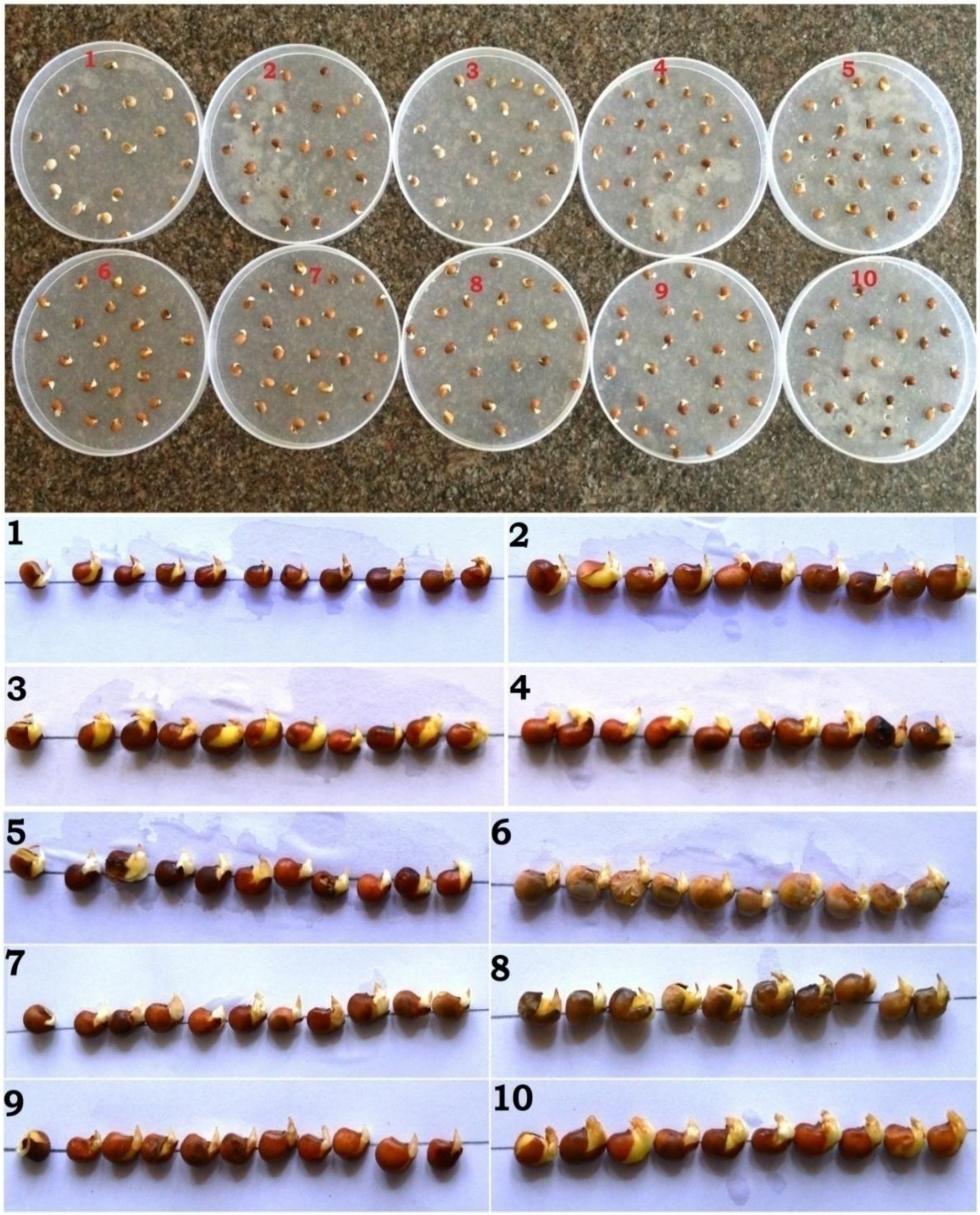


☆ **The seeds in the left side are the control (without Kinetin treatment)**

Legend -

1: MARUTI 2: TS3R 3: GRG-811 4: ASHA 5: BSMR-736
6: GRG-833 7: GC-11-39 8: WRP-1 9: ICPL-87 10: BANNUR LOCAL

Fig.7 Response of seedlings to 2-4-D Chemical test



☆ **The seeds in the left side are the control (without 2-4-D treatment)**

Legend -

1: MARUTI **2: TS3R** **3: GRG-811** **4: ASHA** **5: BSMR-736**

6: GRG-833 **7: GC-11-39** **8: WRP-1** **9: ICPL-87** **10: BANNUR LOCAL**

These results were in line with observations in paddy (Tiwari *et al.,* 2013). Though no individual chemical test was able to distinguish all the varieties, different chemical tests in conjunction were useful in identification of varieties. The effect of GA_3 coleoptile growth of seedling was found to be variable among varieties studied.

Seedling growth response to Kinetin

The percent increase in coleoptile length during seedling response to kinetin was recorded as Very low response (< 25%), Low response type (25 to 50%), Medium response type (50 to 75%), High response type (75 to 100%) and Very high response type (> 100%). Among the ten varieties, three varieties GRG-811 (-10) and Asha (-4) were not responded to kinetin application and recorded less coleoptile length than the control (0.5 cm). The varieties TS3R (12 percent), BSMR-736 (3 percent), GRG-833 (22 percent) and GC-11-39 (10 percent) were recorded as very low response types.

The varieties Maruti (28 percent), WRP-1 (44 percent) and ICPL-87 (26 percent) were recorded as low response types. The variety Bannur local (66 percent) was recorded as medium response. These tests were similar to observations in soya bean (Roopa *et. al*., 2008) and tomato (Vishwanath *et. al.*, 2013).

Seedling growth response to 2-4D

The percent decrease in coleoptile length during seedling response to 2-4D was recorded as susceptible (< 60 per cent) and highly susceptible (> 60 per cent). Among the ten varieties, only one variety TS3R (66 percent decrease) was recorded as highly susceptible.

All the remaining varieties, Maruti (2 percent decrease), GRG-811(21 percent decrease), Asha (31 percent decrease)), BSMR-736 (43 percent decrease), GRG-833 (11 percent decrease), GC-11-39 (5 percent decrease), WRP-1 (36 percent decrease), ICPL-87 (8 percent decrease) and Bannur local (2 percent) were recorded as susceptible.

The mean, range, standard deviation and co-efficient of variation for three chemical tests were analysed. The co-efficient of variation for the increase in coleoptile length over the control ranged from 0.20 (Kinetin test) to 0.37 (GA_3 test). These tests were similar to observations in ground nut (Sambasivarao *et al.*, 2002) cotton (Ponnuswamy *et al.*, 2003; Kirankumar Reddy, 2004) and safflower Biradarpatil *et al.*, (2006).

Descriptors in chemical tests based on the chemical tests

Among the ten varieties, Maruti can be distinguishable based on low absorbance of seed solution in Peroxidise Test (based on the absorbance). TS3R can be distinguishable based on less susceptibility of seedling to 2-4D. GRG-811 can be distinguishable based on seed colour change to dark brown in sodium hydroxide (NaOH) test. Asha can be distinguishable based on less response of seedling to seedling growth response to GA_3. BSMR-736 can be distinguishable based on highest peak luminance in Ferrous Sulphate test ($FeSO_4$). GRG-833 can be distinguishable based on highest absorbance of seed solution in Peroxidise Test (based on the absorbance). GC-11-39 can be distinguishable based on low response of seedling to seedling growth response to GA_3. WRP-1 can be distinguishable based on seed colour change to light brown in sodium hydroxide (NaOH) test. ICPL-87 can be distinguishable based on seed colour change to saddle brown in sodium hydroxide (NaOH) test and Bannur local can be distinguishable based on medium response of Seedling growth response to Kinetin**.** A list of descriptors was developed for all the test used in the present investigation were presented in Table 4. Based on the results of present study, it is clear that genetic purity testing using chemical methods were more efficient and faster compared to field grow out test. These tests can differentiate pigeon pea varieties with greater precision compared to field grow out test based on morphological characters. Despite of this, there are certain issues like standardization of seed sample size for chemical methods and guidelines for use of these tests in genetic purity testing were not refined or standardized as a legal tender. Hence these methods should be refined to adapt in to the routine seed testing and variety identification in the new IPR regime.

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