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CHARACTERIZATION OF WHEAT VARIETIES (*TRITICUM* SPP.) THROUGH CHEMICAL TESTS

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ABSTRACT
An experiment was carried out at the Department of Seed Science and Technology, Junagadh Agricultural University, Junagadh, to characterize 28 wheat varieties of different species (17 of *Triticum aestivum*, 9 of *Triticum durum* and 2 of *Triticum dicoccum*) 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 varieties. Based on the seed colouration with phenol, varieties were grouped into absent (6), light brown (9), brown (5) and dark brown (8) in colour. Based on the colour of the solution due to peroxidase activity, varieties were grouped into four categories *viz*., absent (4), light brown (11), brown (8) and dark brown (5) coloured types. Based on the colour of the seed coat due to NaOH solution, the varieties were grouped into straw coloured (26) and orange (2) in colour reaction. However, the KOH test did not differentiate any wheat varieties studied. All the varieties were negative (no colour to the solution) in response. Therefore, it can be concluded that varieties can be identified on the basic of chemical tests like phenol, peroxidase and NaOH tests.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most staple and second most important food crop after rice in the country, which contributes nearly one-third of the total food grains production. It is consumed mostly in the form of bread as "*Chapati*". Wheat straw is used for feeding cattle. Wheat contains more protein than other cereal and has a relatively high content of niacin and thiamine. It is basically concerned in providing the characteristics substance "Glutin" which is very essential for bakers. The area under wheat was increased since the start of Green revolution in 1967 and production and productivity were also increased from 12.8 million hectare in 1966-67 to 257.4 m ha in 2011-12. In this period, production has also increased from 11.4 to 88.3 mt and productivity has gone up 887 kg ha^{-1} to 3140 kg ha^{-1} (Maurya *et al.,* 2014).

The present trend of continuous release of wheat varietiesby Central and State Varietal Release Committee has warranted developing techniques of varietal identification at the laboratory level particularly when the seeds have been submitted for seed purity analysis. Maintenance of genetic purity of varieties is of primary importance for preventing varietal deterioration during successive regeneration and for ensuring varietal performance at an expected level. The use of morphological traits in varietal identification and purity testing is time consuming and needs more area. Hence, there is a need for some quick tests for varietal purity testing in wheat. The chemical tests revealed differences in seeds and seedlings different crop varieties (Agarwal and Pawar, 1990). Various scientists *viz*., Pieper (1922), Chemelar and Mostovoj (1938), Joshi and Banerjee (1970), Gupta *et al*. (2007), El-Kalla *et al*. (2010) and Mansing (2010) used various chemical tests for the varietal/genotype identification/characterization in wheat. These tests do not much require virtually no technical expertise or training and can be completed in a relatively short time. The results of these tests are usually distinct, easily interpreted and help in grouping of the genotypes. Therefore, an investigation has been carried out to study the response of wheat genotypes to various chemical tests for effective utilization in varietal characterization and purity analysis. In the light of the above facts, the present study was planned to study the suitability of chemical tests for the identification of wheat varieties.

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* 2014 to study the varietal characterization in 28 wheat varieties *viz*.,MP 4010, HI 1500, HI 1531, HI 1544, GW 1, GW 503, DL 788-2, HD 2932, GW 11, GW 173, GW 190, GW 273, LOK 1, GW 322, MP 3288, GW 366, GW 496, HI 8381, HI 8498, HI 8627, HI 8713, A 28, A 206, GDW 1255, GW 1139, RAJ 1555, DDK 1025 and DDK 1029, of different species (17 of *Triticum aestivum*, 9 of *Triticum durum* and 2 of *Triticum dicoccum*) released for general cultivation in Gujarat at state level as well as at the National level in Central India based on 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 petridishes 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 varieties were classified into four categories *viz*., no change in colour, light brown, brown and dark brown or black colour of the seed coat (Jaiswal and Agarwal, 1995).

Peroxidase Test

The Peroxidase test was carried out as per the procedure given by Agarwal and Pawar (1990) with slight modification. Twenty seeds were soaked in distilled water for two hours and decanted. Seeds of each of varieties 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 varieties 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 varieties were classified into two group's *viz*., no change in colour and reddish brown (Mckee, 1973).

Sodium hydroxide (NaOH) test

Hundreds seeds in four replications 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 varieties 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 (Agarwal, 1987 and Ashwani Kumar *et al*., 1995), very often these tests provide supportive evidence for the morphological evaluation of the seeding (Vanderburg and Vanzwol, 1991).

The seeds were subjected to phenol, peroxidase, NaOH and KOH test for differentiating the varieties. Based on the seed colouration with phenol, varieties were grouped in to absent (6 varieties), light brown (9 varieties), brown (5 varieties) and dark brown (8 varieties) in colour. Based on the colour of the

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

Varieties	Grain Colouration with Phenol	Peroxidase Enzyme Activity Test	Potassium Hydroxide (KOH) Test	Sodium Hydroxide (NaOH) Test
Triticumaestivum L.				
MP 4010	Light brown	Brown	No change	Straw colour
HI 1500	Absent	Absent	No change	Straw colour
HI 1531	Absent	Light brown	No change	Straw colour
HI 1544	Light brown	Light brown	No change	Straw colour
GW 1	Absent	Light brown	No change	Straw colour
GW 503	Brown	Brown	No change	Straw colour
DL 788-2	Brown	Light brown	No change	Straw colour
HD 2932	Light brown	Absent	No change	Straw colour
GW 11	Dark brown	Brown	No change	Straw colour
GW 173	Light brown	Dark brown	No change	Straw colour
GW 190	Dark brown	Light brown	No change	Straw colour
GW 273	Brown	Brown	No change	Straw colour
LOK 1	Dark brown	Brown	No change	Straw colour
GW 322	Dark brown	Dark brown	No change	Straw colour
MP 3288	Dark brown	Light brown	No change	Straw colour
GW 366	Dark brown	Brown	No change	Straw colour
GW 496	Brown	Dark brown	No change	Straw colour
Triticum durum Desf				
HI 8381	Light brown	Light brown	No change	Straw colour
HI 8498	Absent	Light brown	No change	Straw colour
HI 8627	Light brown	Light brown	No change	Straw colour
HI 8713	Light brown	Brown	No change	Straw colour
A 28	Dark brown	Absent	No change	Straw colour
A 206	Dark brown	Absent	No change	Straw colour
GDW 1255	Light brown	Light brown	No change	Straw colour
GW 1139	Absent	Light brown	No change	Straw colour
RAJ 1555	Absent	Dark brown	No change	Straw colour
*Triticumdicoccum*Schrank				
DDK 1025	Brown	Brown	No change	Orange
DDK 1029	Light brown	Dark brown	No change	Orange

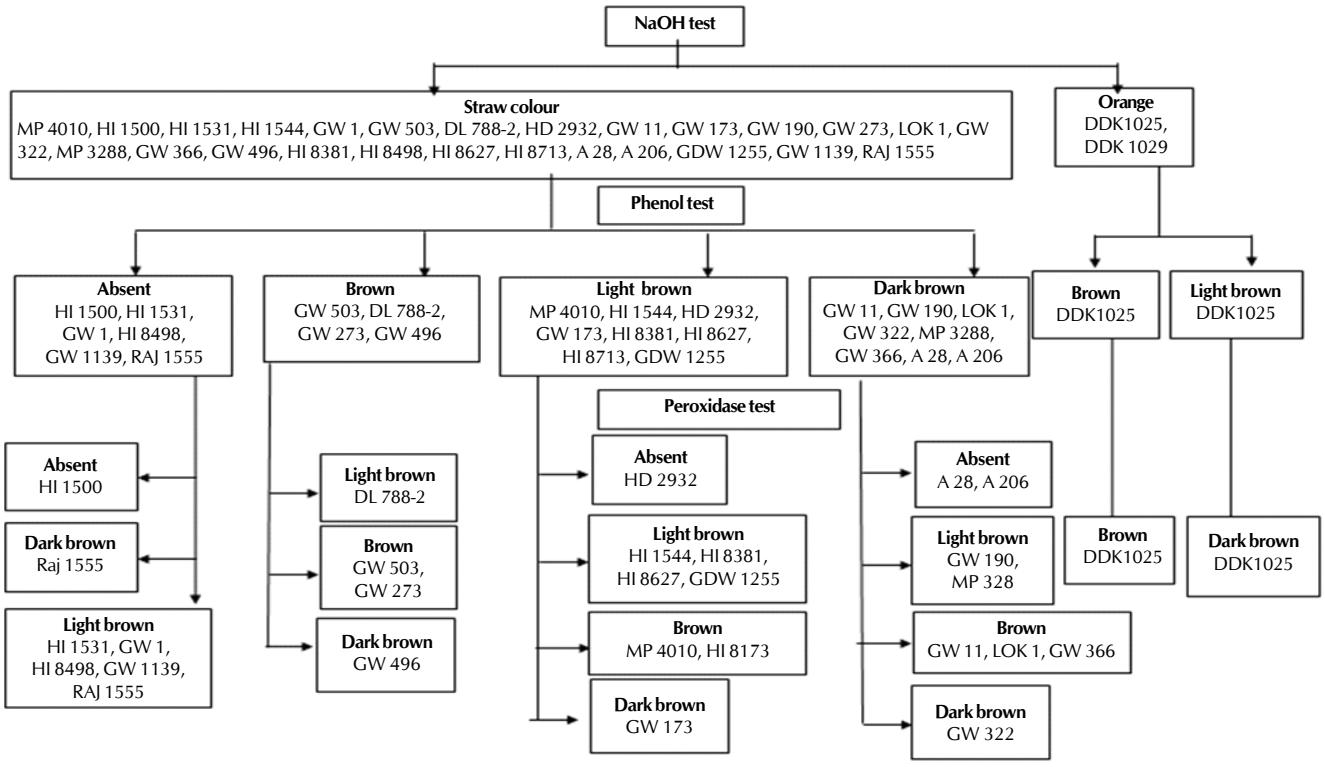

Figure 1: Wheat varieties identification keys on the basis of chemical tests

solution due to peroxidase activity, varieties were grouped in to four categories *viz.,* absent (4 varieties), light brown (11 varieties), brown (8 varieties) and dark brown (5 varieties) coloured types. Based on the colour of the seed coat due to NaOH solution, the varieties were grouped into straw colour (26 varieties) and orange (2 varieties, DDK 1025 and DDK 1029) in colour reaction. However, the KOH test did not differentiate any wheat varieties studied. All the varieties were negative (no colour to the solution) in response (Table 1).

On the basis of various chemical tests, varietal identification keys were prepared (Figure 1). MP 4010 and HI 8713 expressed light brown grain colouration with phenol and brown colour in peroxidase test, while HI 1544, HI 8381, HI 8627 and GDW 1255 expressed light brown grain colouration with phenol and light brown colour in peroxidase test (Table 1). HD 2932 showed light brown grain colouration with phenol and no colouration in peroxidase test. GW 173 and DDK 1029 expressed light brown grain colouration with phenol and dark brown colour in peroxidase test. DDK 1025, GW 503 and GW 273 brown grain colouration with phenol and peroxidase test, while DL 788-2 and GW 496 expressed brown grain colouration with phenol, but expressed light brown and dark brown colouration with peroxidase test, respectively. HI 1531, GW 1, HI 8498 and GW 1139 expressed no grain colouration with phenol and light brown colour in peroxidase test, while HI 1500 and RAJ 1555 expressed no grain colouration with phenol, but expressed no colouration and dark brown colouration with peroxidase test, respectively. GW 11, LOK 1 and GW 366 expressed dark brown grain colouration with phenol and brown colour in peroxidase test; GW 190 and MP 3288 expressed dark brown grain colouration with phenol and light brown colour in peroxidase test; GW 322 expressed dark brown grain colouration with phenol and dark brown colour in peroxidase test; and A28 and A 206 expressed dark brown grain colouration with phenol and no 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. Walls (1965) reported that the phenol colour reaction depends on the quality and quantity of oxidases enzymes present in seeds, whereas Takahashi and Hamza (1983) reported that monophenol oxidase was extremely localized in grains even though it is present in all other plant parts. Phenol colour reaction, which is an index of polyphenol oxidase activity, has been utilized to distinguish the crop varieties by earlier workers, *viz.,* Pieper (1922), Joshi and Banerjee (1970), Gupta *et al*. (2007), El-Kalla *et al*. (2010) and Mansing (2010) in wheat; Chauhan and Nanda (1984), Shin *et al*. (1990), Gupta *et al*. (2007), Vijayalakshmi and Vijay (2009), Anitalakshmi *et al*. (2014) and Sripunitha and Sivasubramaniam (2014)in rice and Vishwanath *et al*. (2013) in tomato.

The results observed in the present study for the peroxidase activity are in conformity with the findings of Buzzell and Buttery (1969), who has reported that, high and low peroxidase activity in soybean seed coat is controlled by a major gene which is designated complete dominance and produces high activity and its recessive allele; (ep) gives low activity. Loverkovich *et al*. (1968) presented some evidences

suggesting that the peroxidase may play a fairly generalized role in resistance of plants to infectious disease. It is possible that, a high level in the seed coat could be involved in resistance for seed infection. Characterization of varieties/genotypes on the basis of peroxydase activity were also carried out by some researchers and presented the similar results *viz.,* Agrawal (1987) and Mansing (2010) in wheat; Mckee (1973) in barley; Ponnuswamy *et al*. (2003), Kirankumar Reddy (2004) and Reddy *et al*. (2008) in cotton; Koszykowski and Burgoon (1983) and Chavan (2010) in soybean; Chakrabarthy and Agrawal (1989) in urdbean and Thawari *et al*. (2014) in sunflower.

Similar classification by NaOH test, as observed in the present study, also reported earlier by Chemelar and Mostovoj (1938), Agrawal (1987) and Mansing (2010) in wheat; Vanagamudi*et al*.(1988), Dhanaraj (2001), Sambasiva Rao *et al*. (2002) and Sripunitha and Sivasubramaniam (2014) in rice; Chakrabarthy and Agrawal (1990) in urdbean; Ponnuswamy*et al*. (2003) and Reddy *et al*. (2008) in cotton; Biradar Patil *et al*. (2006) in safflower; Suhasini (2006) in sesamum; Chavan (2010) in soybean; Sathisha *et al*. (2012) and Kallihal *et al*. (2013) in sunflower; and Vishwanath *et al*. (2013) in tomato. The colour reaction to sodium hydroxide solution was obtained in wheat due to reaction of seeds to secondary metabolites (Vanderburg and Vanzwol, 1991). The difference in colour reaction of seeds seems to be due to difference in genetic background concerning the enzyme system (Chakrabarthy and Agrawal, 1990).

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