Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Assessing the effectiveness of the TaMATE1B and TaALMT1 genes to enhance the Al3+ tolerance of durum wheat (Triticum turgidum) grown under controlled conditions and in the field.

Akitomo Kawasaki
Anton Wasson
Evangeline Kielnhofer
Peter Ryan
Emmanuel Delhaize (manny.delhaize@csiro.au)
CSIRO https://orcid.org/0000-0002-0529-7160

Research Article

Keywords: Durum, Triticum turgidum, aluminum, acid soil, TaALMT1, TaMATE1B

Posted Date: July 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1549253/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Purpose

Durum wheat is sensitive of acid soils because it lacks effective genes for Al^{3+} tolerance. Previous research showed introgression of the bread wheat (*Triticum aestivum*) *TaMATE1B* and *TaALMT1* genes individually increased the Al^{3+} tolerance of durum wheat. Here we aimed to (a) combine the genes into a single durum line, (b) compare the various introgression lines to each another and (c) establish the effectiveness of the introgressions in improving tolerance of acid soils in the field.

Methods

Durum wheat lines homozygous for Al^{3+}-tolerant alleles of *TaMATE1B* and *TaALMT1* were crossed to develop a line that incorporated both genes. The lines comprised of Jandaroi (parental cultivar), *TaMATE1B*-Jandaroi, *TaALMT1*-Jandaroi and *TaMATE1B* + *TaALMT1*-Jandaroi were screened for Al^{3+} tolerance by hydroponic and soil cultures in a growth cabinet. The lines were also assessed for biomass production and grain yield in the field on acid soils.

Results

The durum wheat lines with the various Al^{3+}-tolerance genes introgressed performed better based on root growth than Jandaroi, the parental cultivar, in both hydroponic and soil assays when grown in a cabinet. The various introgression lines were tolerant of acid soils compared to Jandaroi when grown in the field as assessed by shoot biomass and grain yield.

Conclusion

The *TaALMT1* and *TaMATE1B* genes improve the acid soil tolerance of durum wheat with indications that combing the two genes is the most effective strategy. The various lines will be valuable to breeders who wish to enhance the acid soil tolerance of durum germplasm.

Introduction

The high protein content of durum wheat (*Triticum turgidum*) is suitable for pasta production which makes it a lucrative crop and an important option for alleviating poverty in countries such as Ethiopia (Sall et al. 2019; Sissons 2008). However, current durum wheat cultivars are sensitive of acid soils because they lack genes that confer Al^{3+} tolerance. Acid soils are widespread around the world and a common toxin found in these soils is the Al^{3+} released from normally innocuous forms by the low pH (von Uexküll and Mutert 1995). The use of Al^{3+} tolerant germplasm to breed crops able to grow effectively

on acid soils forms part of the strategy for managing these soils (Ryan 2018). Acid soils constrain the effective production of durum wheat and Ethiopia in particular has many regions where acid soils restrict the growth of this preferred crop (Wayima 2022). Inhibition of root growth is the earliest obvious symptom of Al^{3+} toxicity amongst the small grain cereals (Kochian et al. 2015). When root growth is inhibited by Al^{3+} toxicity, plants are compromised in their ability to take up nutrients and water. Durum wheat is one of the most Al^{3+}-sensitive of the small-grained cereals (Bona et al. 1991) and screens of *T. turgidum* germplasm comprising both cultivars and landraces have failed to identify useful levels of Al^{3+} tolerance (Ryan et al. 2010; Wayima et al. 2019). This contrasts with bread wheat (*Triticum aestivum*) which shows a large variation in Al^{3+} tolerance within its germplasm. Although Al^{3+} tolerance in bread wheat is largely conferred by the major genes *TaALMT1* and *TaMATE1B* (Delhaize et al. 2012), other genes of smaller effect confer additive tolerance (Raman et al. 2010). *TaALMT1* encodes an Al^{3+}-activated anion channel that is permeable to malate. In the presence of Al^{3+}, the TaALMT1 protein, which resides on the plasma membrane of root apical cells, is activated allowing the efflux of malate down its electrochemical gradient (Delhaize et al. 2012; Ryan et al. 1997). The malate acts to chelate the Al^{3+} to protect root apices which are particularly susceptible of Al^{3+} toxicity. Although *TaMATE1B* also encodes a membrane protein it transports citrate and, unlike TaALMT1, TaMATE1B function is constitutive such that the protein does not require Al^{3+} for citrate to be released (Ryan et al. 2009; Tovkach et al. 2013). Like malate, citrate chelates Al^{3+} to render it non-toxic. Tolerant alleles of both genes are due to enhanced expression of the respective coding regions. In the case of *TaALMT1* enhanced expression is due to repeated sequences in the promoter whereas for *TaMATE1B* a large transposable element inserted into the promoter confers enhanced expression (Delhaize et al. 2012).

To enhance the acid-soil tolerance of durum wheat, Al^{3+}-tolerant alleles of both *TaALMT1* and *TaMATE1B* were introgressed from bread wheat into durum wheat. Starting with a chromosome 4D substitution line of durum wheat, the *pH1c* mutant of durum was used to introgress a chromosomal fragment of *TaALMT1* of bread wheat to chromosome 4B of durum wheat (Han et al. 2014; Han et al. 2016). Since *TaMATE1B* maps to chromosome 4B, it was possible to undertake direct crosses between durum and bread wheat to introgress *TaMATE1B* into the durum genetic background (Han et al. 2016).

Analysis of durum lines with the introgressed genes found that *TaMATE1B* was more effective gene than *TaALMT1* in conferring acid soil tolerance in short term experiments of about a week (Han et al. 2016). Based on this finding, subsequent research focused on the *TaMATE1B* line which was able to develop an effective root system in an acid subsoil that the control was unable to achieve in a large rhizobox where plants were grown to maturity and grain yield (Pooniya et al. 2020). Durum wheat is grown in regions that often experience a terminal drought (Boussakouran et al. 2019; Habash et al. 2014) and climate change will likely exacerbate the extent and severity of these events (Habash et al. 2010). A subsequent growth study mimicked this situation by growing plants in a reconstituted field soil with an acid subsoil and exposing them to a terminal drought. The results showed that the *TaMATE1B* line grew larger and yielded significantly more grain than the control line (Lui et al. 2020).

Here we describe the development of a new durum line that combines *TaALMT1* and *TaMATE1B*. This "double gene" line was compared to the parental cultivar and durum lines that possessed the individual genes separately. Growth of the lines were compared by hydroponics and in soil pots grown under the controlled conditions of a cabinet. We further compared their performance in field trials on acid soils and demonstrate the benefit of these introgressions to grain yields under field conditions.

Materials And Methods

Germplasm

The development of durum wheat lines with *TaALMT1* and *TaMATE1B* introgressed separately into a Jandaroi genetic background has been described previously (Han et al. 2016; Pooniya et al. 2020). These lines had been backcrossed three times to the cultivar Jandaroi. Here we refer to the various lines as Null (Jandaroi parental line), *TaALMT1*-Jandaroi and *TaMATE1B*-Jandraoi. Since a small chromosomal fragment with *TaALMT1* had been introgressed into the 4B chromosome and *TaMATE1B* is located on chromosome 4B of bread and durum wheat, we needed to identify a recombinant line that possessed both genes on the same chromosome. To develop a line with both genes introgressed we crossed the backcross three lines with the separate genes in the Jandaroi background to one another. To identify suitable recombinants, we first screened seedlings with a co-dominant marker for *TaMATE1B* (Tovkach et al. 2013) to identify seedlings that were homozygous for *TaMATE1B*. Once identified, we screened those lines with a dominant marker for *TaALMT1* (Sasaki et al. 2004). The dominant marker for *TaALMT1* did not allow us to identify seedlings homozygous for this gene but having identified the presence of at least one 4B chromosome that possessed *TaALMT1* in a homozygous *TaMATE1B* background ensured that a recombination had occurred. Selected recombinants were self-fertilised to generate F_2 and resulting F_3 populations were screened for lines that possessed homozygous Al^{3+}-tolerant alleles of both *TaALMT1* and *TaMATE1B*. We named a line identified from the screening as the *TaALMT1* + *TaMATE1B*-Jandaroi line or "double gene" line.

Hydroponics

For both long and short-term experiments seedlings were initially grown using a basket (28 cm diameter × 12 cm height with 2 mm mesh size) that was positioned on a top of a bucket (28 cm diameter × 28 cm height) filled with approximately 10 L of 0.2 mM $CaCl_2$ solution so that the bottom of the basket touched the surface of the solution. Seedling pre-germinated on Petri dishes for 2 days in the dark were placed in the basket so that the roots passed through the mesh into the solution. The $CaCl_2$ solution was aerated with an aquarium air pump, and the basket covered with aluminium foil to keep the plants in the dark. The seedlings were grown in the dark for another 6 days at room temperature until the coleoptiles develop to about10 cm. After the 6 days growth seedlings were transferred to a plastic tank (38.5 cm length × 23.5 cm width × 32.3 cm height) covered with a lid. A total of 12 slits (7 cm length × 1.5 cm width) were made around the lid to hold the plants. The tank was filled with 22 L of a basal nutrient solution

comprising of 500 μM $CaCl_2$, 150 μM $MgSO_4$, 1000 μM KNO_3, 500 μM NH_4Cl, 2 μM FeEDTA, 10 μM KH_2PO_4, 11 μM H_3BO_3, 2 μM $MnCl_2$, 0.35 μM $ZnSO_4$ and 0.2 μM $CuCl_2$. The seedling coleoptiles were wrapped with a piece of sponge and carefully inserted into the slits, so that the roots grew into the nutrient solution while the shoot grew above the tank. The Al^{3+} treatments were prepared by addition of stock 1 M $AlCl_3$ to the final concentration. The tanks were aerated with an air compressor at the rate of 8.8 L air min^{-1} $tank^{-1}$. The plants were grown for a further 7 days (short-term experiment) or 21 days (long-term experiment) in a growth cabinet (Conviron, Canada) set to 16 h day/8 h night cycle (24°C/20°C) and 600 μmol photon m^{-2} s^{-1}. The pH of solutions was monitored and adjusted daily to 4.3 for all treatments. Solutions were not changed for the short-term experiment whereas for the long-term experiment nutrient solutions were replaced on days 8, 11, 14 and 19 after seedlings had been planted in the tanks. At harvest, roots were cut away from shoots and stored in 70% ethanol (v/v) until they were scanned and analysed with WinRHIZO™ software (Regent Instruments Inc., Quebec, Canada). The length of axial roots (roots without lateral roots) was measured with a ruler and this was subtracted from the length of the total root system to obtain the lateral root length.

Soil Experiments

An acid ferrosol described previously (Delhaize et al. 2009) was collected from a farm in Robertson, NSW (latitude 34.63° S; longitude 150.48° E). The soil was air dried, sieved (4 mm) and stored at ambient temperature. The initial soil pH was 4.2, as determined by extraction with 10 mM $CaCl_2$. Prior to use for the experiment, soil moisture was adjusted to 324 mL H_2O kg^{-1} dry soil (90% of the field capacity) with deionised water. Lime ($CaCO_3$) was added (4 g kg^{-1} dry soil) to neutralise the acidity for control treatments. For the long-term soil experiment, nutrients were added as described below. No nutrients were added in the short-term soil experiment as nutrients in the seed were sufficient to supply the developing seedlings over 10 days.

Short-term (10 days) and long-term (3 weeks) pot trials were established to enable comparison of root morphology of young seedlings and more mature plants on acid and limed (control) soils. For the short-term soil experiment, 15 cm tall plastic tubes with an 9 cm diameter were used. A mass of 620 g of moistened soil was packed to 0.88 g cm^{-3} with no added nutrients. The genotypes were tested on the acid and limed (control) soil with 4 replicates per genotype per treatment. Pots were watered daily to weight to maintain the starting moisture content.

For the long-term soil experiment, 50 cm tall plastic pots with a 9 cm diameter were used. Soil was added as two stratified layers. The bottom layer (39 cm) contained 2150 g of soil, compacted to about 0.69 g cm^{-3}, whilst the top 10 cm layer contained 550 g of moist soil compacted to about 0.86 g cm^{-3}. A thin layer of polyethylene pellets was added between the top and bottom layers to allow the layers to be differentiated at subsequent sampling. Since this was a long-term experiment, nutrients were added to sustain plant growth and 400 mg of P as KH_2PO_4 was added only to the top 10 cm layer of the soil profile

to mimic field conditions where the P is typically found in the top 5 to 10 cm of the soil profile. The following nutrients soil (mg kg^{-1}) were added to both layers: $Ca(NO_3)_2.4H_2O$ (400), $(NH_2)_4SO_4$ (250), K_2SO_4 (200), $MgCl_2.H_2O$ (17), $MnSO_4.H_2O$ (10), $ZnSO_4.7H_2O$ (10), $CuSO_4.5H_2O$ (2), H_3BO_3 (0.67) and $Na_2MoO_4.2\ H_2O$ (0.17). Two treatments (with and without lime) were prepared and pots watered daily to weight.

At harvest, roots were washed with running tap water. For the long-term soil experiment, roots were separated into two fractions comprising of the top 10 cm and bottom 39 cm of the profile and analysed separately. The developmental stages of the plants were recorded and roots stored in 70% ethanol (v v^{-1}) for scanning and analysis with WinRHIZO™.

Field Experiments

Field trials were undertaken at three locations on farmer's or research station paddocks that varied in severity for Al^{3+} toxicity and were named after the nearest townships. The Tarcutta site (latitude 35.31° S; longitude 147.62° E; New South Wales, Australia) was rated as moderately toxic whereas the Gunning (latitude 34.68° S; longitude 149.28° E; New South Wales, Australia) and Merredin (latitude 31.46° S; longitude 118.37° E: Western Australia, Australia) sites were rated as severely toxic based on the pH and concentration of extractable Al^{3+} (Table 1). Each genotype was sown with four (Gunning and Tarcutta) or three (Merredin) replications in randomised complete blocks in trials that included other germplasm. For Tarcutta and Merredin plots were 6 m long by 1.75 m wide (6 rows at 25 cm inter-row; approximately 120 plants m^{-2}) that were cut back to 5 m long at harvest. Only Jandaroi-*TaMATE1B* was available for the Tarcutta trial run in 2018 and, due to limited grain, single rows 3 m long were sown at Gunning in 2019. Sufficient grain was available for the Merredin trial in 2019 to sow 6 m by 1.8 m plots of each genotype but the trial experienced severe drought. Nitrogen was supplied as urea prior to sowing and was top-dressed again at stem elongation with urea (timed with rainfall), or liquid N fertilisers (e.g. urea and ammonium nitrate liquid formulations). Muriate and sulphate of potash (KCl and K_2SO_4) were applied as required to ensure adequate K and S, as were micronutrients. Foliar diseases were managed with prophylactic fungicide and pesticide applications. Weeds were managed with district herbicide application practices at the recommended rates. Quadrat cuts comprising of 1 m^2 from each plot were collected close to anthesis for the Tarcutta and Merredin trials whereas total shoot biomass of plants collected at Gunning was measured at harvest. The Tarcutta and Merredin trials were machine-harvested whereas the Gunning trial was hand-harvested and grain cleaned with a table-top thresher.

Table 1

Summary of field trials selected to assess the Al^{3+}-tolerant durum lines. Soil samples were collected from each trial at three depths for assay of pH and extractable Al^{3+}. Values are the means with the standard error shown in parentheses (n = 209 for Tarcutta; n = 29 for Gunning; n = 219 for Merredin).

Location and year	Depth (cm)	pH	Extractable Al^{3+}
Tarcutta 2018	0–5	4.61 (0.01)	2.1 (0.1)
	5–10	4.42 (0.02)	4.9 (0.3)
	10–20	4.17 (0.01)	7.0 (0.3)
Gunning 2019	0–10	3.78 (0.02)	15.3 (0.8)
	10–20	4.02 (0.02)	16.8 (1.3)
	20–30	4.19 (0.03)	11.9 (1.4)
Merredin 2019	0–5	4.56 (0.01)	0.6 (0.1)
	5–10	4.24 (0.01)	4.3 (0.4)
	10–20	4.02 (0.01)	14.2 (0.5)

Results

Hydroponics

The Al^{3+} tolerance of the various lines was first assessed in a hydroponic experiment over ten days growth in acidic nutrient solutions that contained Al^{3+}. Figure 1 shows that all the lines that possessed either *TaALMT1* or *TaMATE1B* individually or both genes together had roots that were more tolerant of Al^{3+} than the parental cultivar Jandaroi (Null line). The enhanced tolerance measured as root length was particularly evident for axial seminal roots (Fig. 1B) whereas lateral roots for all lines were more sensitive of the Al^{3+} although, even here, the lines still differed from one another and maintained their relative tolerance rankings (Fig. 1C). Lines that possessed *TaALMT1* either by itself or in combination with *TaMATE1B* performed best with the double gene line showing the greatest Al^{3+} tolerance of lateral roots at 2.5 and 5.0 μM $AlCl_3$.

We then compared the three Al^{3+}-tolerant lines to each other in a longer-term 21 day growth experiment in solutions that contained Al^{3+}. In this experiment, Jandaroi was omitted as its extreme sensitivity of Al^{3+} would have resulted in very short roots and the aim was to establish if the Al^{3+}-tolerant lines differed from one another. The three Al^{3+}-tolerant lines performed similarly to one another for total root length (Fig. 2A) and axial root length (Fig. 2B) at 2.5 μM Al^{3+} whereas lines that possessed *TaALMT1* performed better than the *TaMATE1B* line at the highest Al^{3+} treatment. The differences in root growth at 7.5 μM M Al^{3+}

were largely driven by differences in lengths of lateral roots (Fig. 2C). In this experiment the double gene line did not perform better than the single gene line containing *TaALMT1* only.

Pot trials

In contrast to the hydroponics experiments, lines with *TaMATE1B* only or in combination with *TaALMT1*, generally performed best when grown on acid soil in pots (Figs. 3 and 4). In the short-term experiment, both *TaMATE1B*-containing lines had the most tolerant roots although the *TaALMT1* line still performed better than the Null line (Fig. 3). The double gene line did not perform better than the line with *TaMATE1B* alone. When the soil had been limed to detoxify the Al^{3+}, root growth of all four lines, including the Null line, was similar to one another (Fig. 3).

For the long-term pot experiments a profile with an acidic subsoil was constructed that approximated the field. The pots were 50 cm tall and P fertiliser was applied only in the top 10 cm reflecting the stratification of P in the soil profile that is commonly found in soils. Phosphate can bind with Al^{3+} and the application of K phosphate in the top 10 cm served to detoxify toxic Al^{3+}. This was clearly demonstrated by measuring root weights in the upper soil layer as they were the same for all lines with or without lime addition (Fig. 5A). By contrast, the *TaMATE1B* lines had the longest roots in the lower acid layer with the double gene line tending to have the longest roots (Fig. 4A). All lines had similar root lengths to one another in the limed soil and although the Al^{3+} tolerance genes enabled roots to grow in the acid layer they did not grow as well as in soil that had lime incorporated throughout the pot as determined by root length (Fig. 4A and 4B). The *TaMATE1B* lines had the greatest shoot dry weights (Fig. 5B) in the acid soil treatment although this was not as marked as the differences in root growth found in the bottom 39 cm of the tube (Fig. 4A).

Field experiments

Although we expected that pot experiments would more closely approximate the field than hydroponics experiments, they still only approximate field conditions. For instance, water was applied to pots daily and Al^{3+}-sensitive roots of the Null line grew well in the top 10 cm of the P-fertilised layer which had been detoxified for Al^{3+} as discussed above and stayed moistened for the duration of growth (Fig. 5A). To assess the lines in the field, Jandaroi-*TaMATE1B* and Jandaroi lines were first compared in a moderately acidic site at Tarcutta in 2018 and all lines were subsequently grown together at two other sites where acidity was more severe (Merredin and Gunning in 2019).

The *TaMATE1B* line produced about 40% more grain than Jandaroi when grown at Tarcutta in 2018 (Fig. 6A). Shoot biomass estimated with a quadrat cut pre-anthesis showed a similar trend to grain yield although it was not statistically significant at $P < 0.05$ (Fig. S1A). A pronounced benefit of *TaMATE1B* alone or in combination with *TaALMT1* was evident at Gunning in 2019 (Fig. 6B; note that data for Gunning are from single rows whereas data for Tarcutta and Merredin are from whole plots). There was a tendency for the double gene line to produce the most grain whereas the *TaALMT1* line was similar to the

Null line. Lines that possessed *TaMATE1B* (lines *TaMATE1B* and *TaALMT1 + TaMATE1B*) had a significantly greater grain yield than lines that lacked *TaMATE1B* (lines Null and *TaALMT1*) when assessed by Student's t-test ($P < 0.001$). For the Gunning trial the Null and *TaALMT1* lines produced about 50% of the shoot biomass of the *TaMATE1B* lines (Fig. S1B) although they produced almost no grain (Fig. 6B). Merredin in 2019 was severely droughted with the Null line producing little or no grain (Fig. 6C). Although grain yields were considerably lower than would have been expected for a normal year, the Al^{3+} tolerance genes still enabled plants to produce some grain with an indication that line Jandaroi *TaMATE1B + TaALMT1* produced the most grain. Although the Al^{3+} tolerance genes enabled durum to set grain whereas the Jandaoi Null line produced almost no grain, the large variability and only three replicates meant grain data for all lines were not significantly different from the Null line at $P < 0.05$ (Fig. 6C). Total biomass prior to anthesis showed similar trends to grain yields in the Merredin trial although in this case the *TaMATE1B + TaALMT1* line was significantly larger than the Null line (Fig. S1C).

Discussion

Here we show that *TaMATE1B* and *TaALMT1* derived from bread wheat conferred Al^{3+} tolerance to root growth of durum wheat when grown in hydroponics and acid soils. Although *TaALMT1* was more effective than *TaMATE1B* in conferring Al^{3+} tolerance when grown in hydroponics (Figs. 1 and 2), the opposite was apparent when the lines were grown in soil (Figs. 3 and 4). This inverse relationship of the tolerance of Jandaroi-*TaMATE1B* and Jandaroi-*TaALMT1* in hydroponics and soil was previously described for young seedlings (Han et al. 2016) and here we extend the work to find a similar relationship in more mature plants. Lateral roots were generally more sensitive of the Al^{3+} than the axial roots for all lines when grown in both hydroponics and acid soil (Figs. 1 to 3) even though Al^{3+}-tolerant wheat lines are capable of citrate and malate release from tips of lateral roots (Kawasaki et al. 2018). Lateral roots comprised the largest proportion of root system length and had the greatest influence on total root length for both hydroponics and soil experiments. The benefit in shoot growth of lines having the *TaMATE1B* gene was evident for the long-term soil experiment but it was a relatively small advantage (~ 1.3-fold) when compared to the benefit on root growth in the acid layer of the pot experiment (~ 4-fold). This subdued effect on shoots can be attributed to (a) the top 10 cm of the soil having been detoxified by the application of P-fertiliser and (b) water being applied daily to the surface of the pots. The daily watering ensured that the surface soil remained moist and allowed root growth to be maintained for all lines including the Null line (Fig. 5A). Similarly, previous work (Pooniya et al.) found that when the *TaMATE1B* line was grown to maturity in a severely acid soil in large rhizoboxes, grain yield was not increased compared to a Null line even though root growth was considerably improved. Similar to the current experiment, the top 10 cm of soil was not Al^{3+} toxic and plants were watered daily on the surface. When the *TaMATE1B* line was grown in large pots and experienced a terminal drought the benefit of *TaMATE1B* on grain yield became apparent as a result of an ability to access soil moisture at depth (Lui et al. 2020). Importantly, there was no evidence of introgression of deleterious genes linked to *TaALMT*1 or *TaMATE1B* on the basis of the long-term pot trial. Here we show that shoot biomass and root growth (Fig. 5) on the limed treatments where Al^{3+} toxicity was eliminated were similar for all lines.

The benefit on grain yield of durum lines with introgressed Al^{3+} tolerance genes was apparent in field trials at several sites with acid soils. The *TaMATE1B* gene by itself conferred a greater grain yield than Jandaroi on the moderately acid site of Tarcutta (Fig. 6A). The better performance of *TaMATE1B* than *TaALMT1* was evident in the field trials undertaken at the Gunning 2019 and Merredin 2019 sites. There was a tendency for the double gene line to produce the most grain yield at these two very acidic sites suggesting an additive effect of the two genes on Al^{3+} tolerance which was most evident in the severely-droughted site in Merredin. Previous work comparing the *TaMATE1B* and *TaALMT1* lines in young seedlings grown for a week found that *TaMATE1B* was more effective in conferring Al^{3+} tolerance to root growth in acid soil than *TaALMT1* (Han et al. 2016) and hence subsequent research has focussed on the *TaMATE1B* line (Lui et al. 2020; Pooniya et al. 2020). Here we show that that the *TaMATE1B* line performed better for root growth than the *TaALMT1* line in pot trials with acid soil indicating that short term experiments were able to predict performance in longer term experiments and in the field. However, we suggest the double gene line warrants further research as it may be the best option on severely acid soils particularly when experiencing drought. An improved drought tolerance of the double gene line compared with the single gene lines might be a consequence of longer roots than the single gene lines in enabling access deep water as suggested by root lengths in the acid layer of the long-term pot trial (Fig. 4A).

From a breeding perspective it is simpler to introgress a single genetic locus for the desired trait instead of two or more loci. The *TaMATE1B*-Jandaroi line performed best under controlled conditions and this was also evident in the field indicating that introgression of this gene alone could be sufficient to obtain effective Al^{3+} tolerance in the field. Although we suggest the double gene line could be the best option for production on acid soils but this shouldn't complicate breeding efforts. Both genes have been introgressed onto the 4B chromosome of durum wheat and are linked such that they can be transferred together to generate new lines. Molecular markers are available for each gene to verify that a recombination event in progeny has not dissociated the genes from one another. Although the Al^{3+}-tolerant durum lines enabled improved growth on acid soil, liming practices should still be maintained. Note that the most effective root and shoot growth was found in the limed treatments of the long-term pot trials (Figs. 4B and 5B). In that experiment the soils were well-mixed with the lime and the acid pH was effectively neutralised throughout the profile. Liming is likely to provide the most effective solution to soil acidity in the field if it can be incorporated throughout the soil profile which may take years with repeated applications (Azam and Gazey 2021). Lime application can be costly particularly if it needs to be transported long distances and is typically applied to the surface soil. Incorporating the lime to depth incurs additional cost that can be prohibitive. The cost of transporting and incorporating lime is a particular challenge for developing countries such as Ethiopia where durum wheat is grown. Furthermore, previous research on bread wheat has shown that combining liming practices with Al^{3+}-tolerant bread wheat germplasm provides an effective strategy for managing acid soils (Scott et al. 2001; Tang et al. 2003) and a similar argument can be made for durum wheat. The lines generated from this study warrant further assessment in field trials on a range of acid soils. Our initial field trials provide supportive evidence that the *TaALMT1* and *TaMATE1B* genes can be used to enhance the Al^{3+} tolerance of

commercial durum germplasm. When combined with liming practices, these lines will have the potential of greatly improving durum production on acid soils.

Declarations

Acknowledgements: The authors thank Dr Xiaoqing Li (CSIRO Agriculture and Food) for assistance in setting up the hydroponics experiments.

Funding: The research was supported by internal CSIRO funds with Evangeline Kielnhofer as the recipient of a CSIRO studentship award and Akitomo Kawasaki as the recipient of an OCE Postdoctoral Fellowship.

Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Author contributions: All authors contributed to the manuscript preparation. Evangeline Kielnhofer carried out practical work with growth cabinets, Anton Wasson designed and supervised field trials, Aki Kawasaki planned and supervised growth cabinet experiments, Peter Ryan assisted with writing the manuscript and Emmanuel Delhaize developed the germplasm and contributed to the planning of all experiments.

Conflict of Interests: No conflicts of interest

References

1. Azam G, Gazey C (2021) Slow movement of alkali from surface-applied lime warrants the introduction of strategic tillage for rapid amelioration of subsurface acidity in south-western Australia. Soil Res 59:97–106. doi: https://doi.org/10.1071/SR19329
2. Bona L, Wright RJ, Baligar VC (1991) A rapid method for screening cereals for acid soil tolerance. Cereal Res Commun 19:465–468
3. Boussakouran A, Sakar EH, El Yamani M, Rharrabti Y (2019) Morphological traits associated with drought stress tolerance in six Moroccan durum wheat varieties released between 1984 and 2007. J Crop Sci Biotechnol 22:345–353. doi: 10.1007/s12892-019-0138-0
4. Delhaize E, Ma JF, Ryan PR (2012) Transcriptional regulation of aluminium tolerance genes. Trends Plant Sci 17:341–348. doi: 10.1016/j.tplants.2012.02.008
5. Delhaize E, Taylor P, Hocking PJ, Simpson RJ, Ryan PR, Richardson AE (2009) Transgenic barley (*Hordeum vulgare* L.) expressing the wheat aluminium resistance gene (*TaALMT1*) shows enhanced phosphorus nutrition and grain production when grown on an acid soil. Plant Biotech J 7:391–400
6. Habash DZ, Baudo M, Hindle M, Powers SJ, Defoin-Platel M, Mitchell R, Saqi M, Rawlings C, Latiri K, Araus JL, Abdulkader A, Tuberosa R, Lawlor DW, Nachit MM (2014) Systems responses to progressive water stress in durum wheat. PLoS ONE 9:e108431. doi: 10.1371/journal.pone.0108431
7. Habash DZ, Kehel Z, Nachit M (2010) Genomic approaches for designing durum wheat ready for climate change with a focus on drought. J Exp Bot 61:1249–1249. doi: 10.1093/jxb/erq024

8. Han C, Ryan PR, Yan Z, Delhaize E (2014) Introgression of a 4D chromosomal fragment into durum wheat confers aluminium tolerance. Ann Bot 114:135–144. doi: 10.1093/aob/mcu070
9. Han C, Zhang P, Ryan PR, Rathjen TM, Yan ZH, Delhaize E (2016) Introgression of genes from bread wheat enhances the aluminium tolerance of durum wheat. Theor Appl Genet 129:729–739. doi: 10.1007/s00122-015-2661-3
10. Kawasaki A, Okada S, Zhang C, Delhaize E, Mathesius U, Richardson AE, Watt M, Gilliham M, Ryan PR (2018) A sterile hydroponic system for characterising root exudates from specific root types and whole-root systems of large crop plants. Plant Methods 14:114. doi: 10.1186/s13007-018-0380-x
11. Kochian LV, Pineros MA, Liu JP, Magalhaes JV (2015) Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. Annu Rev Plant Biol 66:571–598. doi: 10.1146/annurev-arplant-043014-114822
12. Lui L, Bai C, Chen Y, Palta JA, Delhaize E, Siddique KHM (2020) Durum wheat with the introgressed *TaMATE1B* gene shows resistance to terminal drought by ensuring deep root growth in acidic and Al^{3+}-toxic subsoils Plant Soil. 447:73–84. 10.1007/s11104-021-04961-6
13. Pooniya V, Palta JA, Chen Y, Delhaize E, Siddique KH (2020) Impact of the *TaMATE1B* gene on above and below-ground growth of durum wheat grown on an acid and Al^{3+}-toxic soil. Plant Soil 447:73–84
14. Raman H, Stodart B, Ryan PR, Delhaize E, Emebiri L, Raman R, Coombes N, Milgate A (2010) Genome-wide association analyses of common wheat (*Triticum aestivum* L.) germplasm identifies multiple loci for aluminium resistance. Genome 53:957–966. doi: 10.1139/g10-058
15. Ryan PR (2018) Assessing the role of genetics for improving the yield of Australia's major grain crops on acid soils. Crop Pasture Sci 69:242–264. doi: 10.1071/cp17310
16. Ryan PR, Raman H, Gupta S, Horst WJ, Delhaize E (2009) A second mechanism for aluminum resistance in wheat relies on the constitutive efflux of citrate from roots. Plant Physiol 149:340–351
17. Ryan PR, Raman H, Gupta S, Sasaki T, Yamamoto Y, Delhaize E (2010) The multiple origins of aluminium resistance in hexaploid wheat include *Aegilops tauschii* and more recent *cis* mutations to *TaALMT1*. Plant J 64:446–455
18. Ryan PR, Skerrett M, Findlay GP, Delhaize E, Tyerman SD (1997) Aluminum activates an anion channel in the apical cells of wheat roots. Proceedings of the National Acadamy of Sciences USA 94: 6547–6552
19. Sall AT, Chiari T, Legesse W, Seid-Ahmed K, Ortiz R, vG M, Bassi FM (2019) Durum wheat (*Triticum durum* Desf.): origin, cultivation and potential expansion in sub-Saharan Africa. Agronomy MDPI 9.
20. Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, Ahn SJ, Ryan PR, Delhaize E, Matsumoto H (2004) A wheat gene encoding an aluminum-activated malate transporter. Plant J 37:645–653
21. Scott BJ, Fisher JA, Cullis BR (2001) Aluminium tolerance and lime increase wheat yield on the acidic soils of central and southern New South Wales. Aust J Exp Agric 41:523–532
22. Sissons M (2008) Role of durum wheat composition on the quality of pasta and bread Food. 2:75–90

23. Tang C, Rengel Z, Diatloff E, Gazey C (2003) Responses of wheat and barley to liming on a sandy soil with subsoil acidity. Field Crops Res 80:235–244
24. Tovkach A, Ryan PR, Richardson AE, Lewis DC, Rathjen TM, Ramesh S, Tyerman SD, Delhaize E (2013) Transposon-mediated alteration of *TaMATE1B* expression in wheat confers constitutive citrate efflux from root apices. Plant Physiol 161:880–892. doi: 10.1104/pp.112.207142
25. von Uexküll HR, Mutert E (1995) Global extent, development and economic impact of acid soils. Plant Soil 171:1–15
26. Wayima EF (2022) Classification of Ethiopian soils with pH. Journal of Soil Science and Environmental Management In press
27. Wayima EF, Ligaba-Osena A, Dagne K, Tesfaye K, Machuka EM, Mutiga SK, Delhaize E (2019) Screening of diverse Ethiopian durum wheat accessions for aluminum tolerance. Agron MDPI 9:440

Figures

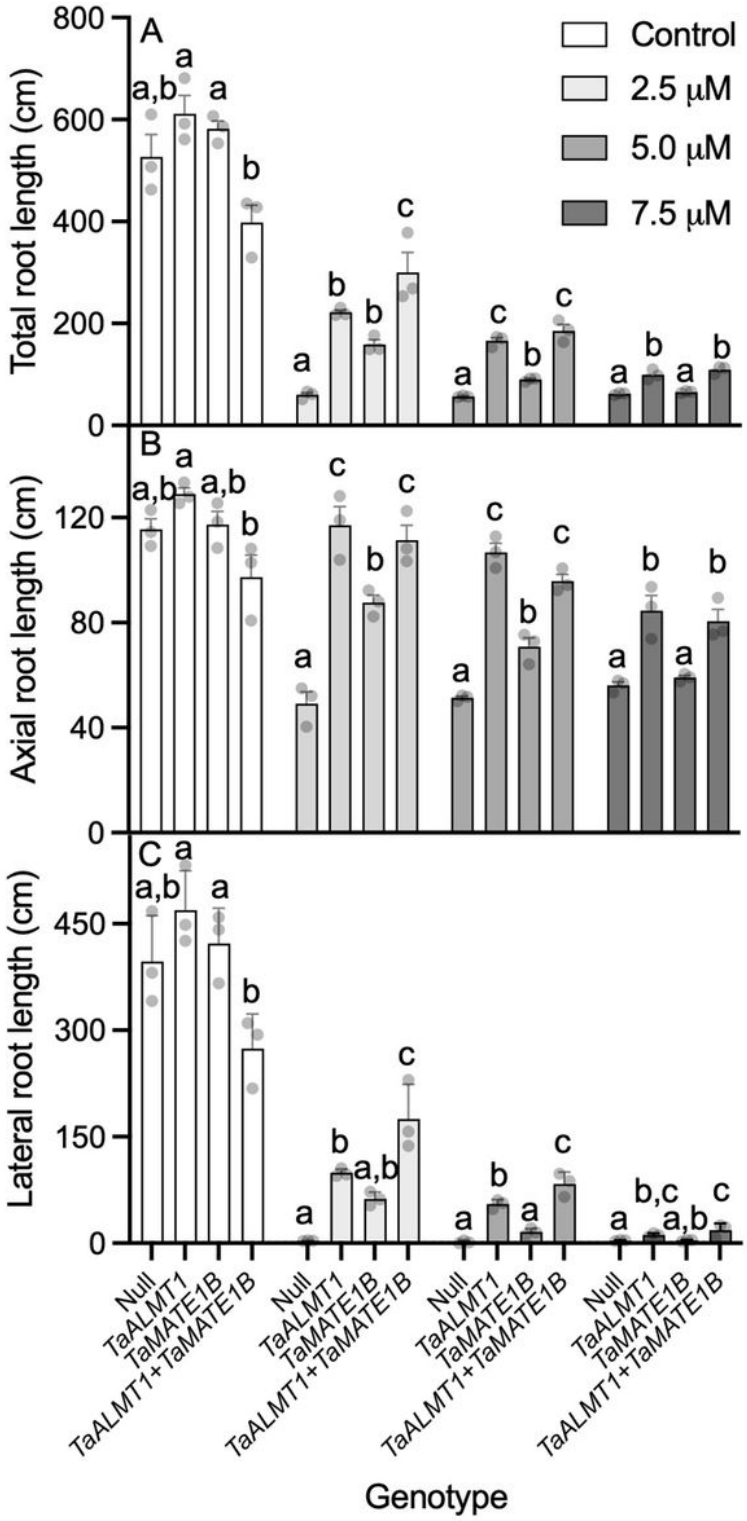


Figure 1

TaALMT1 and *TaMATE1B* introgressed from bread wheat confer Al^{3+} tolerance to durum wheat in short-term hydroponic assays. Seedlings were grown in hydroponic culture for seven days after which roots were collected and scanned to determine total root length (a), axial root length (b) and lateral root length (c). Data were analysed by one way ANOVA for each concentration with different letters indicating significant differences at $P < 0.05$ (n = 3; grey circles for individual values, error bars indicate the standard

error of the mean and in some cases error bars are obscured by the circles). The null line indicates Jandaroi as the parental genotype for all lines.

Figure 2

Comparison of Al^{3+} tolerance in long-term hydroponic assays of durum wheat lines with *TaALMT1*, *TaMATE1B* and *TaALMT1* + *TaMATE1B* introgressed from bread wheat. Seedlings were grown in hydroponic culture for 21 days after which roots were collected and scanned to determine total root length (a), axial root length (b) and lateral root length (c). Data were analysed by one way ANOVA for each concentration with different letters indicating significant differences at $P < 0.05$ (n = 8; grey circles for individual values and error bars indicate the standard error of the mean). The null line Jandaroi was not included due to its extreme sensitivity of Al^{3+} as determined in the short-term assay.

Figure 3

TaALMT1 and *TaMATE1B* introgressed from bread wheat confer Al^{3+} tolerance to durum wheat in short-term soil assays. Seedlings were grown in soil culture for ten days after which roots were washed out and analysed to determine total root length (a), axial root length (b) and lateral root length (c). The acid soil was a highly acid ferrosol that was treated with $CaCO_3$ to neutralise the acidity for the limed treatment. Data were analysed by a two-way ANOVA for factors genotype and treatment with different letters indicating significant differences at $P < 0.05$ (n = 8; grey circles for individual values and error bars indicate the standard error of the mean). The null line indicates Jandaroi as the parental genotype for all lines.

Figure 4

TaMATE1B + *TaALMT1* introgressed from bread wheat confers Al^{3+} tolerance to durum wheat in a long-term soil assay. Seedlings were grown in soil culture with two layers comprising of soil supplemented with phosphate in the top 10 cm while the lower layer (39 cm) had all nutrients supplied except for P. After 27 days roots were washed out from the lower layer and scanned to determine total root length in the acid soil (A) and the same soil that had been limed (B). Data were analysed by one way ANOVA for each soil type (acid or limed) with different letters indicating significant differences at $P < 0.05$ (n = 6 for the acid soil; n = 4 for the limed soil; grey circles show individual values and error bars indicate the standard error of the mean). The null line indicates Jandaroi as the parental genotype for all lines and there were no significant differences between genotypes found in the limed soil.

Figure 5

Root growth in a surface soil that had been supplemented with phosphate did not differ between lines whereas *TaMATE1B* lines had improved shoot growth. Seedlings were grown in soil culture with two layers comprising of soil supplemented with phosphate in the top 10 cm while the lower layer (39 cm) had all nutrients supplied except for P. After 27 days roots were washed out from the upper layer and shoots collected. Roots (A) and shoots (B) were dried and weighed. Data for roots (A) and shoots (B) were analysed by two-way ANOVAs with no significant differences between genotypes at $P < 0.05$ for either soil ($n = 6$; grey circles show individual values and error bars indicate the standard error of the mean). Shoot growth of the lines possessing the *TaMATE1B* gene were combined and compared with Student's t-test to the lines that did not possess *TaMATE1B* (null and *TaALMT1*) and found to be significantly greater at $P < 0.05$ whereas analysis with a one-way ANOVA where each soil was treated separately did not detect differences. The null line indicates Jandaroi as the parental genotype for all lines.

Figure 6

Effect of *TaALMT1* and *TaMATE1B* introgressed from bread wheat into durum on grain yields when grown on acid soils in the field. Jandaroi-*TaMATE1B* and Jandaroi were grown on an acid site at Tarcutta (A) and grain yield data of genotypes compared with Student's t-test ($n = 4$, * $P < 0.05$). All lines were grown on acid sites at Gunning (B) and Merredin (C). A one-way ANOVA detected differences between genotypes at $P < 0.05$ for Gunning with different letters indicating significant differences ($n = 4$ except for *TaMATE1B* which had $n = 3$). No significant differences between genotypes were detected for Merredin at $P < 0.05$. For all experiments grey circles show individual values and error bars indicate the standard error of the mean.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- KawasakietalFigS1.docx