Evaluation of spelt germplasm for polyphenol oxidase activity and aluminium resistance

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INTRODUCTION

Spelt, *Triticum aestivum* ssp *spelta* (L.) Thell. (2n=6x=42) is one of the oldest cereals of the modern world. More recently, it is becoming a valuable resource due to its high protein content, resistance to several diseases and suitability for speciality products such as bread, pastry, noodles [1] and licorice preparation. In most parts of the world, spelt is being grown as a marginal crop with low inputs. In Australia, it is being grown mainly for export markets. A limited number of genotypes have been exploited for commercial cultivation by farmers in NSW. At this stage, this crop is not being targeted for systematic breeding in Australia and no definitive objectives for improvement have been identified. Helyar [2] reported that over 40% of agricultural land which received >500 mm average annual rainfall was affected by soil acidity. Aluminium (Al) toxicity is one of the major constraints limiting plant growth and crop productivity in these acidic soils. The selection of Al-resistant spelt genotypes is of agronomic importance.

In common wheat, polyphenol oxidase (PPO) has been implicated in time-dependent discolouration of processed dough and its products, especially Asian noodles, pan and steam breads, and pasta [3, 4, 11]. PPO catalyses the oxidation of endogenous phenolics present in the wheat flour. Therefore, low PPO may be a desirable attribute in spelt wheat.

In order to test the suitability of spelt for cultivation on acid soils and to make speciality products, we evaluated 125 spelt accessions, collected from various parts of the world, for resistance to aluminium, and for low PPO.

MATERIALS AND METHODS

The 125 spelt accessions were obtained from the Australian Winter Cereal Collection (NSWDPI, Tamworth) and from other parts of the world. These accessions were dehulled (if required) and used sequentially for estimation of PPO activities and evaluation for Al resistance.

Estimation of polyphenol oxidase activity

PPO activities in wheat kernels were measured using the L-DOPA method as described previously [10]. Five seeds from each accession were incubated in 1.5ml solution containing 10mM L-DOPA [L-3, 4-dihydroxyphenylalanine] in 50mM MOPS [3-(N-morpholino) propane sulfonic acid] buffer (pH 6.5) and Tween-20 for 1hr at room temperature. Kernels were scored visually, and their colour intensities were measured in a spectrophotometer at 490 nm with a Milenia Kinetic Analyser (Molecular Devices, USA). The same wheat kernels were then grown and the plants subsequently evaluated for Al resistance. Check genotypes were Arrivato (Durum, low PPO) and Excalibur (high PPO).

Evaluation for Aluminium resistance

Seedlings of the spelt accessions plus the standard check varieties Diamondbird (Al resistant) and Banks (Al sensitive), were grown in a nutrient solution. Seedlings were then transferred to aerated nutrient solution containing 360µM Al ($AlCl_3.6H_2O$) for 18 h, under the same growth conditions. The pH of all solutions was maintained at 4.2 ± 0.1 throughout the experiment. Roots were stained with a haematoxylin solution containing 0.2% (w/v) haematoxylin and 0.02% (w/v) KIO_3 for 15 min [6]. The roots were rinsed thoroughly with deionised water and scored visually. Seedlings with root-tips showing stain, were rated as Al sensitive and those root-tips showing no stain or only lightly stained were rated as Al resistant. The experiment consisted of two replications designed with spatial balance and conducted over two periods.

Prediction of desirable alleles for low PPO and Al resistance using molecular markers

DNA was extracted from 5-7 days-old fresh leaves from the seedlings previously scored for PPO activity and Al resistance, using a modified CTAB method. Molecular markers associated with loci conditioning PPO activity and aluminium resistance have been identified previously in wheat populations [7-10, 12]. Given that

common wheat and spelt wheat have evolved from the same ancestors; *Triticum urartu* (AA), *T. speltoides* (BB) and *Aegilops tauschii* (DD), significant synteny between marker loci and related traits is expected. Both functional and genic markers (PPO18 and WMC170 for PPO; TaALMT1-SSR and TaALMT-1 Long Fragment Promoter markers for aluminium malate transporter gene) were tested to confirm their association with the observed phenotypes in spelt accessions. The 5' end of the forward primer from each of the SSR primer-pairs was tailed with 19-nucleotide long M13 sequence. These modified primers were labelled with a fluorescent dyes (D2, D3 or D4, Beckman Coulter Inc., Fullerton, USA). Amplicons were separated using capillary electrophoresis on a CEQ8000 platform as described by manufacturer (Beckman Coulter Inc.).

Determination of molecular diversity

A total of 137 DArT markers were detected as polymorphic among the 125 accessions of spelt (Triticarte Pty. Ltd., Canberra, Australia). DArT alleles of 120 accessions, having high 'P' and 'Q' values, were binned in binary format for analysis. Genetic distance matrices between all possible pairs of spelt accessions were computed using DARwin 5 software [5]. Factorial coordinate analysis was conducted using DARwin 5.

RESULTS AND DISCUSSION

Twenty-seven spelt accessions exhibited low PPO activity (not significantly different to the check cultivar Arrivato, mean=0.11). PPO activity for spelt genotypes ranged from 0.11 to 1.41 (Fig 1). The analysis of variance did not show any significant difference between replications, but revealed highly significant differences among the spelt lines for PPO activity ($P < 0.05$).

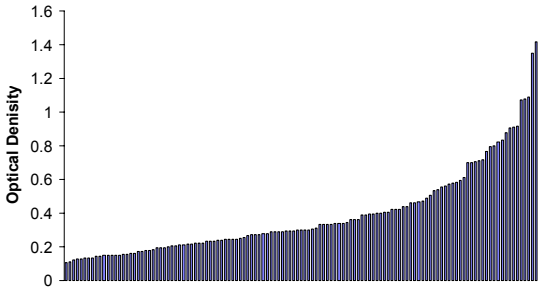

Fig 1: Ranked average PPO activities of 125 spelt genotypes measured using L-DOPA substrate in whole kernels.

Visual ratings of PPO activity were positively correlated with optical density, indicating that both methods are suitable for measuring PPO activity in spelt kernels. All the Al sensitive genotypes accumulated higher Al as compared to Al resistant genotypes as seen by increased intensity of haematoxylin stain (Fig 2). The haematoxylin staining test of root-tips revealed that 37 accessions were resistant to Al.

Fig 2: Relative root growth and intensity of haematoxylin stained seedlings of Al-resistant genotype (A) and Al-sensitive genotype (B) of spelt wheat grown in a nutrient solution.

Molecular markers associated with loci conditioning Al resistance gene and PPO activity in bread wheat were confirmed for their association with target phenotypes within spelt accessions. The majority of spelt accessions having PPO18 alleles of 876-bp exhibited low PPO, and the lines with 685-bp allele displayed high PPO activity. Similar associations between PPO activity and PPO18 alleles have been reported previously in common wheat varieties [10, 12]. The existence of the similar alleles in common and spelt wheat confirms that PPO alleles were originated from the same ancestral species. WMC170 marker allele of 222-bp was frequent in the spelt accessions exhibiting high PPO activity. Some of the accessions carried both alleles associated with high and low PPO activities, indicating that these accessions were either mixed or heterozygous at marker loci. None of the *TaALMT1* alleles was found to be diagnostic for Al resistance in spelt wheat. Similar observations were made previously in common cultivated varieties, landraces and some subspecies of wheat [8].

Molecular analyses using DArT markers showed that these spelt accessions are highly diverse (Fig 3). There are clear, closely-related groups of accessions and these groupings are related to geographic origin, parentage and selection history. Some genotypes were highly diverse and rare in this collection (e.g. accessions indicated with arrows; 91 and 117, Fig 3). This diversity

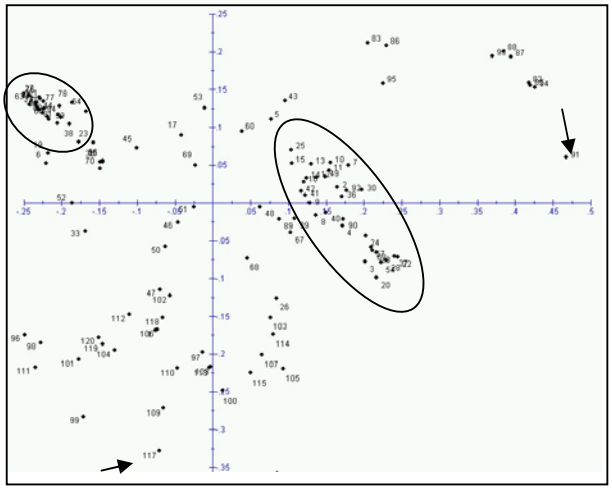

Figure 3: A plot of principal coordinate 1 versus principal coordinate 2 from an analysis of DArT data of 120 accessions of spelt wheat.

analysis will enable optimal use of the germplasm for subsequent improvement of spelt wheat.

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