

Seed Characters Based Identification Key for Important Varieties of Indian Mustard (*Brassica juncea* L. Czern & Coss)

HIMANSHU RAI^{1*}, PK SINGH² SS JAKHAR¹ AND OS DAHIYA

¹Department of Seed Science & Technology, CCS Haryana Agriculture University, Hisar, Haryana, India

²ICAR- Indian Institute of Sugarcane Research, Lucknow-226002, Uttar Pradesh, India

*himanshurai.ag@gmail.com

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Indian mustard (*Brassica juncea* L. Czern & Coss), belonging to Brassicaceae family, is an important oil seed crop, grown across the world for its rich source of oil and potential meal. In India, it is cultivated in the states of Assam, Bihar, Gujarat, Haryana, H.P., J&K, M.P., Odisha, Punjab, Rajasthan, Uttar Pradesh and West Bengal as Rabi crop. At present large number of Indian mustard varieties are available for commercial cultivation. The present trend of continuous release of new Indian mustard varieties from central and state varietal release committees has warranted to developed suitable techniques for varietal identification at the Seed Testing Laboratory level particularly when the seeds are submitted for the purity testing. Maintenance of genetic purity of varieties is of primary importance for preventing varietal deterioration during successive regeneration cycles and for ensuring varietal performance at the expected level. The purity of seed material can be maintained by combining the morphological and chemical test based characters of the seeds. The chemical tests reveal differences among the varieties as reported by various workers such as [1-4] etc. The results of these tests are usually distinct and can be easily interpreted for identification of the varieties. Therefore, an investigation was carried out to study the various seed characters and chemical testbased characters of Indian mustard varieties for effective utilization in varietal characterization during purity testing.

The experiment was conducted in the Seed Testing Laboratory of the Department of Seed Science & Technology, CCS Haryana Agriculture University, Hisar, (Haryana) during 2014-16. The study material comprised of twenty Indian mustard varieties viz., RH30, RH8812, RH8113, RH0749, Rb50, RH0406, RB24, RH0119, RH9304, RH9801, RH819, RH781, Varuna, NRCDR02,

NRCDR601, NRCHB101, DRMRIJ31, NPJ112, RGN73 and Kranti released for commercial cultivation from State and Central Variety Release Committees. The characters observed during the study were seed colour, test weight, oil content and chemical tests viz., phenol test, modified phenol test, potassium hydroxide test, Peroxidase activity test and 2, 4-D test. The procedures for recording observations are as under:

Seed colour: Visual group observations were recorded on the seed lots and grouped into Dark Brown, Brown and Reddish Brown.

Seed Size (Test weight): Thousand seed weight (in g). The groups were made as Small, Medium and Bold.

Oil content: The oil content of each seed lot was estimated by using Nuclear Magnetic Resonance (NMR) MK111 Newport Analyser equipped with 2.0 ml sample viol assembly. The varieties were grouped into Low and Medium oil content groups.

Phenol test: Soaking of the seeds in water for 16 hr. under ambient conditions, followed by transferring 50 seeds in 15 cm petri-dishes between two layers of filter paper soaked in 1% phenol solution. Care was taken to keep the hilum region on the lower side. The final observation on the colour was marked as Dark Brown, Dark Red and Dark Grey [5].

Modified phenol test: Seeds were soaked for 4 hr in 0.40% solution of CuSO₄ for adding Cu⁺⁺ ions and another set in 0.60% Na₂CO₃ for adding Na⁺ ions. Then the seeds were overnight placed in 2.0% phenol solution for colour development. Based on the colour they were marked as Dark Brown, Brown, Reddish Brown, Strong Brown [6].

Potassium hydroxide test: Seeds were soaked in 5.0% KOH solutions for 2 hr. at room temperature and marked

for colour development into three groups viz. Dark Brown, Brown and Light Brown [7].

Peroxidase activity test: Seeds were soaked in water for 24 hr. followed by incubating them in 0.05% of Guaiacol solution for 20 minutes and then in 0.10% H₂O₂. The Reddish Brown coloured was quantified by D-64 spectrophotometer at 480nm [8].

2, 4-D auxin test: Seeds were grown by placing them on two layers of filter paper moistened in 5.0 ppm solution of 2, 4-D auxin in the petridishes at 25 °C. Seedlings

were evaluated after 7 days and 10 seedlings were selected randomly for measuring length (in cm).

Varietal identification at the time of seed testing stage requires precise tests conducted in a series to obtain conclusive results. The results obtained in the series of tests conducted on the seeds of different varieties can be used to make an 'identification key' for the concerned varieties, which is quick, easy and reproducible [7&9].

The seeds of the twenty Indian mustard varieties were subjected the series of tests under laboratory conditions to obtain the results as presented in table 1.

Table 1. Characterization of Indian mustard varieties based on seed characters

Variety	Phenol test	Modified Phenol test	KOH test test	Peroxidase activity test	2,4-D auxin	Seed colour	Seed: Size (Weight of 1000 seeds)	Oil content
RH30	Dark brown	Brown	Light brown	Low	High susceptible	Dark Brown	Medium (5.95)	39.37 Medium
RH8812	Dark brown	Dark brown	Light brown	High	Susceptible	Dark Brown	Medium (5.12)	38.88 Medium
RH8113	Dark brown	Dark brown	Dark brown	Low	Susceptible	Dark Brown	Medium (5.29)	38.81 Medium
RH0749	Dark brown	Brown	Brown	Medium	Tolerant	Dark Brown	Medium (5.41)	39.08 Medium
RB50	Dark grey	Brown	Light brown	High	High susceptible	Dark Brown	Medium (5.30)	38.26 Medium
RH0406	Dark grey	Dark brown	Dark brown	Low	Tolerant	Brown	Bold (6.01)	38.77 Medium
RB24	Dark red	Brown	Brown	Medium	High susceptible	Dark Brown	Medium (5.74)	38.22 Medium
RH0119	Dark brown	Reddish brown	Light brown	High	Susceptible	Dark Brown	Medium (5.87)	38.10 Medium
RH9304	Dark grey	Brown	Brown	Medium	Susceptible	Reddish Brown	Medium (5.89)	38.20 Medium
RH9801	Dark brown	Reddish brown	Light brown	Medium	High susceptible	Dark Brown	Medium (5.95)	38.40 Medium
RH819	Dark brown	Strong brown	Dark brown	High	Tolerant	Brown	Medium (5.82)	38.34 Medium
RH781	Dark red	Reddish brown	Light brown	High	Tolerant	Dark Brown	Medium (5.73)	37.69 Low
Varuna	Dark grey	Brown	Brown	High	High susceptible	Brown	Medium (5.58)	38.22 Medium
NRCDR02	Dark grey	Dark brown	Dark brown	High	Susceptible	Dark Brown	Medium (5.67)	38.20 Medium
NRCDR601	Dark brown	Strong brown	Dark brown	Medium	High susceptible	Dark Brown	Medium (5.84)	37.92 Low
NRCHB101	Dark grey	Dark brown	Brown	High	High susceptible	Brown	Small (4.42)	37.75 Low
DRMRIJ31	Dark brown	Brown	Light brown	High	Susceptible	Brown	Medium (5.98)	38.30 Medium
NPJ112	Dark brown	Dark brown	Dark brown	Medium	Susceptible	Dark Brown	Small (4.84)	38.02 Medium
RGN73	Dark brown	Reddish brown	Light brown	Medium	Susceptible	Dark Brown	Medium (5.72)	38.28 Medium
Kranti	Dark brown	Strong brown	Light brown	Medium	High susceptible	Brown	Small (4.77)	38.28 Medium

Based on the variation in the seed characters, both morphological and chemical treatment based response, an effort was made to group the varieties to distinguish them from each other in a systematic way, which ultimately resulted in the Key to Identification. The key is based on the above Laboratory test's based characters in the following sequence:

Seed Colour → Seed Size (Test Weight) → Oil Content → Phenol Test → Modified Phenol Test → Potassium hydroxide Test → Peroxidase Test → 2,4-D auxin Test.

A. Seed Colour: DARK BROWN

[RH30, RH8812, RH8113, RH0749, RB50, RB24, RH0119, RH9801, RH781, NRCDR02, NRCDR601, NPJ112, RGN73]

1. Seed Size (Test Weight): SMALL

[NPJ112]

2. Seed Size (Test Weight): MEDIUM

[RH30, RH8812, RH8113, RH0749, RB50, RB24, RH0119, RH9801, RH781, NRCDR02, NRCDR601, RGN73]

i. Oil Content: LOW

[RH781, NRCDR601]

a. Phenol Test: DARK BROWN

[NRCDR601]

b. Phenol Test: DARK RED

[RH781]

c. Phenol Test: DARK GREY

[Nil]

ii. Oil Content: MEDIUM

[RH30, RH8812, RH8113, RH0749, RB50, RB24, RH0119, RH9801, NRCDR02, RGN73]

a. Phenol Test: DARK BROWN

[RH30, RH8812, RH8113, RH0749, RH0119, RH9801, RGN73]

I. Modified Phenol Test: DARK BROWN

[RH8812, RH8113]

KOH Test: DARK BROWN

[RH8113]

KOH Test: LIGHT BROWN

[RH8812]

II. Modified Phenol Test: BROWN

[RH30, RH0749]

KOH Test: BROWN

[RH0749]

KOH Test: LIGHT BROWN

[RH30]

III. Modified Phenol Test: REDDISH BROWN [RH0119, RH9801, RGN73]

KOH Test: LIGHT BROWN [RH0119, RH9801, RGN73]

Peroxidase Test: HIGH

[RH0119]

Peroxidase Test: MEDIUM [RH9801, RGN73]

2,4-D auxin Test: HIGHLY SUSCEPTIBLE

[RH9801]

2,4-D auxin Test: SUSCEPTIBLE

[RGN73]

IV. Modified Phenol Test: STRONG BROWN

[Nil]

b. Phenol Test: DARK RED

[RB24]

c. Phenol Test: DARK GREY

[RB50, NRCDR02]

I. Modified Phenol Test: DARK BROWN

[NRCDR02]

II. Modified Phenol Test: BROWN

[RB50]

3. Seed Size (Test Weight): BOLD Nil

B. Seed Colour: BROWN

[RH0406, RH819, VARUNA, NRCHB101, DRMRIJ31, KRANTI]

1. Seed Size (Test Weight): SMALL

[NRCHB101, KRANTI]

i. Oil Content: LOW

[NRCHB101]

ii. Oil Content: MEDIUM

[KRANTI]

2. Seed Size (Test Weight): MEDIUM

[RH819, VARUNA, DRMRIJ31]

i. Oil Content: LOW

[Nil]

ii. Oil Content: MEDIUM

[RH819, VARUNA, DRMRIJ31]

a. Phenol Test: DARK BROWN

[RH819, DRMRIJ31]

I. Modified Phenol Test: DARK BROWN

[Nil]

II. Modified Phenol Test: BROWN

[DRMRIJ31]

III. Modified Phenol Test: REDDISH BROWN

[Nil]

IV. Modified Phenol Test: STRONG BROWN

[RH819]

b. Phenol Test: DARK RED

[Nil]

c. Phenol Test: DARK GREY

[VARUNA]

3. Seed Size (Test Weight): BOLD

[RH0406]

C. Seed Colour: REDDISH BROWN

[RH9304]

This Identification Key based on differential response of the seeds of varieties towards different laboratory based tests and observations clearly indicate the possibility of varietal identification of Indian mustard varieties in Laboratory also. The protocol can include many more varieties, as several slots are still vacant in the Key. Further, this Key can provide a potent way to save one full crop season which otherwise will be required in field identification of varieties.

Laboratory based observations based on the physical and chemical tests on the seeds of the varieties can be used to structure an Identification Key for these varieties to easily identify them. The present study based on 08 characters was successful in identifying 20 varieties of Indian Mustard in laboratory.

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