Assessment of aluminium stress tolerance of triticale breeding lines in hydroponics

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ABSTRACT

The aluminium (Al) stress tolerance of 41 Waite breeding lines was characterised in a hydroponic system. In this experimental protocol root regrowth provided the most useful index of Al stress tolerance. Highly significant genotypic differences were found with root regrowth ranging from nil to 3.1 cm. Eleven lines (27%) had similar root regrowth to the Al-tolerant check Tahara (2.4 cm), whilst 27 Waite lines produced either shorter or significantly shorter regrowth root lengths. Based on root regrowth, entries could be classified into four groups, i.e. Al-sensitive (S), Al-tolerant (T), moderately Al-sensitive (MS) or moderately Al-tolerant (MT). Heterogeneity of within variety/line seedling Al response suggested that useful improvement in Al tolerance could be obtained from within-line selection.

KEY WORDS

Aluminium stress tolerance, hydroponic system, regrowth root length, selective breeding, triticale, Waite lines.

INTRODUCTION

Both basic and applied research on aluminium (Al) stress tolerance in triticale has indicated directional selection for improved Al tolerance based on larger root regrowth in nutrient solutions can effectively enhance plant Al tolerance (2, 4, 6). Continued progress in a selective breeding program depends, in part, on the availability of wide genetic variability in the population from which selections are made. The current Australian triticale breeding program involves, to a considerable extent, the evaluation and utilisation of introduced germplasm (1). The potential for improvement in acidic stress tolerance (and presumably Al stress tolerance) in this material was evaluated by comparing the Al tolerance response of a group of new triticale lines from the Adelaide component of the national triticale breeding program (1).

MATERIALS AND METHODS

A total of 41 new triticale breeding varieties or lines supplied by Dr K. Cooper at the Waite Campus of the University of Adelaide (referred to as Waite lines in Table 1) were screened for Al tolerance response. Due to space limitations in the controlled environment facility, these 41 Waite lines were divided into two groups for screening. Group 1 contained 21 Waite lines that were screened first, followed by the remaining 20 Waite lines (as Group 2). In both groups Tahara was included as a check. The test for Al tolerance response was undertaken in a hydroponic system at the University of New England, Armidale of NSW in 1999, with the procedures described previously by Zhang and Jessop (3) and Zhang et al. (4).

The stock nutrient solution had the following composition: 0.65 m*M* KNO_3, 0.40 m*M* $CaCl_2$, 0.25 m*M* $MgCl_2$, 0.10 m*M* $(NH_4)_2SO_4$ and 0.04 m*M* NH_4NO_3. During stages of seedling growth and Al treatment, the solution was maintained at pH 4.5 by daily pH adjustment using 1 *N* HCl and 1 *N* NaOH. Ten germinated seeds were placed on floats for seedling culture in a nutrient solution without Al stress for 4 days; seedlings were then transferred to an Al treatment solution of 10 mg.L^{-}? Al (as $AlCl_3.6H_2O$) stress for 24hr duration. Following treatment Al-treated roots were thoroughly washed for 2 minutes in running tap water. Floats with seedlings were placed back on fresh nutrient solution (pH 4.5 without Al) with daily pH adjustment for 2 days re-culture to allow the regrowth of seedlings. Roots were then stained with *Eryochrome cyanine R* (1.5g/L aqueous solution*)* for 8 minutes, and the excess dye after staining was removed by washing in running tap water for 2 minutes. The root regrowth after Al shock was easily

assessed from the existence of a stained ring along the root tips (3). Throughout the solution culture, temperature was maintained at 25/25?C during 12/12 hr light/darkness regime in a controlled growth chamber. A two-replicate RCB design was used in both groups, with ten individual seedlings per replicate being measured for (a) root length, (b) regrowth root length (if any), and (c) total root length (i.e. root length plus regrowth root length). Regrowth root length is reported here since this character has been confirmed to be a consistently good indicator of apparent Al tolerance response.

As uniform experimental conditions were used for both groups; it was assumed that the relative performance among the Waite lines tested would be comparable in both tests. In support of this assumption, comparable levels of Al tolerance (i.e. similar absolute extension of root regrowth and a similar relative level of Al tolerance) were expressed in the Tahara controls of both experiments. In addition, a statistical test (Chi-square: χ?) indicated the homogeneity of error variances of the two sample groups. An ANOVA was therefore performed on a plot mean basis to test genotypic differences, with multiple comparisons being made when a significant *F*-test was found. Coefficients of genotypic variation and broad sense heritability estimates were determined.

Table 1. Root regrowth length (cm) and Al tolerance response ratings of 41 Waite triticale lines.

Code	Variety/cross	RRL †	Type ‡	Code	Variety/cross	RRL †	Type ‡
	Group 1				*Group 2*		
G01	Credit	2.25	T	G23	TX94-39-1	2.70	T
G02	Treat	2.05	T	G24	TX94-42-1	2.45	T
G03	HX84-534-1	1.70	MT	G25	TX94-43-1	1.90	MT
G04	TX84-470-1	1.40 **	MS	G26	TX94-50-1	2.10	T
G05	OX83-25-1	0.95 **	S	G27	TX94-69-1	2.30	T
G06	HX87-260-1	2.35	T	G28	TX94-87-2	2.40	T
G07	OX83-34-1	1.90	MT	G29	TX94-117-1	1.45 **	MS
G08	TX93-19-1	2.55	T	G30	TX95-14-2	1.80	MT
G09	TX93-78-1	2.25	T	G31	TX95-20-1	1.40 **	MS

G10	TX93-19-1	2.50	T	G32	TX94-37-2	1.45 **	MS
G11	HX84-714-2	2.50	T	G33	TX94-22-1	0.65 **	S
G12	TX93-19-4	2.30	T	G34	TX94-34-1	1.70	MT
G13	Kiewiet-2	1.45 **	MS	G35	TX94-37-2	1.55	MT
G14	TX87-390-12	2.45	T	G36	TX94-40-1	2.55	T
G15	Tahara (check)	2.40	T	G37	Tahara (check)	2.45	T
G16	HX86-164-2-C	2.15	T	G38	TX94-41-3	2.00	T
G17	TX87-338-6CC	0 **	S	G39	TX94-46-1	0.95 **	S
G18	TX87-371-4CC	0.80 **	S	G40	TX94-65-1	2.50	T
G19	TX88-74-3CCN	2.55	T	G41	TX94-67-1	2.85	T
G20	TX88-75-6CCN	2.80	T	G42	TX93-59-1-1	3.05	T
G21	TX92-5-7CCN	2.25	T	G43	TX94-119-2	2.60	T
G22	TX94-36-1	1.90	MT				

† RRL = mean regrowth root length (cm); ** highly significant difference ($p < 1\%$) from the check.
‡ Al tolerance responses are classified into four types as Al-tolerant (T), moderately Al-tolerant (MT), moderately Al-sensitive (MS), or Al-sensitive (S).

RESULTS

Marked variation in Al tolerance response

There was a wide range in root regrowth among the 41 Waite lines, as shown in Table 1. The Al-tolerant check variety Tahara (G15 and G37) produced similar mean values of regrowth root length (2.40 and 2.45 cm, respectively) when tested with both groups of Waite lines. Eleven Waite lines (e.g. G20, G41 and G42) had numerically though not statistically larger root regrowth than Tahara, whilst 27 Waite lines (e.g. G4, G5, G17 and G33) produced either shorter or much shorter regrowth root length. Regrowth root length ranged from nil (in G17) to 3.05 cm (in G42), with a mean of 2.00 cm. The ANOVA revealed highly significant varietal differences in Al tolerance, indicating marked variation in Al tolerance response among these Waite lines.

Classification of the 41 Waite lines for Al tolerance response types

Multiple comparisons indicated significant or highly significant differences between some of the Waite lines and Tahara as well as among the Waite lines themselves. In terms of varietal differences from Tahara, 10 out of 41 Waite lines produced significantly smaller root regrowth; these included G04, G05, and G28. The remaining 31 Waite lines were statistically similar to Tahara in regrowth root length. Based on the absolute values of regrowth root length, the response types of Al tolerance of the Waite lines could be determined relative to the Al-tolerant check variety Tahara.

To simplify the ranking, the criteria used to separate the lines were the critical values representing a significant difference between Tahara and any Waite line given in the multiple comparison. Thus the 10 Waite lines which produced a significantly shorter regrowth root length were broadly classified as Al-sensitive (S) whilst all other 31 Waite lines were broadly classified as Al-tolerant (T). Further separation (of moderate types) is possible among both Al-sensitive types and Al-tolerant types. Lines for which the absolute RRL value fell between 1.40−1.45 cm were classified as moderately Al-sensitive (MS), whilst those lines with an absolute RRL value between 1.55−1.90 cm were classified as moderately Al-tolerant (MT). Based on this classification, a large proportion of Waite lines were found to be Al-tolerant or moderately Al-tolerant (Figure 1), and they would be expected to show a similar magnitude of Al tolerance to Tahara.

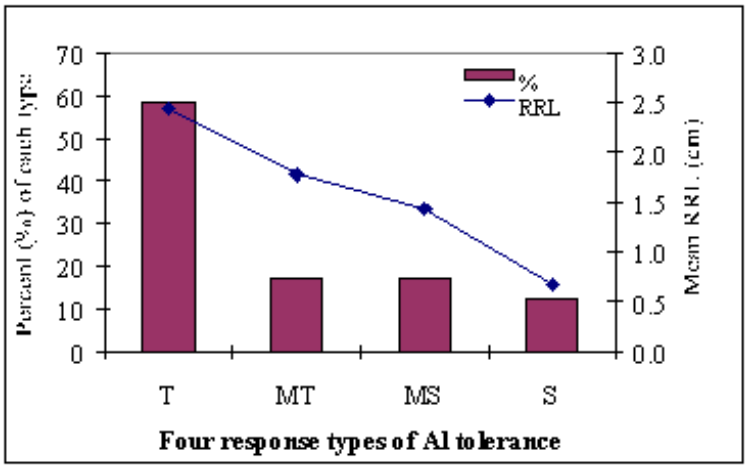

Figure 1. Grouping of 41 Waite lines (excluding check Tahara) into four response types for Al tolerance based on the information given in Table 1. The histogram shows the percentage of each type and the line the respective mean regrowth root length (RRL in cm).

DISCUSSION

Evaluation of the genotypic variance from the ANOVA indicated the importance of genotypic variation in contributing to the phenotypic variation in regrowth root length, and hence in Al tolerance response, among these Waite lines. The genotypic coefficient of variation (GCV) was 31.2%; the highest so far recorded in our experiments with Australian triticale germplasm (3, 4, 5). This high genetic variability in Al tolerance suggests that further genetic improvement in Al stress tolerance can be expected to occur through selection. A direct comparison and selection of lines showing greater root regrowth appears to be an effective breeding approach (6). Further improvement could be made through hybridisation (among high root regrowth Waite lines) and selection using a pedigree breeding method, since there was a high estimate of heritability (h_b? = 84.6%) for Al tolerance among these lines.

Additionally, within-variety/line selection seems to have promise among some of the Waite lines, as these displayed a wide range of regrowth root length (and hence large variation) in their 20 seedling samples. These lines, which included G03, G04, G05, G13, G18, G29, G34 and G38, were all less Al-tolerant than Tahara; however they contained some seedlings with superior root regrowth. This within-line variability may indicate seedling response heterogeneity and hence the opportunity to select for improved Al stress

tolerance. Individual within-line selections for desirable seedlings may result in the identification of elite lines with superior Al tolerance and increased plant productivity in Al-stressed field conditions.

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