



Characterization and genetic variability of wheat genotypes for polyphenol oxidase (PPO) activity

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Abstract

Noodles and chapatti having white or slightly yellow colour are preferred by consumers. Polyphenol oxidase (PPO) enzyme is responsible for discoloration of wheat products. One hundred wheat genotypes were screened using L-DOPA and phenol assays for PPO activity. Activity at 30 min and 90 min of incubation ranged from 13.78 to 100.27 and 5.38 to 72.18 au/min/g, respectively. Genotypes RAJ3766 (99.38), RAJ4266 (76.44) and DWR9507 (72.37) exhibited highest PPO activity, whereas NIAW2349 (18.05), BANSI988-18 (19.72) and NIAW2348 (19.94) showed lowest PPO activity. Interaction data indicated significant differences between genotypes and different seasons, emphasizing role of seasons on expression of PPO. Strong correlation was observed between PPO at 30 min and 90 min incubation. However, PPO30 could serve as a reliable estimate than PPO90. The correlation between PPO30 and phenol were not consistent and varied from strong ($r=0.7304$) to weak ($r=0.2377$) in different years.

Keywords: L-DOPA, Polyphenol oxidase, Wheat

INTRODUCTION

Wheat is the second largest crop grown in India after rice and occupies an area of ~30.55 m.ha with production of 107.18 m. t. (IIWBR Annual Report, 2020). It is consumed in different forms like chapati, noodles, bread, biscuits, pasta, pan and flat bread, cake, rolls etc. The organoleptic quality of any food material depends on its taste, color and appearance. In Asian subcontinent, wheat is largely consumed as noodles, chapati and unleavened breads. The physiochemical properties, color and taste of these wheat products are determined by the wheat flour used for dough preparations which are in turn dependent on textural and structural properties of the kernel. Wheat based products as determined by grain quality parameters have resulted in identification of key factors governing the product color (Tikoo *et al.*, 1973; Singh and

Sheoran, 1972). Various protocols have been established for screening of wheat genotypes for dough color, which is responsible for color of the finished products, such as noodles and chapati. Polyphenol oxidase (PPO) is an enzyme located in bran layer of the wheat kernel that exists in 12 isozyme forms (Okot *et al.*, 2001), responsible for discoloration of the dough and ultimately the end-product (Kruiger *et al.*, 1994). It's a copper-containing metallo-protein, catalyzes the hydroxylation of monophenols to *o*-diphenols and dehydrogenation of *o*-dihydroxyphenols to *o*-quinones. These *o*-quinones undergo auto-oxidation and in turn react with proteins and other cell constituents to produce dark colored secondary products (melanins) and contribute to browning in various tissues and food products (Anderson and Morris, 2001). Discoloration

of flat bread has also been attributed to PPO activity (Tikoo *et al.*, 1973). Polyphenol oxidase enzyme is a major cause of the time dependent discoloration in noodles, chapattis, and other wheat products (Morris *et al.*, 2000).

The activity of PPO is genetically regulated and also influenced by environment (Ge *et al.*, 2003). It's activity is manifested into a physio-biochemical trait that can not be easily evaluated based on phenotype (Gaicomo *et al.*, 2014). The genes encoding wheat PPOs are members of two paralogous PPO families viz., PPO-01 and PPO-02 located on the group 2 chromosomes (Beecher *et al.*, 2002). Among the PPO genes, PPO A1, PPO A2, PPOD1 and PPOD2 are expressed at substantial levels in developing kernels, whereas PPO B1 and PPO B2 are not expressed (Zhang *et al.*, 2005). Interaction of these genes and influence of environment contribute to variation in their response and levels of PPO in grain.

Genetic improvement of crops for quality traits is necessary for the overall improvement in quality of wheat and wheat products. In mutation breeding experiments, very large M_2 population is required to identify mutants with desired traits. For evaluation of such a large population and identifying low PPO mutants, simple, reproducible and fast screening techniques are required. PPO is known to be located in bran layer of kernel and therefore, substrate based protocols in which seeds are soaked in water and then analyzed for PPO activity not only gives fair results but also allows using same seeds for raising further generations as these protocols are non-destructive and does not hamper seed germination. Various substrate based protocols have been established to study the PPO activity in wheat. The substrates were L-DOPA, catechol, pyrocatechol, methylcatechol, phenol, chlorogenic acid and tyrosine. Anderson and Morris (2001) studied PPO activity using tyrosine, catechol, methylcatechol, phenol, cafferic acid and L-DOPA and found that cafferic acid and tyrosine showed poor solubility and intermediate reaction kinetic. Methyl catechol was observed to get auto-oxidized at pH 6.5 and showed intermediate kinetics. Seeds incubated in catechol were found to exhibit reduced germination and phenol also demonstrated poor kinetics. L-DOPA was found to show excellent kinetics, good solubility, least oxidation at pH 6.5 and no significant effect on seed viability. Phenol colour test has also been applied for rapid testing of grain lots and correlating it with noodles or bread quality (Wrigley, 1976). Conversely, these PPOs are also associated with protecting the plants against various biotic and abiotic stresses (Rai *et al.*, 2011) and hence a balance has to be achieved in determining the optimal activity without affecting the agronomical value of the plants.

In this study, 100 wheat genotypes (stable advanced breeding lines and varieties) grown in central and peninsular zones of India were screened using L-DOPA and phenol substrate based assays. Efforts were made

to identify the fast and reproducible screening techniques and their utilization in cost effective screening of large breeding populations.

MATERIALS AND METHODS

One hundred genotypes of wheat including released varieties and advanced lines were selected based on their stability, agronomical traits and yield levels. These lines were collected from Wheat Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra and Agriculture Research Station, Mahatma Phule Krishi Vidyapeeth, Niphad Nasik, Maharashtra. They were tested for their purity, agronomical performance and maintained at Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Center Mumbai. Selected genotypes were grown in *rabi* season of 2016-17 and 2017-18. All the recommended agronomic practices were followed to raise successful crop. Grains were harvested from each of the genotype and threshed to get the grains for biochemical analysis.

The genotypes were evaluated by two different substrate based protocols. Substrates were L-DOPA and phenol. The two assays were compared to study the efficiency of the protocols and correlate the results for easy, reproducible and fast screening of large number of accessions.

Assay developed by Anderson and Morris (2001) was followed to determine the PPO activity using freshly prepared L-DOPA solution (LOBA Chemie, Mumbai, India) as substrate. Three seeds of each genotype were weighed and placed in 2 ml centrifuge tubes. L-DOPA substrate solution (1.5 ml) was added to tubes and placed on constant shaking (Bangalore Genei, India). L-DOPA substrate assay is amenable for single seed screening but 3 - 5 seeds are preferred (Kruger *et al.*, 1994). Absorbance was recorded using spectrophotometer (Jasco, USA) at 475 nm across two time intervals i.e., after 30 and 90 min. of incubation.

Phenol colour test previously reported by Pieper (1922) and further modified by Joshi *et al.* (1969) and Anderson and Morris (2001) was used to characterize the wheat genotypes for phenol reaction. Grading of the seeds based on their phenol reaction into four classes was followed as described by (Watanabe *et al.*, 2004). Five seeds were soaked in distilled water for 18 hrs and later incubated in 1% phenol solution for 4 hrs. Phenol treated seeds were then dried for 30min. and graded on 1 to 4 scale as per the intensity of color development. (1:Off white, 2:Light Brown, 3:Brown, 4:Dark Brown).

The ANOVA for phenol test at different time intervals and its seasonal interactions were carried out using RBD variability analysis module of TNAU STAT software (Manivannan, 2014). In addition, PCV, GCV, broad sense heritability and Pearson's (r) correlation coefficients

were also determined using the same software. Genetic advance (GA) was estimated as per formula given by Allard (1960), $GA = K \times \sigma_p \times h^2(b)$ Where, K = Selection differential at 5% selection intensity which accounts to a constant value 2.06, σ_p = Phenotypic standard deviation and $h^2(b)$ represents broad sense heritability. Cluster analysis was done using PAST software.

RESULTS AND DISCUSSION

Breeding for quality traits need easy and fast screening techniques, such that large number of genotypes or populations could be screened effectively within a short span of time. Effective and fast screening techniques enable proper study of the genetics and in advancement of desired genotype for yield improvement or for using the selected genotype in hybridization programmes. In this context, present study aimed at screening of Indian wheat genotypes for PPO activity.

On pooled mean for both the years, genotypes exhibited significant differences for PPO activity with F-value of 33.92 (at $p < 0.001$). Data on interaction effect between genotypes and both the seasons indicate that significant differences were observed among the genotypes in both the season (at $p < 0.001$). This indicates that seasons play important role in expression of PPO activity in wheat germplasm. Wheat genotypes showing high value in one season may exhibit low values in another season. Kruger *et al.* (1994) also observed that a number of CPS wheat genotypes showed colour variations during phenol test. However, their subjective ranking between the degrees of darkening was difficult to make due to quantitative nature of the trait. Similarly, role of non-PPO darkening compounds may be involved in the genotypes showing very high seasonal variation for PPO activity and needs further testing (Table 1). Substantial darkening would occur even at zero PPO activity in the kernel PPO assay. Darkening of alkaline noodles is probably due to the cultivar-specific level of PPO activity and the presence of at least one additional darkening mechanism. Hence, it is also required to identify the phenolic discoloration agent(s) and potential roles of non-PPO discoloration

mechanisms, both enzymatic and non-enzymatic, in wheat products (Fuerst *et al.*, 2006).

ANOVA for PPO at 30 minutes (PPO30) indicated that genotypes vary significantly (at $p < 0.05$) for both the seasons. However, PPO estimation at 90 minutes (PPO90) indicated significant variation among the genotypes (at $p < 0.01$) for both the seasons. The interaction effect between genotypes and season indicated high significant variation for data of PPO taken both at 30 minutes and 90 minutes (at $p < 0.001$). Thus, it could be postulated that seasonal variation played important role in PPO activity in wheat grains (Table 1). Anderson and Morris (2001) also observed similar seasonal variation for PPO activity in wheat.

In this study, the enzymatic assay using L-DOPA as substrate showed wide variation among the genotypes for PPO activity. The PPO activity measured at 30 min and 90 min of incubation ranged from 14.04 to 90.49 au/min/g and 5.38 to 44.69 au/min/g, respectively for the season 2016-17. Whereas, the PPO activity was 78 to 100.27 au/min/g and 5.72 to 72.18 au/min/g, respectively for 2017-18 season. Mean PPO activity across both the years ranged from 14.92 to 99.38 au/min/g and 6.8 to 52.91 au/min/g at 30 min and 90 min of incubation, respectively. Naqvi *et al.* (2013) studied PPO activity using caffeic acid, catechol, L-DOPA, phenol and tyrosine as substrate found that the sum of PPO activities of these substrates fell in a range of 34.15-150.27 units/min/g. In the present study, phenol test, a qualitative test to estimate the PPO activity which gives an indirect estimate of PPO activity ranged from 1.12 to 4 and 0 to 4 for both the years respectively (Table 2). In addition, wide diversity among wheat genotypes using phenol as substrate for PPO activity was observed (Fig.1). Similarly, Anderson and Morris (2001) also reported L-DOPA values for PPO from the Aberdeen environment ranging from 0.013 to 0.097 AU for 'Carifan'. Considering significant environmental impact, genotypes can be classified into three groups: (1) PPO activity less than 25AU, (2) PPO activity between 25 to 60AU and (3) PPO activity more than 60AU, after 30 min of

Table 1. Analysis of variance for phenol test at different time intervals in wheat germplasm lines

SOURCE	DF	Mean sum of square		
		Phenol test	MPA at 30# min	MPA at 90 min
Replication	2	0.045	12.97	38.88
Treatments (T)	99	3.930**	900.49**	292.12**
Seasons (S)	1	22.20**	713.93**	5.74
S x T	99	1.17**	127.11**	74.36**
Error	198	0.11	44.66	44.66
TOTAL	399	-	-	-
MEAN		40.83	18.61	2.79
CV (%)		16.74	15.93	15.46

**Significance at 0.1%

#MPA: Mean PPO activity

Table 2. Pooled mean of PPO activity estimated by L-DOPA and phenol based colorimetric test across wheat genotypes

S. No.	Genotype	Mean PPO activity at 30min	Mean PPO activity at 90min	Phenol color test
W1	GW2008-161	33.60	14.02	1.06
W2	GW 2010-288	39.10	12.73	1.37
W3	GW 2010-285	25.66	10.45	1.37
W4	NIAW 2304	43.53	17.73	3.75
W5	NIAW 1343	38.19	13.66	3.43
W6	NIAW 345	28.51	11.91	1.18
W7	NIAW 1049	23.47	11.57	1.12
W8	NIAW 2273	31.76	15.31	1.00
W9	NIAW 2302	18.93	10.65	1.31
W10	NIAW 1121	35.58	15.97	0.87
W11	NIAW 514	25.14	12.44	2.25
W12	NIAW 179	36.02	13.89	1.75
W13	NIAW 1258	26.16	16.16	3.25
W14	NIAW 2059	29.82	11.13	3.75
W15	NIAW 1045	53.88	33.73	3.50
W16	NIAW 1395	16.12	10.00	1.87
W17	NIAW 1415	14.92	10.83	2.12
W18	NIAW 612	40.28	20.46	3.87
W19	NIAW 2349	18.05	16.52	1.43
W20	NIAW 2346	43.10	19.34	3.68
W21	NIAW 2255	45.71	20.32	3.87
W22	NIAW 2348	19.94	7.66	0.93
W23	NIAW 9406	42.02	11.64	3.33
W24	MP 3336	66.81	38.47	4.00
W25	MP3097	65.59	39.95	3.87
W26	JS-6-31	40.76	20.01	1.62
W27	DBW 14	63.31	34.60	2.87
W28	DBW 17	38.87	20.78	1.5
W29	VW 916	65.13	27.99	3.00
W30	PBW 452	59.24	35.61	3.87
W31	PHS 0622	68.74	25.58	1.31
W32	DWR 9518	42.83	18.74	1.12
W33	DWR 9507	72.37	38.48	3.00
W34	FLW 1	65.51	40.54	2.62
W35	FLW 6	65.87	45.24	4.00
W36	RAJ 3063	53.99	16.85	3.37
W37	RAJ 4176	44.02	19.25	1.87
W38	RAJ 4274	40.83	17.76	2.06
W39	RAJ 4205	46.14	12.96	2.25
W40	RAJ 3766	99.38	52.91	3.50
W41	RAJ 4083	32.61	11.19	1.87
W42	RAJ 4037	33.90	8.38	2.37
W43	RAJ 4266	76.44	28.73	3.87
W44	RAJ 4268	45.46	17.56	4.00
W45	FLW 3	52.62	26.62	4.00
W46	NIAW 1279	35.43	6.80	2.10
W47	NIAW 34	35.53	21.19	3.00
W48	NIAW 2247	54.79	18.72	3.62
W49	NIAW 2075	30.03	21.49	1.50
W50	HD2998	57.74	28.05	3.87

Table 2. Continued

S. No.	Genotype	Mean PPO activity at 30min	Mean PPO activity at 90min	Phenol color test
W51	HD2932	38.5	20.29	2.37
W52	NIPHAD 4	32.79	17.33	1.75
W53	NI343	56.43	27.70	3.62
W54	NI 747-19	46.24	17.66	3.62
W55	SAMRUDHI	38.22	25.84	1.75
W56	BANSI 288-18	19.72	8.40	1.25
W57	BOB WHITE	48.20	23.83	3.75
W58	HI 1552	35.61	21.45	2.75
W59	HI 977	61.88	25.36	4.00
W60	HI 1500	36.57	16.07	2.62
W61	HS 490	59.72	16.26	3.62
W62	HS 345	45.46	14.83	3.75
W63	HS 522	36.70	12.93	2.50
W64	HW 3027	33.38	12.79	2.12
W65	JOMBHOR 1002	33.39	17.16	2.25
W66	SONALIKA	51.00	16.63	3.37
W67	AKAW 4210-6	64.98	26.69	4.00
W68	AKAW 4537	29.03	17.35	1.87
W69	LBPY 2011-10	44.03	15.52	3.81
W70	LBPY 2010- 11	19.43	8.55	1.25
W71	LBPY 2011-1	32.82	11.23	3.12
W72	LBPY 08-09	23.45	12.29	1.62
W73	LBRL-4	37.81	15.15	3.06
W74	MACS 622	51.74	16.37	3.62
W75	MACS 2496	42.80	22.72	3.87
W76	UP 2783	34.85	13.96	3.93
W77	LOK 1	53.13	24.50	3.87
W78	HW 2002	41.68	16.50	3.87
W79	PBW 343	37.29	15.46	2.93
W80	K 8027	26.80	13.71	3.25
W81	VL 852	20.11	14.47	1.87
W82	DBW 54	26.10	12.05	2.68
W83	DBW 60	37.01	12.30	3.87
W84	DBW 62	43.90	15.80	3.62
W85	NIAW 301	25.52	12.79	1.68
W86	AKAW 2344	31.45	12.44	3.18
W87	AKAW 2571	27.49	14.09	2.12
W88	AKAW 2862-2	44.27	15.11	3.87
W89	AKAW 3997	48.9	19.94	3.12
W90	AKAW 4627	30.31	21.83	2.37
W91	AKAW 4800	31.60	24.71	3.31
W92	AKAW 4842	46.98	19.93	3.50
W93	AKAW 4843	41.65	15.34	4.00
W94	AKAW 4839	41.07	15.45	3.56
W95	AKAW 3722	29.53	12.76	3.62
W96	AKAW 4730	29.97	15.60	3.62
W97	AKW 1071	23.67	8.56	2.06
W98	AKW381	31.30	12.69	3.37
W99	AKW 65-1	46.46	13.68	3.75
W100	PKV WASHIM	28.83	13.79	3.12

incubation in L- DOPA. The genotypes showing very low PPO activity can be used in quality breeding programmes for integrating the trait in high yielding background. Genotypes showing very high PPO activity can be maintained for genetic and molecular studies. Information will also be useful in eliminating high PPO genotypes and their progenies at an early stage of breeding program. From the study over both the seasons it is evident that the genotypes RAJ3766 (99.38), RAJ4266 (76.44), DWR9507 (72.37) and MP3336 (66.81) were found to

have high PPO activity whereas genotypes NIAW2349 (18.05), BANSI988-18 (19.72), NIAW2348 (19.94), VL852 (20.11) and NIAW1049 (23.47) were found to have low PPO activity. Genotypes having low PPO activity has potential to be used in quality breeding programs for incorporation of low PPO trait in otherwise high yielding genotypes. Total phenol and PPO were found lowest in RSP-566, PBW-550 and RSP-81. RSP-561 and RSP-566 had low total phenol and PPO (Mallick *et al.*, 2013).

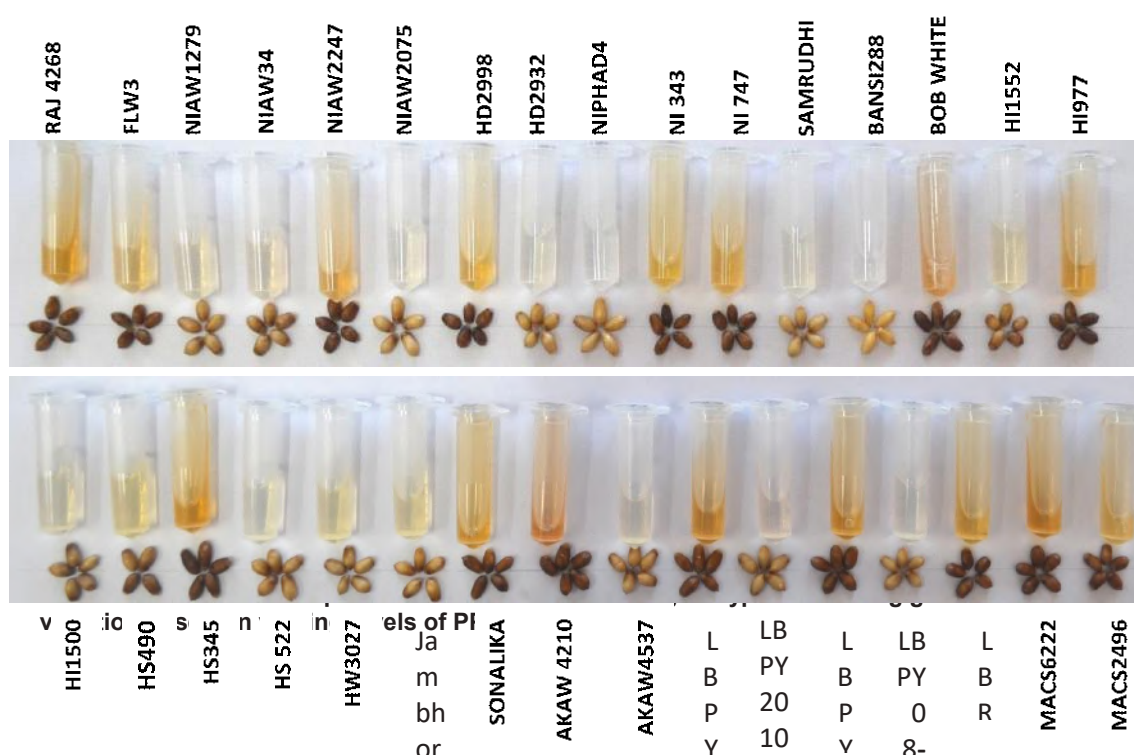


Fig. 1. Color reaction for phenol content among wheat genotypes exhibiting genetic variation based on varying levels of PPO.

The estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and heritability (h^2) are presented in **Table 3**. It is apparent that phenotypic coefficients of variation were invariably greater than the corresponding genotypic coefficient of variation though the trend of both GCV and PCV was the same. PCV for 30 min incubation was recorded as 41.21% (2016) and 40.54% (2017) and for 90 min as 41.74% (2016) and 64.34% (2017) (**Table 3**). Similarly, the GCV followed a similar trend with 37.28% and 37.71% for 30min and 37.92% and 56.67% for 90 min test in 2016 and 2017, respectively. This indicated that the variation was consistent for 30 min test in both the years. However, it varied significantly for 90 min test in both the years. For phenol test, the PCV values in 2016 and 2017 were 29.69% and 53.18%, respectively. Similarly,

the GCV values were 26.70% and 52.07% for the year 2016 and 2017, respectively. This indicated that PPO based on phenol test varied significantly over the years. Heritability estimates indicated that the PPO evaluation by L-DOPA and phenol tests were highly heritable with h^2 ranged from 80.87 to 82.51 and 77.55 to 95.88 per cent in the years 2016 and 2017, respectively. High heritability for these traits is due to high genetic variance component which indicates low deviation for PPO attributes in different replications among different genotypes. However, these heritability estimates need to be confirmed over several generations. Genetic advance, degree of gain for a trait under selection pressure, ranged from 13.11 to 29.29 for PPO activity and 1.5 to 2.69 for phenol test. PPO -30 recorded high values of genetic gain than PPO-90.

Table 3. Genetic parameters for PPO activity at 30 and 90 min. intervals in wheat genotypes

Parameters	PPO 30		PPO 90		Phenol test	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
PCV (%)	41.21	40.54	41.74	64.34	29.69	53.18
GCV (%)	37.28	37.71	37.92	56.67	26.70	52.07
Heritability (h^2)	81.83	86.55	82.51	77.55	80.87	95.88
Genetic advance	29.29	28.54	13.11	19.24	1.50	2.69

Estimation of PPO using L-DOPA at 30 and 90 min and doing phenol test are essential to see the activity of polyphenol oxidase enzyme. However, a method which is easier to do and robust will help in efficient screening of germplasm and selection. Higher correlation among these traits will help us to decide type of test to be used in screening. In the present experiment, strong correlation was observed between PPO at 30 min and PPO at 90 min in 1st year ($r=0.7773$) and 2nd year ($r=0.785$) (Table 4). This indicated that PPO30 and PPO90 can be used for estimation of PPO activity and PPO30 could serve as a better estimate than PPO90 and will help to save time. The correlation between PPO30 and phenol were not consistent and varied from strong ($r=0.7304$) to weak ($r=0.237$) in different years. Anderson and Morris (2001) also found simple linear correlation of $r = 0.56$ ($P < 0.0001$) between environments for L-DOPA values. Poor correlation was found between PPO90 and phenol test. Naqvi *et al.* (2013) also concluded that there are more than one PPO activities (isozymes) present in wheat grain, which show different preference for different substrates. Phenol score may not serve as a suitable indicator of chapatti quality (Kumar *et al.*, 2018). This indicated that PPO30 or PPO90 can serve as a better estimate of PPO activity. Kruger *et al.* (1994) studied the PPO activity among whole wheat seeds and ground wheat seeds using catechol as substrate and found Pearson correlations of 0.85 ($P < 0.001$) and concluded that whole seed assay could be used to give approximations of total PPO activity. In turn, it indicated the possibility of higher influence of seasonal or environmental factors resulting in highly variable values of phenol test and hence, resulted in poor correlation with PPO30 and PPO90 in different years. Monophenolic substrates like cathetol and phenol are less satisfactory as compared to diphenolic substrates like L-DOPA in terms of low rate of colour production. After overnight steep in distilled water, a reaction time of 30

min was sufficient for adequate discrimination of steeped seeds (Kruger *et al.*, 1994).

This clearly indicated that expression of PPO is influenced by the environment. Hence, presence of genes and congenial environment play important role in the expression of PPO and therefore, affect the resulting genetic variation. Selection for such traits is very difficult as it is influenced by environment. In the present study, using L-DOPA as substrate and measuring the absorbance after 30 min gave consistent results for PPO activity over years among genotypes and seemed to be less influenced by the environment. Phenol test exhibited significant variation among genotypes in different seasons indicating profound effect of environment and cannot serve as a reliable estimate for PPO activity but it is quite useful for very large scale preliminary screening and eliminating genotypes with high PPO activity and further confirmatory studies can be done by L-DOPA assay. Low or high PPO genotypes based on phenol test need to be confirmed over seasons. Higher correlation between PPO30 and PPO90 and relatively lower influence of seasons/environment make PPO30 a reliable assay for PPO estimation.

Cluster analysis was performed based on the PPO activity wheat genotypes following paired group method and Euclidean similarity measures. Cluster analysis for the PPO traits resolved all the wheat genotypes in to six clusters (Fig. 2). RAJ 3766 (W-40) genotype showed high PPO activity at 30 and 90 min and was diverse from rest of the genotypes with a separate cluster. Cluster-V grouped most of the wheat genotypes (40), whereas cluster-VI included only eight genotypes. The PPO activity measured after 30 min of incubation ranged between 36.57 (HI1500) to 76.44 (RAJ4266). Average PPO activity in these clusters was 63.42 au/min. In cluster 3 which

Table 4. Pairwise correlation coefficients for PPO activities (at 30 and 90 min.) and Phenol estimates

Tests	Correlation coefficient for	
	2016-17	2017-18
PPO30 V/s PPO 90	0.7773**	0.7851**
PPO30 V/s Phenol color	0.7304**	0.2377
PPO90 V/s Phenol color	0.5674*	0.1768

Significance at 5% (*) and 1% (**)

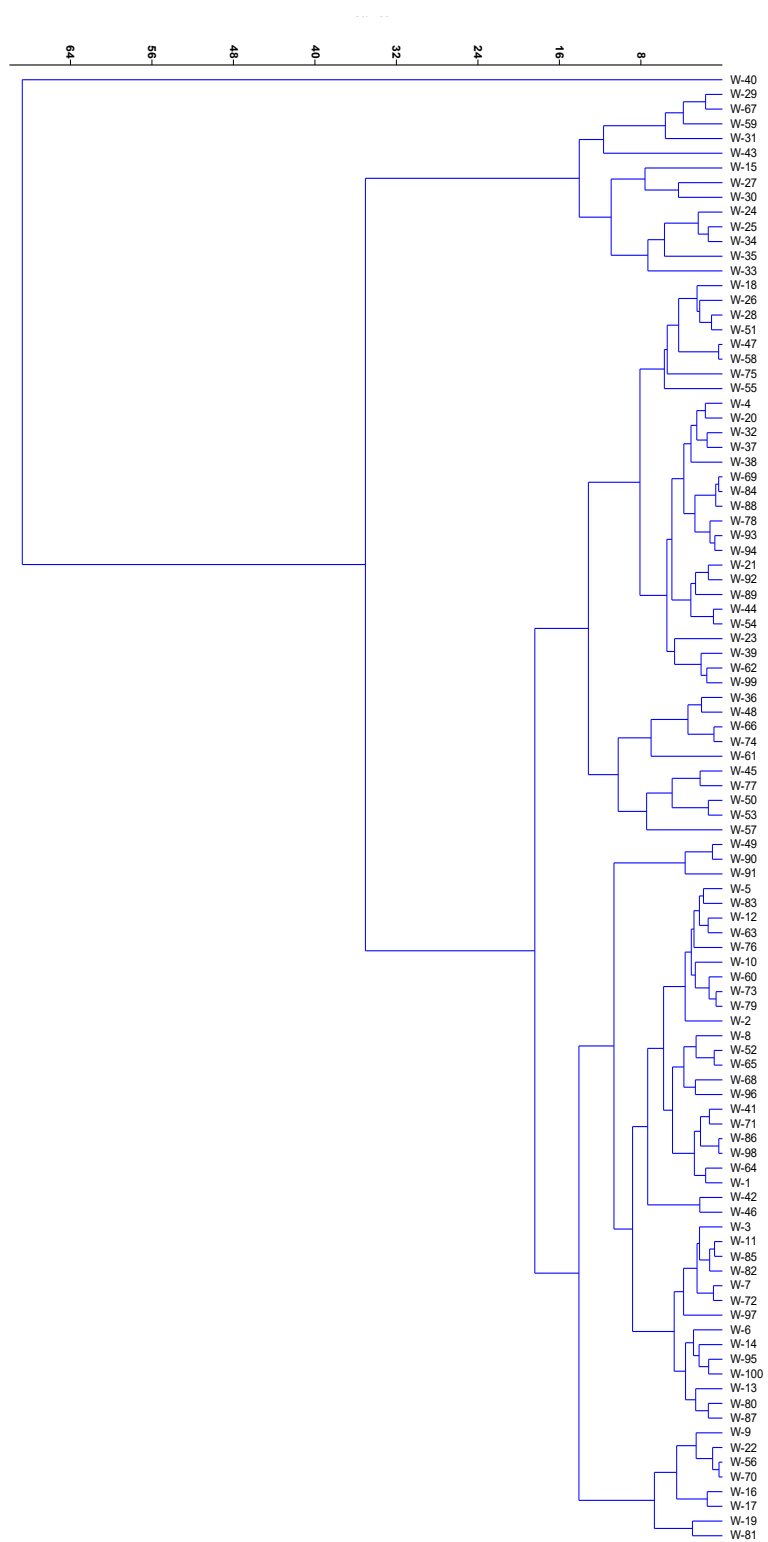


Fig. 2. Dendrogram based on Cluster analysis of wheat genotypes PPO activity

Table. 5 Cluster mean and range values of wheat genotypes for PPO activity and phenol estimation

Cluster Number	Range	Mean	Genotype
I	99.38	99.38	W40
II	36.57-76.44	63.42	W60,W15,W30,,W27,W67,W29,W34,W25,W35,W24,W31,W33,W43
III	35.53-48.90	42.67	W47,W58,W55,W51,W28,W18,W26,W38,W94,W93,W78,W23,W75,W32,W20,W4,W84,W37,W69,W88,W44,W62,W21,W39,W54,W99,W92,W89
IV	30.03-59.72	51.17	W49,W57,W66,W74,W45,W77,W36,W48,W53,W61
V	23.45-61.88	32.62	W72,W7,W97,W11,W85,W3,W82,W13,W80,W87,W6,W100,W68,W95,W14,W96,W90,W98,W86,W91,W8,W41,W52,W71,W64,W65,W1W42,W76,W46,W10,W12,W63,W83,W79,W73,W5,W2,W50,W59
VI	14.92-20.11	18.40	W17,W16,W19,W9,W70,W56,W22,W81

comprises of 28 genotypes, the PPO activity after 30 min ranged between 35.53 (NIAW34) to 48.90 (AKAW3997), while Cluster 4 is relatively small cluster consisted of 10 genotypes with PPO activity ranged between 30.03 (NIAW2075) to 59.72 (HS490). Wide range values for PPO activity were evident in cluster-6 ranged from 23.45 (LBPY08-09) to 61.88 (HI 977). Genotypes in this cluster showed least average PPO activity after 30 min. of incubation indicating their suitability for developing high yielding genotypes with low PPO content.

Extensive screening of wheat genotypes for PPO activity and phenol test indicated wide variation across different seasons. Strong correlations among these traits would help in better selection of wheat genotypes. This provides a quick and cost-effective screening of large screening of wheat genotypes.

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REFERENCES

- Allard, R.W. 1960. Principles of plant breeding. John Willey and Sons. Inc. New York, 485.
- Anderson, J.V. and Morris, C.F. 2001. An improved whole-seed assay for screening wheat germplasm for polyphenol oxidase activity. *Crop Science*, **41**:1697-1705. [Cross Ref]
- Annual Report 2020, ICAR - Indian Institute of Wheat and Barley Research, Karnal-132001, Haryana, India
- Beecher, B., Bettge, A., Smidansky, E. and Giroux, M. 2002. Expression of wild-type pinB sequence in transgenic wheat complements a hard phenotype. *Theoretical and Applied Genetics*, **105** (6): 870-877. [Cross Ref]
- Fuerst, E.P., Anderson, J.V. and Morris, C.F. 2006. Delineating the role of polyphenol oxidase in the darkening of alkaline wheat noodles. *Journal of Agricultural and Food Chemistry*, **54** (6): 2378-2384. [Cross Ref]
- Ge, X.X., He, Z.H. and Yang, J. 2003. Polyphenol oxidase activities of Chinese winter wheat cultivars and correlations with quality characteristics. *Acta Agronomica Sinica*, **29** (4): 481-485.
- Joshi, M.G., Banerjee, S.K. and Naik, M.S. 1969. Tyrosinase activity as a quantitative indicator of genetic control of phenol colour reaction in tetraploid triticums. *Indian journal of experimental biology*, **7**:189 – 190.
- Kanimozhi, S., Kumaresan, D., Latha, T.K.S. and Thiruvengadam, T. 2021. Morphological and biochemical characteristics associated with powdery mildew resistance in M3 population of blackgram (*Vigna mungo* (L.) Hepper). *Electronic Journal of Plant Breeding*, **12** (3):983-989.
- Kruger, J.E., Hatcher, D.W. and DePauw, R. 1994. A whole seed assay for polyphenol oxidase in Canadian prairie spring wheats and its usefulness as a measure of noodle darkening. *Cereal chemistry*, **71** (4):324-326.
- Kumar, S., Sohu, V.S., Gupta, S.K., Singh, R.P. and Bains, N.S. 2018. Understanding the chapati making attributes of the Indian wheats: The physico-chemical basis. *Journal of Applied and natural science*, **10** (2):572-592. [Cross Ref]
- Mallick, S.A., Azaz, K., Gupta, M., Sharma, V. and Sinha, B.K. 2013. Characterization of grain nutritional quality in wheat. *Indian journal of plant physiology*, **18** (2):183-186. [Cross Ref]
- Manivannan, N. 2014. TNAU-STAT-Statistical package. Retrieved from <https://sites.google.com/site/tnaustat>.
- Morris, C.F., Jeffers, H.C. and Engle, D.A. 2000. Effect of processing, formula and measurement variables

- on alkaline noodle color—Toward an optimized laboratory system. *Cereal Chemistry*, **77** (1):77-85. [\[Cross Ref\]](#)
- Mukhtar, M., Arul, L., Jayamani, P. and Kokiladevi, E. 2020. Understanding the role of different biochemical compounds responsible for inducing resistance in pigeon pea towards *Helicoverpa armigera* infestation. *Electronic Journal of Plant Breeding*, **11** (03): 860-866.
- Naqvi, S.M.S., Batool, I., Farooq, M.U., Deebea, F., Hyder, M.Z. and Mahmood, T. 2013. Polyphenol oxidase activities in wheat (*Triticum aestivum* L.) grain. *Pak. J. Bot.*, **45** (2):407-410.
- Okot-Kotber, M., Liavoga, A., Yong, K.J. and Bagorogoza, K. 2001. Activity and inhibition of polyphenol oxidase in extracts of bran and other milling fractions from a variety of wheat cultivars. *Cereal chemistry*, **78** (5):514-520. [\[Cross Ref\]](#)
- Pieper, H. 1922. Ein Mittel zur Unterscheidung von Weizensorten am Korn. Deutsche Landwirtschaftlinche Presse, **49** (67):438-439.
- Rai, G.K., Kumar, R., Singh, J., Rai, P.K. and Rai, S.K. 2011. Peroxidase, polyphenol oxidase activity, protein profile and phenolic content in tomato cultivars tolerant and susceptible to *Fusarium oxysporum* f. sp. *lycopersici*. *Pak. J. Bot.*, **43** (6):2987-2990.
- Singh, R. and Sheoran, I.S. 1972. Enzymic browning of whole wheat meal flour. *Journal of the Science of Food and Agriculture*, **23** (1):121-125. [\[Cross Ref\]](#)
- Tikoo, S., Singh, J.P., Abrol, Y.P. and Sachar, R.C. 1973. Studies on polyphenol oxidase in wheat grains. *Cereal Chemistry*, **50**:520-528.
- Watanabe, N., Takeuchi, A. and Nakayama, A. 2004. Inheritance and chromosomal location of the homoeologous genes affecting phenol colour reaction of kernels in durum wheat. *Euphytica*, **139** (2):87-93. [\[Cross Ref\]](#)
- Wrigley, C.W. 1976. Single-seed identification of wheat varieties: Use of grain hardness testing, electrophoretic analysis and a rapid test paper for phenol reaction. *Journal of the Science of Food and Agriculture*, **27** (5):429-432. [\[Cross Ref\]](#)
- Zhang, L.P., Ge, X.X., He, Z.H., Wang, D.S., Yan, J., Xia, X.C. and Sutherland, M.W. 2005. Mapping QTLs for polyphenol oxidase activity in a DH population from common wheat. *Acta Agronomica Sinica (Zuowu Xuebao)*, **31** (1):7-10.