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Research Article

Characterization of Bread Wheat (*Triticum aestivum* L.) Genotypes through Chemical Tests

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ABSTRACT

The investigation was undertaken at the Department of Seed Science and Technology, Junagadh Agricultural University, Junagadh, during rabi 2017-18 to characterize 30 bread wheat genotypes released for general cultivation in Gujarat at state level as well as at the National level in central India based on the chemical tests. The seeds were subjected to phenol, peroxidase, NaOH and KOH test for differentiating the genotypes. Based on the seed colouration with phenol, genotypes were grouped into dark brown (12 genotypes), light brown (11 genotypes) and brown (7 genotypes) in colour. Based on the colour of the solution due to peroxidase activity, genotypes were grouped into four categories viz., brown (15 genotypes), light brown (9 genotypes), no change (4 genotypes) and dark brown (2 genotypes) coloured types. The KOH and NaOH test did not differentiate any wheat genotypes studied.

Keywords: *Bread wheat, Characterization, Chemical test*

INTRODUCTION

Wheat is a type of grass grown all over the world for its highly nutritious and useful grain. It is one of the annual or biennial grass having erect flower spikes and light brown grains. It is the world's largest cereal crop. It has been described as the 'King of cereals' because of the acreage it occupies, high productivity and the prominent position it holds in the international food grain trade.

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. The aspects of Distinctness, Uniformity and Stability (DUS) are fundamental for characterization of varieties. In countries having Plant Breeder's Right (PBR) in operation, a new variety is registered only, if it is distinct from other varieties, uniform in its characteristics and genetically stable Laboratory procedures furnish several additional characteristics useful for genotype identification. These chemical tests are very quick, easy to do, reproducible and can be undertaken throughout the year under controlled conditions.

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In chemical tests, the chemical agents react with the seed and help in genotype identification. Some of the sensitive analytical techniques employed in the laboratory are phenol test, potassium hydroxide (KOH) test, sodium hydroxide (NaOH) test and peroxidise enzyme activity test.

MATERIALS AND METHODS

The experiment was conducted in the Seed Testing Laboratory of the Department of Seed Science and Technology, Junagadh Agricultural University, Junagadh, during *rabi* 2017-18 to study the genotype characterization in 30 bread wheat genotypes *viz.,* AKAW 4899, DBW 154, DBW 88, DWAP 1530, DWAP 1531, GW 2014-562, GW 451, GW 455, GW 463, HD 2967, HI 1600, HPW 360, HUW 666, HUW 677, KBRL 78-2, KBRL 79-2, LBPY 2013-3, MP 3336, NIAW 1994, NIAW 2349, PBW 681, PBW 698, PHSL 11, RAJ 4238, RAJ 4350, UP 2891, UP 3000, VL 967, VL 977 and WS 1503 released for general cultivation in Gujarat at state level as well as at the National level in central India based on the chemical tests *viz.,* Grain colouration with phenol, Peroxidase enzyme activity test, Potassium hydroxide (KOH) test and Sodium hydroxide (NaOH) test following procedure as given below:

Phenol Test

Two hundred (50 x 4) seeds were presoaked in distilled water for 16 hours at 25± 1º C. Then they were transferred on two layer filter paper saturated with two per cent phenol solution. The petri dishes were covered and incubated at 25±1ºC and the colour reactions were noted after four hours. Based on the development of seed coat colour, the genotypes were classified into four categories *viz.,* no change in colour, light brown, brown and dark brown or black colour of the seed coat (Jaiswal & Agrawal, 1995).

Peroxidase Test

The Peroxidase test was carried out as per the procedure given by Agrawal and Pawar (1990) with slight modification. Twenty seeds were soaked in distilled water for two hours and decanted. Seeds of each of genotypes were soaked in 10 mL of 0.5 per cent guaicol solution for one hour. Then 0.5 mL of 0.1 per cent hydrogen peroxide solution was added. The change in colour of the solution was observed within two minute and the genotypes were classified on the basis of no change, light brown, brown and dark brown or black colour of solution.

Potassium hydroxide (KOH) test

Hundred seeds in four repetitions were soaked in five per cent KOH solution for three hours at room temperature. Change in colour of the solution and seeds were observed after three hours. Based on the intensity of the colour, the genotypes were classified into two group's *viz.,* no change in colour and reddish brown (Mckee, 1973).

Sodium hydroxide (NaOH) test

Hundreds seeds in four repetitions were soaked in five per cent NaOH solution for one hour at room temperature. Changes in colour of the seeds were observed after one hour. Based on the colour intensity of the seed, the genotypes were classified into three group's *viz.,* orange, brown and straw types (Agrawal, 1987).

RESULTS AND DISCUSSION

Varietal identification by morphological characters is laborious, time consuming, tedious, cumbersome and costly affair. A number of chemical tests have been developed for varietal identification such as phenol test, sodium hydroxide test and potassium hydroxide test, these chemical tests are very quick, easy and reproducible (Agrawal, 1987), very often these tests provide supportive evidence for the morphological evaluation of the seedling (Vanderburg & Vanzwol, 1991).

The seeds were subjected to phenol, peroxidase, NaOH and KOH test for differentiating the genotypes (Table 1). Based on the seed colouration with phenol, genotypes

were grouped into dark brown (12 genotypes), light brown (11 genotypes) and brown (7 genotypes) in colour. Based on the colour of the solution due to peroxidase activity, genotypes were grouped into four categories *viz.*, brown (15 genotypes), light brown (9 genotypes), no change (4 genotypes) and dark brown (2 genotypes) coloured types. The KOH and NaOH test did not differentiate any wheat genotypes studied.

On the basis of various chemical tests, genotype identification keys were prepared (Figure 1). The genotypes AKAW 4899, HUW 677, NIAW 1994 and UP 3000 expressed brown grain colouration with phenol and light brown colour in peroxidase test, while the genotypes DBW 154, GW 451 and HPW 360 expressed brown grain colouration with phenol and brown colour in peroxidase test. The genotypes DBW 88, DWAP 1530, GW 463, HD 2967, HUW 666 and VL 967 expressed dark brown grain colouration with phenol and brown colour in peroxidase test; GW 2014-562, KBRL 79-2 and PBW 681 expressed dark brown grain colouration with phenol and light brown colour in peroxidase test; HI 1600 and PHSL 11 expressed dark brown grain colouration with phenol and no colouration in peroxidase test; and DWAP 1531 expressed dark brown grain colouration with phenol and dark brown colour in peroxidase test. The genotypes KBRL 78-2, LBPY 2013-3, MP 3336, UP 2891, VL 977 and WS 1503 expressed light brown grain colouration with phenol and brown colour in peroxidase test; NIAW 2349 and RAJ 4238 expressed light brown grain colouration with phenol and light brown colour in peroxidase test; GW 455 and PBW 698 expressed light brown grain colouration with phenol and no colouration in peroxidase test; and RAJ 4350 expressed light brown grain colouration with phenol and dark brown colour in peroxidase test.

Seed colouration with phenol is one of the important qualitative character which is not affected by environmental condition. The result of phenol test is usually distinct and easily interpreted. Gupta et al. (2007) studied that phenol test which is the index of polyphenol oxidase activity is a simple, quick and accurate test for grouping of wheat varieties. Chandusingh et al. (2017) observed that phenol test, which is an index of polyphenol oxidase activity, has been reported to be associated with intra-varietal diversity and have been used in ascertaining varietal purity. This reaction caused melanin formation by oxidizing phenol via orthoquinones and hydroxyquinones. This reaction is controlled by single gene (monogenically), which is localized in seed coat.

The results obtained in the present study for the peroxidase activity are in conformity with the findings of Reddy et al. (2008), he concluded that peroxidase activity is controlled by a major gene (Ep) with complete dominance and produces high activity and its recessive allele (ep) was responsible for low activity. Loverkovich et al. (1968) reported that peroxidase may play a fairly generalized role in resistance of plant to infectious disease. It is possible that, a high level in the seed coat could be involved in resistance for seed infection.

Mckee (1973) reported that potassium hydroxide solution could be useful for separating white grain wheat varieties from red grain wheat varieties.

The colour reaction to sodium hydroxide solution was obtained in wheat due to reaction of seeds to secondary metabolites (Vanderburg & Vanzwol, 1991). The difference in colour reaction of seeds seems to be due to difference in genetic background concerning the enzyme system (Chakrabarthy & Agrawal, 1989).

Table 1: Identification and grouping of wheat genotypes based on phenol, peroxidase enzyme activity, NaOH and KOH tests

Genotypes	Grain Colouration with Phenol	Peroxidase Enzyme Activity Test	Potassium Hydroxide (KOH) Test	Sodium Hydroxide (NaOH) Test
AKAW 4899	Brown	Light brown	No change	Straw colour
DBW 154	Brown	Brown	No change	Straw colour
DBW 88	Dark brown	Brown	No change	Straw colour
DWAP 1530	Dark brown	Brown	No change	Straw colour
DWAP 1531	Dark brown	Dark brown	No change	Straw colour
GW 2014-562	Dark brown	Light brown	No change	Straw colour
GW 451	Brown	Brown	No change	Straw colour
GW 455	Light brown	No change	No change	Straw colour
GW 463	Dark brown	Brown	No change	Straw colour
HD 2967	Dark brown	Brown	No change	Straw colour
HI 1600	Dark brown	No change	No change	Straw colour
HPW 360	Brown	Brown	No change	Straw colour
HUW 666	Dark brown	Brown	No change	Straw colour
HUW 677	Brown	Light brown	No change	Straw colour
KBRL 78-2	Light brown	Brown	No change	Straw colour
KBRL 79-2	Dark brown	Light brown	No change	Straw colour
LBPY 2013-3	Light brown	Brown	No change	Straw colour
MP 3336	Light brown	Brown	No change	Straw colour
NIAW 1994	Brown	Light brown	No change	Straw colour
NIAW 2349	Light brown	Light brown	No change	Straw colour
PBW 681	Dark brown	Light brown	No change	Straw colour
PBW 698	Light brown	No change	No change	Straw colour
PHSL 11	Dark brown	No change	No change	Straw colour
RAJ 4238	Light brown	Light brown	No change	Straw colour
RAJ 4350	Light brown	Dark brown	No change	Straw colour
UP 2891	Light brown	Brown	No change	Straw colour
UP 3000	Brown	Light brown	No change	Straw colour
VL 967	Dark brown	Brown	No change	Straw colour
VL 977	Light brown	Brown	No change	Straw colour
WS 1503	Light brown	Brown	No change	Straw colour

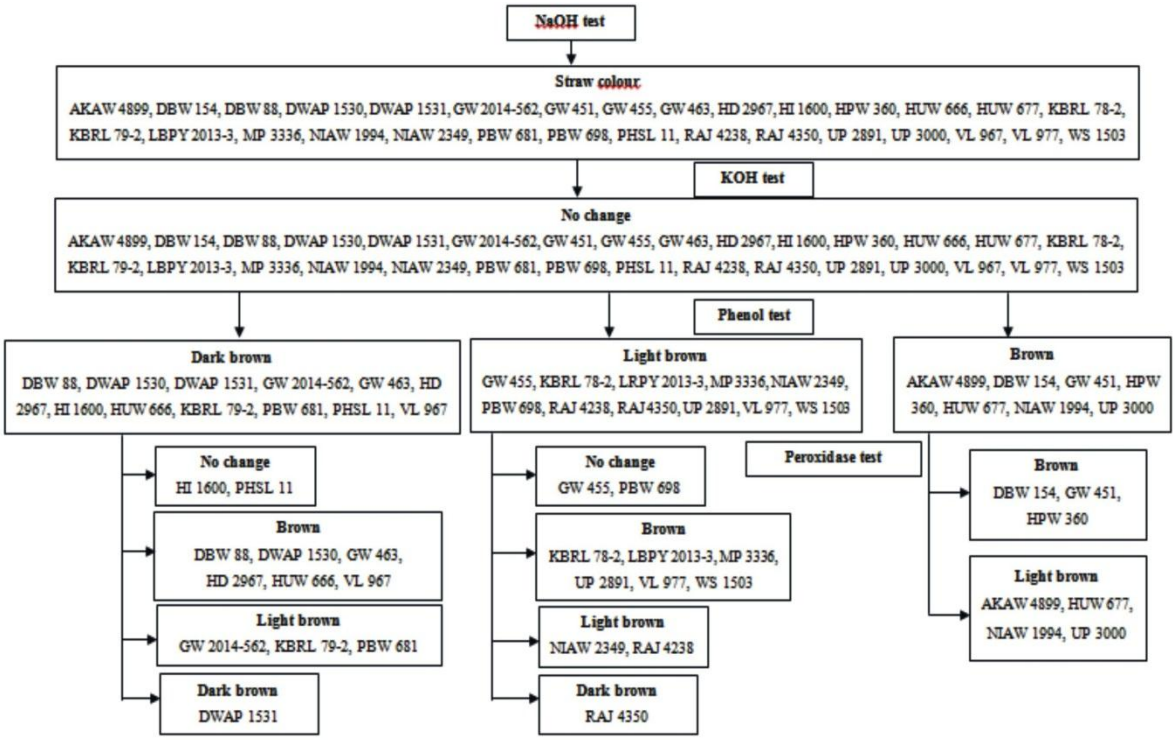

Fig. 1: Wheat genotypes identification keys on the basis of chemical tests

CONCLUSION

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 wheat 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 wheat genotypes and also in genetic purity testing.

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