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GENETIC MAPPING OF POLYPHENOL OXIDASE IN TETRAPLOID WHEAT

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Abstract: Pasta colour is one of the main factors influencing pasta quality. It is the product of a desirable yellow component, an undesirable brown component and, under some drying conditions, a red component. The brown colour depends on enzymatic and chemical factors. Polyphenol oxidase (PPO; E.C. 1.14.18.1) is one of the enzymatic factors. It is mainly localised in the peripheral part of the wheat kernel, and is involved in the oxidation of endogenous wheat phenolic compounds resulting in the production of highly coloured products. Therefore, a knowledge of the genetic control of PPO activity could enable the developing of better strategies in breeding programs to reduce pasta darkening. The aim of this study was to map the gene(s) affecting PPO activity using a set of recombinant inbred (RI) lines, derived from a cross between *Triticum turgidum* L. var. *durum* cultivar Messapia and the accession MG4343 of *Triticum turgidum* L. var. *dicoccoides*. After performing linkage analysis, the gene for high PPO activity was mapped on the long arm of the chromosome 2A and its characteristic was found highly associated to the RFLP marker *Xutv1427-2A*, with a value of LOD equal to 29.84. The identification of molecular markers linked to loci controlling the PPO activity may potentially accelerate wheat breeding since the selection of plants can be carried out by genotype rather than phenotype.

Key Words: *Triticum turgidum* L. var. *durum*, Polyphenol Oxidase, Recombinant Inbred Lines, Mapping, Brownness.

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INTRODUCTION

Pasta colour is one of the main factors influencing pasta quality. Although it does not affect the nutritional and technological value of the product, it strongly influences the consumer's choice. The brown component of its colour is undesirable and depends on enzymatic and chemical factors. Polyphenol oxidase (PPO; E.C. 1.14.18.1) is one of the enzymatic factors. It is mainly localised in the peripheral part of the wheat kernel, and is involved in the oxidation of endogenous wheat phenolic compounds resulting in the production of highly coloured products. The presence of PPO in wheat has already been assessed by many authors [1-5], and there is evidence of a correlation between the activity of this enzyme and the brown colour both in grains and in end-products [6-8].

The knowledge of the genetic control of PPO activity could enable the developing of better strategies in breeding programs to reduce pasta darkening. Up to now, studies conducted in this area indicated the localisation of the major gene affecting PPO activity in homeologous group 2 in wheat. [9-11]. Further studies are necessary to map the loci involved in the regulation of PPO activity on a genetic map of durum wheat, as well as to identify molecular markers associated with this trait. The identification of molecular markers linked to loci controlling PPO activity may potentially accelerate wheat breeding, since the selection of plants can be carried out by genotype rather than phenotype.

In genetic mapping, it is necessary to set up segregating populations obtained from the cross of parental lines characterised by extremely different values for the character object of study. We have already created a set of recombinant inbred lines (RILs) obtained from a cross between the *Triticum turgidum* L. var. *durum* cultivar Messapia and the accession MG4343 of *Triticum turgidum* L. var. *dicoccoides*. This set of RILs was characterised by 418 genetic and molecular markers (RFLPs, microsatellites and AFLPs) developing a linkage map [12-14] used to achieve the genetic mapping of several useful technological traits such as grain protein content [15], SDS sedimentation volume [16], and productivity and its components [17]. The aim of this study was to identify the genetic factors affecting PPO activity in tetraploid wheats using a set of recombinant inbred lines derived from the same cross.

MATERIALS AND METHODS

Plant materials and field experiments

We assessed a set of 65 recombinant inbred lines (RILs) of durum wheat (*Triticum turgidum* L. var. *durum*), which were developed at the Institute of Plant Breeding, University of Bari, Italy, from a cross between the durum wheat cv. Messapia and the wild accession MG4343 of var. *dicoccoides* by advancing random individual F₂ plants to the F₇ generation by single-seed descent. Both the parents and the recombinant inbred lines were cultivated in Valenzano (Bari) in 1995, following a randomised complete block design with four replications with

two different nitrogenous treatments (N50, N100). For each treatment, two replications were analysed for PPO activity.

Determination of polyphenol oxidase activity

The methods proposed by Bernier and Howes (1994) [18] and by Mahoney and Ramsay (1992) [19] were used, with minor modifications. Thirty whole seeds for each line were incubated in 0.01 M disodium tyrosinate solution with 0.2% Tween 80 at 37°C for 19 hours, and, after leaving of the seeds, the absorbance of the solution was measured at 405 nm.

Linkage mapping

The PPO activities were translated into a binomial code (attributing code 0 to the lines with values in the range 1.0-2.2 and code 1 to those in the range 2.4-3.4), and linkage analysis was performed using the QGENE software, version 3.05 [20].

RESULTS AND DISCUSSION

An evaluation of PPO activity in a set of durum wheat (*Triticum turgidum* L. var. *durum*) Italian cultivars and wild accessions of *Triticum* was performed [21] in a previous work. The enzymatic activity was found to be higher in the wild accessions than in cultivars and was particularly high in the MG4343 accession of *Triticum turgidum* L. var. *dicoccoides*. This accession of *Triticum* and the durum wheat cultivar Messapia are the parents of a set of RILs characterised by 418 genetic and molecular markers (RFLPs, microsatellites and AFLPs), used to develop a linkage map [12-14].

The PPO activity was then assessed on both the parental and RI lines essentially following the methods proposed by Bernier and Howes (1994) [18] and by Mahoney and Ramsay (1992) [19], both based on a spectrophotometric assay in the presence of tyrosine as a substrate. The difference was that the number of seeds was increased to 30 in order to reduce the variation coefficient to 5%.

The parental lines, Messapia and MG4343, showed extremely different values for the examined trait: the PPO activity measured in Messapia had a mean of 1.32, while in accession MG4343, the mean value found was higher – 3.00.

Figure 1 shows the strongly different coloration of the solution of tyrosinate where the parental line seeds were suspended to assess PPO activity. The analysis of variance revealed highly significant differences ($P \leq 0.001$) between the RILs as regards PPO activity, while no significant differences were found for different treatments.

Figure 2 shows the frequency distribution of the trait the RI population, as well as the mean value. The graph shows the typical profile of a qualitative trait with two different groups of lines, each group scattered on one of the parents. The trait is therefore regulated by a single major gene. The heritability of the trait was very high (94.4%).

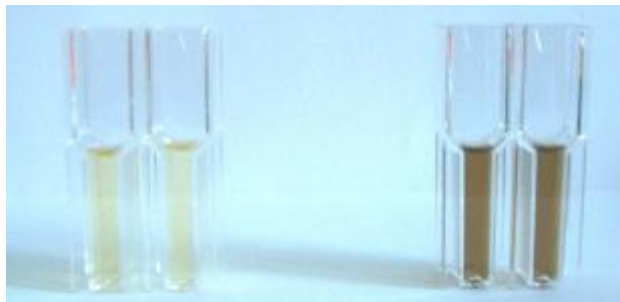


Fig. 1. Test tubes containing 0.01M disodium tyrosinate solution after 19 hours of incubation of Messapia (left) and MG4343 (right) seeds.

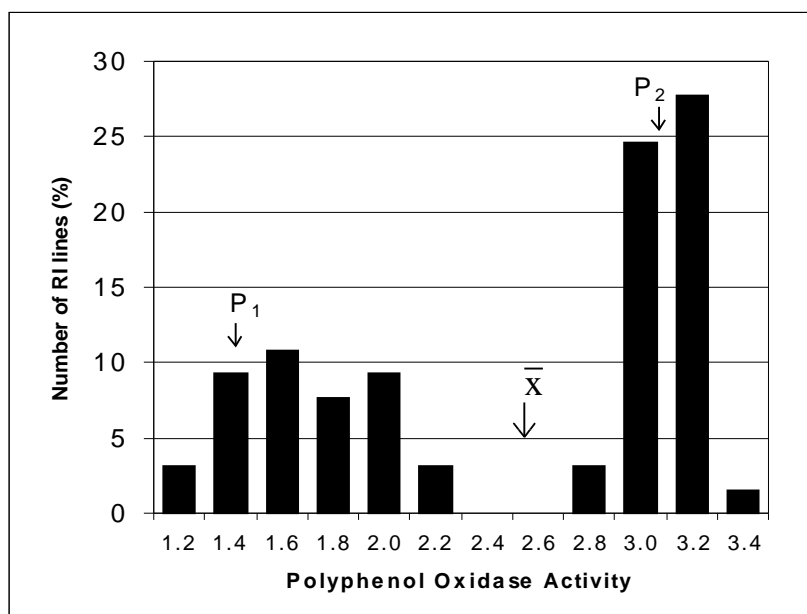


Fig. 2. Distribution of polyphenol oxidase activity among 65 RILs grown in replicated trials at Valenzano in 1995. Arrows indicate the mean values of the population (\bar{X}) and the two parental lines: P_1 = Messapia; P_2 = MG 4343

After performing the linkage analysis, the gene for high PPO activity was mapped on the long arm of chromosome 2A (Figure 3) and the trait was found to be highly associated with the RFLP marker *Xutv1427-2A*, with a LOD value of 29.84. This molecular marker could be used for a faster and easier selection of plants during breeding for low PPO activity.

Other authors found that genes located in homeologous group 2 play a major role in the activity of PPO in wheat. Substitution lines of chromosome 2A of the

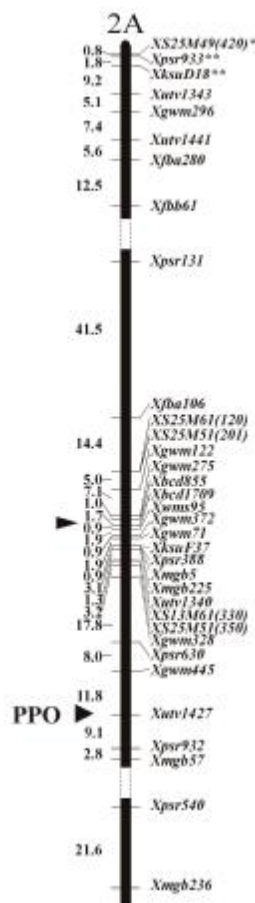


Fig. 3. Linkage map of the durum wheat chromosome 2A. The approximate location of the centromere is indicated by a small arrow (▶). Map distances in cM and marker names are shown on the left and right side of the chromosome, respectively. The approximate location of the locus for high PPO activity is indicated by a larger arrow (▶).

hexaploid varieties Cheyenne, Thatcher and Timstein in Chinese Spring showed significantly higher PPO activity than all other substitution lines of the same variety; moreover, substitution lines of chromosome 2A of *Triticum turgidum* var. *dicoccoides* and of chromosome 2D of Chinese Spring in the tetraploid variety Langdon showed a significant increase in PPO activity. Finally, the gene(s) responsible for high PPO activity in chromosome 2D from Chinese Spring was localised on the long arm within a deletion that represents 24% of the distal part of the arm [9].

Udall [10], by means of quantitative trait loci analyses in the cross between NY6432-18 and Clark's Cream, pointed out a major effect on PPO associated with an RFLP marker from homeologous group 2 and smaller effects associated

with RFLP markers from homeologous group 3 and 5. Souza *et al.* [11] mapped the RFLP marker from homeologous group 2 on the chromosome 2D. Li *et al.* [22], using nullisomic-tetrasomic lines of Chinese Spring and a PPO maize probe, localised PPO loci on 5B and 7D chromosomes and on homeologous group 6.

In conclusion, in this study, a locus responsible for high PPO activity was mapped on the long arm of chromosome 2A using a set of recombinant inbred lines, derived from a cross between the cultivar Messapia of durum wheat and the accession MG4343 of the var. *dicoccoides*. These findings concurred with the results of and advanced the research of other authors, who had found evidence that the localisation of the gene responsible for high PPO activity is on chromosome 2A in durum wheat [9]. The identification of a molecular marker (*Xutv1427-2A*) linked to high PPO activity has the potential to accelerate wheat breeding to reduce pasta darkening by carrying out a negative selection of this trait.

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