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Characterization of Black Sesame (*Sesamum indicum* L.) Genotypes through Chemical Tests

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ABSTRACT: A knowledge of different genotype is a prerequisite for any successful improvement programme. A study was conducted to characterize 40 black sesame genotypes based on the chemical tests during the summer of 2020 at the Department of Seed Science and Technology, Junagadh Agricultural University. For the purpose of discriminating the genotypes, the seeds were subjected to the NaOH, KOH, seedling growth response to GA_3, and 2, 4-D test. The genotypes were divided into three colour categories based on the seed coloration with NaOH: dark brown (12 genotypes), light brown (11 genotypes), and brown (7 genotypes). Genotypes were divided into four categories based on the colour of the solution caused by peroxidase activity: brown (15 genotypes), light brown (9 genotypes), no change (4 genotypes), and dark brown (2 genotypes). None of the examined sesame genotypes could be distinguished using the KOH and NaOH tests. So, this study is helpful for easy identification of genotype based on chemical test which are negate cumbersome morphological identification.

Keywords: Black sesame, Characterization, Chemical test.

INTRODUCTION

Sesame (*Sesamum indicum* L., 2n = 26) is a very old oilseed crop grown after peanut and mustard in India. It comes under order *Tubiflorae*, family *Pedaliaceae*. It is basically considered a crop of tropical and sub-tropical regions, but it has also expanded to the temperate parts of the world. Sesame is said to have its origin in Africa, and it travelled quickly through West Asia to countries like India, China, and Japan before becoming distributed further (Weiss, 1983). In India it is cultivated in an area of 15.8 lakh ha with production of 7.92 lakh tonnes (Patel *et al.,* 2022).

An economically significant crop, sesame is traded extensively in local, regional, and global markets (Myint *et al.,* 2020). Sesame consumption worldwide is steadily rising as a result of high consumer demand for its distinctive nutritional qualities, which include higher contents of vitamins (such as A and E), minerals, fibre, and healthy fatty acids (such as oleic acid and linoleic acid), as well as carbohydrate (about 13.5%) and protein (about 24%) (Myint *et al.,* 2020). The demand for sesame products has also grown because of growing populations, urbanisation, and changing lifestyles (Myint *et al.,* 2020).

Due to its high oil yield, excellent oil quality, and high economic value, sesame is known as the "queen of oilseed crops" (Dossa *et al.,* 2018). In general, sesame oil content ranges from 34% to 63%. (Were *et al*., 2006). Environmental and genetic variables affect the fatty acid contents and oil levels of sesame (Carlsson *et al.,* 2009). According to Yermanos *et al.* (1972), late-maturing cultivars have more oil content than early-maturing ones, while indeterminate cultivars produce more oil than determinate ones (Uzun *et al.,* 2002).

The process of morphologically identifying a variety is time-consuming, tedious, difficult, and expensive. For varietal identification, a number of chemical assays including the sodium hydroxide test, potassium hydroxide test, gibberellic acid response test and 2, 4-D soak test have been established. These chemical tests are quick, simple, and reproducible (Agrawal, 1987), and frequently they offer evidence to corroborate the morphological assessment of the seeds (Vanderburg and Vanzwol 1991).

The chemical tests are spot checks that can be used to identify substances by changes in the colour of the seeds or the solution as a result of chemical additions. Basic biochemical tests, including as the phenol colour reaction, the NaOH test, the KOH test, and the seedling response to different chemicals, such as growth regulators and herbicides, have also been found to be highly helpful in identifying varietal combinations and

classifying a large number of genotypes (Chakrabarthy and Agrawal 1990).

MATERIALS AND METHODS

During the summer of 2020, the experiment was carried out in the Seed Testing Laboratory of the Department of Seed Science and Technology, Junagadh Agricultural University, Junagadh, to explore genotype characterization in 40 black sesame genotypes like *viz.,* IC 43063, Malvan 1, Vinimik 81, TNAU 12, RJS 190, Jira 24, Bhuva 2, Khadkala 1, Khadkala 5, Khadkala 7, Nana bhamodara 5, Hathigadh 1-3, Mota Liliya 2, Lalavadar 6, Ansodar 3, Lathi 3, Keriya 5, Keriya 6, Keriya 8, Keriya 11, Liliya 1, Nana Rajkot 1, IC 96127, IC 322186, IC 132281, IC 204653, IC 204666, IC 204681, IC 204983, IC 204496, IC 199435-E, IC 204526, IC 204528, IC 127278, IC 199433, NIC 8486, NIC 17326, KR 77, NIC 17336 and NIC 17598. Potassium hydroxide (KOH) test, Sodium hydroxide (NaOH) test GA_3 test and 2, 4-D test following procedure as given below:

Sodium hydroxide (NaOH) test. Sesame seeds (one gramme) were washed in distilled water and then steeped in 10 ml of 5% NaOH solution in a test tube for one hour at room temperature. The solution was drained and examined visually. The genotypes were classified as light brown, brown, or dark brown based on the change in colour of the solution.

Potassium hydroxide (KOH) test. One gramme of the seeds from different sesame genotypes washed in distilled water before being placed in a test tube and left to soak for an hour at room temperature in 10 ml of a 6% KOH solution. For visual inspection, the solution was filtrated. The genotypes were divided into three groups based on how the colour of the solution changed: light brown, brown, and dark brown.

GA_3 test. Sesame genotype seeds were washed in distilled water to surface sterilise them. According to the ISTA method, fifty seeds per replication will be put on two layers of blotter paper that has been moistened with a 25 ppm GA_3 solution and incubated at 25±10 °C (Anon., 1996). The control was made up of blotting sheets that had been wet. Twenty-five randomly chosen seedlings had their coleoptiles measured on the seventh day, and the growth response was expressed as a percentage increase in coleoptile length compared to the control.

2, 4-D test. According to the ISTA technique, fifty seeds in each of three replications were put on two layers of blotter paper that had been moistened with a 2 ppm 2, 4-D solution (Anon., 1996). The control was made up of the blotter sheets that had been wet. Coleoptile length of 25 randomly chosen seedlings was measured on the seventh day, and the genotypes' sensitivity response was recorded as a percent reduction in coleoptile length compared to control.

RESULTS AND DISCUSSION

It takes a lot of work, time, effort, tedium, and money to identify a variety based on its morphological characteristics. Many chemical tests, including the phenol test, sodium hydroxide test, and potassium hydroxide test, have been developed for varietal identification (Agrawal, 1987). These chemical tests are quick, simple, and reproducible, and frequently provide supporting evidence for the morphological evaluation of the seedling (Vanderburg and Vanzwol 1991).

The seeds were subjected to NaOH, KOH, gibberellic acid response and 2, 4-D soak test for differentiating the genotypes. Based on the seed colouration with NaOH, genotypes were grouped into dark brown (19 genotypes), light brown (2 genotypes) and brown (19 genotypes) in colour. Based on the colour of the solution due to KOH activity, genotypes were grouped into three categories *viz*., brown (16 genotypes), light brown (7 genotypes) and dark brown (17 genotypes) coloured types. The varied coleoptile growth response of sesame genotypes to gibberellic acid (25 ppm) has been observed in the present study. Based on the differential response of coleoptile length to GA_3, the genotypes were grouped into two categories as low response (10-30%) with (25 genotypes and moderate response (>30%) with (15 genotypes). The per cent increase in coleoptile length over control ranged from 13.82 per cent (IC 43063) to 25.75 per cent (NIC 17598). The genotypes showed varied response to 2, 4-D application (2 ppm). The per cent decrease in coleoptile length over control ranged from 93.20 per cent (IC 43063) to 94.46 per cent (NIC 17598). Based on this, the genotypes were grouped into two groups as susceptible (>85%) with (12 genotypes) and highly susceptible (>85%) with (28 genotypes).

The genotypes *viz*., Malvan 1, Vinimik 81, Bhuva 2, Khadkala 5, Khadkala 7, Hathigadh 1-3, Mota Liliya 2, Lathi 3, Keriya 6, Liliya 1, Nana Rajkot 1, IC 204526, IC 204528, IC 127278 and NIC 17598 were having similar response to chemical tests *viz*., brown colour in NaOH test, except dark brown in (Bhuva 2, Khadkala 7, Liliya 1, Nana Rajkot 1, IC 204496 and NIC 17598), brown colour in KOH test, moderate response to GA_3, except low response in (Lathi 3, Liliya 1, Nana Rajkot 1, IC 204526, IC 127278 and NIC 17598), highly susceptible to 2, 4-D test, except susceptible in (Khadkala 7, Lathi 3 and IC 204526). The genotypes IC 43063, TNAU 12, Khadkala 1, Nana bhamodara 5, Keriya 11, IC 96127, IC 204653, IC 204666, IC 204681, IC 204983, IC 204496, IC 199435-E, KR 77 and NIC 17336 were having similar response to chemical tests *viz*., dark brown colour in NaOH test, dark brown colour in KOH test, except light brown in Nana bhamodara 5, low response to GA_3, except moderate response in (TNAU 12, IC 204496 and Nana bhamodara 5), highly susceptible to 2, 4-D test, except susceptible in (TNAU 12, Khadkala 1, IC 199435-E and NIC 17336). The genotypes Jira 24, Lalavadar 6, Ansodar 3, Keriya 5, Keriya 8, IC 132281, IC 199433, NIC 8486 and NIC 17326 were having similar response to chemical tests *viz*., brown colour in NaOH test, light brown colour in KOH test, except dark brown in (Ansodar 3, Keriya 8, IC 199433 and NIC 8486), low response to GA_3, except moderate response in (Jira 24, Lalavadar 6 and Ansodar 3), highly susceptible to 2, 4-D test, except susceptible in (Jira 24, Lalavadar 6, Ansodar 3 and NIC 8486). The genotypes RJS 190 and IC 322186 were having similar response to light brown colour in NaOH test, light brown

colour in IC 322186 and brown colour in RJS 190, low response to GA_3, susceptible to 2, 4-D in RJS 190 and highly susceptible in IC 322186.

The finding of the present investigation (NaOH and KOH test) which are simple, quick and cheap for determining the varietal differences in black sesame genotypes could be used as routine genetic purity test. Observations and grouping was earlier reported by Suhasini (2006); Mesfin *et al*. (2013); Donga *et al*. (2018) in sesame; Rao *et al*. (2013) in groundnut; Ponnuswamy *et al*. (2003); Reddy *et al*. (2008); Harish (2015) in cotton; Chavan (2010) in soybean; Sathisha *et al*. (2012) in sunflower; Rai *et al*. (2019) in mustard; Raut *et al*. (2019) in wheat and Palaniswami *et al*. (1998); Rao *et al*. (2002); Sripunitha and Sivasubramaniam (2014); Hiremath (2016); Chandusingh *et al*. (2017); Nagendra *et al*. (2020) in rice. Based on the seedling response to GA_3, observation and grouping made by Suhasini (2006); Mesfin *et al*. (2013); Donga *et al*. (2018) in sesame; Rao *et al.* (2013) in groundnut; Sripunitha and Sivasubramaniam (2014) and Nagendra *et al*. (2020) in rice; Raut *et al*. (2019) in wheat and Rakesh *et al*. (2019) in pigeon pea.

Based on the seedling response to 2, 4-D, observation and grouping made by Suhasini (2006); Mesfin *et al*. (2013); Donga *et al*. (2018) in sesame; Rao *et al.* (2013) in groundnut; Rai *et al.* (2019) in mustard; Raut *et al*. (2019) in wheat and Nagendra *et al*. (2020) in rice and Patel *et al*. (2022) use various biochemical marker for identification of various chili genotype.

From the above discussion, it can be stated that the assessment of genetic purity is an important criterion in seed production programme. Therefore, simple and reliable techniques need to be developed for genetic purity assessment and variety characterization. The identified morphological characteristics of black sesame genotypes could be utilized in DUS testing, seed production programme and genetic purity testing. The result of chemical test is useful in identifying and grouping of black sesame genotypes and also in genetic purity testing.

Table 1: Identification and grouping of black sesame genotypes based on sodium hydroxide (NaOH) and potassium hydroxide (KOH) test.

Genotypes	Sodium hydroxide (NaOH) test	Potassium hydroxide (KOH) test
IC 43063	Dark brown	Dark brown
Malvan 1	Brown	Brown
Vinimik 81	Brown	Brown
TNAU 12	Dark brown	Dark brown
RJS 190	Light brown	Brown
Jira 24	Brown	Light brown
Bhuva 2	Dark Brown	Brown
Khadkala 1	Dark Brown	Dark brown
Khadkala 5	Brown	Brown
Khadkala 7	Dark Brown	Brown
Nana bhamodara 5	Dark brown	Light brown
Hathigadh 1-3	Brown	Brown
Mota Liliya 2	Brown	Brown
Lalavadar 6	Brown	Light brown
Ansodar 3	Brown	Dark brown
Lathi 3	Brown	Brown
Keriya 5	Brown	Light brown
Keriya 6	Brown	Brown
Keriya 8	Brown	Dark brown
Keriya 11	Dark Brown	Dark brown
Liliya 1	Dark Brown	Brown
Nana Rajkot 1	Dark Brown	Brown
IC 96127	Dark brown	Dark brown
IC 322186	Light Brown	Light brown
IC 132281	Brown	Light brown
IC 204653	Dark Brown	Dark brown
IC 204666	Dark brown	Dark brown
IC 204681	Dark Brown	Dark brown
IC 204983	Dark Brown	Dark brown
IC 204496	Dark brown	Dark brown
IC 199435-E	Dark Brown	Dark brown
IC 204526	Brown	Brown
IC 204528	Brown	Brown
IC 127278	Brown	Brown
IC 199433	Brown	Dark brown
NIC 8486	Brown	Dark brown
NIC 17326	Brown	Light brown
KR 77	Dark brown	Dark brown
NIC 17336	Dark brown	Dark brown
NIC 17598	Dark Brown	Brown

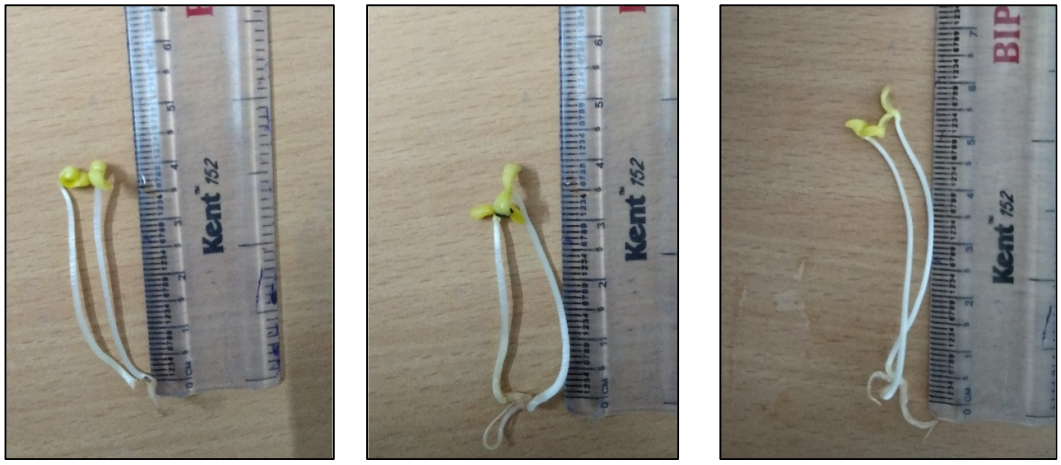
Table 2: Identification and grouping of black sesame genotypes based on coleoptile growth response to GA_3.

Genotypes	Coleoptile growth (cm)		Per cent increase in coleoptile length over control	Groups
	Control	GA_3		
IC 43063	4.51	5.13	13.82	Low response
Malvan 1	4.13	5.53	33.98	Moderate response
Vinimik 81	4.22	5.50	30.49	Moderate response
TNAU 12	4.12	5.80	40.86	Moderate response
RJS 190	4.13	5.30	28.49	Low response
Jira 24	3.57	5.80	62.65	Moderate response
Bhuva 2	4.12	5.41	31.31	Moderate response
Khadkala 1	4.68	5.21	11.40	Low response
Khadkala 5	3.45	4.92	42.61	Moderate response
Khadkala 7	3.91	5.13	31.29	Moderate response
Nana bhamodara 5	3.2	5.50	71.98	Moderate response
Hathigadh 1-3	3.42	4.51	32.07	Moderate response
Mota Liliya 2	3.34	5.42	62.28	Moderate response
Lalavadar 6	3.51	5.50	56.79	Moderate response
Ansodar 3	3.73	6.22	66.85	Moderate response
Lathi 3	3.64	4.51	24.08	Low response
Keriya 5	3.53	4.36	23.70	Low response
Keriya 6	3.41	4.70	37.93	Moderate response
Keriya 8	4.17	4.86	16.71	Low response
Keriya 11	3.64	4.31	18.50	Low response
Liliya 1	4.21	4.80	14.17	Low response
Nana Rajkot 1	4.17	4.63	11.11	Low response
IC 96127	3.91	4.66	19.35	Low response
IC 322186	4.26	5.23	22.77	Low response
IC 132281	4.53	5.31	17.37	Low response
IC 204653	4.61	5.46	18.44	Low response
IC 204666	4.58	5.62	22.78	Low response
IC 204681	3.95	4.70	19.07	Low response
IC 204983	3.87	4.71	21.79	Low response
IC 204496	3.61	5.37	48.94	Moderate response
IC 199435-E	3.77	4.31	14.50	Low response
IC 204526	4.11	5.11	24.33	Low response
IC 204528	4.32	6.52	51.00	Moderate response
IC 127278	4.46	5.61	25.86	Low response
IC 199433	4.35	5.32	22.38	Low response
NIC 8486	4.54	5.42	19.38	Low response
NIC 17326	4.18	4.93	18.02	Low response
KR 77	3.55	4.53	27.70	Low response
NIC 17336	3.64	4.42	21.52	Low response
NIC 17598	3.43	4.31	25.75	Low response
Mean	3.96	5.12	29.19	

Table 3: Identification and grouping of black sesame genotypes based on coleoptile growth response to 2, 4-D.

Genotypes	Coleoptile growth (cm)		Per cent decrease in coleoptile length over control	Groups
	Control	2, 4 -D		
IC 43063	4.51	0.31	93.20	Highly susceptible
Malvan 1	4.13	0.51	87.65	Highly susceptible
Vinimik 81	4.22	0.61	85.55	Highly susceptible
TNAU 12	4.12	0.89	78.40	Susceptible
RJS 190	4.13	0.81	80.39	Susceptible
Jira 24	3.57	1.1	69.19	Susceptible
Bhuva 2	4.12	0.32	92.23	Highly susceptible
Khadkala 1	4.68	0.93	80.06	Susceptible
Khadkala 5	3.45	0.12	96.62	Highly susceptible
Khadkala 7	3.91	0.63	83.97	Susceptible
Nana bhamodara 5	3.2	0.10	96.98	Highly susceptible
Hathigadh 1-3	3.42	0.21	93.86	Highly susceptible
Mota Liliya 2	3.34	0.12	96.31	Highly susceptible
Lalavadar 6	3.51	0.77	78.16	Susceptible
Ansodar 3	3.73	0.63	83.02	Susceptible
Lathi 3	3.64	0.67	81.68	Susceptible
Keriya 5	3.53	0.40	88.67	Highly susceptible
Keriya 6	3.41	0.16	95.31	Highly susceptible
Keriya 8	4.17	0.32	92.25	Highly susceptible
Keriya 11	3.64	0.32	91.30	Highly susceptible
Liliya 1	4.21	0.13	96.99	Highly susceptible
Nana Rajkot 1	4.17	0.31	92.49	Highly susceptible
IC 96127	3.91	0.39	90.11	Highly susceptible
IC 322186	4.26	0.63	85.21	Highly susceptible
IC 132281	4.53	0.23	94.85	Highly susceptible
IC 204653	4.61	0.25	94.58	Highly susceptible
IC 204666	4.58	0.51	88.79	Highly susceptible
IC 204681	3.95	0.34	91.31	Highly susceptible
IC 204983	3.87	0.37	90.35	Highly susceptible
IC 204496	3.61	0.36	90.12	Highly susceptible
IC 199435-E	3.77	1.25	66.84	Susceptible
IC 204526	4.11	0.75	81.67	Susceptible
IC 204528	4.32	0.11	97.53	Highly susceptible
IC 127278	4.46	0.30	93.27	Highly susceptible
IC 199433	4.35	0.17	96.17	Highly susceptible
NIC 8486	4.54	0.92	79.74	Susceptible
NIC 17326	4.18	0.36	91.47	Highly susceptible
KR 77	3.55	0.18	94.84	Highly susceptible
NIC 17336	3.64	0.60	83.52	Susceptible
NIC 17598	3.43	0.19	94.46	Highly susceptible
Mean	3.96	0.46	88.51	

Coleoptile growth response to GA_3



Control: Khadkala 7 | Low response: IC 43063 | Moderate response: Jira 24

Coleoptile growth response to 2, 4-D

Control: Khadkala 7 | Susceptible: Jira 24 | Highly Susceptible: Vinimik 81

Plate 1: Identification of black sesame genotypes on the basis of coleoptile growth response to GA_3 and 2, 4-D.

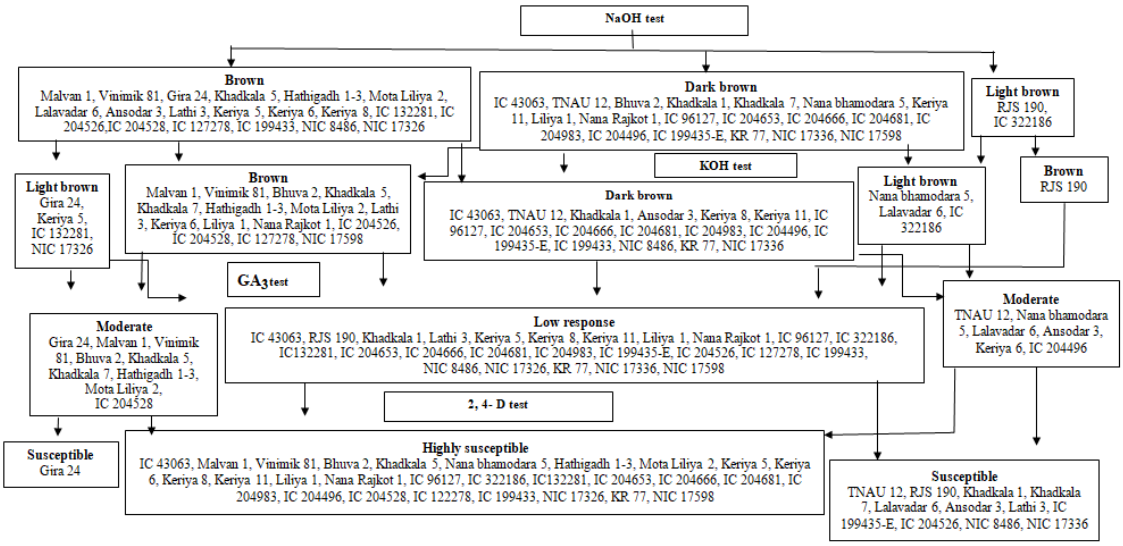


Fig. 1. Black sesame genotypes identification keys on the basis of chemical test.

CONCLUSIONS

It can be said that a crucial factor in any programme for producing seeds is the evaluation of genetic purity. Thus, methods for determining genetic purity and characterising varieties must be made simple and trustworthy. The DUS test, seed production programme, and genetic purity testing could all benefit from the identified morphological traits of wheat genotypes. The outcome of a chemical test is helpful for both classifying and identifying wheat genotypes as well as for determining genetic purity.

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Conflict of Interest. Authors have declared that no conflict of interest exist.

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