Characterisation of durum germplasm for aluminium resistance using nutrient solution culture

Raman H[1], Hare R[2], Graham K[1], Coombes N[1] and Raman R[1]

[1]*NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, PMB, Wagga Wagga, NSW 2650, Australia.* [2]*NSW Department of Primary Industries, Tamworth Agricultural Institute, Calala, NSW 2340, Australia*

INTRODUCTION

Soil acidity limits the growth and productivity of various crops in parts of the world. At low pH, toxicities of aluminium and manganese and deficiencies of phosphorus, nitrogen, potassium, calcium, magnesium, sulphur, zinc and molybdenum generally occur [10]. These deficiencies and toxicities may act independently or together to reduce overall growth [3]. Acid soils can be ameliorated with an application of lime. However, this approach is undesirable due to high inputs costs especially in low-yielding environments. Furthermore, this approach is not practical because of the slow movement of lime especially into the deeper soil layers [4, 7]. Heavy application of lime may also have adverse effects on some crops in the rotation or cause deficiencies of certain nutrients [13].

Genetic variation for Al resistance exists within key crops [5, 9-12, 15, 17] and has been used in developing Al resistant varieties. However, only limited genetic variability for aluminium resistance has been reported in tetraploid wheats. Durum wheat (*Triticum turgidum* L ssp *durum*, genome AABB; 2n=4×=28) is the most Al sensitive member of the Triticeae and is a close relative of common hexaploid wheat (genome: AABBDD; 2n=6x=42). The relative Al resistance of key cereals is rye > oats > millet > bread wheat > barley > durum wheat (Bona *et al.*, 1993). In order to extend cultivation of durum into acid soils, it is imperative to identify genetic variation for Al resistance. Al resistance has been evaluated using different methods based upon nutrient screening [1, 12, 14], hematoxylin staining [8] and soil based assays [16] in wheat. Recently, a new measure, 'Incremental crop tolerance' (ICT) to weeds, has been used for selecting competitive ability in Australian wheats [6]. In the present study, ICT is the deviation from the regression of the Al treated root length on the nil Al (control) root length for all genotypes. This reflects the incremental root growth between genotypes associated with Al resistance, over and above difference in underlying root vigour and is based upon statistical methods. In this study, we measured ICT and compared it with 'relative root growth' to evaluate durum germplasm for Al resistance.

MATERIALS AND METHODS

Four hundred and twenty tetraploid (2n=4X, AABB) genotypes of the subspecies *turgidum* (36 accessions), *durum* (351 accessions), *dicoccon* (21 accessions), *carthlicum* (2 accessions) and 'standard check varieties for Al resistance and Al sensitivity of hexaploid wheats', were used to determine genetic variation for Al resistance. These accessions, derived from 27 countries, were procured from the Australian Winter Cereal Collection, Tamworth. The screening was performed in a nutrient solution using two replicates of each accession, with each replicate split between three 40L tubs. Each tub held 4 strips of 21 accessions, each represented by seven seeds. An incomplete block design for strips nested within tubs was employed and was spatially optimised using DiGGer, allowing for row and column effects and positive correlations between locations within tubs. Seedlings were initially grown in a nutrient solution [10] in the dark for 48 h. Control and Al treated (10 µM of $AlCl_3.6H_2O$) seedlings were then grown for a further 48 hrs. The longest seminal root from each seedling was then measured. Relative root growth was measured as root elongation with Al treatment/root elongation without Al (control) × 100. ICT was calculated as described previously [6]. Ten per cent of the genotypes having high ICT indices were further evaluated for aluminium resistance using a nutrient solution containing 20 µM of Al ($AlCl_3.6H_2O$). Al resistant wheat varieties Carazinho, Dollarbird, Wyalkatchem and Atlas66 were used as standard checks, and Banks, Rosella and Bellaroi (durum) were used as standard Al sensitive checks.

To confirm whether the identified sources were tetraploid (specifically, not hexaploid), 'D' genome specific gamma gliadin gene based marker, located on the long arm of chromosome 1D [18] was used for molecular analyses. DNA was isolated from 5-6 day old seedlings from the Al resistance (+Al) assays. Forward primer was labelled with a fluorescent dye (D4, Beckman Coulter Inc., USA). PCR reactions were carried-out in 10µL, following touch-down PCR protocol (Raman *et al.* 2005). Amplicons were separated on a CEQ8000 DNA sequencer (Beckman Coulter, Inc.) ad their allele sizes were measured using fragment analysis software as described previously (Raman *et al.* 2005).

RESULTS AND DISCUSSION

Residual maximum likelihood analysis of indices of root growth of tetraploid accessions of wheat in control

solution (minus Al) and root growth of tetraploid accessions in Al solution (10µM) indicated that the majority of genotypes were sensitive to Al.

Al resistant genotypes were expected to have positive ICT indices since these should have greater root growth than the Al sensitive genotypes in the presence of toxic levels of Al^{3+} ions. A number of tetraploid genotypes and the Al resistant checks Carazinho, Dollarbird, Wyalkatchem and Atlas 66 exhibited high ICT to aluminium across replicates, while the Al sensitive tetraploid accessions and negative controls Bellaroi (4X), Rosella (6X) and Banks (6X) had a low ICT (Fig 1). To validate the usefulness of the ICT approach, we compared the relative root growth (mm) with the ICT indices of 420 genotypes. Results showed a high correlation (0.988) between the two measures (Figure 2). Ten per cent of the total genotypes (42) having higher ICT indices at 10µM were considered as Al-resistant (Fig2).
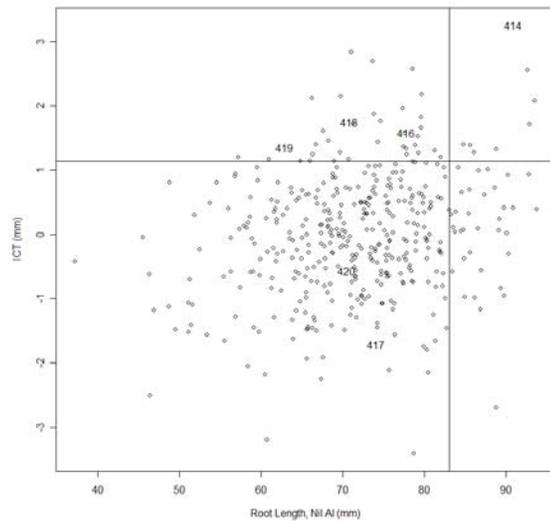
Figure 1 Incremental crop tolerance (ICT-mm) indices of 420 genotypes grown in a solution culture solution supplemented with 10µM of Al. IDs 414, 416, 418, 419 refer to Carazinho, Dollarbird, Wyalkatchem and Atlas66 respectively, while 417 and 420 refer to Banks and Bellaroi.

These accessions were further evaluated for aluminium resistance using a nutrient solution containing 20 µM of Al. ICT of these accessions ranged from -3.14 to 5.03 (Fig 2). Standard check varieties: Carazinho, Wyalkatchem, Dollarbird and Atlas66 along with tetraploid durum accessions 267, 202 and 249 exhibited higher ICT index (Fig 3).

These three durum accessions were 'rated' as Al-resistant and originated from Iran, Turkey and Spain, respectively. It is worthwhile to mention that some common wheat genotypes like Al resistant Atlas66 (accession 419) had poor root growth but still had a positive ICT index. This was because of the poor germination of the seeds used in this experiment. Our

preliminary results suggest that useful genetic variability for Al resistance exists within durum germplasm. These results are based on the performance of standard check
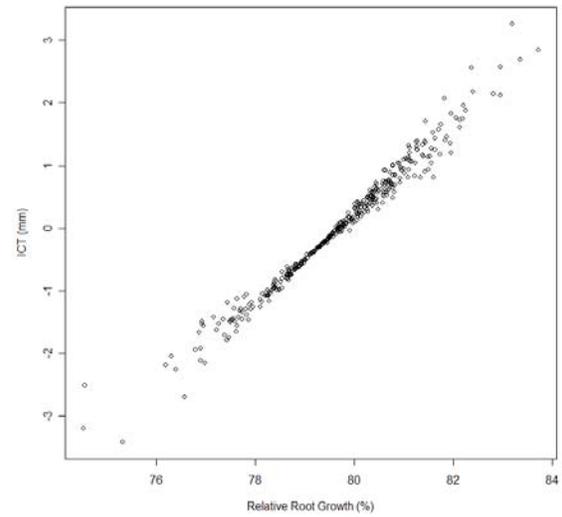
Fig 2: Relationship between relative root growth (%) and Incremental crop tolerance (ICT-mm) among 420 accessions evaluated for aluminium resistance in a nutrient solution containing 10µM Al. IDs 414, 416, 418, 419 refer to Carazinho, Dollarbird, Wyalkatchem and Atlas66, and 417, 420 refer to Banks and Bellaroi, respectively.
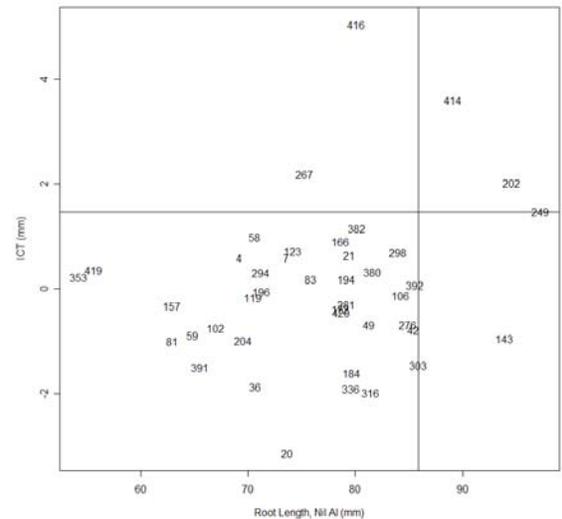
Figure 3: Incremental crop tolerance (ICT-mm) indices of 42 genotypes grown in a solution culture solution supplemented with 20µM of Al. IDs 414, 416, 418, 419 refer to Carazinho, Dollarbird, Wyalkatchem and Atlas66, and 417, 420 refer to Banks and Bellaroi respectively.

varieties and tetraploid accessions in nutrient solutions, measured with the ICT index. ICT index relies on the fact that the slope is expected to be positive since Al

resistant genotypes grow well under acidic condition (plus Al). In several previous studies, Al resistant wheat and barley varieties possessing a higher root growth and relative root growth as compared to the Al sensitive under Al stress were reported [10, 11]. General correlation/ranking of wheat genotypes for Al resistance was also found, when they were screened with different methods in solution culture, hematoxylin and soil [11, 12, 16]. None of the tetraploid genotypes approached the level of resistance of the highly tolerant hexaploid wheat (*T. aestivum* ssp. *aestivum* L) cultivar Carazinho. The genetic identity of all the 42 Al-resistant genotypes including the check varieties was confirmed reliably using the gamma gliadin gene specific marker. All the hexaploid wheat genotypes amplified a fragment of 357-bp. No such fragment was amplified from tetraploid accessions. Identification of Al-resistant genotypes will allow us to develop improved durum germplasm for Al resistance, suitable for cultivation on acidic soils. The selection of aluminium resistance in durum wheat is likely to improve the rate of yield gain, as demonstrated in other crops.

ACKNOWLEDGMENTS

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