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Varietal characterization based on chemical methods in different varieties of wheat (*Triticum* spp.)

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Abstract
This experiment was conducted at Seed Science and Technology Section, Department of Genetics of Plant Breeding, CCSHAU, Hisar Haryana to differentiate wheat varieties based on different chemical tests. Three chemical tests (Standard phenol, Modified phenol and NaOH test) used to differentiate and make group of twenty eight wheat varieties/ genotypes. Among chemical test standard phenol tests and modified phenol test were observed as reliable test for varietal characterization in wheat. it is revealed that these test are less time consuming, easy to perform, economical and reproducible. So can be used as routine tests for varietal identification of wheat genotype in laboratories.

Keywords: Modified phenol and NaOH test, characters, chemical methods, wheat standard phenol

Introduction
Wheat is thought to have been originated in the Fertile Crescent area of the south-western Asia. Wheat is the largest cereal crop species of the world. It is also known 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. In our country many crop oriented many crop improvement Programme is running due to this so many new varieties of crop are being developed. So varietal identification is essential to maintain the genetic purity and identity of the varieties.
Distinctness Uniformity Stability (DUS) is fundamental aspect for Varietal characterization. Accurate identification of varieties is essential for DUS testing, but also required for the production of quality seed. Maintenance of genetic purity is of primary importance of cultivated varieties for preventing varietal deterioration generation after generation and for ensuring performance of varieties at an expected level. Seeds can be differentiated in different color with the help of chemical tests. Study of chemical based characters along with phenotypic characters and biochemical techniques are useful for producing more authentic result. These chemical tests are very quick, easy to perform, reproducible, economical and can be conducted throughout the year at room temperature. In chemical tests, the chemical agents which react with the seed develops different color. These colors helps in varietal identification. Most popular chemical tests used in wheat for varietal characterization are phenol test, modified phenol Test ($CuSo_4$ and Na_2CO_3) and sodium hydroxide (NaOH) test.

Materials and Methods
Freshly harvested seed (Rabi 2013-14) of 28 Wheat varieties (WH 542, WH 711, WH 730, WH 1021, WH 1025, WH 1063, WH 1080, WH 1105,WH 1081, WH 1124, WH, 1138, PBW 343, PBW 550, PBW 373, PBW 509, PBW, 590, HD 2733, HD 285, HD 2932, HD 2967, HD 3043, DBW 17, DBW 71, DPW 621-50, KRL 19, C 306, K 307, RAJ 3765) were collected from the Wheat and Barley Section, Department of Genetics and Plant breeding, CCS HAU Hisar, Haryana. Collected seed of these varieties was subjected for varietal characterization of wheat varieties.

Phenol Test: The phenol test procedure for varietal identification in wheat was followed which was suggested by Walls (1965) [7]. In three replication fifty seeds in each were counted and presoaked in distilled water for 16 h. at 25±1 °C. Then these presoaked seed were transferred over two layers of filter paper which was drenched with 1% phenol solution in a Petridis. The lid of the Petridish was closed tightly and kept at 25±1 °C for 4 h.

After four hours on the basis of development of seed coat colour observations were recorded and varieties/cultivars were classified into four categories as No change in colour (- -), Light brown (+), Brown (++) and Dark brown or black (+++).

Modified Phenol Test (Na_2CO_3 0.6% and $CuSO_4$ 0.4%): The Modified Phenol test (Na_2CO_3 and $CuSO_4$) procedure for varietal purity testing with slight modification was followed as suggested by Banerjee and Chandra (1977) [9]. Three replications of 50 seeds each were soaked in 50 ml of 0.6% Na_2CO_3 and 0.4% $CuSO_4$ and for 16 h. for both the tests. The presoaked seeds were then placed in Petri dishes containing filter paper moistened with 4 ml of 1% phenol solution and kept at room temperature (25±1 °C). The seeds were examined after staining 4 h. and grouped as No change in colour (- -), Light brown (+), Brown (++) and Dark brown or black (+++).

NaOH Test: Three replications of hundred seeds were soaked in 5% solution of NaOH for one hour at room temperature. After 1 hr. colour of the seeds changes. Based on the intensity of colour of the seed, the varieties were classified into two group's *viz.*, light orange and straw yellow Types (1), (8).

Table 1: Colour response of wheat varieties toward chemical tests

S. No.	Varieties	Phenol Test	Mod Phenol $CuSO_4$	Mod Phenol Na_2CO_3	NAOH
		After 4 hr.	After 4 hr.	After 4 hr.	4 hr.
1.	WH 542	Dark brown	Dark Brown	Light Brown	yellow
2.	WH711	Dark Brown	Brown	Light Brown	yellow
3.	WH 730	Dark Brown	Dark Brown	Brown	yellow
4.	WH1021	Dark brown	Dark Brown	Dark Brown	yellow
5.	WH1025	Light Brown	Light Brown	Light Brown	yellow
6.	WH1063	Dark Brown	Dark Brown	Dark Brown	yellow
7.	WH 1080	Dark Brown	Brown	Light Brown	yellow
8.	WH 1081	Dark Brown	Dark Brown	Dark Brown	Yellow
9.	WH1105	Dark Brown	Dark Brown	Dark Brown	Yellow
10.	WH1124	Dark Brown	Dark Brown	Dark Brown	Yellow
11.	WH1138	Dark brown	Dark Brown	Light Brown	Yellow
12.	PBW 343	Dark brown	Dark Brown	Light Brown	Yellow
13.	PBW 373	Dark brown	Brown	Dark Brown	Yellow
14.	PBW 509	Dark Brown	Brown	Light Brown	Yellow
15.	PBW 550	Dark Brown	Brown	Brown	Yellow
16.	PBW 590	Brown	Brown	Dark Brown	Yellow
17.	HD 2733	Brown	Brown	Dark Brown	Yellow
18.	HD 2851	Brown	Brown	Light Brown	Yellow
19.	HD 2932	Light Brown	Light Brown	Light Brown	Yellow
20.	HD 2967	Brown	Brown	Dark Brown	Yellow
21.	HD 3043	Dark Brown	Dark Brown	Brown	Yellow
22.	DBW 17	Brown	Brown	Light Brown	Yellow
23.	RAJ 3765	Light Brown	Light Brown	Light Brown	Yellow
24.	DPW 621-50	Dark Brown	Dark Brown	Light Brown	Yellow
25.	KRL 19	Light Brown	Light Brown	Light Brown	Yellow
26.	C 306	Light Brown	Brown	Brown	Yellow
27.	K 307	Brown	Dark Brown	Brown	Yellow
28.	DPW71	Brown	Brown	Brown	Yellow

Results and Discussion

Phenol test showed great variation among genotypes and differentiate varieties into various genotypes *viz*. light brown, brown and dark brown group. This test is highly specific for wheat varieties. Phenol reaction is mono-genically controlled response which is present in seed coat (2). An enzyme Polyphenol Oxidase (PPO) is responsible for the oxidation of externally supplied phenol into quinones and their further polymerization yield melanin like pigments which have resulted in development of black coloration in seeds. So wheat seed coat colour development in wheat seed coat by phenol colour reaction is detected and varieties were differentiated as dark brown, brown, light brown and no reaction. Out of 28 varieties sixteen varieties WH 542, WH 711, WH 730, WH 1021, WH 1063,WH 1080, WH 1081, WH 1105, WH 1124, WH 1138, PBW 343, PBW 373, PBW 509, PBW 550, HD 3043 and DPW 621-50 showed dark brown colour, seven varieties i.e. PBW 590, HD 2733, HD 2851, HD 2967, DBW 17, K 307 and DPW 71 were brown colour and rest five varieties i.e. WH 1025, HD 2932, RAJ 7365, KRL 19 and C 306 was light brown colour (Table-1). No variety showed negative or no colour reaction. The results were in conformity with findings of (2, 3).

Further modified phenol test ($CuSO_4$ 0.4% and Na_2CO_3 0.6% as an inhibitor) was used for getting better result and categorize the varieties in sub groups. In modified phenol ($CuSO_4$ 0.4%) test out of five light brown group varieties one variety (C 306) sub grouped as Brown, from seven Brown group varieties one variety (K 307) sub grouped as Dark brown while out of 17 Dark brown varieties five varieties (WH 711, WH 1080, PBW 373, PBW 509 and PBW 550) was sub grouped in Brown (Figure-1). Modified phenol (Na_2CO_3) also shows similar result as under phenol but some verities show variation and out of five light brown varieties one variety (C 306) sub grouped as brown. Out of 7 Brown varieties two varieties (HD 2851 and DBW 17) sub grouped into light Brown while three varieties (PBW 590, HD 2967 and HD 2733) sub grouped in Dark brown. From Dark brown

group of seventeen varieties four (WH 730, PBW 373, HD 3043 and PBW 550) sub grouped under in Brown and seven varieties (WH 711, WH 1080, PBW 509, WH 542, WH 1138, PBW 343 and DPW 621-50) sub grouped into light brown while rest varieties remained dark brown in colour (Fig-1).

These sub groups ware made due to inhibiting effect of $CuSO_4$ and Na_2CO_3. Both phenol and modified phenol is emerging as a stable and uniform method of wheat variety grouping. The similar observations were recorded by (4) in rice. Cultivated Indian durum wheat varieties (5) and genotypes in wild species, bread wheat cultivars, durum wheat and synthetic hexaploids (SH) (6) have been characterized for phenol reaction and found the related results.

On the basis of colour reaction with sodium hydroxide solution the wheat varieties were grouped into orange and yellow colour. This test is very effective for groping of red and white wheat varieties. However in present study in all the 28 varieties produces yellow colour after reacting with NaOH as they were of white group no variety produced orange colour. Similar results were reported by (1).

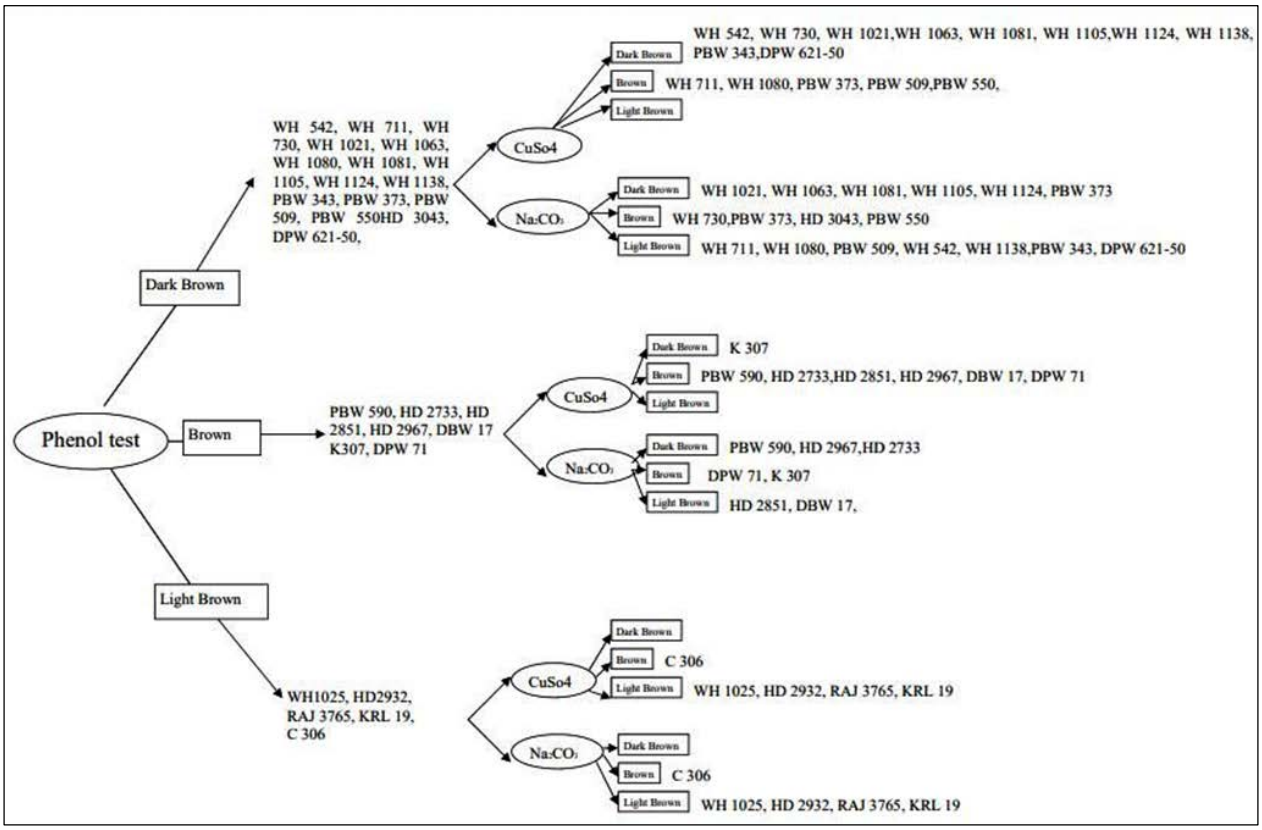

Fig 1: Schematic diagram for varietal/genotype identification on the basis of chemical tests in wheat varieties

Conclusion

So from this study it was concluded that no single chemical test is helpful in identification of wheat varieties hence phenol and modified phenol tests are important diagnostics chemical tests which can provide an excellent system of characterization of wheat variety in simple, rapid, reliable and cost effective way.

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