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Varietal Identification Based on Chemical Methods in Different Varieties of Indian Mustard (*Brassica juncea* (L.) Czern. & Coss.)

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A B S T R A C T

The study was conducted during 2014-15 and 2015-16 at the laboratories of the Department of Seed Science & Technology, CCS HAU, Hisar, to distinguish twenty Indian mustard varieties/genotypes based on chemical tests (phenol, modified phenol, KOH, NaOH, Peroxidase and 2, 4-D Auxin). Phenol test grouped variety into three distinct groups *viz*., dark reddish brown (12 varieties), dark grey (6 varieties) and Dark red (2 varieties). With the help of modified phenol test these varieties were further sub grouped. KOH test grouped varieties into three distinct groups *i.e.*, dark brown (6 varieties), brown (5 varieties) and light brown (9 varieties). These tests clearly differentiated the varieties of one group to that of another groups on the basis of seed coat colour. NaOH test was not reliable for the differentiation of these varieties because all these varieties showed dark brown colour after treating with NaOH solution. Peroxidase test categorized varieties into three groups viz., high (9 varieties), medium (8 varieties), and (3 varieties) while 2, 4-D Auxin test grouped the varieties into three categories viz., tolerant (4 varieties), susceptible (8 varieties) and highly susceptible (8 varieties).

Introduction

In India crop specific large number of crop improvement programmes are running and with the result of this a large number of varieties are being developed.

Thus varietal identification becomes an essential issue to maintain the genetic purity and identity of each variety. Indian musturd (*Brassica juncea*) belongs to the Cruciferae (Brassicaceae) family. In India, the *Brassica* oilseed is collectively referred to as rapeseed-mustard, which is the most important *Rabi* oilseed crop and occupies an important position in the rain fed agriculture of our country.

The aspect of Distinctness, Uniformity and Stability (DUS) is fundamental for characterization of varieties. Accurate identification of varieties is not only a pre requisite for DUS testing, but is critical for the production of quality seed also. Maintenance of genetic purity of varieties is of primary importance for preventing varietal deterioration during successive regeneration cycles and for ensuring varietal performance

at an expected level. Laboratory tests have several additional benefits for varietal identification. These chemical tests are very quick, easy to do, reproducible and can be conducted throughout the year under controlled conditions.

Some of the popular chemical tests used in Indian mustard for varietal characterization are phenol test, modified phenol test ($CuSO_4$ and Na_2CO_3), sodium hydroxide (NaOH) test, peroxidase test, potassium hydroxide (KOH) test and 2, 4-D auxin test. The chemical tests reveal differences of colour among the seeds. Study of phenotypic characters along with chemical and biochemical techniques have additional benefits for producing more authentic result. In these chemical tests, the chemical agents react with the seed and help in varietal identification.

Materials and Methods

The freshly harvested Seed of all the twenty Indian mustard varieties were used for varietal identification. The experiment was conducted at the laboratories of Department of Seed Science and Technology during the period of 2014-15 and 2015-16. The list of varieties and their source is given below:

Phenol test

The Standardized phenol test for varietal purity testing as suggested by walls (1965) was followed. The procedure consisted of soaking the seed in water for 16 h under ambient condition and then 50 seeds in 15 cm petridishes in two layers of filter soaked in 1% phenol solution in three replications. The seeds were placed on filter paper with hilum region on the down side. The petridishes were immediately covered. A final observation was made after 6 h. The following three distinct phenol colour reaction group were made dark reddish brown, dark grey and dark red.

Modified phenol test

Modified phenol test was followed as described by Banerjee and Chandra (1977). 50 seeds were soaked in 0.4 per cent solution of $CuSO_4$ for adding Cu^{++} ions and another set in 0.6 per cent Na_2CO_3 for adding Na+ ions for 4 h. Then the seeds were placed in 2 per cent phenol solution after removing from the $CuSO_4$ and Na_2CO_3 solution overnight. Based on the colour development in both the tests groups were made and classified in to three groups dark brown, brown and reddish brown colour in $CuSO_4$ soaked seed, were classified in to three groups brown, dark brown and strong brown for Na_2CO_3 soaked seeds.

Potassium Hydroxide (KOH) test

Hundred seeds in three replications were soaked in five per cent KOH solution for two h at room temperature. Changes in colour of the seeds were observed after one h. Based on the colour intensity of the seed, the genotypes were classified into three group's *viz.,* dark brown, reddish brown and light brown (Agrawal and Pawar, 1990).

Sodium Hydroxide (NaOH) test

Hundred seeds in three replications were soaked in five per cent NaOH solution for one h at room temperature. Changes in colour of the seeds were observed after one h. Based on the colour intensity of the seed, the genotypes were classified into three group's *viz.,* Dark brown, Light brown and Brown.

Peroxidase test

Under this test, 60 seed were soaked in water for 24 h after that 15 seeds were incubated in 2.5 ml of guaiacol solution (0.05%) for 20 minutes. 2ml of guaiacol was taken out and 0.2 ml of H_2O_2 (0.1%) was added. In this reddish brown coloured appeared which was

quantified by DU 64 spectrophotometer at 480 nm. The reading was taken after two minutes of adding the H_2O_2 in guaiacol. The whole test was carried out at 25^0C on basis of transmittance per cent.

2, 4-D Auxin test

The effect of 2, 4-D test at 5ppm concentration on seedling was studied. For this 20 seeds were grown by placing them on two layers of filter paper moistened in 2, 4-D auxin in the petridishes. The petridishes were kept in germinator at 25^0C. Seedlings were evaluated after 7days and ten seedling were selected at random and seedling length (shoot length + root length) was measured in Centimetres.

Results and Discussion

In the present experiment, twenty Indian mustard varieties were characterized on the basis of different chemical tests (Table 1).

Phenol test and modified phenol test

Phenol test showed great variation among varieties into light brown, brown and dark brown group (Table 1). This test is highly specific for varieties. Phenol reaction is monogenically controlled response, which is present in seed coat (Joshi and Banerjee, 1970).

An enzyme polyphenol oxidase (PPO) is responsible for the oxidation of externally supplied phenol into quinones and their further polymerization yield melanin like pigments which have resulted in development of brown colouration in seeds. So seed coat colour development in Indian mustard seed coat by phenol colour reaction is detected and varieties were differentiated as dark reddish brown, dark grey, dark red. Out of 20 varieties twelve varieties viz., RH30, RH8812, RH8113, RH0749, RH0119, RH9801, RH819, NRCDR601, DRMRIJ31, NPJ112, RGN73 and Kranti showed dark reddish brown, six *i.e.*, RB50, RH0406, RH9304, Varuna, NRCDR02, and NRCHB101showed dark grey and rest two varieties RB24 and RH781 had dark red colouration.

The results are in conformity with findings of Jawaharlal (1994), Ezhilkumar (1999), Ponnuswamy *et al.*, (2003) and Reddy (2004) in cotton and Rana (2006) in cluster bean.

Further modified phenol ($CuSO_4$ 0.4% and NA_2CO_3 0.6% as a inhibitor) is used for better result and sub grouping of the varieties in different groups.

Both phenol and modified phenol is emerging as a stable and uniform method for grouping of Indian mustard varieties. Similar observations were recorded by Gupta *et al.*, (2007) in wheat and Anitalakshmi *et al.*, (2014) in rice.

Potassium hydroxide (KOH) test

On the basis of colour reaction with potassium hydroxide solution, the Indian mustard varieties were grouped into dark brown, brown and light brown (Table 1).

Among the 20 varieties, six varieties, RH8113, RH0406, RH819, NRCDR02, NRCHB601 and NPJ112 showed dark brown colour, five varieties, RH0749, RB24, RH9304, NRCHB101 and Varuna showed brown colour and nine, varieties, RH30, RH8112, RB50, RH0119, RH9801, RH781, DRMRIJ31, RGN73 and Kranti had light brown colouration.

Same type of results was revealed by Sivakumar (2002) in cluster bean, Sambasiva Rao *et al.*, (2002) in groundnut and Biradarpatil *et al.*, (2006) in safflower.

Table.1 Categorization of Indian mustard varieties on the basis of Chemical approach (pooled data)

Variety	Phenol test (1%) after 6 hours	Modified phenol ($CuSo_4$ 0.4%) after 4 hours.	Modified phenol (NA_2CO_3 0.6%) after 4 hours	Potassium hydroxide (0.5%) after 4 hours	Sodium hydroxide (0.5%) After 4 hour
RH30	Dark reddish brown	Brown	Brown	Light brown	Dark brown
RH8812	Dark reddish brown	Dark brown	Dark brown	Light brown	Dark brown
RH8113	Dark reddish brown	Dark brown	Dark brown	Dark brown	Dark brown
RH0749	Dark reddish brown	Brown	Brown	Brown	Dark brown
RB50	Dark grey	Brown	Brown	Light brown	Dark brown
RH0406	Dark grey	Dark brown	Dark brown	Dark brown	Dark brown
RB24	Dark red	Brown	Brown	Brown	Dark brown
RH0119	Dark reddish brown	Reddish brown	Light brown	Light brown	Dark brown
RH9304	Dark grey	Brown	Brown	Brown	Dark brown
RH9801	Dark reddish brown	Reddish brown	Light brown	Light brown	Dark brown
RH819	Dark reddish brown	Strong brown	Strong brown	Dark brown	Dark brown
RH781	Dark red	Reddish brown	Light brown	Light brown	Dark brown
Varuna	Dark grey	Brown	Brown	Brown	Dark brown
NRCDR02	Dark grey	Dark brown	Dark brown	Dark brown	Dark brown
NRCDR601	Dark reddish brown	Strong brown	Strong brown	Dark brown	Dark brown
NRCHB101	Dark grey	Dark brown	Dark brown	Brown	Dark brown
DRMRIJ31	Dark reddish brown	Brown	Brown	Light brown	Dark brown
NPJ112	Dark reddish brown	Dark brown	Dark brown	Dark brown	Dark brown
RGN73	Dark reddish brown	Reddish brown	Light brown	Light brown	Dark brown
Kranti	Dark reddish brown	Strong brown	Strong brown	Light brown	Dark brown

Variety	Peroxidase test (%)	Groups	2,4-D auxin test (cm)	Groups
RH30	39.00(38.63)	Low	1.4	High susceptible
RH8812	51.00(45.56)	High	1.8	Susceptible
RH8113	38.00(38.03)	Low	1.6	Susceptible
RH0749	48.00(43.84)	Medium	2.6	Tolerant
RB50	69.00(56.18)	High	1.3	High susceptible
RH0406	38.00(38.04)	Low	2.3	Tolerant
RB24	41.00(39.80)	Medium	1.4	High susceptible
RH0119	69.50(56.46)	High	1.5	Susceptible
RH9304	47.00(43.26)	Medium	1.7	Susceptible
RH9801	48.00(43.84)	Medium	1.3	High susceptible
RH819	61.00(51.34)	High	2.5	Tolerant
RH781	51.50(45.84)	High	2.4	Tolerant
Varuna	55.00(47.85)	High	1.4	High susceptible
NRCDR02	61.00(51.35)	High	1.7	Susceptible
NRCDR601	43.00(40.96)	Medium	1.3	High susceptible
NRCHB101	62.00(51.93)	High	1.4	High susceptible
DRMRIJ31	56.00(48.43)	High	1.8	Susceptible
NPJ112	41.00(39.79)	Medium	1.6	Susceptible
RGN73	42.50(40.67)	Medium	1.5	Susceptible
Kranti	44.50(41.83)	Medium	1.4	High susceptible
Mean	50.30	-	1.7	-
Range	38-69.50	High- 51-69.50 Medium-41-48 Low- 38-39	1.3 to 2.6	Tolerant-2.3-2.6 Susceptable-1.5-1.8 High susceptible-1.3-1.4
SE (m)	1.30 (0.77)		0.1	
C.D.	3.72 (2.20)		0.3	

Source of Seed: Seed of 20 Indian mustard varieties

Variety	Source	Variety	Source	Variety	Source
RH30	CCSHAU, Hisar	RH0119	CCSHAU, Hisar	NRCDR601	DRMR Bharatpur
RH8812	CCSHAU, Hisar	RH9304	CCSHAU, Hisar	NRCHB101	DRMR Bharatpur
RH8113	CCSHAU, Hisar	RH9801	CCSHAU, Hisar	DRMRIJ31	DRMR Bharatpur
RH0749	CCSHAU, Hisar	RH819	CCSHAU, Hisar	NPJ112	IARI, New Delhi
RB50	CCSHAU, Hisar	RH781	CCSHAU, Hisar	RGN73	RAU, Sriganganagar
RH0406	CCSHAU, Hisar	Varuna	CSAUA&T Kanpur	Kranti	GBPUA&T, Pantnagar
RB24	CCSHAU, Hisar	NRCDR02	DRMR Bharatpur		

Sodium hydroxide (NaOH) test

The colour reaction with sodium hydroxide solution grouped the Indian mustard varieties into dark brown colour (Table 1). The seeds soaked in NaOH solution reacted variedly based on the chemical compositions of the seed, which is determined by the genetic makeup of the varieties and hence variation in colour was observed. Among all varieties (RH30, RH8812, RH8113, RH0749, RB50, RH0406, RB24, RH0119, RH9304, RH9801, RH819, RH781, Varuna, NRCDR02, NRCDR601, NRCHB101, DRMRIJ31, NPJ112, RGN73 and Kranti) showed dark brown colouration. Similar results were reported by Biradarpatil *et al.*, (2006) in safflower, Singh (2001) in chickpea and Ali (2005) in soyabean.

Peroxidase test

The general mean value for peroxidase was 50.30 (50%) with a range varied from 38-69.50.Three groups were made on the basis of peroxidase activity (Table 1). Nine *i.e.*, RH0119 (69.50), RB50 (69.00), NRCHB101 (62.00), RH819 (61.00), NRCDR02 (61.00), DRMRIJ31 (56.00), Varuna (55.00), RH781 (51.50) and RH8812 (51.00), eight *i.e.*, RH0749 (48.00), RH9801 (48.00), RH9304 (47.00), Kranti (44.50), NRCDR601 (43.00), RGN73 (42.50), RB24 (41.00) and NPJ112 (41.00) and three varieties *i.e.*, RH30 (39.00), RH8813 (38.00) and RH0406 (38.00) had high, medium and low peroxidase activities respectively (Table 1).

2, 4-D auxin test

Variation in seedling growth response to 2, 4-D was due to inhibition of seedling growth and other activity. Significant differences were observed among the varieties with respect to 2, 4-D application and classified into tolerant, susceptible and highly susceptible (Table 1). Out of 20 varieties four *i.e.*, RH0749 (2.6), RH819 (2.5), RH781 (2.4) and RH0406 (2.3) were tolerant, eight *i.e.*, RH8812 (1.8), DRMRIJ31 (1.8), RH9304 (1.7), NRCDR02 (1.7), RH8113 (1.6), NPJ112 (1.6), RH0119 (1.5), and RGN73 (1.5) were susceptible and eight *i.e.*, RH30 (1.4), RB24 (1.4), Varuna (1.4), NRCHB101 (1.4), Kranti (1.4), RB50 (1.3), RH9801 (1.3) and NRCDR601 (1.3) were highly susceptible. The differences in seedling growth reduction among the varieties might be due to differences in ethylene production because of application of 2, 4-D. Similar findings were reported by Biradarpatil (2006) in

safflower, Shivakumar. (2000) in rapeseed and mustard and Sambasivarao (2002) in groundnut.

References

Agrawal, R.L., Pawar, A. 1990. Identification of soybean varieties based on seed and seedling characteristics. *Seed Research*, 18:77-81.

Ali, M.N. 2005. Cultivar identification in soybean *M.Sc. Thesis*, CCS HAU, Hisar.

Anitalakshmi, V., Gowda, R, C.S., Sathisha and Prasad, Rajendra. 2014. Varietal response to various chemical tests for their characterization in rice. *Indian Journal of Plant Sciences*, 3 (2): *177- 179.*

Banerjee, S.K. and Chandra, S. 1977. Modified phenot test for the varietal identification of wheat seed, *Seed Science & Technology*. 5: 53-60.

Biradarpatil, N.K., sangeeta, Macha, Motagi, B.N., Vijaykumar, A.G. and Hanchinal, R.R. 2006. Characterization of safflower varieties through chemical tests. *Abstract XII National Seed Seminar*, 24-26 February, ANGRAU Hyderabad, pp. 168.

Ezhilkumar, S. 1999. Studies on varietal identification in hybrids, parents and varieties in cotton (*Gossypium* spp.). *M. Sc. (Agri.) Thesis*, Tamil Nadu Agric. Univ., Coimbatore (India).

Gupta, Namarta, Joshi, Monika, A., Sarao, Navraj K. and Sharma, R.C. 2007. Utility of phenol test in varietal characterization. *Crop Improvement* 34 (1): 77-81.

Jawaharlal. 1994. Studies on varietal characterization in inbred, hybrids and varieties of cotton (*Gossypium* spp.) through physical, physiological and biochemical methods. *M.Sc. (Agri.) Thesis*, Tamil Nadu Agric. Univ.,

Joshi, M.G. and Banerjee, S.K. 1970. Genetics of phenol colour reaction in emmer wheat. *Proceeding International. Seed Association.* 35: 207.

Ponnuswamy, A.S., Bhaskaran, M. and Sastri, G. 2003, Variety characterization in cotton by physical, chemical and bio-chemical methods. *Training Mannual, Variety characterization by image analysis and electrophoresis*, pp. 106-120.

Rana, R., 2006. Characterization and vigour assessment of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] M.Sc. Thesis, CCS HAU, Hisar.

Reddy, K. 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).

Sambasivarao, P. Muralimohanreddy, B., Bharathi, M. and Bayyapu Reddy, K., 2002. Varietal identification in groundnut (*Arachis hypogaea* L.) by chemical tests and electrophoresis of total soluble seed proteins. *Seed Tech News*, 32(1): 93.

Shivakumar, 2000. Characterization of rapeseed and mustard (*Brassica* spp.) cultivars using field and laboratory techniques. *Seed Tech News*, 31 (1):31.

Singh, R., 2001. Characterization of chickpea cultivars by field and laboratory techniques. Ph.D. Thesis. CCS Haryana Agricultural University, Hisar.

Sivakumar, S., 2002. Characterization of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. M.Sc. Thesis, CCS HAU, Hisar.

Walls, F.W., 1965. A standardized phenol method for testing wheat for varietal purity. Handbook on seed testing, AOSA, Contribution No. 28.

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