Austin Journal of Analytical and Pharmaceutical Chemistry

Open Access

Austin Publishing Group

Research Article

Association of Polyphenol Oxidase Activities with Molecular Markers and Yellow Rust Resistance in Diverse Bread Wheat Genotypes

Asghar MN[1#*], Bajwa AA[2#], Ali A[3], Muhammad A[2], Sami A[2] and Saqlan Naqvi SM[2]

[1]Department of Medical Biology, University of Quebec, Trois Riveres, Canada

[2]University Institute of Biochemistry and Biotechnology, PMAS-UAAR Rawalpindi, Pakistan

[3]Centre for Plant Sciences and Biodiversity, University of Swat, KPK, Pakistan

[#]Equal Contributed to this Work

***Corresponding author:** Muhammad Nadeem Asghar, Department of Medical Biology, University of Quebec, Trois Riveres, Canada

Received: July 31, 2021; **Accepted:** August 27, 2021; **Published:** September 03, 2021

Abstract

Polyphenol Oxidase (PPO) catalyses the undesirable browning of wheat products which is of significant concern in consumer acceptance perspectives. Another important yield-limiting cause for wheat crops is wheat rust (e.g., yellow rust), a source of great economic loss worldwide. The purpose of the current research was to screen conventional and synthetic bread wheat genotypes for their PPO activity and yellow rust resistance. Different genotypes differed significantly in total PPO activity and in their activities against different substrates (L-DOPA and Catechol). The synthetically derived bread wheat genotypes 1-279, showed the lowest (39.2 units/min/g) cumulative PPO activity. Ten genotypes each with the highest and lowest PPO activities were selected for testing their association with seven reported molecular markers. Intriguingly the PPO markers reported in literature could not clearly differentiate between contrasting cultivars. Association with yellow rust resistance was also investigated. Interestingly, the rust-resistant genotypes, including 1-263, Ch-43, 1-57 and Emat (all synthetic-derived), exhibited low PPO activity. The current study underpins that there is a need to search for more reliable PPO markers and to further validate association of low PPO activity with yellow rust.

Keywords: Browning; Polyphenol oxidase activity; PPO; Yellow rust resistance; Synthetic-derived wheat

Introduction

Wheat is the second major staple crop of the world. Improved wheat products will ensure continuing international cooperativeness and sustainability of wheat industry. Routinely used wheat varieties are Durums; which are allotetraploid (AABB; 2n = 4x = 28) and common wheats; which are allohexaploids (AABBDD; 2n = 6x = 42) [1]. Resistance to stress and disease is of major concern to wheat breeders. Synthetic hexaploid wheat is better adapted to high temperature, drought, salinity, waterlogging and soil micronutrient imbalances. However, the most important hurdle in the up-gradation of the wheat industry is browning wheat-derived products that affect their market value. The leading cause of this undesirable browning is Polyphenoloxidases (PPO), therefore considerable efforts are required to reduce levels of PPO for prevention of economic loss [2,3]. There are other methods and combinations to inhibit the browning of wheat [4], and further tools are searched to limit the browning [5].

PPO is a nuclear-encoded copper-containing enzyme that is distributed from bacteria to mammals. PPO is enzymatically complex as it catalyzes the hydroxylation of monophenols to o-diphenols and oxidation of o-diphenols to o-quinones. The quionones covalently modify amines, thiols and phenolics forming colored polymers, which are responsible for browning of food products. PPO was first reported in wheat bran [6] and has been extensively studied in other plants [7]. PPO also has some wide applications in the industry such as its use as a biosensor in industrial waste waters [8].

PPO has been found in multiple molecular forms (isozymes) in various plants, at least 12 isoforms of PPO have been identified in a wheat kernel during different stages of development [9,10]. The existence of PPO isoforms has been attributed to modification of enzyme during the isolation process or it could also occur during processing of nuclear coded proteins or due to differential expression of different members of a gene family [11]. Jukanti et al. [12] hypothesized that the PPO gene in wheat has evolved by gene duplication into a multigene family. The total PPO activity of any plant is difficult to access because PPO exists in both latent and active forms, PPO activity is also quite variable in the presence of different phenolic substrates, it depends upon the nature of the side chain, number of hydroxyl groups and its position in benzene ring of substrate [11]. Due to its broad substrate specificities, PPO has an array of names such as phenol oxidase, monophenol oxidase and tyrosinase.

PPO activity in wheat grain is influenced by environment and genotype [13,14]. Location of PPO, analyzed through QTL analysis, showed that the chromosomes 2A, 2B, 2D, 3D and 6B contains PPO genes [15-18]. 2D chromosome was found to be associated with elevated levels of PPO activity in kernels of hexaploid wheat [19] as compared to tetraploid wheat, which lacks the genome-D and have high activity associated with the long arm of chromosome 2A [17,18]. PPO enzyme is located in the aleuron layer of wheat, which is removed during milling; however, the contamination by bran layer is enough to cause enzymatic browning [20]. Development of

Citation: Asghar MN, Bajwa AA, Ali A, Muhammad A, Sami A and Saqlan Naqvi SM. Association of Polyphenol Oxidase Activities with Molecular Markers and Yellow Rust Resistance in Diverse Bread Wheat Genotypes. Austin J Anal Pharm Chem. 2021; 8(2): 1134.

wheat varieties having low PPO activity is one of the priorities today because discolored food products are not preferred by consumers. PPO activity is a physiological and biochemical trait so it cannot be accessed based on morphological characteristics. However, the use of molecular markers linked to major QTLs of PPO can facilitate our search for low PPO varieties.

Another important yield-limiting cause for wheat crops that occur throughout the world is wheat rust. Three wheat rust diseases, stem rust, leaf rust and stripe (yellow) rust are a source of great economic loss worldwide [21,22]. Genome wide association studies are carried out in India, Kenya, Mexico and Egypt identified QTLs associated with Yellow rust [23,24]. Severe infection of stripe rust is caused by *Puccina striiformis* with yield losses in susceptible cultivars of up to sixty percent [25]. It is referred to as yellow or stripe rust because of the powdery yellow-orange masses of pustules arranged in stripes along the venation of leaf during spring and summer [26] giving characteristic striped appearance. Breeding for resistance is the most economical, environment friendly and the effective way than the use of fungicides for controlling the disease. Fungal (*Alternaria triticinia*) inoculation on resistant wheat cultivar is associated with the production of more PPO activity and this increase in enzyme concentration is well documented [27]. Several reports suggest a positive correlation between PPO activity and disease resistance [28,29]. Fungal infection on a range of tomato cultivar, from highly resistant to highly susceptible, suggested a considerable positive correlation between resistance, PPO activity and accumulation of phenol. Inoculated resistant cultivars showed higher PPO activity and accumulation of phenol than the inoculated susceptible cultivar. A higher PPO activity and phenol content were found in mature leaves of inoculated resistant cultivar. Disease index, PPO activity and phenol concentration were found to be directly correlated in the tissue [29].

The present study was carried out to screen the synthetic-derived bread wheat genotypes on the basis of their PPO activity; discover the correlation/association with resistant/susceptible cultivars to stripe rust and to check the association of molecular markers with the PPO activity in selected wheat lines.

Materials and Methods

The experimental plant materials consisted of wheat diverse genotypes comprising of total of 95 synthetic derived lines and check cultivars. Synthetic derived wheats were produced earlier from crosses of primary synthetic hexaploid wheats with advanced and improved cultivars and was obtained for research purpose from international maize and wheat improvement center (CIMMYT) through wheat wide crosses and cytogenetics program at National Agriculture Research Center (NARC), Islamabad, Pakistan. Their PPO activities were determined on L-DOPA and Catechol substrates.

Measurement of PPO activity with catechol and L-DOPA

The PPO activity with L-DOPA and catechol substrate was determined according to the method described by Fuerst et al. [30] with little modification. Experiments were performed in triplicate for each variety and substrate. Substrates were prepared in a buffer of 50mM[3]-(N-morpholino) propane sulfonic acid (MOPS) and pH was adjusted to 6.5. In each test tube containing seeds, 1.5mL (10mM) of phenolic substrate was added and tubes were incubated at 25°C for about 2 hours with rotation at 250rpm. Following incubation, absorption was measured through spectrophotometer and the change in absorbance was compared with a control containing only substrate. Absorbance was measured at 410nm and 475nm for catechol and L-DOPA respectively. One unit of PPO is defined as a change in absorbance by 10^{-3}/min/g seed. The PPO activity with all the substrates was calculated as; Enzyme activity=ΔOD/min/g seed.

Selection of seed varieties for molecular markers study

The top ten high and low PPO activity varieties were selected to investigate further their association with molecular markers and rust resistance (Table 1 and 2). Selected varieties were germinated in autoclaved Petri dishes. Seeds were placed on filter paper under moist conditions. Genomic DNA was extracted from leaves after a week by the CTAB method described by Richards.

Selection of molecular marker

The molecular markers (PPO18, PPO16, PPO33, PPO43, BQ161439, WP2-2, WP3-3) based on the previous study [31-34] (Table 3) were used to check whether these molecular markers can differentiate between high and low PPO synthetic-derived bread wheat genotypes.

Polymerase chain reaction conditions

PCR conditions were optimized for various sets of primers used

Table 1: Low PPO genotypes characterized according to PPO activity and rust resistance score.

S. No.	Genotype	Catechol	L-DOPA	Cumulative PPO Activity	Rust
1	1-279	17.505	21.681	39.185	5 MR
2	1-27	21.644	20.064	41.707	10 RMR
3	1-180	17.408	24.363	41.771	5 RMR
4	1-263	14.197	28.272	42.468	0
5	Aryana	18.651	25.462	44.113	10 RMR
6	Tf-24	26.32	18.396	44.716	5 MS
7	Ch-43	19.428	30.684	50.112	0
8	1-18	22.965	28.191	51.156	5 RMR
9	1-57	24.347	27.442	51.788	0
10	Emat	21.875	30.075	51.95	0

Table 2: High PPO genotypes characterized according to PPO activity and rust resistance score.

S. No.	Genotype	Catechol	L-DOPA	Cumulative PPO Activity	Rust
11	Tf-61	47.544	72.491	120.035	40S
12	Sb-14	50.533	70.548	121.081	50S
13	9 × 2-14	20.218	102.931	123.148	80S
14	So-19	70.389	53.539	123.927	70S
15	1-247	48.864	75.079	123.943	70S
16	So-31	66.318	58.864	125.182	70S
17	Tf-21	58.329	67.968	126.297	40MRMs
18	12 × 2-22	57.673	69.458	127.131	0
19	So-5	65.628	62.976	128.604	70S
20	Sc-28	26.049	109.858	135.906	80S

Table 3: Primers with their target gene and optimized annealing temperatures.

Name	Primer Sequences	Target Gene	Optimized Annealing Temperature	References
PPO18	Forward: AACTGCTGGCTCTTCTTCCCA Reverse: AAGAAGTTGCCCATGTCCGC	Ppo-A1a and Ppo-A1b (2A)	60°C	[31]
PPO16	Forward: TGCTGACCGACCTTGACTCC Reverse: CTCGTCACCGTCACCCGTAT	Ppo-D1a (2D)	59°C	[32]
PPO33	Forward: CCAGATACACAACTGCTGGC Reverse: TGATCTTGAGGTTCTCGTCG	Ppo-A1a and Ppo-A1b (2A)	60°C	[32]
PPO43	Forward: GCAGCATGGAGAGCAGTCGC Reverse: GCAGTTGTGCACCTGGATCTCA	Ppo-D1 (2D)	64°C	[32]
BQ161439	Forward: GGACGTGAACTT TGTCTGAAC Reverse: AGAGCGGCGTTCAGGATATC	XTc1 and XPPO-LDOPA (2A)	62°C	[33]
WP2-2	Forward: CGACGCTGAGGGAGACGGT Reverse: GTTCACCGTTCCGATTGTTCT	TaPPO-2	66°C	[34]
WP3-2	Forward: AGGTTCTACGTCTACTTCCAC Reverse: CCGCCGAGAAGAAGTTGC	TaPPO-3	58°C	[34]

for the amplification of required products. General recipe for 10µL PCR reaction mixture contained 1µL of DNA (50ng), 0.6µL of 1.5mM $MgCl_2$, 1µL of Taq buffer, 0.2µL of dNTPs (200µM), 0.5µL of each forward and reverse primer (25pM), 0.4µL of Taq DNA polymerase (1.5 units) and 5.8µL of PCR water. Thermal profile comprises of pre-amplification denaturation at 94°C for 3min, followed by 35 cycles of denaturation at 94°C for 20sec, annealing at an optimized temperature for each primer (Table 3) for 20sec, extension at 72°C for 40sec and final extension was carried out at 72°C for 20min. Amplified PCR products were electrophoresed at 1% agarose, stained with ethidium bromide and visualized by UV transilluminator.

Statistical analysis

Two-factor factorial ANOVA was applied by the Statistix 8.1 data analysis software in order to check the significance between the varieties, substrates and PPO activities. The phylogenetic relationship between genotypes was determined using PAST [35]. Discrete Scoring was done for bands of the gel that were obtained.

Scoring rust

Scoring was done according to the modified Cobb scale. It is recorded in the form of percentage severity and field response. Field response is recorded using the following letters; (O) No visible infection on the plants, (R) Resistant, (MR) Moderately Resistant, (M) Intermediate, (MS) Moderately Susceptible and (S) Susceptible. Severity and field response readings are usually combined [36].

Results

Effect of substrate on PPO activity

The Polyphenol oxidase activity was determined for 95 wheat genotypes with two substrates namely L-DOPA and Catechol. The activity of PPO with these substrates fell in a range of 39.186-135.907 units/min/g. The highest and the lowest cumulative values were shown by the accession (Acc.) Sc-28 and 1-279 respectively. When L-DOPA was used as a substrate, PPO activity ranged from 18.396 to 109.858 units/min/g. The lowest activity was shown by Acc. Tf-24 followed by Acc. 1-27 and 1-279 while the highest PPO activity were observed for Acc. Sc-28 followed by Acc. 9× 2-14 and 9× 2-20. When catechol was used as substrate the enzyme activity fell in the range of 12.072 to 70.389 units/min/g. It was found that Acc.1-284 has the lowest (12.072 units/min/g) activity followed by Acc. Kingbird and 1-263. Highest PPO activity was attained by Acc. So-19 followed by Acc. So-31 and So-5 respectively (Table 1 and 2). Different genotypes differed significantly in total PPO activity and in their activities against different substrates.

Assessing association of molecular markers for grain polyphenol oxidase activity

Seven pairs of molecular markers were employed on the selected genotypes having low and high PPO activities to establish the association. Wp2-2 amplified a fragment of 600 base pair in some low PPO cultivars (Tf-24, Ch-43, 1-18, Emat, 1-279, 1-263, 1-27) and in some high PPO cultivars (So-31, So-19, Tf-21, Sb-14). Whereas, no amplification was observed in 1-180, 1-57, Aryana of low PPO wheat lines and Sc-28, So-5 9 ×2-14,12 × 2-22, Tf-61, 1-247 of high PPO lines (Figure 1). Whereas Wp3-2 amplified up to three fragments of sizes 450, 550 and 650 bp. A 450 bp fragment was amplified in all PPO cultivars, while 550 bp was amplified in some low PPO cultivars (Tf-24, Ch-43, Emat, Aryana, 1-279) and in some high PPO cultivars (Sc-28, So-31, So-19, Tf-21, Sb-14, 12×2-22, Tf-61, 1-247). The third fragment of 650bp was amplified in only two low PPO genotype cultivars (Aryana, 1-279) (Figure 2). PPO 16 amplified a fragment of 713bp in some low PPO cultivars (Tf-24, Ch-43, Emat, Aryana, 1-263, 1-27) and also in some high PPO cultivars (So-19, 9×2-14, Tf-21, Sb-14, 12×2-22, 1-247). No band was detected in 1-180, 1-18, 1-57, 1-279 of low PPO wheat lines and Sc-28, So-5, So-31, Tf-61 of

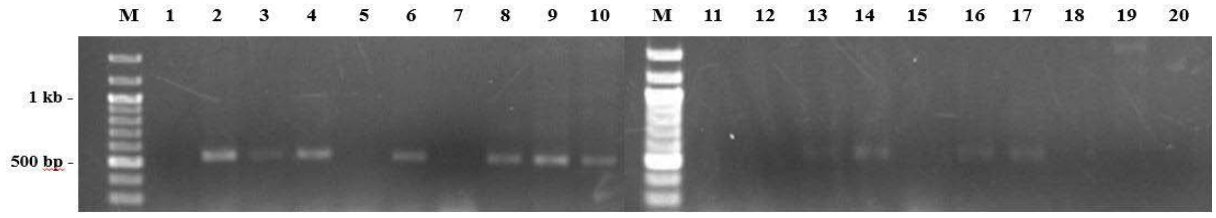

Figure 1: Gel analysis of twenty selected low and high PPO cultivars with Wp2-2 molecular marker.
1-10 wells have amplified products of 1-180, Tf-24, Ch-43, 1-18, 1-57, Emat, Aryana, 1-279, 1-263, 1-27 low PPO varieties respectively. 11-20 wells have amplified products of Sc-28, So-5, So-31, So-19, 9×2-14, Tf-21, Sb-14, 12×2-22, Tf-61, 1-247 high PPO genotypes respectively.

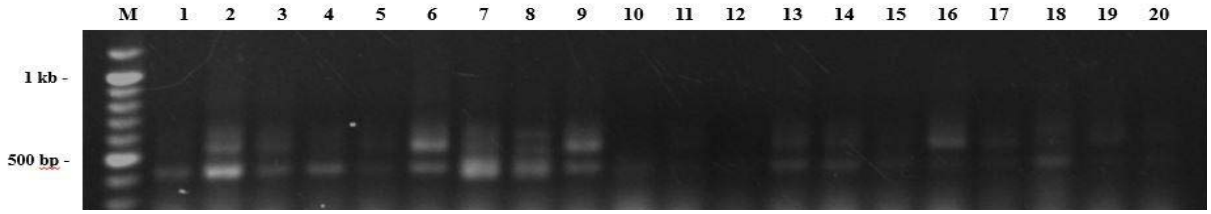

Figure 2: Gel analysis of twenty selected low and high PPO cultivars with Wp3-2 molecular marker.
1-10 wells have amplified products of 1-180, Tf-24, Ch-43, 1-18, 1-57, Emat, Aryana, 1-279, 1-263, 1-27 low PPO varieties respectively. 11-20 wells have amplified products of Sc-28, So-5, So-31, So-19, 9×2-14, Tf-21, Sb-14, 12×2-22, Tf-61, 1-247 high PPO genotypes respectively.

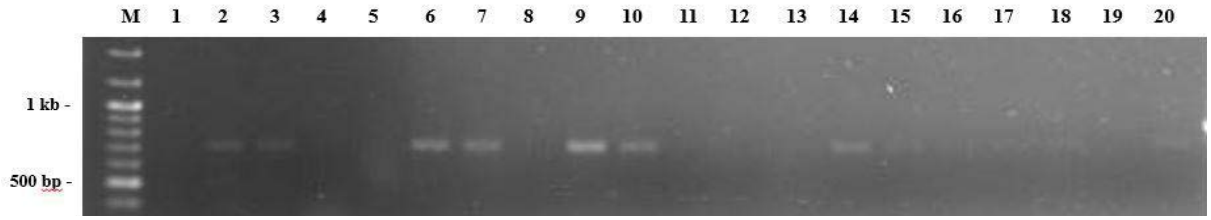

Figure 3: Gel analysis of twenty selected low and high PPO cultivars with PPO16 molecular marker.
1-10 wells have amplified products of 1-180, Tf-24, Ch-43, 1-18, 1-57, Emat, Aryana, 1-279, 1-263, 1-27 low PPO varieties respectively. 11-20 wells have amplified products of Sc-28, So-5, So-31, So-19, 9×2-14, Tf-21, Sb-14, 12×2-22, Tf-61, 1-247 high PPO genotypes respectively.

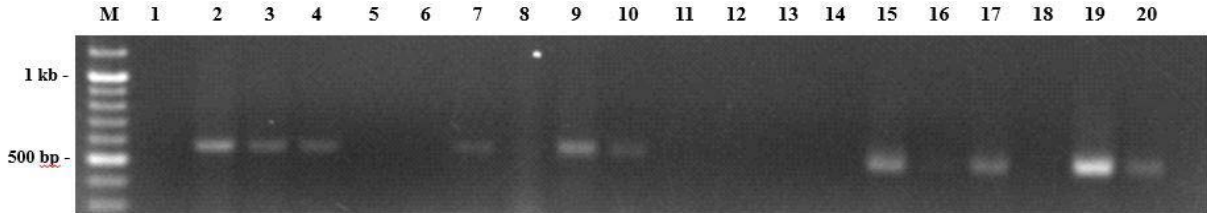

Figure 4: Gel analysis of twenty selected low and high PPO cultivars with PPO43 molecular marker.
1-20 wells have amplified products of 1-180,Tf-24,Ch-43, 1-18, 1-57, Sc-28, So-5, So-31,So-19, 9×2-14,Tf-21, Sb-14, 12×2-22, Tf-61, Emat, 1-247, Aryana, 1-279, 1-263, 1-27 PPO genotypes respectively.

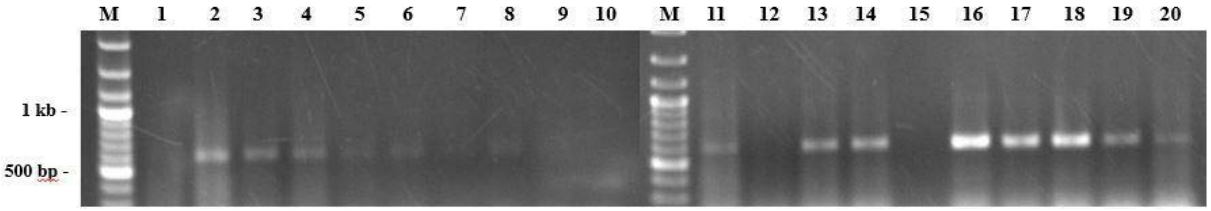

Figure 5: Gel analysis of twenty selected low and high PPO cultivars with PPO30 molecular marker.
1-10 wells have amplified products of 1-180, Tf-24, Ch-43, 1-18, 1-57, Emat, Aryana, 1-279, 1-263, 1-27 low PPO varieties respectively. 11-21 wells have amplified products of Sc-28, So-5, So-31, So-19, 9×2-14, Tf-21, Sb-14, 12×2-22, Tf-61, 1-247 high PPO genotypes respectively.

high PPO lines (Figure 3). PPO 43 amplified a fragment of 578bp in most low PPO cultivars (Tf-24, Ch-43, 1-18, Emat, Aryana, 1-263, 1-27) and in some high PPO cultivars (So-5, So-19, 9×2-14). No band was detected in 1-180, 1-57, 1-279 low PPO wheat genotypes and in most Sc-28, So-31, Tf-21, Sb-14, 12×2-22, Tf-61, 1-247 high PPO genotypes (Figure 4). PPO 30 amplified a fragment of 615bp in low PPO cultivars (Tf-24, Ch-43, 1-18, 1-57, Emat, Aryana, 1-279) and in some high PPO cultivars (Sc-28, So-31, So-19, Tf-21, Sb-14, 12×2-22, Tf-61, 1-247) (Figure 5). No band was detected in 1-180, 1-263, 1-27 of low PPO wheat lines and So-5, 9×2-14 of high PPO lines. A 500bp fragment was amplified by STS marker BQ161439 (Figure 6). PPO18 amplified a 300 bp band in all varieties except few genotypes (1-180, Tf-24, Ch-43, 1-18) (Figure 7).

Assessing association of PPO and rust resistance

Genotypes selected on the basis of their PPO activities (Top ten low and high PPO genotypes) were scored for their rust prevalence. Genotypes 1-27 and Aryana displayed a score of 10RMR which are resistant to moderately resistant, whereas a zero response was observed for 1-263, Ch-43, 1-57 and Emat, hence depicting that they are rust-resistant. A score of 5RMR was shown by 1-279, 1-180 and 1-18 (Table 1). High PPO genotypes displayed a score from 40S to

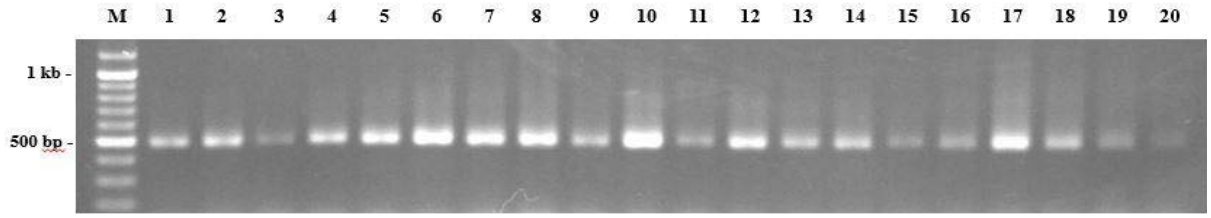

Figure 6: Gel analysis of twenty selected low and high PPO cultivars with BQ161439 molecular marker.
1-20 wells have amplified products of 1-279,1-27,1-180,1-263,Aryana, Tf-24, Ch-43, 1-18, 1-57, Emat, Tf-61, Sb-14, 9×2-14, So-19, 1-247, So-31, Tf-21,12×2-22,So-5,Sc-28 PPO genotypes respectively.

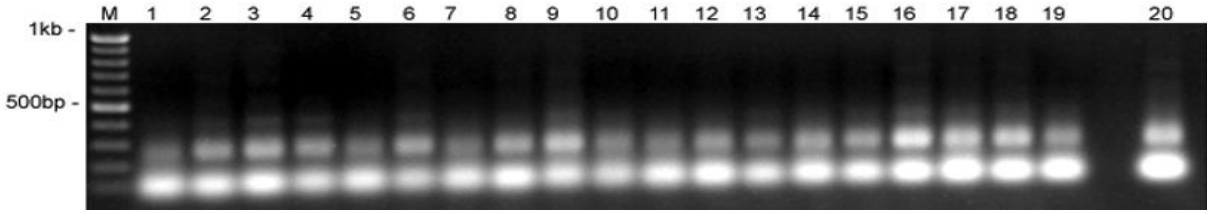

Figure 7: Gel analysis of twenty selected low and high PPO cultivars with PPO 18 molecular marker.
1-10 wells have amplified products of 1-180, Tf-24, Ch-43, 1-18, 1-57, Emat, Aryana, 1-279, 1-263, 1-27 low PPO varieties respectively. 11-20 wells have amplified products of Sc-28, So-5, So-31, So-19, 9×2-14, Tf-21, Sb-14, 12×2-22, Tf-61, 1-247 high PPO genotypes respectively.

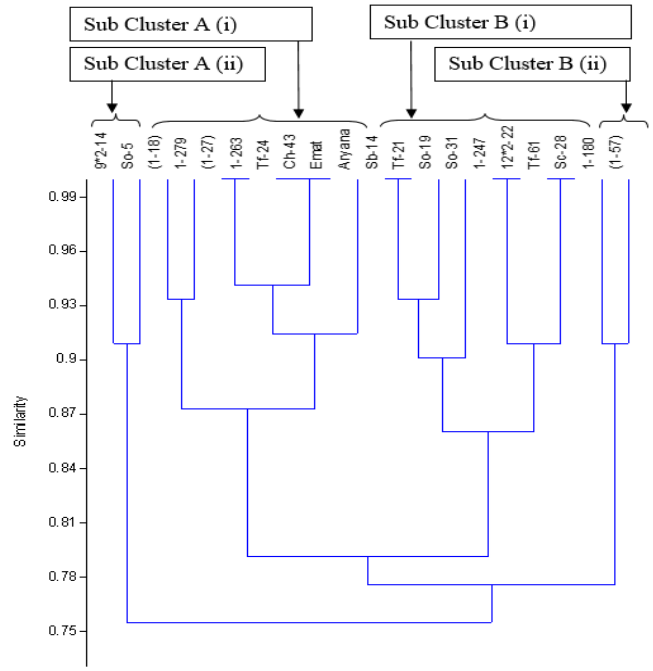

Figure 8: Dendogram based on UPGMA analysis of genetic similarity obtained from the molecular marker data and rust resistance.

80S except genotypes Tf-21 and 12×2-22 having scores of 40MRMs and O respectively (Table 2). Phylogenetic relationship was studied by scoring the data obtained by gel analysis of molecular markers and rust and analyzing it by UPGMA (Unweighted Pair Group Method Arithmetic mean). The Dendogram obtained showed that the varieties Emat, Ch43 are closely related. Low PPO genotypes form one group as depicted in Subcluster (Ai), whereas in Subcluster (Bi) high PPO genotypes form another group (Figure 8).

Discussion

Polyphenol Oxidase (PPO) has gathered much attention from researchers due to its role in lowering the quality of wheat-derived food products. L-DOPA showed the highest PPO turnover when used as substrate as compared to Catechol. Results have shown that ranking based on one enzyme activity for L-DOPA is not valid for catechol. This observation is in accordance with the findings by Anderson et al. [37] which leads to the hypothesis that total PPO activity in one genotype is the sum of activities of more than one isozyme. Two Factor Factorial ANOVA (Analysis of variance) was applied to check the significance of the numerical differences among means of various genotypes and PPO activities against the substrates used. A highly significant relationship was observed between genotype and substrate, which was evident in the Genotype and PPO interaction. ANOVA results for PPO activity of selected high PPO cultivars showed that the genotypes were not displaying significant variations (P value=0.8682). However, their activities in different substrates (L-DOPA and Catechol) were significantly different. The verifiability of results being valid is described by the value of Coefficient of Variation that is (19.52%). A similar trend was observed for low PPO genotypes (Table 4 and 5).

Further, seven pairs of molecular markers (WP2-2, WP3-2, PPO16, PPO43, PPO30, BQ161439 and PPO18) were employed on the selected genotypes to establish association with PPO with an aim to differentiate between high and low PPO lines. The present study results showed that molecular markers failed to differentiate between low and high PPO lines. The molecular marker WP2-2 amplified same band in low and high PPO varieties (Figure 1), our results were in accordance with the previous findings by Chang et al. [34] for the WP2-2 marker. Similarly, with WP3-2 marker, a fragment of the same size was observed in high and low PPO cultivars as was reported previously by Chang et al. [34]. PPO16 amplified a fragment of 713bp in some low & high PPO cultivars, whereas He et al. [32]

Table 4: Analysis of Variance for High PPO varieties only.

Source	Degree of Freedom	Sum of Squares	Mean Square	F value	Probability
Genotype	9	618.8	68.76	0.5	0.8682
Substrate	1	7043.2	7043.16	50.83	0
genotype*substrate	9	16785.2	1865.02	13.46	0
Error	40	5542.4	138.56		
Total	59	29989.6			

Coefficient of Variation = 19.06.

Table 5: Analysis of Variance for Low PPO varieties only.

Source	Degree of Freedom	Sum of Squares	Mean Square	F value	Probability
Genotype	8	295.86	36.982	2.14	0.0575
Substrate	1	557.14	557.141	32.18	0
Genotype*Substrate	8	761.37	95.172	5.5	0.0001
Error	36	623.31	17.314		
Total	53	2237.68			

Coefficient of Variation = 17.72.

reported an amplified fragment of the same size in low PPO cultivars only. However, in the present study, this molecular marker could not establish any association. PPO43 has according to the previous study by He et al. [32] did not provide differentiating results for high and low PPO wheat genotypes, similarly in our study a fragment of 578bp was observed in all genotypes. Another molecular marker PPO30 in our study amplified a fragment of 615bp in most high and low PPO cultivars but could not differentiate between contrasting PPO genotypes.

STS marker BQ161439 could not differentiate between contrasting genotypes in the present study while in a previous study by Raman [33] it amplified a fragment of 146bp in all low PPO except high PPO cultivars. PPO 18 also gave the same banding pattern in most PPO genotypes (Figure 7), previous results by Sun et al. [31] showed that PPO18 gave fragments of 876bp and 685bp in low and high PPO genotypes respectively. However, in the present study, PPO18 did not show any differentiation between contrasting cultivars. Hence, keeping in view the results obtained, it may be concluded that none of the seven pairs of molecular markers employed in our study are alone efficient and reliable for evaluating PPO activity.

Another important objective of our study was to assess the association between rust resistance and PPO activity. Wheat being the most important cereal crop is under threat of diseases, especially the rust disease. There have been many Rust epidemics in the history and often cause severe yield losses. With the increasing trend in population, there is great concern about food security and the threats posed by different biotic and abiotic stresses. Among biotic stresses is the diseases of stripe rust, leaf rust and stem rust caused by *Puccinia triticina* different strains which have been the widely vicious diseases of wheat in the world and there is a need for development of rust free wheat varieties. Selected genotypes were scored for its rust prevalence. Interestingly, it was found that genotypes possessing low PPO activities were also displaying resistance to rust disease. Although the degree of resistance varied among low PPO genotypes. But according to the previous studies, there is a positive correlation of PPO with resistance [28,29]. Hence, the most suitable varieties according to our investigation are 1-263, Ch-43, 1-57 and Emat, to be recommended to plant breeders based on rust resistance and PPO activities.

So, it is reasonable to speculate that synthetic-derived bread wheat genotypes have improved resistance by other mechanisms than PPO or PPO is not positively correlated with resistance to rust. Further, the isozyme pattern may be checked from the leaves of inoculated resistant and susceptible varieties to establish this relationship. The current research aims to improve the standards of food quality and will reduce the economic losses faced by developing countries like Pakistan. The current study also underpins the need to investigate more reliable PPO markers for evaluating PPO activity, which could be utilized in wheat breeding programs to improve the quality of wheat-derived food products.

Acknowledgments

The research was financed by Higher Education Commission of Pakistan.

References

1. Morris CF, Rose SP. Wheat. In: Henry RJ, Kettlewell PS editors. Cereal grain quality, Chapman and Hill, London. 1996: 4-5.
2. Martin JM, Berg JE, Fischer AM, Jukanti AK, Kephart KD, Kushnak D, et al. Divergent Selection for Polyphenol Oxidase and Its Influence on Agronomic, Milling, Bread, and Chinese Raw Noodle Quality Traits. Crop Sci. 2005; 45: 85-91.
3. Taranto F, Pasqualone A, Mangini G, Tripodi P, Miazzi MM, Pavan S, et al. Polyphenol oxidases in crops: Biochemical, physiological and genetic aspects. Int J Mol Sci. 2017.
4. Brütsch L, Rugiero S, Serrano SS, Städeli C, Windhab EJ, Fischer P, et al. Targeted Inhibition of Enzymatic Browning in Wheat Pastry Dough. J Agric Food Chem. 2018; 66: 12353-12360.
5. Sikora M, Swieca M, Franczyk M, Jakubczyk A, Bochnak J, Zlotek U. Biochemical properties of polyphenol oxidases from ready-to-eat lentil (*Lens culinaris medik*.) sprouts and factors affecting their activities: A search for potent tools limiting enzymatic browning. Foods. 2019; 8: 154.
6. Bertrand G, Muttermilch W. Sur l'existence d'une tyrosinasedans le son de furmet. Compt. Rend. 1907; 144: 1285-1288.
7. Steffens JC, Harel E, Hunt MD. Polyphenol oxidase. In: Recent Advances in Phytochemistry; Ellis, BE Kuroki, GW Stafford, HA, Editors. Genetic Engineering of Plant Secondary Metabolism. 1994; 28: 275- 313; Plenum Press: New York.

8. Gul I, Ahmad MS, Naqvi SMS, Hussain A, Wali R, Farooqi AA, et al. Polyphenol oxidase (PPO) based biosensors for detection of phenolic compounds: A Review. J Appl Biol Biotechnol. 2017; 5: 72-85.

9. Kampatsikas I, Bijelic A, Rompel A. Biochemical and structural characterization of tomato polyphenol oxidases provide novel insights into their substrate specificity. Sci Rep. 2019; 9: 4022.

10. Kruger JE. Changes in the polyphenol oxidases of wheat during kernel growth and maturation. Cereal Chem. 1976; 53: 201-213.

11. Yoruk R, Marshall MR. Physicochemical properties and function of plant polyphenol oxidase. Journal of food biochemistry. 2003; 27: 361-422.

12. Jukanti AK, Bruckner PL, Fischer AM. Evaluation of wheat polyphenol oxidase genes. Cereal Chem. 2004; 81: 481-485.

13. Park WJ, Shelton DR, Peterson CJ, Martin TJ, Kachman SD, Wehling RL. Variation in polyphenol oxidase activity and quality characteristics among hard white wheat and hard red winter wheat samples. Cereal Chem. 1997; 74: 7-11.

14. Baik BK, Czuchajowska Z, Pomeranz Y. Comparison of polyphenol oxidase activities in wheats and flours from Australian and U.S. cultivars. J Cereal Sci. 1994; 19: 291-296.

15. Jimenez M, Dubcovsky J. Chromosome location of affecting polyphenol oxidase activity in seeds of common and durum wheat. Plant Breed. 1999; 118: 395-398.

16. Demeke T, Morris CF, Campbell KG, King GE, Anderson JA, Chang HG. Wheat polyphenol oxidase distribution and genetic mapping in three inbred line populations. Crop Sci. 2001; 41: 1750-1757.

17. Simeone R, Pasqualone A, Clodoveo LM, Blanco A. Genetic mapping of polyphenol oxidase in tetraploid wheat. Cell Mol Biol Lett. 2002; 7: 763-769.

18. Raman R, Raman H, Johnstone K, Lisle C, Smith A, Matin P, et al. Genetic and insilico comparative mapping of the polyphenol oxidase gene in bread wheat (*Triticumaestivum L*.). Funct Integr Genomics. 2005; 5: 185-200.

19. Anderson JV, Morris CF. An improved whole-seed assay for screening wheat germplasm for poly oxidase activity. Crop Sci. 2001; 41: 1697-1705.

20. Rani KU, Rao P, Leelavathi JS, Rao HP. Distribution of enzymes in wheat flour mill streams. J Cereal Sci. 2001; 34: 233-242.

21. Singh RP, Huerta-Espino J. Global monitoring of wheat rusts and assessment of genetic diversity and vulnerability of popular cultivars. 2004: 38-40.

22. Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN. The past, Present and future of breeding rust resistant wheat. Front Plant Sci. 2014; 5: 1-13.

23. Elbasyoni IS, El-Orabey WM, Morsy S, Baenziger PS, Ajlouni Z Al, Dowikat I. Evaluation of a global spring wheat panel for stripe rust: Resistance loci validation and novel resources identification. PLoS One. 2019; 14: 1-20.

24. Juliana P, Singh RP, Huerta-Espino J, Bhavani S, Randhawa MS, Kumar U, et al. Genome-wide mapping and allelic fingerprinting provide insights into the genetics of resistance to wheat stripe rust in India, Kenya and Mexico. Sci Rep. 2020; 10: 1-16.

25. Beresford RM. Stripe rust (*Puccinia striiformis*), a new disease of wheat in New Zealand. In: Cereal Rust Bulletin. 1982: 35-41.

26. Rominger R. Stripe rust strikes several central valley wheat fields. 'California Farm Bureau'. 2003.

27. Tyagi M, Kayastha AM, Sinha B. The role of phenolics and peroxidase in resistance to *Alternaria triticina* in bread wheat (*Triticum aestivum L*). J Agron Crop Sci. 1981; 181: 29-34.

28. Retig N. Changes in peroxidase and polyphenol oxidase associated with natural and induced resistance of tomato to *Fusarium wilt*. Physiol Plant Pathol. 1974; 4: 145-150.

29. Bashan Y, Okon Y, Henis Y. Peroxidase, polyphenol oxidase and phenols in relation to resistance against *Pseudomonas syringae pv. tomato* in Tomato plants. Can J Bot. 1987; 65: 366-372.

30. Fuerst EP, Anderson JV, Morris CF. Polyphenol Oxidase in Wheat Grain: Whole Kernel and Bran Assays for Total and Soluble Activity. Cereal Chem. 2006; 83: 10-16.

31. Sun DJ, He ZH, Xia XC, Zhang LP, Morris CF, Appels R, et al. A novel STS marker for polyphenol oxidase activity in bread wheat. Mol Breed. 2005; 16: 209-218.

32. He XY, He ZH, Zhang LP, Sun DJ, Morris CF, Fuerst EP, et al. Allelic variation of polyphenol oxidase (PPO) on chromosome 2A and 2 D and development of functional markers for the PPO genes in common wheat. Theor Appl Genet. 2007; 115: 47-58.

33. Raman R, Raman H, Martin P. Functional gene markers for polyphenol oxidase locus in bread wheat (*Triticumaestivum L*.). Mol Breed. 2007; 19: 315-328.

34. Chang C, Zhang HP, Xu J, You MS, Li BY, Liu GT. Variation in two PPO genes associated with polyphenol oxidase activity in seeds of common wheat. Euphytica. 2007; 154: 181-193.

35. Hammer Ø, Harper DAT, Ryan PD. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica. 2001; 4: 4-9.

36. Rust scoring guide. Mexico, DF (Mexico). CIMMYT. 1986: 11.

37. Anderson JV, Fuerst EP, Hurkman WJ, Vensel WH, Morris CF. Biochemical and genetic characterization of wheat (*Triticum spp*.) kernel polyphenol oxidases. Cereal Sci. 2006; 44: 353-367.