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## Chemical methods in varietal identification based on different varieties of Indian mustard (*Brassica juncea* (L.) Czern. & Coss.)

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### Abstract

The study was conducted during 2018-19 and 2019-20 at the laboratories of the Department of Seed Science & Technology, CSAU, Kanpur, to distinguish twenty One Indian mustard varieties/genotypes based on phenol & KOH chemical Tests phenol. On the basis of phenol test varieties/genotypes were categorized into three distinct groups viz., dark reddish brown (12 varieties), dark grey (7 varieties) and Dark red (2 varieties). KOH test grouped varieties/genotypes into three distinct groups i.e., dark brown (6 varieties), brown (6 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.

**Keywords:** Chemical, varietal, identification, mustard, *Brassica juncea* L.

### 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 mustard (*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/genotypes. Accurate identification of varieties/genotypes 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. The study was conducted during 2018-19 and 2019-20 at the laboratories of the Department of Seed Science & Technology, CSAU, Kanpur, to distinguish twenty One Indian mustard varieties/genotypes based on phenol & KOH chemical tests. Phenol test all varieties/genotypes categorized into three distinct groups viz., dark reddish brown (12 varieties), dark grey (7 varieties) and Dark red (2 varieties). This test clearly differentiated the 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 & KOH tests. 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 One Indian mustard varieties/genotypes used for varietal identification. The experiment was conducted at the laboratories of Department of Seed Science and Technology during the period of 2018-19 and 2019-20. procured from DUS Test unit CSAUA& Tech. Kanpur.

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.

Potassium Hydroxide (KOH) test Hundred seeds in three replications were soaked in 5% 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) [1]

## Results and Discussion

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

### Phenol test

Phenol test exhibited great variation among varieties/genotypes 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) [11]. 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 21 varieties twelve varieties viz., RH30, PM-27, PM-25, Potni, Jagay Mani-1, Purni, Tejan, Lotni Gol, Dangi, Araak, Giridhar and Tanru-M showed dark reddish brown, seven i.e., Anmol, Pusa Bold, Pomi, Bathani, Bullet, Kali and Boori showed dark grey and rest two varieties Sita Ram Rai and Prerna had dark red colouration.

The results are in conformity with findings of Jawaharlal (1994) [10], Ezhilkumar (1999) [8], Ponnuswamy *et al.*, (2003)

[12] and Reddy (2004) [14] in cotton and Rana (2006) [13] in cluster bean.

Phenol is emerging as a stable and uniform method for grouping of Indian mustard varieties. Similar observations were recorded by Gupta *et al.*, (2007) [9] in wheat and Anitalakshmi *et al.*, (2014) [3] in rice. Combination of different chemical tests B. Vijaya lakshmi & D. Vijay 2009- 23 genotypes in rice tested for Phenol, Modified Phenol, NaOH, FeSo<sub>4</sub> and KOH tests. Nagendra *et al.*, (2020) [7] Identification and characterization of crop varieties are crucial for ensuring the genetic purity of seeds.

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 21 varieties/genotypes, six varieties, Anmol, PM-27, Pusa Bold, Potni, Purni and Araak showed dark brown colour, and six, varieties, Bathani, Bullet, Sitaram Rai, Kali, Boori and Giridhar had brown colouration. nine varieties/genotypes, RH-30, PM-27, Pomi, Jagay mani-1, Tejan, Lotni gol, Dangi, Prerna and Tanru-M showed Light brown colour. Same type of results was revealed by Sivakumar (2002) [18] in cluster bean, Sambasiva Rao *et al.*, (2002) [15] in groundnut and Biradarpatil *et al.*, (2006) [5] in safflower. Same type of results were revealed by Sivakumar (2002) [18] in cluster bean, Sambasiva Rao *et al.*, (2002) [15] in groundnut and Biradarpatil *et al.*, (2006) [5] in safflower. P. Raju, S.B. Patil, S.N. Vasudevan, Mohammad Ibrahim and R.C. Mathad *et al.*, (2017) Investigations were carried out to study the varietal characterization of paddy hybrid and its parents through chemical tests and using iamge analyzer. In case of FeSO<sub>4</sub>, KOH and NaOH tests both parents and hybrid showed similar colour reaction. PC Raut, KA Gawali and AV Nagmote *et al.*, (2019) The wheat varieties viz., AKW-381, AKW-1071, AKAW-3722, AKAW-2997.16, AKAW-4627, PDKV WASHIM, AKAW-4210-6 were grouped on the basis of chemical test Peroxidase test, NaOH (0.5%), KOH (0.5%), GA3 (100 ppm) and 2-4 D (5 ppm). E. Sudeep Kumar, S. N. Vasudevan, N. M. Shakuntala, S. R. Doddagoudar, B. G. Masthan Reddy and K. Mahantashivayogayya *et al.*, (2021).

**Table 1:** Categorization of Indian Mustard Varieties/Genotypes on the Basis of Chemical Approach (pooled data)

Variety/Genotypes	Phenol Test (1%) after 6 hours	KOH Test (Potassium Hydroxide Test) 0.5% After 4 hours
RH30	Dark reddish brown	Light Brown
ANMOL	Dark grey	Dark Brown
PM-27	Dark reddish brown	Light Brown
PM-25	Dark reddish brown	Dark Brown
PUSA BOLD	Dark grey	Dark Brown
POMI	Dark grey	Light Brown
BATHANI	Dark grey	Brown
POTNI	Dark reddish brown	Dark Brown
JAGAY MANI-1	Dark reddish brown	Light Brown
PURNI	Dark reddish brown	Dark Brown
BULLET	Dark grey	Brown
SITARAM RAI	Dark red	Brown
TEJAN	Dark reddish brown	Light Brown
LOTNI GOL	Dark reddish brown	Light Brown
KALI	Dark grey	Brown
DANGI	Dark reddish brown	Light Brown
ARAAK	Dark reddish brown	Dark Brown
PRERNA	Dark red	Light Brown
BOORI	Dark grey	Brown
GIRIDHAR	Dark reddish brown	Light Brown
TANRU-M	Dark reddish brown	Light Brown

**Table 2:** Seed Source of 21 Different Indian mustard Varieties/Genotypes.

Variety	Source	Variety	Source	Variety	Source
RH30	Seed was procured from DUS Test unit, CSAUAT, Kanpur	JAGAY MANI-1	Seed was procured from DUS Test unit, CSAUAT, Kanpur	KALI	Seed was procured from DUS Test unit, CSAUAT, Kanpur
PUSA MUSTARD-27	Seed was procured from DUS Test unit, CSAUAT, Kanpur	PURNI	Seed was procured from DUS Test unit, CSAUAT, Kanpur	DANGI	Seed was procured from DUS Test unit, CSAUAT, Kanpur
PUSA MUSTARD-25	Seed was procured from DUS Test unit, CSAUAT, Kanpur	ANMOL	Seed was procured from DUS Test unit, CSAUAT, Kanpur	ARAK	Seed was procured from DUS Test unit, CSAUAT, Kanpur
PUSA BOLD	Seed was procured from DUS Test unit, CSAUAT, Kanpur	BULLET	Seed was procured from DUS Test unit, CSAUAT, Kanpur	PRERNA	Seed was procured from DUS Test unit, CSAUAT, Kanpur
POMI	Seed was procured from DUS Test unit, CSAUAT, Kanpur	SITARAM RAI	Seed was procured from DUS Test unit, CSAUAT, Kanpur	BOORI	Seed was procured from DUS Test unit, CSAUAT, Kanpur
BATHNI	Seed was procured from DUS Test unit, CSAUAT, Kanpur	TEJAN	Seed was procured from DUS Test unit, CSAUAT, Kanpur	GIRIDHAR	Seed was procured from DUS Test unit, CSAUAT, Kanpur
POTNI	Seed was procured from DUS Test unit, CSAUAT, Kanpur	LOTNI GOL	Seed was procured from DUS Test unit, CSAUAT, Kanpur	TANRU-M	Seed was procured from DUS Test unit, CSAUAT, Kanpur

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