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SHORT COMMUNICATION

Biochemical Characterization of Wheat Polyphenol Oxidase Activity in Genebank Accessions

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A selected set of wheat accessions received at the National Genebank, were analyzed for the extent of variation in seed *Polyphenol oxidase* activity. Analysis of seed vigour data confirmed the non-destructiveness of the protocol. The method is proposed to be used for germplasm screening prior to sowing.

Key Words: Genebank, Germplasm, *Polyphenol oxidase*, Seed vigour, Wheat

Polyphenol oxidases are a group of enzymes having ubiquitous presence in the plant kingdom. Functionally, it catalyzes the hydroxylation and dehydrogenation of phenolic compounds to form *o*-quinones, which rapidly polymerize through non-enzymatic reactions, to form brownish melanin compounds (Taranto *et al.,* 2017). Wheat seeds are known to possess significant PPO content which is primarily localized in the outer caryopsis, in an insoluble form (Fuerst *et al.,* 2006). During grain processing for flour, the cells lose the compartmentalization due to which PPOs come in contact with the phenolic substrates which are otherwise localized within vacuoles, leading to the browning reaction in the processed products. Such browning caused by the seed PPO is an undesirable trait for the food processing industry since it has negative impact on the colour and flavor of the final product (Kruger *et al.,* 1992; Hatcher *et al.,* 1993 and Mc Caig *et al.,* 1999). Hence, there is a specific demand for varieties having lower PPO content in mature grains. However, it has also been established that PPO is a major defense enzyme, which contributes to the protective mechanism of the plant, against a broad spectrum of pathogens. The enzyme gets induced in response to plant-pathogen interaction and its extrinsic presence enables effective protection (Fuerst *et al.,* 2014). At least four to five distinct PPOs are reported to be expressed in seeds of hexaploid wheat (Massa *et al.,* 2007 and Beecher *et al.,* 2012) and there is significant diversity in the level of PPO content within wheat genotypes (Demeke and Morris, 2002). Precise information on the level of PPO activity will help in categorizing the genotypes for specific end-use and it will also facilitate their utilization in various breeding programmes. A high PPO genotype may not be desirable in the pedigree of a variety which is being developed for superior biscuit/pasta/noodle quality, due to the browning factor. But an optimum level of seed PPO would be essential to maintain the required defensive role against pathogens. In this context one should also consider the recent change in consumer preference and the surge in demand for whole wheat products that are considered to be the healthier choice. In such instances where retention of the bran is essential, a balanced PPO content will enable development of more diverse products without compromising on the flavor and shelf life. Hence, an attempt has been made to assess the range of PPO content in the wheat germplasm collection held in National Genebank, India.

A random group of 339 accessions that were received in the genebank as a single lot, after multiplication at a single location, were identified and subjected to whole kernel enzyme assay. Out of these 339 accessions, 50 accessions were of exotic origin and 289 accessions were indigenous. A non-destructive five-seed assay was used for the PPO estimation (James and Morris, 2001). From each accession, 5 seeds, in triplicate sets, were randomly selected and placed in 2 ml microcentrifuge tubes containing 1.5 ml of phenolic

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substrate 3,4 dihydroxyphenyalanine (L-DOPA) prepared in 50 mM 3-(*N*-morpholino) propane sulfonic acid (MOPS) buffer of pH 6.5. The tubes were incubated for 2 hours at room temperature, with intermittent shaking. After 2 hours, the substrate was decanted and the change in absorbance was recorded at 475 nm, using a UV-VIS spectrophotometer. The control consisted of the substrate alone. One unit of PPO activity was calculated as change in 0.001 absorbance unit/min/ml. 120 accessions were randomly selected from within the 339 accessions, for seed vigour test. The test was aimed at validating the non-destructiveness of the protocol. It was verified by comparing the seed vigour prior to and after the assay, for 120 accessions. Seed vigour was estimated through the calculation of Vigour index, according to the protocol proposed by Abdul Baki and Anderson, 1973. After the germination test (fourteen days), 10 normal seedlings from both treatments and each replication were selected at random and their average seedling length (plumule tip to root tip) was recorded. The Vigour index were calculated using the formula as mentioned below:

Vigour Index = Germination (%) × Average seedling length of 10 seedlings (cm)

For analyzing the diversity in PPO activity amongst the wheat genotypes, the experiments were laid out in a completely randomized design. The data were subjected to analysis of variance using SAS9.3 software and significant effects ($p< 0.05$) were noted. Further, Tukey's HSD Test was done for pair-wise comparison of various effects. ANOVA tables along with critical differences at $P\leq 0.05$ for each set of analysis were generated. As the Tukey's subsets were extensive in number, only the extreme categories were represented in the manuscript.

The PPO activity showed significant variability amongst the analysed genotypes. Based on the enzyme activity, wheat genotypes were categorized into very high, high, medium and low activity groups. For this categorization, the Tukey's homogeneous subsets were analysed and the pattern of notations indicating the significant difference in mean values were used to demarcate the activity levels. Out of the 339 accessions that were analysed, 44 accessions were in low category (less than 1.0 abs/gm), 227 accessions were in medium category (having 1.1 – 5.0 abs/gm), 57 accessions were in high category (having 5.1 – 8.0 abs/gm) and 11 accessions were in very high category (more than 8.0 abs/gm; maximum absorbance value was 10.6). These categories were further corroborated with visual scoring, after seed staining.

In the very high category, six accessions were indigenous improved cultivars and five accessions were of exotic origin. Maximum absorbance value was recorded for IC111714 (elite line developed by IARI RS Pusa, Bihar), followed by IC290185 and IC111731(elite line developed by IARI RS Shimla). The exotic accessions with maximum PPO content were of Sudanese origin (EC174785 & EC174788). In the high category, eight accessions were exotic and 48 accessions were indigenous. In the medium category, 32 accessions were of exotic origin and 195 accessions were indigenous (99 accessions were elite lines that were part of varietal trials and 96 accessions were breeding lines). Under the low category, five accessions were of exotic origin and 39 accessions were indigenous (eight accessions were elite lines and 31 accessions were breeding lines). The lowest PPO activity was recorded in IC290204. Post hoc comparisons using the Tukey's test for least square means (LS Mean) is depicted in Table 1. The analysis categorized the accession with the maximum PPO absorbance value (IC111714) as a separate group, significantly different from all other accessions (Table 1A). Similarly, the accession IC290204, having lowest PPO activity categorized as a single entity subset (Table 1B).

Genotypes in the Low PPO activity group can be potential contributors for this trait in processed product industry. The Very High category genotypes need to be further analysed for the presence of any enhanced mechanism of biochemical defense, since PPO has a prominent role in protection of the seed against pathogens. The data reveals that a large proportion of accessions within this experimental material are in the medium PPO activity category. These PPO lines, as per passport information, are genotypes generated as a part of various varietal development programmes and were identified for conservation due to certain superior agronomic traits. The medium level of activity is ideal for varietal development in countries like India, where wheat consumption is basically in the form of whole grain products, rather than processed forms.

The seed vigour data was analysed in case of 120 accessions, to ascertain the non-destructiveness of the assay protocol. For confirming the non-destructiveness of a seed protocol, seed vigour is a more convincing parameter as compared to the routine germination test

Table 1. Post hoc table for accessions in the Very High and Low activity groups
(Standard error± 0.29819)

A. Very High activity group (descending order of enzyme activity)

S.No	National Identity	LS Mean	Tukey's subsets
1.	IC111714	10.38516582	A
2.	IC290185	10.05184798	AB
3.	IC111731	9.404829683	ABC
4.	EC174788	9.000309805	BCD
5.	EC174785	8.871267312	CDE
6.	IC111708	8.849107704	CDE
7.	EC174118	8.72549106	CDEF
8.	IC111722	8.547185198	CDEF
9.	EC174123	8.517820857	CDEFG
10.	EC177756	8.436340541	CDEFG
11.	IC290238	8.350422711	CDEFGH

B. Low activity group (10 accessions with lowest activity listed in ascending order)

S.No	National Identity	LS Mean	Tukey's subsets
	IC290204	0.28609544	(6) I
	IC28086	0.298078899	(6) HI
	IC290256	0.307416481	(6)GHI
	IC290178	0.328218535	(6)FGHI
	IC290188	0.346549349	(6)EFGHI
	IC290052	0.358376191	(6)EFGHI
	IC427824	0.395221434	(6)EFGHI
	IC290250	0.444000108	(6)DEFGHI
	IC290205	0.475852933	(6)CDEFGHI
	IC28501	0.485525754	(6)CDEFGHI

(6) in the Tukey's subsets stand for the sixth cycle of A-Z grouping, owing to large number of sub-set groups

because, loss of vigour during the experiment will have long-lasting implications while re-using the tested seeds. In order to verify whether the content of PPO plays any inhibitory role in radical emergence, correlation analysis was carried out. The analysis revealed that seed vigour is positively correlated with PPO activity in Very high (r = 0.480), high (r = 0.208) and medium(r = 0.289) PPO categories, whereas it was negatively correlated in the low PPO category (r = ¯0.447). This correlation trend indicates superior germination and rate of growth in genotypes with higher PPO content (except for Low PPO genotypes). Thereby, it can be stated that the presence of PPO in the seed coat and its phenol-oxidation capacity is not a hindrance to seed germination. ANOVA carried out on initial and final seed vigour data are represented in Tables 2 & 3. The coefficient of variation values in both the tables indicates highly significant relative vigour variability amongst the accessions.

Table 2. ANOVA for initial seedling vigour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	1171.463	30.037	6.05	<.0001
Error	80	397.173	4.964		
Corrected Total	119	1568.636			

R-Square	Coeff Var	Root MSE	seedling_vigour Mean
0.746803	10.64443	2.228153	20.93258

Table 3. ANOVA for final seedling vigour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	39	2513.73	64.45	5.34	<.0001
Error	80	965.55	12.069		
Corrected Total	119	3479.28			

R-Square	Coeff Var	Root MSE	seed_vigour_after_ppo Mean
0.722486	15.10430	3.474101	23.00075

The Tukey's HSD test was carried out within each category of PPO activity, for the seed vigour parameter, before and after the PPO estimation. There was a general increase in seed vigour values after the PPO analysis. This can be attributed to the effect of seed hydration that has occurred during the course of the experiment. But, there was no difference in the subset grouping pattern of the genotypes, thus indicating that the vigour parameter remained same, prior to and after the enzyme estimation (the representative table (Table 4) of Tukey's subsets in the high PPO category is depicted for reference). Thus, the non-destructability of the PPO assay could be conclusively proven. This test can hence, be conducted on a wide range of material since sample size will not be a limiting factor. Germplasm characterization experiments, where seed quantity is restricted, can include this parameter along with other

Table 4. *Post hoc* table of seed vigour parameter for representative accessions from the high PPO category

S.No	National Identity	LS Mean (Initial seed Vigour)	Tukey's subsets (common for initial and final vigour)	LS Mean (Final seed vigour)
1	IC228518	25.23	A	31.55
2	IC28542	25.0	A	30.79
3	IC26730	24.08	AB	29.35
4	IC26721	21.96	ABC	25.18
5	IC28526	21.78	ABC	24.87
6	IC26743	19.58	ABC	22.55
7	EC174786	19.58	ABC	22.13
8	EC177696	18.77	ABC	21.83
9	EC177687	17.89	BC	20.80
10	EC177682	15.9	C	19.03

descriptors, since the analyzed seeds can be subsequently sown without loss of viability. A positive correlation between PPO and seed vigour is also having significance in the context of conservation agriculture, wherein both the parameters will synergistically contribute to better field establishment.

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