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Phenol colour reaction and its biochemical basis in cultivated wheat genotypes

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ABSTRACT

For seed to play a catalytic role, it should reach farmers in a good quality state, i.e. high genetic purity and identity. So, varietal purity testing plays a critical role in preventing varietal admixtures. Phenol colour reaction is being used as a rapid varietal identification technique for a long time. PPO has been implicated to cause this enzymatic browning. Polyphenol oxidase activity (PPO) must be reduced or eliminated if wheat quality is to be improved. This research was undertaken for development of quick genetic purity testing method in wheat varieties and to study the effect of PPO activity during storage in cultivated wheat. The biochemical studies of the pathway which gives phenol colouration has also been studied.

Key words : *Phenol, PPO, Varieties, Cultivated, Wheat*

Introduction

Wheat is the first important and strategic cereal crop for the majority of world's populations. It is the most important staple food of about two billion people (36% of the world population). Maintenance of genetic purity of varieties is primary importance for preventing varietal deterioration during multiplication in successive generations and for ensuring varietal performance at an expected level. Hence, there is a need for some quick tests for varietal purity testing in Wheat.

The phenol test is long established as a useful procedure for checking the varietal purity and identity of cereal grain. The test is ascribed to the enzymic oxidation of phenol to dark brown melanins. The phenotype appears to be simply inherited and characteristic of genotype. So, it can be reliably used for varietal identification in early stages of seed development. The method of phenol testing is standardised by ISTA. The ISTA (1966) uses these grouping: (1) black, (2) dark brown, (3) brown, (4) light brown, and (5) negative.

Presence of Polyphenol oxidase (PPO) in wheat was reported by Abrol and Uprety (1970). Several studies have investigated not only variations of PPO activity with quality characteristics or with cultivars and growing locations (Park *et al.*, 1997).

Colour is a key quality trait of hexaploid (*Triticumaestivum* L.) and durum (*T. turgidum* ssp. *durum* [Desf.] Husn.) wheat products. In this regard, the prevention or minimization of discolouration is an important component to attaining good colour in consumer products. Polyphenol oxidase (PPO) has been implicated as a leading cause of discolouration of Asian wheat noodles, chapattis and other wheat products (Kruger *et al.*, 1994). Therefore, many wheat breeding programs are focusing on developing wheat cultivars with significantly low PPO activity especially in mature grain.

With this objective, we selected 15 wheat varieties belonging to varying phenol colour reaction

groups and subsequently studied the biochemical basis by estimating phenol content and other intermediates which impart phenol colour to grains. We also studied the effect of storage on PPO activity.

Material and Method

ISTA approved method of phenol testing was used. Four replicates of 100 seeds each are used. The seeds are soaked in distilled water for 16 hrs., then flushed with tap. The seeds are then placed on two layers of filter paper in a container moistened with a 1% phenol solution. After 4 hrs of incubation, colour reaction developed by the seed is recorded at room temperature

Total Phenol content was estimated using the method developed by Singleton *et al.* (1965). Estimation of L-Tyrosine and Pyrocatechol (Phenol intermediate) by whole seed assay using method developed by Anderson and Morris (2001).

Results and Discussion

An analysis of these compounds in both fresh and one-year old seeds of the 15 varieties belonging to five colour groups showed that black colour reaction varieties had higher values as compared to that of all other groups. There was a progressive decrease

Table 1. Cultivated varieties of different phenol colour groups studied at different stages of growth and maturity

Black	Dark brown	Brown	Light brown	No colour
HD2967	MACS-9	C-306	HD-2894	HI-8627
HD-2733	HD-7483	HD-2932	HD-2888	MACS-2694
DWR137	HD-2851	K-8027	HD-4530	HD-4672

Table 2. Total phenol content (mg/g), tyrosine and pyro-catechol activity in fresh and one-year old seeds in the different phenol colour group varieties

	Total phenol content(mg/g)			Tyrosine activity (unit/ml/min)			Pyrocatechol activity (unit/ml/min)		
VAR	FRESH	OLD	MEAN	FRESH	OLD	MEAN	FRESH	OLD	MEAN
HD-2967	8.2	8.6	8.4	87	95.3	91.1	119	124.3	121.6
HD-2733	8.1	8.7	8.4	87.3	94.6	91	119	125.6	122.3
DWR137	8.0	8.6	8.3	88.6	95.6	92.1	117.3	127.6	122.5
CD(P=0.05)	Var:N/S;Year:0.5; Year× var:N/S			Var:N/S;Yr:3.8; Var*Yr:N/S			Var:N/S;Yr:3; Var*Yr:N/S		
MACS-9	7.2	7.7	7.5	67.6	76.3	72	82.6	90.3	86.5
HD-7483	7.1	7.6	7.3	69	77.3	73.1	82.3	88.6	85.5
HD-2851	7.0	7.8	7.4	68	75	71.5	83	89.3	86.1
CD(P=0.05)	Var:N/S; Year: 0.4; Year× var:N/S			Var:N/S;Yr:2.8; Var*Yr:N/S			Var:N/S;Yr:2.6; Var*Yr:N/S		
C-306	6.6	7.2	6.9	45.6	54.3	50	55	63.6	59.3
HD-2932	6.6	6.9	6.7	47.6	53	50.3	55	65	60
K-8027	6.4	6.8	6.6	44	54	49	55.3	62.6	59
CD(P=0.05)	Var:N/S; Year: 0.31; Year× var:N/S			Var:N/S;Yr:2.4; Var*Yr:N/S			Var:N/S;Yr:3.2; Var*Yr:N/S		
HD-2894	6.0	6.6	6.3	25.6	34	29.8	40.3	49.3	44.8
HD-2888	6.1	6.6	6.4	28	32	30	43.6	48.3	46
HD-4530	6.2	6.7	6.4	27.3	30.3	28.8	42.3	51	46.6
CD(P=0.05)	Var:N/S; Year: 0.24; Year× var:N/S			Var:N/S;Yr:2.7; Var*Yr:N/S			Var:N/S;Yr:2.2; Var*Yr:N/S		
HI-8627	5.4	5.8	5.6	14	15	14.5	15.3	17	16.1
HD-4672	5.4	5.7	5.6	13.6	17.6	15.6	16	16	16
MACS-2694	5.2	5.6	5.4	14.6	18.3	16.5	15	17.3	16.1
CD(P=0.05)	Var:N/S; Year: 0.28; Year× var:N/S			Var:N/S;Yr:1.6; Var*Yr:N/S			Var:N/S;Yr:2.1; Var*Yr: N/S		
Range	8.1-5.2	8.6-5.6		88.6-13.6	95.6-15		119-15	127.6-16	
Mean	6.62	7.12		48.5	54.8		62.7	69	

in the content of these compounds in the seeds of cultivated varieties studied. Varieties in different colour reaction groups differed significantly while varieties in one colour group did not show significant variation among them.

In the cultivated varieties, there was a significantly higher values of all these three chemicals in the one-year old seeds as compared to the respective fresh seeds. Phenol content ranged from 5.2-8.2 mg/g with an average of 6.62 mg/g among the varieties in fresh seed, whereas it ranged from 5.5 to 8.6 mg/g with an average of 7.12 mg/g in one-year old seed (Table 2). The old seed lots had significantly higher phenol content values as compared fresh seed lots in all the varieties across the colour groups.

Similarly, for tyrosine, its activity ranged from 14 – 89 unit/ml/min, with a mean of 48.5 unit/ml/min and its activity ranged from 15 – 95 unit/ml/min with an average of 54.8 unit/ml/min among the varieties in fresh and one-year old seed, respectively (Table 2). The old seed lots showed significantly higher tyrosine activity as compared to that in fresh seed lots across the varieties. In case of Pyrocatechol, its activity in fresh seeds ranged from 15 – 119 unit/ml/min with a mean of 62.7 unit/ml/min. Its activity ranged from 16 - 127 with an average of 69 unit/ml/min among the varieties in one-year old seed (Table 2).

Pyrocatechol was the intermediate found in highest amount in wheat seeds. Gradual decrease in Pyro-catechol in the phenol colour group was found in the varieties in the two seed lots.The mean Pyrocatechol in the various colour groups were 118.4, 82.6, 55.1, 42.1 and 15.4(unit/min/ml), respectively for black, dark brown, brown, light brown, and no colour varieties in the fresh seed lots while these were 125.8, 89.4, 63.7, 49.5 and 16.7 (unit/min/ml) for one year old seed, respectively (Table 2). One year old seeds in each phenol colour group showed a significantly higher pyrocatechol activity as compared to the fresh seeds (Table 2).

Conclusion

In view of the fact that phenol colour reaction is due to PPO activity of the husk in the grain, we intended to measure it in some varieties in each phenol colour group. A rapid, non-destructive test with as few as three wheat seeds was used to study the phenolic intermediates. Total phenol content was also studied. A large variation in phenol content, Pyrocatechol and Tyrosine activity was recorded across the varieties studied. However, our studies were limited to three varieties in each colour group. No overlapping in these values in varieties of two different colour groups was recorded. The varieties included in a particular reaction group fairly showed non-significant difference for the phenol content along with other biochemical factors studied.

This study can be used to distinguish wheat varieties on basis of their phenol colouration. Poly Phenol Oxidase (PPO) activity significantly increased in stored seeds. PPO activity can be estimated with as few as 3 seeds and has a potential to be used as rapid testing kit for wheat varietal identification in farmer's field. This research might be expanded to include a large wheat germplasm and breeding population in order to better understand genetics and, as a result, aid in the development of low PPO cultivars.

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