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Seed characters based identification key for important varieties of Indian mustard (*Brassica juncea* L. Czern and Coss)

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Abstract
Indian mustard (*Brassica juncea* L. Czern & Coss) is an important economical oilseed crop worldwide. An experiment was carried out at the Department of Seed Science & Technology, CCS Haryana Agriculture University, Hisar, Haryana to prepare an identification key for mustard varieties. It was based on seed characters of twenty Indian mustard varieties release for general cultivation from the various organizations. The characterization of varieties was based on the morphological characters as well as response of chemical seed tests. Mustard varieties were distinguished from each other on the basis of seed colour, test weight, oil content, phenol test, modified phenol test, potassium hydroxide test, peroxidase test and 2, 4-D auxin test. These seed characters based identification key can be an effective tool for maintaining the genetic purity of the varieties during seed certification process.

Keywords: Indian mustard, seed, variety, identification, characterization

Introduction
Indian mustard (*Brassica juncea* L. Czern & Coss) belonging to family *Brassicaceae* is an important economical oilseed crop, grown across the world for its rich source of oil and potential meal. In India, it is mainly cultivated in the states of Assam, Bihar, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Odisha, Punjab, Rajasthan, Uttar Pradesh and West Bengal during *Rabi* season. At present large number of Indian mustard varieties are available for commercial cultivation in our country. 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 seed characteristics and chemical tests reveal differences among the varieties as reported by various workers [1-4]. 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 know the various seed characters and chemical test based characters of Indian mustard varieties for effective utilization in varietal characterization during purity testing.

Materials and Methods
The experiment was conducted in the Seed Testing Laboratory of the Department of Seed Science & Technology, CCS Haryana Agriculture University, Hisar, (Haryana) during 2014 to 2016. The investigation materials 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. These varieties were released from State and Central Variety Release Committees for commercial cultivation of the Indian mustard. The characters observed during the investigation 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 auxin test. The procedures for recording observations were considered 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)
Based on thousand seed weight (in gm), 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 hours 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 and 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 hours 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 480 nm [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 ^{0}C. Seedlings were evaluated after 7 days and 10 seedlings were selected randomly for measuring length (in cm).

Results and Discussions
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 'identification key' for the concerned varieties, which is quick, easy, and reproducible [7, 9]. Distinctness means the variety should be clearly distinct from existing varieties. On the basis of seed size, all the genotypes were categorized into three groups, i.e. small, intermediate and bold type [11]. Uniformity indicates the similarity of characteristics features among the plants of selected variety. Stability means that the variety should produce stable results year after year. Seventy eight genotypes of mustard were grouped by using DUS test traits [10]. 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.

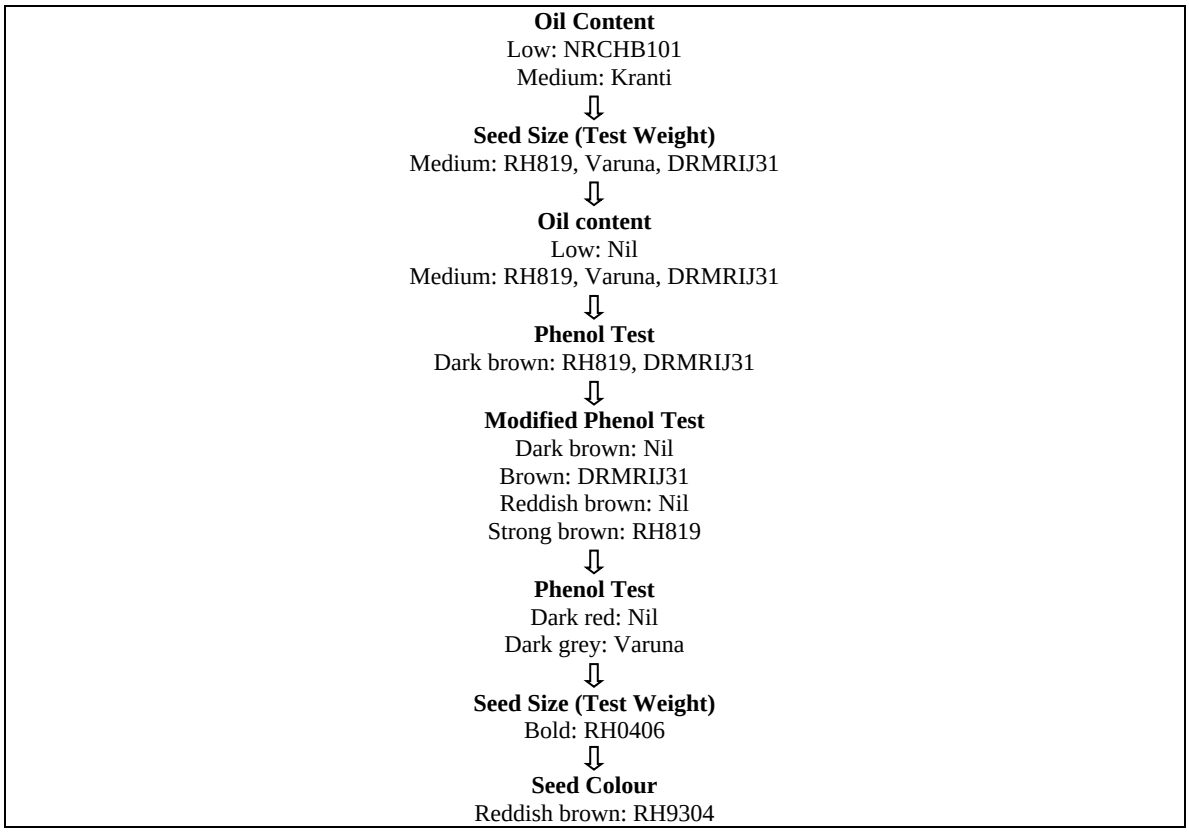
Table 1: Characterization of Indian mustard varieties based on seed characters

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

Based on the variation in the seed characters by morphological and chemical treatments based response, an effort was made to categorize the varieties to distinguish them from each other in a systematic way, which ultimately resulted in the Key for Identification. The key is based on the above morphological and 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.

Seed colour
Dark brown: RH30, RH8812, RH8113, RH0749, RB50, RB24, RH0119, RH9801, RH781, NRCDR02, NRCDR601, NPJ112, RGN73
⇩
Seed Size (Test Weight)
Small: NPJ112
Medium: RH30, RH8812, RH8113, RH0749, RB50, RB24, RH0119, RH9801, RH781, NRCDR02, NRCDR601, RGN73
⇩
Oil Content
Low: RH781, NRCDR601
Phenol Test
⇩
Dark brown: NRCDR601
Dark red: RH781
⇩
Oil Content
Medium: RH30, RH8812, RH8113, RH0749, RB50, RB24, RH0119, RH9801, NRCDR02, RGN73
⇩
Phenol Test
Dark brown: RH30, RH8812, RH8113, RH0749, RH0119, RH9801, RGN73
⇩
Modified Phenol Test
Dark brown: RH8812, RH8113
⇩
KOH Test
Dark brown: RH8113
Light brown: RH8812
⇩
Modified Phenol Test
Brown: RH30, RH0749
⇩
KOH Test
Brown: RH0749
Light brown: RH30
⇩
Modified Phenol Test
Reddish brown: RH0119, RH9801, RGN73
⇩
KOH Test
Light brown: RH0119, RH9801, RGN73
⇩
Peroxidase Test
High: RH
Medium: RH9801, RGN73
⇩
2, 4-D auxin Test
Highly susceptible: RH9801
Susceptible: RGN73
⇩
Modified Phenol Test
⇩
Strong brown**:** Nil
⇩
Phenol Test
Dark red: RB24
Dark grey: RB50, NRCDR02
⇩
Modified Phenol Test
Dark brown: NRCDR02
Brown: RB50
⇩
Seed Size (Test Weight)
Bold: Nil
⇩
Seed Colour
Brown: RH0406, RH819, Varuna, NRCHB101, DRMRIJ31, Kranti
⇩
Seed Size (Test Weight)
Small: NRCHB101, Kranti
⇩



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.

Conclusion

Laboratory based observations based on the morphological and chemical tests on the seeds of the mustard can be used to structure an Identification key for easily characterization the varieties. The present study based on eight characters of the seeds was successfully analysed for identifying twenty varieties of Indian mustard in laboratory.

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