Exudation of organic acids from roots of triticale

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Abstract

Several triticale genotypes differing in Al tolerance, along with an Al-tolerant wheat line (ET3) were used to investigate the root exudation of organic acids. An enzymic assay method was used to detect malate release from both the whole root system and root tips. Organic acid exudates from Al-stressed root tips were measured by HPLC. The enzymic assay method revealed some association between Al tolerance and malate efflux from Al-stressed roots, but Al-tolerant triticale genotypes were found to rele ase only a small amount of malate. HPLC analysis showed that malate and citrate were not the main exudates related to different levels of Al tolerance. A yet to be identified organic acid showed significant concentration differences among genotypes. Thi s preliminary study does not support the putative Al tolerance mechanisms proposed for wheat, highlighting the importance and the necessities of elucidating the biochemical mechanisms involved in Al tolerance in triticale.

Key words: Aluminium tolerance, enzymic assay, high performance liquid chromatography (HPLC), malate, organic acids, root apex, triticale (Triticosecale Wittmack)

Aluminium (Al) toxicity is one of the major factors limiting the productivity of crop plants in acid soils (5). The initial response to Al toxicity is inhibition of root elongation, which occurs by Al-induced disruption of the root apex (12). Crop species and cultivars exhibit genetic variability in their response to Al stress (6, 14), and this variability serves as the basis for a considerable amount of recent research on Al tolerance mechanisms (7).

Numerous Al tolerance mechanisms have been proposed although specific mechanisms of Al tolerance are still poorly understood in higher plants. They can be grouped into two categories: exclusion of Al from roots; and detoxification of Al ions in the plan t (5, 13). ?Putative exclusion mechanisms proposed include bind-ing of Al in the cell wall, a plant-induced rhizosphere pH barrier, and root exudation of Al-chelating com-pounds (7, 13). Organic acids have been suggested to play a role in exclusio n via release from roots and Al detoxification in the symplasm, where organic acids such as citrate and malate could chelate Al and reduce or prevent its toxic effects at the cellular level (6, 14).

The idea that root exudation of organic acids may function as an Al tolerance mechanism is supported by the work of Delhaize and co-workers (4), who demonstrated that Al stimulates the exudation of malate in near iso-genic wheat lines exhibiting differential Al tolerance and found that malate is released only from the root apex (the primary site of Al toxicity) of Al-tolerant lines (12). The association between Al-induced exudation of malate and Al tolerance has been reinforced in wheat by other investigators (2, 10, 11). In contrast, Miyasaka et al. (8) observed that an Al-tolerant snapbean genotype (but not an Al-sensitive one) exudes citric acid into the medium, and thus provides Al tolerance possibly by chelating Al^{3+} and de toxifying Al in the apoplasm or the rhizosphere. In Al-tolerant and Al-sensitive maize varieties and lines, Pellet et al. (9) also found that Al rapidly triggers exudation of citrate from the root apex of Al-tolerant maize genotypes. At present, root exudat-ion of organic acids appears to be the most promising mechanism of Al tolerance yet studied (3). However, there is no direct evidence that this mechanism of root exudation of organic acids could also operate in triticale.

In this study we investigated the possible relationship between Al tolerance and Al-stimulated release of malate or other organic acids from roots of some triticale genotypes differing in Al tolerance (in terms of root regrowth characteristics in nutrient solution). We tested the hypothesis that Al-induced changes in organic acid exudation from roots could be involved in the mechanisms conferring Al tolerance.

Materials and methods

Plant materials and seedling growth

Several triticale cultivars, along with an Al-tolerant wheat line (ET3) were used. Seeds of triticale cultivars were self-pollinated in the previous growing season. ?Seeds of ET3 were kindly provided by Dr. P. R. Ryan (Division of Plant Industry, CSIRO, Canberra, Australia).

Seeds were surface sterilised for 10 min in 4% NaOCl and then rinsed several times with distilled water; disinfected seeds were then germinated aseptically in a petri dish.

In experiments designed to detect malate release from whole roots, ten pre-germinated seeds were transferred to 125 mL flasks containing 40 mL of control solution (0.2 mMol $CaCl_2$, pH 4.4). The flasks were incubated on a shaker (120 r pm) in a growth cabinet at 24^{o}C for 4 days. Prior to Al treatment, solutions were decanted from the flasks and the seedlings were rinsed twice with 40 mL of the control solution and then once with the different Al treatment solutions (0.2 m< EM>Mol $CaCl_2$ plus 0, 0.37, or 0.74 mMol $AlCl_3$?6H_2O, pH 4.4). The flasks were then refilled with the appropriate Al treatment solutions for duration of 24 hr or 48 hr.

In experiments designed to measure exudation of malate and some other organic acids from root tips, seedlings were removed from flasks or floats (which were put on nutrient solutions) and the apical 3.0 mm of each root was excised with a surgical blade into the control solution. About 25 apices were collected from each genotype x treatment combination and transferred to a series of 5.0 mL vials containing 1.0 mL of control solution, sealed with Parafilm, and placed horizontally on a shaker (70 rpm) f or 30 min to wash out the malate or other organic acids released from the wounded tissue. The washing solution was discarded and the apices re-suspended in either 1.0 mL of control solution or 1.0 mL of respective Al treatment solutions. The vials were re-sealed and incubated on a shaker for 60 min.

Organic acid assays

The enzymic assay for malate and the procedures to extract malate from root tissues as described by Delhaize et al. (4) were followed. A 0.68 mL aliquot of samples was incubated with 0.75 mL buffer (hydrazine, pH 9.0) and 50 mL NAD. The reaction mixture was pre-incubated for 30 min to obtain a stable A340 reading on a spectrophotometer (Model: Shimadzu, UV-120-02) before the addition of 4 mL malate dehydrogenase (MDH). Since the increase in A340 due to production of NADH is directly proportional to the amount of malate in the sample the malate content released from roots was able to be determined.

Organic acid exudates from root tips were measured using a Waters HPLC (high performance liquid chromatography) by reverse phase ion-suppression on a C18 column with 0.05 M KH_2PO_4 buffer (pH 2.5). At this time components w ere unable to be identified with certainty by comparison of retention times to a range of acid standards.

Determination of Al tolerance in nutrient solution

Sterilised seeds pre-germinated as indicated above were grown in floats on 7 L of nutrient solution (without Al) for 4 days followed by Al treatment (0.37 mMol $AlCl_3$?6H_2O at pH 4.4) for 24 hr in a growth cabinet. & nbsp;The differential Al tolerance response exhibited by the cultivars was assessed after 2 days of regrowth in terms of total root length and regrown root length following the Al pulse method as proposed by Aniol (1).

Results

Root growth and malate release from roots

Al-tolerant genotypes were able to produce longer roots and show root regrowth even after the imposition of moderate Al stress which is toxic to Al-sensitive lines but not the Al-tolerant lines. Based on seedling root length, particularly the regrown ro ot length, ET3 was confirmed as Al-tolerant at 0.37 mMol Al in nutrient solution; two triticale cultivars (Tahara and G4-95A) were also found to be Al-tolerant, whilst Empat was considered Al-sensitive at this level of Al stress (Table 1).
There was a reasonable agreement between Al tolerance as indicated by the regrown root length in nutrient solution and malate efflux. However, the Al-tolerant wheat line (ET3) exuded a much higher malate content from its root tips of seedlings (2 to 4 days old) than the Al-tolerant triticale cultivars. Despite Al-tolerant G4-95A and/or Tahara exuded more malate than the Al-sensitive cultivar Empat (in most cases it could not be detected), considerable variability existed between the two Al-tolerant t riticale cultivars and among the different Al treatments imposed.

Table 1. Root growth and malate efflux from whole roots or root tips of three triticale cultivars and ET3, an Al-tolerant wheat line

Cultivar or line	Root length (cm) at 0.37 m*Mol* Al[a]		Malate efflux from root tips at 0.37 m*Mol* Al for 60 min			Malate efflux from whole root system[b]			
						at 0.37 m*Mol* Al		at 0.74 m*Mol* Al	
	total	regrown	2-day-old	3-day-old	4-day-old	24 hr	48 hr	24 hr	48 hr
Tahara	9.4	1.8	0.12	0.07	-	0.06	0.02	-	-
Empat	7.6	0.5	-	0.05	-	0.02	-	-	-
G4-95A	10.8	2.0	0.46	0.39	0.09	0.12	0.05	0.10	0.04
ET3	8.5	1.3	0.69	0.86	0.59	-	-	-	-

[a] Measurement of regrown root length was made after 2 days' re-culture in nutrient solution following imposition of Al stress.
[b] Malate efflux (*nmol*/apex/hr) was calculated on the supposition that the 10 seedlings each develop 3 roots. Data are mean values from several and similar tests at different times, with blank ones showing negligible values (but ET3 was not tested from its whole root system).

Detection of organic acids from roots

Following the imposition of Al stress at 0.74 mMol Al, HPLC analysis indicated the Al-induced changes in the organic acid components of the exudates from the three genotypes (two triticale cultivars, Empat and G4-95A; and one Al-tolerant wheat line, ET3) compared with their samples treated in the control solution (0.2 mMol CaCl2, pH 4.4). It appeared that the release of a yet to be identified organic acid was triggered by Al treatment only in the Al-tolerant triticale cultivar (G4-95A). The exudates were compared with the standard retention times of a few commonly detectable organic acids which include acetic, citric, malic, lactic, ascorbic and tartaric acid. No peaks were identified as citrate in ET3, the Al-tolerant wheat line. There appeared t o be some differences in the pattern of peaks relating to organic acids between ET3 and Empat, the Al-sensitive triticale cultivar.

Discussion

Genetic variability for Al tolerance in triticale has been reported and Al-tolerant triticale cultivars have been used on acidic soils and in other marginal situations. However, the physiological and/or biochemical mechanisms responsible for this genetic variability are unknown. Root exudation of organic acids which can chelate and detoxify Al in the apoplasm or rhizosphere has been reported in snapbean (8) and in wheat (4, 11). ?But in triticale, information concerning the root exudates of organic acids is not available. A better understanding of such mechanisms leading to Al tolerance in triticale should have important implications for breeding Al-tolerant triticale.

The Al-stimulated efflux of malate from root apices has been implicated in the mechanism of seedling Al tolerance of wheat and some other crops (9). Although root exudation of malate was detected in the established Al-tolerant wheat line (ET3) and two triticale genotypes showing Al tolerance, the malate efflux from even the Al-tolerant wheat line seemed to be much lower than those reported (4, 11), let alone the Al-tolerant triticale genotypes. Several cultural conditions and experimental factors, such as the

seedling ages and Al stress imposed may account for the differences in the results. On the other hand, Al-tolerant triticale genotypes were able to produce longer regrown roots following Al stress in nutrient solution than the Al-tolerant wheat line, but they exuded a much lower level of malate. This led to the conclusion that malate exudation may not be the mainstay of Al tolerance in triticale though it exerts and functions well in such crops as wheat.

As Al-stimulated malate release from roots could not account for much of the tolerance exhibited by these genotypes tested, particularly for triticale; and Al tolerance is a multi-genic nature which is controlled by many minor genes in plants (6, 14), i t remains likely that different mechanisms of Al tolerance operate in different crops or even in cultivars of the same crop depending on the diversity of their genetic backgrounds. Differences of root exudates did exist among the three genotypes, particularly between the two triticale cultivars differing in Al tolerance, but the chromatogram could not demonstrate the efficacy of separation and positive identification of the exudates compared to available organic acids used as standards. Further work is in progress to try to improve identification.

Failure of our results for triticale to agree with those for other plant species may reflect true differences in Al toxicity/tolerance mechanisms involved in different species or differences in experimental conditions. Similar discrepancies have been re ported by Foy et al. (6) when investigating organic acid exudates related to differential Al tolerance in wheat. It appears necessary to verify whether the hypothesis that Al-stimulated efflux of malate or other organic acids from root tips is a general mechanism for Al tolerance in triticale and other crop plants.

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