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RESEARCH ARTICLE

Variation in phenol content in grains of wheat (*Triticum* spp.) in relation to phenol colour reaction

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Abstract

For a long time, phenol colour reaction on grains has piqued researchers' curiosity all around the world. Bread wheat (*Triticum aestivum* L., 2n=6x=42) and macaroni (*T. turgidum* subpp. *durum* [Desf.] Husn., 2n=4x=28) wheat products include colour as an important quality feature. Browning caused by polyphenol oxidase activity must be reduced or eliminated if wheat quality is to be improved. A study was, therefore, undertaken to identify different cultivated varieties with low phenol coloration and subsequently low phenol content at various stages of growth and maturity since its induction after flowering. A few wild species having diverse genomes were also studied to identify the potential donor for phenolic activity in grains. Results indicated that phenol colour was induced post-fertilization and phenol content increased with intensity of coloration. Generally, wild species showed higher phenolic activity than that in cultivated varieties. The information accrued may be useful in breeding low phenol content in wheat.

Keywords: Phenol colour, Phenol content, Variation, Variety, Wild species.

Introduction

The countries of eastern Asia are major wheat users, with as much as 50% of the flour consumed in the form of noodles (Miskelly and Gore 1991). Two of these leading types of noodles in these markets are white salted and yellow alkaline. Colour is a key quality trait for both of these types of noodles, and can vary dramatically among different wheat cultivar and grain lots (Morris et al. 2000). Consequently, any means of predicting noodle color from grain is highly desirable. The darkening takes place after the noodles have been made. This darkening process in noodles is associated with polyphenol oxidase (PPO) activity and its corresponding products (Miskelly 1984; Kruger et al. 1992; Baik et al. 1995).

Chapatti is the staple diet in Indian subcontinent. Wheat varieties with positive phenol colour reaction are not desirable for chapatti and bread making. Recently, a surge in demand for good chapatti quality wheat in Indian market is noticed. One of the important chapatti quality traits pertains to the time-dependent darkening of whole meal dough and its subsequent effect on browning of chapattis and other end use products. This is the result of high levels of activity of enzyme PPO present in the pericarp (bran layer) of wheat kernel (McCaig et al. 1999). It has been observed that a high PPO activity is responsible for whole meal dough darkening (Abrol et al. 1972). In this regard, the prevention or minimization of discolouration is an important component to attaining a desired good colour in consumer product (Kruger et al. 1994; Morris 1998).

Voss (1936) and Esbo (1945) discovered tyrosinase for the first time. Quality and amount of the enzyme determines the phenol colour reaction which is present in seeds (Walls 1965). Joshi and Banerjee (1970) clearly demonstrated that the reaction oxidizing phenol to melanin (dark colour developed in the seed coat) via orthoquinone and hydroxyquinone was catalyzed by means of copper containing polyphenol oxidase, *i.e.*, tyrosinase with the possible contribution of other phenolic substances present in the seeds and also of amino acids such as tyrosine and phenylalanine.

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Phenol test is a rapid variety identification technique that employs phenol to cause different colour reaction in seeds based on which the varieties can be categorized (Friedberg 1933). Friedberg (1933) and Wrigley et al. (1975) proposed five colour classes that included no colour, light brown, brown, dark brown and mixed coloration. The colour categories proposed by the AOSA (Walls 1965) were ivory, fawn, light brown, brown and black brown. The ISTA (1966) uses phenol colour groupings as: (1) black, (2) dark brown, (3) brown, (4) light brown, and (5) negative. Hermann (1928) suggested that in wheat the phenol colouration was caused by a chemical reaction of unidentified nitrogen compounds using metal and oxygen by Csala (1972). Site of phenol colour reaction in wheat grain was the seed coat (pericarp) as observed by Friedberg (1933) and Joshi and Banerjee (1969).

PPO based phenol colour test is a simple method for classifying rice varieties in different colour groups (Oka 1958; Abrol and Uprety 1972; Vanangamudi et al. 1988; Sivasubramanian and Ramakrishnan 1974). In this test, phenol is oxidized into dark a colour melanin pigment primarily catalyzed by the tyrosinase enzyme present on the seed coat and it is under simple genetic control in wheat (Joshi and Banerjee 1970; Bhowal et al. 1969; Nair and Tomar 2001). Phenol colour response has been extensively used as a rapid laboratory test for assessing varietal purity in wheat. Based on systematic and congruous results in the literature (Casla 1972) on phenol colour responses in wheat, it was considered by ISTA for grouping and assessing genetic purity of wheat varieties. Wheat varieties of Indian origin and accessions of different ploidy levels have been tested earlier (Singhal and Prakash 1988; Singhal et al. 1991; Nair and Tomar 2001) for phenol reaction on grains and glumes and classified those into four groups as black, dark brown, brown and light brown colour; none of the genotype showed a negative reaction to phenol. The notified Indian durum wheat varieties (Kundu et al. 2006; Singh et al. 2009) and genotypes in wild species, bread wheat cultivars, durum wheat and synthetic hexaploids (SH) (Niranjana et al. 2018) have been characterized for phenol reaction. The inheritance study showed that development of phenol colour in grains was governed by three dominant alleles (Niranjana et al. 2018).

The aspects of Distinctness, Uniformity and Stability (DUS) are fundamental for characterization and protection of varieties. Phenol colour of a variety is an approved characteristic for DUS testing of wheat varieties for its protection. According to DUS test guidelines, wheat varieties are classified into five groups (as discussed in the present paper) based on intensity of phenol colour reaction on the seed coat of grains. Information on phenol content in varieties of different phenol colour groups and further in grain at different maturity period and ear head positions as well as in different ear heads in a plant in wheat are not known. Information available on wild species as potential donor of PPO based browning is not available. Keeping this information in mind, a study was, therefore, undertaken to identify different cultivated varieties and wild species with diverse genomes with low phenol colouration and subsequently low phenol content at various stages of growth and maturity since its induction after flowering.

Materials and methods

Materials

The following varieties and wild species either collected (grown in 2018-19) or fresh seeds produced in *rabi*, 2019-20 in the field of ICAR-Indian Agricultural Research Institute, New Delhi were used for the phenol colour studies (Tables 1 and 2). The seeds of wild species were collected from the Division of Genetics, ICAR-IARI, New Delhi. The materials used have been referred to as fresh seeds (produced in *rabi*, 2019-20). Part of the seeds collected from the ear heads of the varieties was kept at ambient conditions and colour reaction was studied after about one year after harvest (referred to as stored seeds). The cultivated varieties belonging to different phenol colour reaction groups which were grown in winter season of 2020 are mentioned in

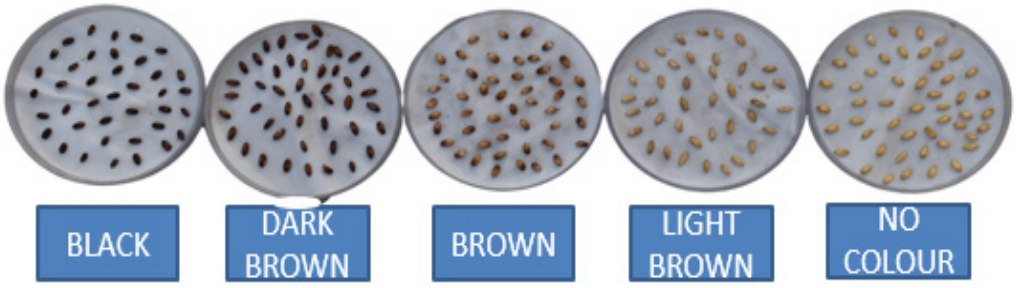

Fig. 1. Wheat varieties classified in 5 colour groups based on phenol colour reaction

Table 1. Bread wheat and macaroni wheat (d) varieties belonging to different phenol colour reaction groups grown in *rabi* 2019-20

Black	Dark brown	Brown	Light brown	No colour
HD 2967	MACS 9 (d)	C 306	HD 2894	HI 8627 (d)
HD 2733	HI 7483 (d)	HD 2932	HD 2888	MACS 2694 (d)
DWR 137 (d)	HD 2851	K 8027	HD 4530 (d)	HD 4672 (d)

D = durum

Table 2. The wild diploid *Triticum* spp. (A, S/B and D genomes) belonging to different phenol colour reaction groups (harvest of *rabi* 2018-19) to study phenol content and the effect of storage on it

Black	Dark brown	Brown	Light brown	No colour
T. monococcum acc. IARI67 (AA)	*T. urartu* EC578249 (AA)	*Ae. spelltoides* acc. IARI133 (SS/BB)	*Ae.squarrosa* acc. 14231 (DD)	*Ae. squarrosa* acc. 14227 (DD)
T. monococcum acc. WLT2808 (AA)	*T. urartu* acc. WLT4049 (AA)	*Ae. spelltoides* acc. IARI253 (SS/BB)	*Ae. spelltoides* acc. IARI118 (SS/BB)	*Ae. squarrosa* acc. 14171 (DD)
T. monococcum acc. IARI64 (AA)	*T. urartu* acc. WLT 4047 (AA)	*Ae. spelltoides* acc. WLT203 (SS/BB)	*Ae. spelltoides* EC383066 (SS/BB)	*Ae. squarrosa* acc. DWR33 (DD)

acc.= Accession number

Table 3. Total phenol content (mg/g) in grains at different stages of growth and maturity in the selected wheat varieties

Phenol colour group	Variety	Total phenol content (mg/g) at stages				Mean
		S-2	S-3	S-4	S-5	
Black	HD 2967	8.756	9.6	8.622	8.222	8.8
	HD 2733	8.756	10.089	9.244	8.178	9.067
	DWR 137	8.844	10.133	8.889	8.044	8.978
Dark Brown	MACS 9	7.822	8.667	7.911	7.289	7.922
	HI 7483	7.6	8.8	7.778	6.978	7.789
	HD 2851	7.6	8.578	7.689	7.022	7.722
Brown	C-306	5.511	7.378	7.067	6.667	6.656
	HD-2932	5.956	7.289	7.111	6.622	6.744
	K 8027	6	7.111	6.978	6.4	6.622
Light Brown	HD 2894	4.844	6.978	6.711	6.089	6.156
	HD 2888	4.978	6.978	6.933	6.178	6.267
	HD 4530	5.022	6.844	6.711	6.311	6.222
No colour	HI 8627	4.444	6.622	6.356	5.6	5.756
	HD 4672	4.311	6.622	6.711	5.422	5.767
	MACS 2694	4	6.4	6.222	5.244	5.467
Mean		6.296	7.873	7.396	6.684	

CD (P=0.05): Variety: 0.517; Stage:0.217; Variety x Stage: NS

Table 1. The wild and related species of wheat belonging to different phenol colour reaction groups which have been studied for phenol content and the effect of storage on it are mentioned in Table 2.

The wheat varieties belonging to different phenol colour reaction groups (Fig.1) have been studied with respect to maturity, inter-ear head and intra-ear head variation and the effect of storage.

Phenol colour reaction

Four replicates of 100 seeds each were used for cultivated varieties and 25 seeds were used for wild species. The seeds were soaked in distilled water for 16 hours then flushed with tap water and the excess water removed from the surface of the seeds. The seeds were then placed on two layers of filter paper in a Petri plate moistened with a 1% phenol solution. After 4 hours of incubation in an incubator with 25°C, colour reaction developed by the seed was recorded (ISTA 1966).

Total phenol content

For estimation of total phenol, kernel was grounded and fine powder was made. It was kept in air tight container with liquid Nitrogen (-196°C) until used for further procedure. Kernel powder was used for phenol content estimation following the procedure suggested by Singleton et al. (1965). The data were analysed following standard statistical procedures (Panse and Sukatme 1967).

Execution and experimentation

The data recording was done at four different stages. Freshly harvested grains in *rabi,* 2019 after drying were tested for phenol colour response. The different species were also used to study variation in phenol colour reaction.

Analysis of phenol colour reaction at different stages of grain development and growth was done. Just before emergence of spike from the boot leaf, about 60 main tillers were tagged. Ten spikes were sampled from each variety in different stages as indicated below: Stage 1 - only the upper spike portion are out (pre-anthesis); Stage 2–15 days after

Table 4. Total phenol content (mg/g) in different spikes of a plant in wheat varieties of phenol colour groups

Phenol colour group	Name of variety	Phenol content (mg/g) in			Mean
		Spike 1	Spike 2	Spike 3	
Black	HD 2967	8.178	8.044	8.133	8.119
	HD 2733	8	8	7.911	7.97
	DWR137	8.044	8.133	8.089	8.089
Dark brown	MACS 9	6.933	7.067	6.889	6.963
	HI 7483	6.844	6.889	6.889	6.874
	HD 2851	7.022	6.933	7.022	6.993
Brown	C 306	6.444	6.533	6.578	6.519
	HD 2932	6.533	6.756	6.711	6.667
	K 8027	6.756	6.756	6.622	6.711
Light brown	HD 2894	6.178	6.222	6.311	6.237
	HD 2888	6.267	6.444	6.222	6.311
	HD 4530	6.178	6.311	6.267	6.252
No colour	HI 8627	5.422	5.422	5.422	5.422
	HD 4672	5.644	5.556	5.511	5.57
	MACS 2694	5.378	5.289	5.422	5.363
Mean		6.65	6.69	6.64	

CD (P=0.05):Variety: 0.234; spike: NS ;Variety x spike: NS

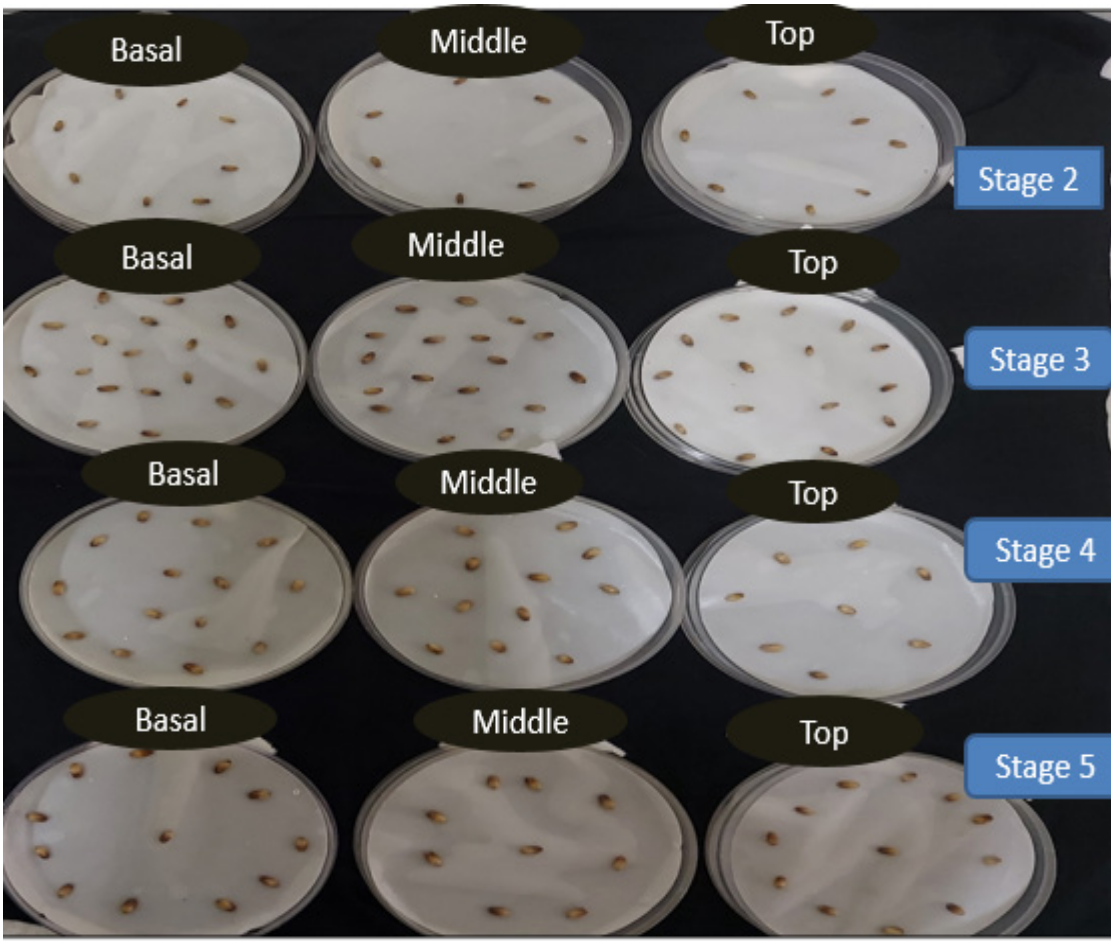

Fig. 2. Intra-spike phenol colour reaction in different stages

Table 5. Total phenol content (mg/g) in grains in top, middle and basal position of a spike at maturity in the selected wheat varieties

Phenol colour group	Name of variety	Phenol content (mg/g) in spike position			Mean
		Top	Middle	Basal	
Black	HD 2967	8.222	8.844	8.089	8.385
	HD 2733	8.178	8.711	8	8.296
	DWR 137	8.044	8.667	7.911	8.207
Dark brown	MACS 9	7.689	8.267	7.644	7.867
	HI 7483	7.778	8.311	7.556	7.881
	HD 2851	7.822	8.311	7.689	7.941
Brown	C 306	7.333	7.644	6.933	7.304
	HD 2932	7.6	7.644	6.756	7.333
	K 8027	7.244	7.733	7.067	7.348
Light brown	HD 2894	6.667	7.067	6.533	6.756
	HD 2888	6.622	7.111	6.4	6.711
	HD 4530	6.667	7.067	6.489	6.741
No colour	HI 8627	6.267	6.4	5.911	6.193
	HD 4672	6.222	6.533	5.778	6.178
	MACS 2694	6.044	6.489	5.733	6.089
Mean		7.227	7.653	6.966	

CD(P=0.05) Variety:0.482; Pos.:0.216; Variety x Pos.: NS

Table 6. Variation in Total phenol content(mg/g) in cultivated varieties and wild species in fresh and one year old seeds

Colour group	Seed lot	Total phenol content(mg/g)	
		Cultivated variety	Wild spp.
Black	Fresh	8.162	8.438
	Old	8.681	8.469
Dark brown	Fresh	7.081	8.09
	Old	7.733	8.12
Brown	Fresh	6.563	7.143
	Old	7.007	7.132
Light brown	Fresh	6.192	5.684
	Old	6.667	5.775
No colour	Fresh	5.466	4.391
	Old	5.733	4.401

CD(P=0.05) Variety: 0.222; seed lot: 0.14 Wild spp.:0.24; seed lot: NA.

the stage 1, full spike exertion (post-fertilization); Stage 3–10 days after the stage 2, dough stage; Stage 4 –10 days after stage 3 and stage 5-harvest maturity.

Spikes were kept in an envelope and dried in an oven at 35˚C for 4–5 days continuously. Subsequently, sampling and drying of grains all the varieties was done, the phenol colour response was examined using the standard procedure mentioned earlier. For analysis of inter- spike and intra- spike variation, ear heads of the selected varieties were sampled. After drying, single was divided into three equal parts and named as top, middle, basal and the seeds were studied for phenol colour response. For analysis of inter-spike variation for phenol content, three spikes of each plant were chosen.

Dried grains collected at different growth and maturity of spike were used for the estimation of total phenol (Singleton et al. 1965).

Results and discussion

Phenol content at different growth stages

Total phenol content in kernel at different stages of growth period was estimated. It was observed that mean of black colour variety was 9.067 mg/g in case of HD 2733 and mean of no colour group was 5.467 mg/g in case of MACS 2694. Among 15 varieties, mean of stage 2 was 6.29 mg/g phenol content, whereas mean total phenol content in stage 3, 4 and 5 were 7.87, 7.39, and 6.68 mg/g, respectively (Table 3). The black coloured varieties showed the highest total phenol (9.067 mg/g) and no coloured varieties showed the lowest (5.467 mg/g). There was a gradual reduction in total phenol content from the black coloured to no coloured varieties. The average phenol content was recorded significantly higher at third stage (Table 3).

Kruger (1976) reported that polyphenol oxidase was formed early in grain development and decreased with its maturation. A large part of the polyphenol oxidase in the immature wheat grain was present in the endosperm. Clancy et al. (1982) concluded that the phenol test could be used for variety identification purposes once the seed reached the hard dough stage and the chlorophyll content had declined. When chlorophyll content of the chaff was high, very little stain in green wheat seed was produced by phenol. The results of present study were supported by this report as far

Table 7. Total phenol content (mg/g) in fresh seeds of different wild species and ploidy level.

Colour group	Diploid	Mean	Tetraploid	Mean	Hexaploid	Mean
Black	*T. monococcum* acc. IARI67	8.387	*T. polonicum* acc. SP 8	8.387	HD 2967	8.267
	T. monococcum acc. WLT2808	8.51	T. carthalicum acc. IARI 194	8.4	HD 2733	8.178
	T. monococcum acc. IARI64	8.417	DWR 137	8.04	*T. spelta* PI 348597	8
Mean		8.49		8.277		8.148
CD(P=0.05): PLOIDY:0.163						
Dark brown	*T. urartu* acc. 578249	8.143	*T. polonicum* acc. IARI 195	7.157	*T. spelta album* IARI acc. acc. 186	6.767
	T. urartu acc. WLT4049	8.055	MACS 9	7.289	*T. vavilovi* acc. IARI 188	6.833
	T. urartu acc. WLT 4047	8.073	HI 7483	6.933	HD 2851	7.022
Mean		8.091		7.126		6.874
CD(P=0.05): PLOIDY:0.136						
Brown	*Ae. spelltoides* acc. IARI 133	7.077	*T. polonicum* acc. LSP 9	7.18	*T. spelta* acc. 348494	6.817
	Ae. spelltoides acc. IARI 253	7.17	*T. polonicum* acc. LSP 10	7.14	C 306	6.667
	Ae. spelltoides acc. WLT203	7.207	*T. turgidum* acc. acc. MSP6	7.087	HD 2932	6.622
Mean		7.151		7.136		6.702
CD(P=0.05): PLOIDY:0.136						
Light brown	*Ae. squarrosa* acc. 14231	5.267	*T. polonicum* acc. IARI 197	6.123	HD 2894	6.089
	Ae. spelltoides acc. IARI 118	5.313	*T. abyssinium* acc. IARI 203	6.127	HD 2888	6.178
	Ae. spelltoides EC383066	5.617	HD 4530	6.311	A-90	6.133
Mean		5.399		6.187		6.133
CD(P=0.05): PLOIDY:0.116						
No colour	*Ae. squarrosa* acc. 14227	4.493	HI 8627	5.6	Sel. 3113	5.4
	Ae. squarrosa 14171	4.2	HD 4672	5.467	Sel. 3139	5.233
	Ae. squarrosa acc. DWR 33	4.48	MACS-2694	5.333	Sel. 3157	5.167
Mean		4.391		5.467		5.267
CD(P=0.05): PLOIDY:0.077						

as phenol content in relation to grain maturity is concerned.

Studies on phenol colour reaction of grain at different growth and maturity stage showed induction of phenol colour reaction occur post-anthesis. In the later stages, *i.e.,* at dough and harvest maturity stages seeds showed similar phenol reaction with low intensity. Phenol content estimated at different stages of growth and maturity of rice grain showed the highest at post-fertilization stage, and it decreased in later stages (Kumar et al. 2021).

Inter- spike variation for phenol content

Total phenol content was analyzed in the seeds of spikes *viz*. early, mid and late (named as spike 1, spike 2 and spike 3, respectively), collected at maturity stage. The results showed that there was no significant difference among the spikes of the same plant in all the colour group varieties. Among the 15 varieties, spike 1 showed 6.65 mg/g, spike 2 had 6.69 and spike 3 showed 6.64 mg/g phenol content (Table 4).

Intra- spike variation for phenol content

A significant difference in the total phenol content in the grain positioned in the top, middle and basal portion in a spike at grain maturity stage of 15 varieties in different phenol colour reaction groups was recorded. In general, the seeds at the middle of a spike showed the highest mean total phenol content (7.653 mg/g). In maturity stage of the grains, the highest mean total phenol content of 8.844 mg/g was found in the middle portion of HD 2967 in black coloured group and the lowest of 5.733 mg/g in the bottom portion of MACS 2964 (Table 5 and Fig.2).

Phenol content and storage

An analysis of total phenol content (mg/g) in cultivated varieties and wild species showed that there was a progressive decrease of phenol content in different colour groups. In cultivated varieties, in black colour group, fresh seeds showed total phenol content of 8.162 mg/g while one year old showed mean of 8.681 mg/g (Table 6). In wild species, fresh seeds showed a mean of 8.438 mg/g as compared to 8.469 mg/g of stored seeds (Table 6). It was found that in cultivated varieties, there was a significantly

higher value of phenol content in old seeds while storage did not affect phenol colouration in wild species indicating that the genes producing phenol remain conserved. However, the phenol content was higher in wild species in black and dark brown groups. While in light brown and no coloured genotypes, wild species showed very less phenolic activity.

Phenol colour reaction and its content in wild species of diverse genome

Effort were also made to understand different genomes of wheat and their response to phenol colour reaction. The mean phenol content in different cultivars and wild species was analysed and estimated. In general, it was observed that phenol content was higher in wild species than that in the cultivated ones. It was found that among black coloured cultivars, phenol content was highest in *T. monococcum* WLT 2808 *i.e.,* 8.51 mg/g and the lowest in *T. spelta* PI 348597 *i.e.,* 8 mg/g. In dark brown group, *T. urartu* 782495 showed highest phenol content (8.143 mg/g) while the lowest value was found in *T. spelta album* (6.767 mg/g). In brown group, highest average phenol content was found *in Ae. spelltoides* WLT203 of 7.207 mg/g and lowest in hexaploid variety, HD 2932 (6.622 mg/g). In light brown group, HD 4530 showed the highest value of 6.311 mg/g and *Ae. squarrosa* 14231 showed lowest value of 5.267 mg/g. In no coloured group, cultivated durum cultivar showed a little higher phenol content while wild. *Ae. squarrosa* 14171 showed the lowest value of 4.222 mg/g (Table 7).

Among the black and dark brown genotypes, highest PPO activity was found in diploid species with AA genome *i.e., T. monococcum* and *T. urartu* and decreased gradually as we moved from diploid to tetraploid to hexaploid genotypes showing that cultivated varieties may have been bred in such a way so as to possess a reduced PPO activity. While this trend deviated significantly as we proceeded in a similar manner in light brown and no coloured genotypes. The cultivated tetraploid durum wheat (AABB) showed higher PPO activities as compared to *Ae. squarrosa* (DD) and *Ae. spelltoides* (SS/BB) genome in light brown and no colour genotypes indicating that DD and SS/BB genotypes may not be the source of gene for higher PPO activity. Variation in colour development indicated that the locus got mutated at diploid level and both alleles producing colour and not producing colour were contributed to tetraploid wheats (Niranjana et al. 2018).

Bhowal et al. (1969) also studied origin and evolution of phenol colour reaction and reported that the gene(s) conferring phenol reaction originated from the A genome in emmer and common wheat. Zeven (1972) provided evidence for two genes in hexaploid wheat located on 2A and 2D chromosomes. Nair and Tomar (2001) genetically analysed the grains of tetraploid and hexaploid wheat and reported that tetraploid wheat varieties Bijaga Yellow and HD 4530 each carrying single gene which are non-allelic indicating two independent genes. Southern blots results suggested that each of the diploid wheat relative has two or more kernel-type PPO genes. There was an apparent correlation between the number of potential kernel-type PPO genes and level of PPO activity. For example, *T. monococcum* accessions all had high PPO activity and appeared to have more kernel-type genes than the other diploid species. *T. monococcum* has very high PPO activity and appears to be the ancestral origin of the high PPO allele, PPO-A1a, of hexaploid wheat (Fuerst et al. 2008). Report on low magnitude of PPO activity in *Ae. speltoides* indicated that B genome also carry gene(s) for phenol reaction (Fuerst et al. 2008). They also proposed an additional loci contributing to PPO activity is located on 7B chromosome (PI481521). Earlier, Watanabe et al. (2006) mapped two genes *Tc1* and *Tc2* for high phenol colour reaction on 2A and 2B chromosomes, respectively, in durum wheat. A gene for high PPO activity has also been mapped on 2D chromosome by Jimenez and Dubcovsky (1999) in Chinese spring. Recently, Sadeque et al. (2017) studied mode of inheritance of polyphenol oxidase activity in doubled haploid population derived from a hybrid between VAW08-A17 and QALB is and reported PPO activity is controlled by three genes located on 2AL, 2BS and 2DL (additive mode of action) chromosomes in wheat.

Therefore, in the present study the tetraploid varieties, namely, HI 8627, HD 4672 and MACS 2694 were identified with no colour reaction varieties with low phenol content. Similarly, among the wild diploid species, *Ae. squarrosa* acc. 14227, *Ae. squarrosa* 14171 *and Ae. squarrosa* DWR33 and bread wheat genotypes, Sel. 3113, Sel. 3139 and Sel. 3157 with no phenol colour reaction and low phenol content. These may be used as potential donor with low phenolic activity in breeding low phenol contents in wheat.

Authors' contribution

Conceptualization of research (SKC, SMST); Designing of the experiments (SKC, PM); Contribution of experimental materials (SKC, NM, SK); Execution of lab experiments (PM); Analysis of data and interpretation (PM, SKC); Preparation of the manuscript (PM, SKC, SMST).

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