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Inheritance of aluminum tolerance in the wheat cultivar Toropi and new findings about the introduction of this trait into the Brazilian wheat germplasm

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ABSTRACT

Aluminum (Al) is the most abundant metal in the earth's crust, and in acidic soils it dissolves into the phytotoxic form Al^{3+}. Analysis of the inheritance of aluminum tolerance in a donor genotype is an important step in the development of new cultivars adapted to acidic soils. The Brazilian wheat cultivars BH1146 and Carazinho have been important donors of aluminum tolerance genes in wheat breeding programs around the world. This trait is under the control of two main genes, *TaALMT1* and *TaMATE1B*. Interestingly, both cultivars have both of these genes, but different alleles. Lineages developed in Brazil from local landraces gave rise to the cultivars BH1146 and Carazinho, as well as other genotypes among which the cultivar Toropi is highlighted herein because of its excellent performance in acidic soils. It is not known if the genes and alleles responsible for aluminum tolerance in Toropi are the same as those carried by BH1146 and Carazinho. We analyzed the recombinant inbred lines (RILs) from Toropi × Anahuac (an Al-susceptible cultivar) and showed that aluminum tolerance in Toropi was controlled by one gene with major effects on the phenotype, although other gene(s) with minor effects also affected this trait. The analysis of RILs from Toropi × BH1146 showed that this major gene was different from the one carried by BH1146. The genealogy and molecular analyses of the *TaALMT1* and *TaMATE1B* genes of the Al-tolerant Brazilian wheat cultivars allowed us to establish that Toropi has the same allele of the *TaMATE1B* gene as Carazinho, but probably originating from a different source.

1. Introduction

Aluminum (Al) is the most abundant metal in the earth's crust, and under low-pH conditions it dissolves into the phytotoxic form Al^{3+}, which inhibits root cell division and elongation in the apical meristem, and also decreases water and essential nutrient uptake and/or translocation (Foy, 1983; Kochian, 1995; Ma and Furukawa, 2003; Kochian et al., 2005; Singh et al., 2017). Phosphorous metabolism is also impaired by aluminum cations because the metal readily precipitates this nutrient in the roots, reducing its root-to-shoot translocation (Alam, 1981; Silva et al., 2010). As an agronomic alternative to mitigate Al toxicity under low-pH conditions, the amount of free aluminum at the soil surface can be reduced by liming. Unfortunately, this practice is too expensive for small-scale farmers and is not very effective at controlling acidity in the deeper layers of the soil (Rao et al., 1993). Therefore, the development and application of Al-tolerant cultivars in fields is a better environmental solution to permit agricultural production in regions with acidic soils.

Wheat is an important staple food produced in a wide range of climatic environments and geographic regions, accounting for 16% of the total dietary calories in the developing world (Dixon et al., 2008; Chaves et al., 2013). When compared to other major cereal crops, such as maize and rice, wheat is more sensitive to aluminum toxicity (Famoso et al., 2010), resulting in restricted growth and productivity of this crop in regions where this metal is more prevalent. Several studies have already shown that wheat contains considerable genetic variation for aluminum tolerance (Hu et al., 2008; Ryan et al., 2009; Raman et al., 2010; Navakode et al., 2014), and the most suitable donor lines

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for breeding programs have arisen in Brazil, where acidic soils are common (Rajaram et al., 1988). Thus, it is not surprising that the cultivars extensively used in studies of aluminum tolerance in wheat are Brazilian (BH1146 and Carazinho) or originated from Brazilian genetic resources, such as Atlas 66, which is descended from the Brazilian cultivar Frondoso.

Previous studies performed on Chinese Spring wheat germplasm stocks identified multiple genetic loci on the chromosome arms 2DL, 3DL, 4BL, 4DL, 5AS, 6 A L, 7AS, and 7D that appear to be critical for tolerance to aluminum stress (Aniol and Gustafson, 1984; Camargo, 1984; Aniol, 1990; Papernik et al., 2001). Nevertheless, it remains unknown whether all of these loci are involved in generating the natural variation in aluminum tolerance observed in wheat. In Brazilian wheat cultivars, different mechanisms of tolerance have been described that are conferred by genes located on different chromosomes. In the cultivar BH1146, a locus with major effects on the phenotype is located on chromosome 4DL (Riede and Anderson, 1996; Rodriguez-Milla and Gustafson, 2001), whereas in the cultivar Carazinho a different locus on chromosome 4BL also contributes to aluminum tolerance (Ryan et al., 2009). In the cultivar Atlas 66 (not a Brazilian cultivar, but one developed from parental cultivars from Brazil), different loci on chromosomes 5D (Kerridge and Kronstad, 1968), 4DL (Ma et al., 2004; Sasaki et al., 2004; Raman et al., 2005), and 3BL (Zhou et al., 2007) were identified as being involved in effective aluminum tolerance. This illustrates that a diverse set of loci that determine plant responsiveness to aluminum stress can be found in the Brazilian wheat germplasm, and reinforces the affirmation of Carver and Garvin (2003) that our understanding of the genetic control of aluminum tolerance in wheat is incomplete.

The Brazilian wheat cultivar Toropi was developed in the 1960s and shows important characteristics demanded by breeders. Noteworthy among these characteristics are the adult plants' resistance to leaf rust (Barcellos et al., 2000; Da-Silva et al., 2012), tolerance to phosphorus starvation (Silva et al., 2008; Espindula et al., 2009), and excellent ability to adapt to acidic soils (Souza, 1998). The outstanding ability of Toropi to grow in acidic soils that has been observed by Brazilian breeders in theirs fields since its release provides evidence for the presence of genes for tolerance to aluminum toxicity in this cultivar. Recently, molecular analyses confirmed the presence of these genes in Toropi (Raman et al., 2008; Ryan et al., 2009; Pereira et al., 2015; Aguilera et al., 2016; Pereira, 2018). Raman et al. (2008) reported that Toropi and BH1146 were aluminum-tolerant genotypes because they have promoter alleles that were correlated with high expression levels of the *TaALMT1* gene. This gene co-segregates with malate efflux and was mapped onto chromosome 4DL of BH1146. On the other hand, Ryan et al. (2009) detected similar levels of citrate efflux in Toropi and Carazinho, while in BH1146 this was 6-fold lower, leading them to propose that citrate efflux is a secondary mechanism of aluminum tolerance in wheat. This mechanism is conferred by a MATE-type transporter gene mapped on chromosome 4BL of Carazinho. Tovkach et al. (2013) isolated and characterized this gene, and named it *TaMATE1B*. These authors also showed that the protein encoded by this gene is a constitutive citrate transporter located in the plasma membranes of the cells in the root apices of Carazinho. Although data are available on the presence of Al tolerance genes in Toropi, the inheritance pattern of this trait in this cultivar is not fully understood.

The goal of this study was to evaluate the genetic inheritance pattern of aluminum tolerance in Toropi and thereby determine whether the gene(s) involved in the expression of this trait in Toropi differ from those in BH1146 and Carazinho. The genetic hypothesis tested was that Toropi has one major gene that is responsible for its Al-tolerance, which is distinct from the major gene in BH1146.

2. Materials and methods

2.1. Plant material

Two populations of recombinant inbred lines (RILs) were used to evaluate the inheritance of Al^{3+} tolerance in wheat. The first population (124 F_7 RILs) was obtained from a cross between the wheat cultivars Toropi (Al^{3+}-tolerant) and Anahuac (Al^{3+}-sensitive), and the second population (79 F_6 RILs) was obtained from a cross between Toropi (Al^{3+}-tolerant) and BH1146 (Al^{3+}-tolerant). Both populations were developed at the National Wheat Research Center - EMBRAPA, Brazil. Toropi is from Brazil with a pedigree of Petiblanco//Frontana/Quaderna A. Anahuac is from Mexico with a pedigree of II12300//Lerma Rojo 64/815613/Norteño67. BH1146 is from Brazil with a pedigree of PG1/Fronteira/Mentana.

The F_7 RILs from the Toropi and Anahuac cross were used to study the inheritance of aluminum tolerance in Toropi. The F_6 RILs from the Toropi and BH1146 cross were evaluated to determine whether aluminum tolerance in Toropi and BH1146 was controlled by the same gene. In all of the experiments performed, the parents were included, as well as the Al-sensitive genotype Anahuac.

2.2. Evaluation of aluminum tolerance

The relative root growth under Al^{3+} stress was evaluated using the relative tolerance index (RTI) method, as previously described by Baier et al. (1995). Seeds from each RIL and its parents were surface sterilized for 3 min in 2% sodium hypochlorite, rinsed with distilled-deionized water three times, maintained on standard germination paper for 24 h at 4 °C, and finally transferred to a germination chamber for 46 h at 23 ± 1 °C. Once germination began, eight seedlings with similar tap root lengths (approximately 1 cm) were selected from each RIL and transferred to pots filled with 2 L of nutrient solution (0.4 mM $CaCl_2$, 0.65 mM KNO_3, 0.01 mM $(NH_4)_2SO_4$, 0.25 mM $MgCl_2$, 0.04 mM NH_4NO_3), properly aerated without phosphorous, and maintained at a pH of 4.0 in a growth chamber at 25 ± 1 °C under a 12/12 light/dark cycle. The hydroponic solution was replaced every 24 h. For Al treatments, increasing concentrations of $AlCl_3$ were added to the hydroponic solution as follows: 0 µM (0 ppm), 74 µM (2 ppm), 148 µM (4 ppm), 222 µM (6 ppm), 370 µM (10 ppm), 550 µM (15 ppm), and 740 µM (20 ppm). After 96 h of initial exposure, the seedlings were removed from the solution and kept at −20 °C until root measurements were taken. Six different experiments were performed (Table 1).

Initially, the RILs were evaluated for their response to Al^{3+} at the concentration of 74 µM. Later, the RILs were evaluated at increasing Al^{3+} concentrations of 148 µM and 222 µM. In all of the experiments

Table 1
Experiments performed to assess the phenotypes of wheat recombinant inbred lines (RILs) when exposed to different Al^{3+} concentrations.

Experiment	Population	Number of RILs	Al^{3+} concentrations (µM)						
			0	74	148	222	370	550	740
I	Toropi x Anahuac	96	✓	✓					
II	Toropi x Anahuac	84	✓		✓				
III	Toropi x Anahuac[a]	107	✓	✓	✓	✓			
IV	Toropi x BH1146[a]	76	✓	✓					
V	Toropi x BH1146[a]	75	✓		✓	✓			
VI	Toropi x BH1146[a]	10	✓				✓	✓	✓

[a] Experiment performed two times.