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Prospecting sugarcane genes involved in aluminum tolerance

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Abstract

Aluminum is one of the major factors that affect plant development in acid soils, causing a substantial reduction in yield in many crops. In South America, about 66% of the land surface is made up of acid soils where high aluminum saturation is one of the main limiting factors for agriculture. The biochemical and molecular basis of aluminum tolerance in plants is far from being completely understood despite a growing number of studies, and in the specific case of sugarcane there are virtually no reports on the effects of gene regulation on aluminum stress. The objective of the work presented in this paper was to prospect the sugarcane expressed sequence tag (SUCEST) data bank for sugarcane genes related to several biochemical pathways known to be involved in the responses to aluminum toxicity in other plant species and yeast. Sugarcane genes similar to most of these genes were found, including those coding for enzymes that alleviate oxidative stress or combat infection by pathogens and those which code for proteins responsible for the release of organic acids and signal transducers. The role of these genes in aluminum tolerance mechanisms is reviewed. Due to the high level of genomic conservation in related grasses such as maize, barley, sorghum and sugarcane, these genes may be valuable tools which will help us to better understand and to manipulate aluminum tolerance in these species.

INTRODUCTION

Aluminum is the most abundant metal in the lithosphere, comprising about 7% by mass of the earth's crust (Delhaize and Ryan, 1995). In soils with a pH higher than 5, aluminum is predominantly bound as insoluble oxides and complex aluminosilicates, while at the lower pH of more acid soils the ionic form (Al^{3+}) is released into the soil solution and becomes available to plants at toxic concentrations (Kinraide and Parker, 1989) and is the major limiting factor for crop production. Acid soils occupy 3.95×10^9 ha (30%) of the world's ice-free land area, but in South America acid soils account for about 66% of the land area (Baligar and Ahlrichs, 1998). Strategies to maintain crop yield in these soils include the application of lime to raise the soil pH and the use of cultivars tolerant to this type of environment. As successive applications of lime lead to runoff pollution and other undesirable side effects, the manipulation of aluminum tolerance in crop species, either by conventional breeding or by genetic engineering, offers an environmentally clean and sustainable solution to improve productivity in acid soils.

The first and principal symptom of aluminum toxicity in plants is the inhibition of root growth, which causes a decrease in water and nutrient uptake and subsequent inhibition of plant growth. Despite a large amount of research in this area the physiological and biochemical mechanisms of both aluminum toxicity and tolerance in plants are far from being completely understood. A great number of hypotheses for aluminum toxicity have been suggested, including alteration of the cation-exchange capacity of cell walls, changes in the potential of the plasma membrane affecting the uptake of Ca^{2+} and/or Mg^{2+}, induction of oxidative stress via lipid peroxidation, replacement of Mg^{2+} or Fe^{3+} by Al^{3+} in cellular reactions, interference with signal transduction, direct binding of aluminum to DNA and/or RNA and changes in the pectin matrix of root cell walls. There are arguments and indirect evidence supporting each of these possibilities (Delhaize and Ryan, 1995; Kochian, 1995) but to date there is little direct evidence favoring one hypothesis over another.

Because aluminum is a strong selective force many plant species have developed tolerance mechanisms to overcome this type of toxicity, yielding species and cultivars very well adapted to acid soils. There are two major groups of aluminum detoxification mechanisms, exclusion (apoplastic) mechanisms and internal (symplastic) mechanisms, the basic difference between the two being the site of detoxification (Taylor, 1991; Kochian, 1995). Exclusion mechanisms prevent aluminum from crossing the plasma membrane and getting inside plant cells (symplasts) while internal mechanisms immobilize, compartmentalize or detoxify this metal when it penetrates into cells (Zheng *et al*., 1998).

Genetic studies on aluminum tolerance have shown that ions of this metal induce the expression of genes involved in the general stress response of wheat, including

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genes coding for phenylalanine ammonia lyase, protease inhibitors and a metalothionein-like protein (Snowden and Gardner, 1993). The nature of the stress induced by aluminum is not completely clear, although there are indications that an oxidative stress is induced in plant tissues exposed to this metal (Richards *et al*., 1998). The expression of several other genes has also been reported to be elicited by treatment with aluminum, showing that several metabolic pathways in the cell are changed in response to the stress caused by this metal.

A great number of aluminum tolerance studies have been carried out in maize, wheat and other grasses, although very few have addressed sugarcane. Landell (1989), working with several sugarcane varieties, observed a wide range of aluminum sensitivity, with 10 ppm of aluminum causing a strong reduction in root growth in some *Saccharum spontaneum* varieties while *Saccharum officinarum* varieties exhibited higher levels of tolerance. Azeredo (1982) has found that in some aluminum-sensitive varieties 1.56 ppm of this metal was able to cause root growth inhibition. However, to our knowledge, there have been no reports on sugarcane genes related to aluminum stress.

During the work presented in this paper we prospected the SUCEST database looking for sugarcane genes related to known genes involved in the response to aluminum stress in plants and yeast. This study is the first initiative to understand the mechanisms by which sugarcane plants deal with aluminum toxicity.

METHODOLOGY

Search for genes related to aluminum tolerance in other species

An extensive search for papers reporting aluminum-related genes from plants and microorganisms was performed using the Web of Science citation database (webofscience.fapesp.br) and aluminum-related genes were searched in the GenBank database (www.ncbi.nlm.nih.gov) by the accession numbers obtained in the literature and by using keywords. Protein sequences from the majority of the genes were obtained, but for a few genes only partial cDNA sequences were available.

Search for sugarcane genes

The cap3 cluster consensi from the SUCEST database sequence were prospected using two algorithms from the BLAST (basic local alignment search tool) family of programs, TBLASTN and TBLASTX, depending on the original source of information (amino acid or nucleotide sequence, respectively). Clusters were accepted as related to a particular gene when presenting e-values below 10^{-5}.

Evaluating similarities

The open reading frames (ORFs) present at the cluster consensi identified above were translated according to the universal translation table, and the deduced proteins were aligned with the entire sequence of the respective protein obtained from the literature. The Clustalw software (Thompson *et al*., 1994) was used to perform the alignments and to check the similarity of the proteins that were matched by the sugarcane genes.

Characterization of aluminum tolerance mechanisms in sugarcane

The aluminum related genes found in the literature were classified into nine groups, of which five groups contained genes with well defined biochemical functions associated with aluminum stress and two groups consisted of genes induced or repressed by aluminum but without further biological evidence for being related to aluminum tolerance mechanisms. Of the remaining two groups, one contained genes of unknown function while the other was made up of genes that exhibited no similarity with sugarcane genes.

In this paper, our discussion of the aluminum tolerance mechanisms that may be present in sugarcane is based on the aluminum-related genes found to be similar to sugarcane genes and on the functions of these genes.

RESULTS

Several sugarcane genes showed similarity to genes known to be related to aluminum tolerance mechanisms in other organisms, with all the genes presenting e-values below 10^{-5} being regarded as related to the genes described in the literature (Table I). The deduced sugarcane proteins were aligned with the ones described in the literature and the percentages of identity and similarity in these alignments were calculated. In some cases, a low percentage of similarity was found, probably indicating that the cap3 cluster consensi do not contain the full gene sequences or that the similarities were restricted to conserved domains.

The genes were classified based on their putative function in the different aluminum tolerance mechanisms (Table I). The first two groups comprise genes of known roles, related to external and internal aluminum tolerance mechanisms, respectively. Five groups include genes with known functions, whose roles in aluminum tolerance were not completely clear. The group of genes with unknown function includes all the remaining sugarcane genes that showed similarity with aluminum stress related genes. The last group comprises all genes that were reported as related to aluminum tolerance but that exhibited no similarity with sugarcane genes sampled in the SUCEST Project (e-value above 10^{-5}).

Table I - Genes putatively involved in aluminum tolerance.

Groups	Gene function	Organism	GI	SUCEST Cluster Consensus showing best similarity	e-value	% Identity*	% Similarity**	Response to aluminum stress***	References
Organic acids pathway	Aconitase	*C. maxima*	1351856	SCCCCL3001A05.g	0.0	88.9	95.1	HK	Hayashi *et al.*, 1995
	Isocitrate dehydrogenase $NADP^+$ dependent	*G. max*	1708401	SCCCCL4017A09.g	0.0	87.7	95.9	HK	Udvardi *et al.*, 1993
	Isocitrate dehydrogenase NAD^+ dependent	*N. tabacum*	3021506	SCCCRZ2004B10.g	1e-171	82.7	92.0	HK	Lancien *et al.*, 1998
	Malate dehydrogenase	*G. max*	1346501	SCCCLR1022D04.g	1e-153	80.7	91.5	HK	Guex *et al.*, 1995
	FUM1 - Fumarase	*A. thaliana*	1769568	SCCCLR1C05F08.g	0.0	83.3	92.5	HK	Behal and Oliver, 1997
	2-oxoglutarate dehydrogenase E1 component	*S. cerevisiae*	1070439	SCEZRZ1012A01.g	0.0	36.1	51.7	HK	Repetto and Tzagoloff, 1989
	Succinate dehydrogenase	*S. cerevisiae*	3392584	SCRFLR1012B07.g	0.0	89.4	94.5	HK	Robinson and Lemire, 1992
	Citrate synthase -mitochondrial matrix	*A. thaliana*	11243	SCCCCL3001C03.g	1e-172	35.0	42.8	OIT	delaFuente *et al.*, 1997
	Succinyl-CoA synthetase, alpha subunit	*A. thaliana*	10177814	SCSGFL1081H08.g	1e-149	77.7	83.9	HK	Nakamura *et al.*, 1997
	Succinate-CoA ligase, beta chain	*A. thaliana*	11272036	SCCCLR1024D12.g	0.0	85.3	93.6	HK	Machuy *et al.*, 1997
Oxidative stress	Superoxide dismutase (Cu-Zn)	*A. thaliana*	930667	SCJFRZ1007G12.g	6e-65 [a]	85.5	90.0	AI	Richards *et al.*, 1998
	prxCb - Peroxidase	*A. thaliana*	405611	SCCCRZ1002B03.g	2e-91	49.3	64.0	AI	Richards *et al.*, 1998
	pAL201 - Anionic peroxidase	*N. tabacum*	-	SCCCRT1001G12.g	7e-52	39.4	56.7	AI OIT	Ezaki *et al.*, 1996 Ezaki *et al.*, 2000
	GST - Glutathione S-transferase	*A. thaliana*	497788	SCCCLR1001A07.g	2e-38	43.3	63.0	AI	Richards *et al.*, 1998
	api2 - Glutathione S-transferase	*N. tabacum*	676880	SCBGLR1003E05.g	4e-46	43.2	64.3	AI OIT	Ezaki *et al.*, 1995 Ezaki *et al.*, 2000
	Phospholipid hydroperoxide glutathione peroxidase – like	*A. thaliana*	2760606	SCCCRZ2C01F01.g	3e-83	85.8	93.5	AI	Sugimoto and Sakamoto, 1997
Pathogen defense	*Glc1* - Beta1,3 Glucanase	*T. aestivum*	924953	SCSGRT2066G06.b	8e-96	39.5	47.3	AI	Cruz-Ortega *et al.*, 1997
	PEARLI5 – Reticuline oxidoreductase	*A. thaliana*	239110	SCJFRT2053H03.b	2e-21	9.5	14.3	AI	Richards *et al.*, 1998
	wali4 –phenylalanine ammonia lyase	*T. aestivum*	170793	SCEQRT1024E12.g	7e-55 [a]	77.3	90.6	AI	Snowden and Gardner, 1993
	War7.2 – phenylalanine ammonia lyase	*T. aestivum*	6996628	SCJFLR1017B11.g	1e-18 [a]	59.8	70.7	AI	Hamel *et al.*, 1998
	War13.2 – oxalate oxidase	*T. aestivum*	6996619	SCCCCL7037E09.g	1e-89 [a]	76.0	80.5	AI	Hamel *et al.*, 1998
	wali 3 putative Bowman-Birk trypsin inhibitor	*T. aestivum*	170791	SCUTSD2028D10.b	7e-07	39.8	52.3	AI	Snowden and Gardner, 1993
	wali5 – putative Bowman-Birk trypsin inhibitor	*T. aestivum*	170795	SCQGRT3045E08.g	7e-07	46.1	58.4	AI	Snowden and Gardner, 1993
	wali6 – putative Bowman-Birk trypsin inhibitor	*T. aestivum*	451195	SCCCSD2002C05.g	3e-06	47.7	56.8	AI	Richards *et al.*, 1994

Cell wall components	*me1* - pectin methylesterase	*P. sativum*	308864	SCSGLR1025E03.g	3e-86	25.8	35.0	OINT	Horst *et al*., 1999
	cfl1 - callose synthase	*G. hirsutum*	4588011	SCCCCL4013E04.g	0.0	27.3	31.6	AI	Sivaguru *et al*., 1999; Larsen *et al*., 1996
Other aluminum induced genes	*wali1* –Metalothionein–like	*T. aestivum*	1171037	SCCCCL3080G03.g	2e-10	73.3	81.3	AI	Snowden and Gardner, 1993
	AtBCB – Blue Copper- binding protein	*A. thaliana*	16203	SCCCRT1004C05.g	1e-16	33.7	50.5	AI OIT	Richards *et al*., 1998 Ezaki *et al*., 1999 Ezaki *et al*., 2000
	sadA – Short chain alcohol dehydrogenase	*P. sativum*	6119723	SCJFLR1017B03.g	2e-40	38.1	58.6	AI	Brosche and Strid, 1999
	sadC – Short chain alcohol dehydrogenase	*P. sativum*	6119844	SCCCRT3004D06.g	2e-31	24.6	35.4	AI	Brosche and Strid, 1999
	Fimbrin/Plastin like	*T. aestivum*	1575595	SCQSAM1030A03.g	1e-108[a]	42.4	50.7	AI	Cruz-Ortega *et al*., 1997
	Fructose-bisphosphate aldolase	*A. thaliana*	2597590	SCSBSB1096D03.g	1e-56[a]	72.5	78.3	AI	Richards *et al*., 1998
Genes repressed by aluminum	Alanine amino transferase	*A. thaliana*	931354	SCCCRZ1002E01.g	5e-34[a]	82.6	88.4	AR	Richards *et al*., 1998
	CAB – Photosystem II type I clorophyll a/b-binding	*A. thaliana*	16366	SCCCST1001B11.g	1e-133	86.8	93.2	AR	Richards *et al*., 1998
Genes of unknown function	*PEARLI1*	*A. thaliana*	871780	SCEZHR1086A11.g	6e-34	39.3	48.8	AI	Richards *et al*., 1998
	PEARLI8	*A. thaliana*	906072	SCSFST1067G09.g	6e-25[a]	35.6	60.7	AI	Richards *et al*., 1998
	wali7	*T. aestivum*	7489671	SCCCLR1024E09.g	1e-108[a]	75.2	80.4	AI	Richards *et al*., 1994
	Sali5-4a	*G. max*	2304954	SCSBHR1051A12.g	1e-20	29.8	51.5	AI	Ragland and Soliman, 1997
	Sali3-2	*G.max*	2317900	SCQSAD1059A04.g	1e-17	14.9	23.9	AI	Ragland and Soliman, 1997
	Bali	*B. napus*	3123745	SCCCRT2002C02.g	9e-91	66.8	80.7	AI	Sohn, 1998
Signal transduction	*SLT2* – MAP – kinase	*S. cerevisae*	730747	SCCCRZ2C04G10.g	2e-90	36.2	53.3	DIS	Schott and Gardner, 1997
	SLK1 – protein – kinase homolog	*S. cerevisae*	417775	SCJLRT1014C03.g	4e-61	4.4	10.4	DIS	Schott and Gardner, 1997
	NtGDI1 – GDP dissociation inhibitor	*N. tabacum*	2501850	SCEPLB1043E09.g	0.0	87.4	95.5	AI OIT	Ezaki *et al*., 1999 Ezaki *et al*., 2000
Genes without similarity with sugarcane genes in the SUCEST project	*HSP150* – heat shock protein	*S. cerevisae*	473374	SCJFRZ2012A04.g	1.1	-	-	AI DIS	Ezaki *et al*., 1998
	Sed1 protein precursor	*S. cerevisae*	4454	SCSFAD1124A06.g	4.5	-	-	AI DNIS	Ezaki *et al*., 1998
	Alr1 – Mg transport	*S. cerevisae*	2498122	SCSGHR1069G08.g	0.13	-	-	OIT	MacDiarmid and Gardner, 1998
	Alr2 – Mg transport	*S. cerevisae*	1175955	SCSGST1072F02.b	5.6	-	-	OIT	MacDiarmid and Gardner, 1998

Genes without similarity with sugarcane genes in the SUCEST project								
phosphatidylserine synthase	*T. aestivum*	4099923	SCUTFL3070F03.b	3.2	-	-	OIT	Delhaize *et al*., 1999
Wali2 – unknown	*T. aestivum*	170789	SCJFRZ3C03D02.b	3.9	-	-	AI	Snowden and Gardner, 1993
War4.2 – peroxidase	*T. aestivum*	6996625	SCAGAM2122B02.g	2.8 [a]	-	-	AI	Hamel *et al*., 1998
War5.2 – cysteine proteinase	*T. aestivum*	6996626	SCJLRZ1018E10.g	0.003 [a]	-	-	AI	Hamel *et al*., 1998
pEARLI4	*A. thaliana*	871781	SCCCCL4009D05.g	8e-05 [b]	-	-	AI	Richards *et al*., 1998
pir1(pAL139) – unknown	*N. tabacum*	676882	SCCCCL6004B02.g	0.003	-	-	AI	Ezaki *et al*., 1995
Alu1 – unknown	*A. viscosus*	2921156	No hits found.	-	-	-	AI	Jo *et al*., 1997
Alu2 – unknown	*A. viscosus*	2827439	SCRUFL4022D11.g	1.2	-	-	AI	Jang *et al*., 1998

*Percentage of amino acids of the protein coded by the known aluminum-related gene (or of the EST translation, when the query was based on an EST sequence) that align with an identical amino acid in the sequence translated from the SUCEST cluster consensi.

**Percentage of amino acids of the protein coded by the known aluminum-related gene (or of the EST translation, when the query was based on an EST sequence) that align with a similar amino acid on the sequence translated from the SUCEST cluster consensi (according to the Blosum 62 amino acid similarity matrix as used by the Clustalw algorithm).

***According to the following keys: AI: Aluminum induces expression of the gene; AR: Aluminum repress expression of the gene; DIS: deletion of the gene increased susceptibility to aluminum; DNIS: deletion of the gene did not increase susceptibility to aluminum; OIT: overexpression of the gene increased aluminum tolerance; OINT: overexpression of the gene did not increase aluminum tolerance; HK: house-keeping genes, known to be related to aluminum-tolerance mechanisms.

[a] The search for a sugarcane gene similar to this gene was carried out using the tblastx algorithm and a partial cDNA sequence which was the only available information. Identity and similarity percentages were calculated based on the length of the deduced protein from the cDNA translation.

[b] This e-value (8×10^{-5}) almost satisfied our criterion, although it could be round up to 1×10^{-4}, although this gene was considered to have no similarity with sugarcane genes because its alignment with the translation of cluster SCCCCL4009D05.g consensi was very poor.

DISCUSSION

A high proportion of the genes involved in all sorts of aluminum tolerance mechanisms in other plants and microorganisms were identified in the SUCEST database, which could indicate that these mechanisms have been conserved among species, helping to explain how sugarcane overcomes aluminum toxicity.

There is strong evidence that secretion of organic acids from the roots is one of the mechanisms involved in aluminum tolerance in higher plants, but the process leading from the synthesis to the secretion of these organic acids are still unknown. For this reason, all key enzymes involved in this metabolic pathway were evaluated and found to be similar in sugarcane, as is to be expected due to their biochemical and physiological importance.

Organic acids are able to form strong complexes with aluminum which act in both major mechanisms of aluminum tolerance, either by chelating aluminum ions present in the apoplast and the rhizosphere (exclusion) or by the internal detoxification of this metal by reversing its toxic effects inside the cell (Ma, 2000). In tea plants (*Camellia sinensis* L.), aluminum is taken up and stored in the central vacuole as complexes with organic acids (Matsumoto *et al*., 1976). Internal detoxification of aluminum in the vacuoles of maize root apical cells has also been identified (Vazquez *et al*., 1999), while an aluminum tolerant variety of wheat was able to externally chelate this metal by the secretion of malic acid into the rhizosphere (Delhaize *et al*., 1993). External detoxification of aluminum by the secretion of organic acids such as citric, oxalic, succinic and/or malic acid from the root apex have also been reported for aluminum-tolerant cultivars of snapbean (Miyasaka *et al*., 1991), maize (Pellet *et al*., 1995) and *Arabidopsis* (Degenhardt *et al*., 1998), under aluminum stress. High aluminum tolerance in buckwheat has been associated with both internal (Ma *et al*., 1998) and external (Zheng *et al*., 1998) detoxification mechanisms due to oxalic acid, which forms a non-phytotoxic complex with aluminum and which is also secreted from the root tips. So, it can be seen that the kind of organic acids as well as the secretion pattern differ markedly between plant species (Ma, 2000). The most convincing evidence for the involvement of organic acid exudation in aluminum tolerance has been presented by Fuente *et al*. (1997) who produced transgenic tobacco and papaya over-expressing citrate synthase. The overproduction and exudation of citrate resulted in a significant increase in the aluminum tolerance of these transgenic plants. In rye citrate synthase activity has also been increased by exposure to aluminum (Li *et al*., 2000).

Another mechanism that may be involved in aluminum toxicity is oxidative stress activated by peroxidation of membrane lipids (Kochian, 1995). Richards *et al* (1998) reported that aluminum induced the expression of oxidative stress genes in *Arabidopsis thaliana*, thereby indicating that stress caused by this metal is closely related to oxida-

tive stress. Among the oxidative stress genes induced by aluminum, sugarcane genes coding for superoxide dismutase, peroxidase, moderately anionic peroxidase, glutathione S-transferase and phospholipid hydroperoxide glutathione peroxidase-like protein were found. Superoxide dismutase is an enzyme that converts superoxide radicals to hydrogen peroxide and oxygen, thus playing a crucial role in antioxidant defense (Tsang *et al*., 1991; Van Camp *et al*., 1994). Peroxidase activities occur mostly at the cell wall, where these enzymes have been suggested to modulate cell wall rigidity and extensibility, thus reducing the rate of aluminum diffusion through the cell wall (Hamel *et al*., 1998). The moderately anionic peroxidases from tobacco are also highly induced in wounded stem tissue (Lagrimini and Rothstein, 1987). These enzymes have a moderate activity toward lignin precursors (Espelie *et al*., 1986) and are likely to be associated with the cell wall. Ezaki *et al*. (1996) speculated that the moderately anionic peroxidase has a function in healing cell membranes damaged by aluminum treatment. The glutathione S-transferase family of proteins catalyzes the conjugation of glutathione to a variety of electrophiles. Glutathione is one of the most important antioxidants and probably prevents the peroxidation of membrane lipids by aluminum ions (Ezaki *et al*., 1995). Sugimoto and Sakamoto (1997) suggested that phospholipid hydroperoxidase glutathione peroxidase-like protein catalyzes the reduction of the hydroperoxides of phospholipids in response to oxidative stress caused by diverse treatments, including exposure to aluminum. Glutathione reductases catalyzes the reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) and has a central role in the cell response during stress, but there are no reports in the literature of the expression of genes coding for this enzyme in response to aluminum. However, we have found that aluminum induces higher levels of glutathione reductase activity in maize roots (Boscolo et al., unpublished results), indicating that the genes coding for this enzyme are also up-regulated by exposure to aluminum.

Genes related to pathogen response have also been reported to be induced by aluminum, and some were found to be similar to sugarcane genes. The expression of the 1,3-β-glucanase gene is often associated with pathogen infection, particularly by fungi. Although its role in aluminum toxicity is unknown, Cruz-Ortega *et al.* (1997) suggested that this protein is synthesized as a protective response, because during aluminum stress roots are more susceptible to pathogen attack. This hypothesis is also a possible explanation for the induction by aluminum of other genes related to pathogen response. Reticuline oxireductase, also induced by aluminum, is involved in the formation of benzophenanthridine alkaloids in the response of plants to pathogenic attack (Richards *et al*., 1998). Phenylalanine ammonia-lyase (PAL) is a well-known defense protein that has been shown to accumulate in several different incompatible plant-pathogen combinations and in response to elicitors (Ebel and Cosio, 1994). This enzyme could play a beneficial role in detoxifying aluminum that has entered the symplasm, since PAL has been shown to catalyze the first step of multi-branched phenylpropanoid metabolism in higher plants (Hamel *et al*., 1998). Oxalate oxidase, an enzyme involved in the degradation of oxalate (accumulated in plant cells as the calcium salt), has been shown to accumulate during the fungal infection of barley (Zhang *et al*., 1995). Hamel *et al*. (1998) reported that PAL, cysteine proteinase, oxalate oxidase and a peroxidase were up-regulated in wheat in proportion to the level of aluminum, suggesting that these proteins may provide protection against ions of this metal by strengthening the cell wall of root cells. Another three genes induced by aluminum in wheat (*wali3, wali5* and *wali6*) have also been found to be similar to some sugarcane genes. These wheat genes encode Bowman-Birk type trypsin inhibitors, an extensively studied family of protease inhibitors (Laskowsky and Kato, 1980), whose homologs in soybean have been shown to have antifungal activity (Chilosi *et al*., 2000) and have been suggested to be a defense against insect feeding (Belzunces *et al*., 1994).

In addition, genes coding for other proteins that participate in the dynamics of the cell wall may be associated with aluminum tolerance or susceptibility. Horst *et al*. (1999) showed that maize and potato mutants over-expressing pectinmethylesterase (PME) had a high negative charge on their pectin matrix and were more susceptible to aluminum stress. The degree of pectin methylation, mainly controlled by pectinmethylesterase, was quantitatively estimated and seemed to especially affect the negative charge density of the pectin matrix in certain defined root zones. However, it is not known how pectin content and the degree of methylation correlates with genotypical differences in aluminum tolerance, and the role of the pectin charge density of root apical cell wall in the tolerance of maize to aluminum needs to be clarified. Callose formation has been induced in the apical root cells of aluminum-sensitive maize cultivars (Sivaguru *et al*., 1999) and *Arabidopsis* mutants with increased sensitivity to aluminum (Larsen *et al*., 1996). A study using immuno-fluorescence and immuno-electron microscopic techniques combined with monoclonal antibodies against callose (Sivaguru *et al*., 2000) showed that aluminum-sensitive wheat root growth inhibition was closely associated with the blockage of plasmodesmata (cytoplasmic channels responsible for the intercellular movement of water, nutrients and for signaling) by callose deposition under aluminum stress, which could effectively block symplastic transport and communication in higher plants. One of the key enzymes acting in callose formation is callose synthase, which showed high similarity with genes in the SUCEST database.

Other stress related genes induced by aluminum were also found to have similarities with some sugarcane genes.

Snowden and Gardner (1993) showed that a wheat gene coding for a metallothioneine-like protein (MLP) was identified as being up-regulated by aluminum and thought that it is highly unlikely that MLPs bind this metal, so it appears that aluminum must induce this gene by interfering with the plant's normal pathway for the uptake or homeostasis of other metal ions. A gene for blue copper binding (*BCB*) protein, induced in wheat by aluminum (Richards *et al*., 1998), belongs to a family of genes that encode many membrane proteins with various suggested functions in plant metabolism, such as the regulation of the uptake of the ions of metals such as calcium, iron, manganese and zinc (Lin and Wu, 1994), redox reductions (VanGysel *et al*., 1993), and lignification of the cell wall (Drew and Gatehouse, 1994). The role of the *BCB* gene in aluminum tolerance is not clear, but Ezaki *et al*. (1999) speculated that the BCB protein might restrict aluminum uptake across the plasma membrane by affecting the structural composition of the membrane.

Aluminum also induces the expression of genes that are not directly related to the stress response or other known defense mechanisms. We found sugarcane genes which are similar to two pea genes (*sadA* and *sadC* genes) that are up-regulated by aluminum and which encode short-chain alcohol dehydrogenases (ADHs). Several genes with high similarity to the *sad* genes are considered to encode proteins involved in steroid metabolism, so it is possible that SAD proteins are involved in the metabolism of phytosteroids (Brosche and Strid, 1999). However, short-chain ADHs are also reported to be involved in oxidation of the hydroxyl groups of diverse substrates such as sugar, acetoacetyl-CoA, mammalian prostaglandins and diols, in addition to steroids (Persson *et al*., 1991). All these compounds, as well as other unidentified hydroxyl-containing chemical species, are possible substrates for SAD proteins. A gene encoding a fimbrin-like cytoskeletal protein, induced in pea by aluminum (Cruz-Ortega *et al*., 1997), also showed similarity with sugarcane genes. Fimbrins are a highly conserved family of actin filament bundling proteins, which are probably utilized by plants to maintain the integrity and functional array of actin filaments in the cell cytoskeleton. Cruz-Ortega *et al*. (1997) suggested that the increased tension of the cytoskeletal actin associated with aluminum toxicity may involve extensive cross-linking of actin filaments by fimbrins, leading to up-regulation of fimbrin gene expression to replenish cellular fimbrin pools. Another gene, induced by aluminum in *Arabidopsis thaliana* and similar to a sugarcane gene, encodes a fructose-bisphosphate aldolase (Richards *et al*., 1998). This protein is involved in both glycolysis and gluconeogenesis in plastids, and catalyzes the formation of fructose-bisphosphate and sedoheptulose-bisphosphate in photosynthesizing chloroplasts (Razdan *et al*., 1992), although its role in aluminum tolerance is not clear.

Two genes have been reported as being down-regulated by aluminum in *Arabidopsis thaliana* (Richards *et al*., 1998), both of which show similarity with sugarcane genes. One of these genes belongs to the chlorophyll a/b-binding (*CAB*) gene family, which is a distinct class of structurally and evolutionary related pigment-binding proteins found in chloroplast thylakoid membranes (McGrath *et al*., 1992). The other gene shows similarity with alanine aminotransferase, a pyridoxal phosphate-dependent enzyme that operates in a wide range of metabolic pathways, catalyzing the reversible transfer of an amino group from alanine to 2-oxoglutarate to form pyruvate and glutamate (Son and Sugiyama, 1992). It has been suggested by Richards *et al*. (1998) that the shutdown of these transcripts might reflect the response of a central metabolic pathway to aluminum stress.

As to be expected, the complex regulation of gene expression and of several metabolic pathways in response to aluminum toxicity also involves genes responsible for signal transduction. Several sugarcane genes were found to be similar to signal transduction genes from other organisms, these genes being a mitogen-activated protein kinase (MAP-kinase, *SLT2* gene), a protein kinase homolog (*SLK1* gene) and a GDP dissociation inhibitor (*NtGDI1* gene). The MAP-kinase functions in a signal transduction cascade downstream of protein kinase C (PKC), and has a known role in regulating the cell cycle under stress conditions (Costigan and Snyder, 1994). *SLK1* belongs to the same pathway of SLT2 MAP kinase (SLT2 pathway) and encodes the corresponding MAP kinase-kinase. Schott and Gardner (1997) have shown that yeasts with a mutant SLT2 pathway are aluminum sensitive, these authors suggesting that this results in failure of the cells properly to cease division in the presence of toxic levels of aluminum. The expression of *NtGDI1* in response to aluminum toxicity seems to cause an increased afflux of aluminum (Ezaki *et al*., 1999). GDI proteins regulate vesicular traffic at many stages of the exocytic and endocytic transport pathways in various organisms (Matsui *et al*., 1990). Ezaki *et al*. (1999) has suggested that the NtGDI1 protein increases aluminum release from yeast cells by stimulating the vesicle transport system.

Sugarcane genes similar to several genes with unknown function, but reported as being regulated by aluminum stress, were also found (Table I). The lack of information on the function of these genes shows how far we are from a complete knowledge of the mechanisms involved in aluminum tolerance in plants. At the end of Table I we have listed all the genes reported to be involved in aluminum-tolerance in other species but which did not show similarity with sugarcane genes. These results suggest that the mechanisms of aluminum tolerance in which these genes are involved may be species-specific, or display high genetic divergence among organisms. It is also possible that some genes may be expressed only under aluminum

stress and since the SUCEST database contains data from plants which were not treated with aluminum specific induced genes may not have been sampled.

The results presented in this paper show that sugarcane has genes similar to most of the genes related to aluminum stress in other species, suggesting that sugarcane may activate a wide array of defenses against aluminum toxicity. However, further experiments are needed to determine the role of these genes in aluminum tolerance in sugarcane and our group is using DNA macroarrays containing the genes we have cataloged (in this and other research projects) to identify changes in sugarcane gene expression in response to aluminum stress. The high level of gene order and conservation of function among related grasses (Guimarães *et al*., 1997; Ming *et al*., 1998) suggest that the genetic resources used by sugarcane can be applied to other species thus increasing the usefulness of the SUCEST project.

RESUMO

Alumínio (Al) é um dos principais fatores que afetam o desenvolvimento de plantas em solos ácidos, reduzindo substancialmente a produtividade agrícola. Na América do Sul, cerca de 66% da superfície do solo apresenta acidez, onde a alta saturação de alumínio é uma das maiores limitações à prática agrícola. Apesar do crescente número de estudos, uma compreensão completa das bases bioquímicas e moleculares da tolerância ao alumínio em plantas está longe de ser alcançada. No caso da cana-de-açúcar, não há nada publicado sobre a regulação gênica induzida durante o stress por alumínio. O objetivo deste trabalho foi identificar genes de cana-de-açúcar relacionados com as várias vias metabólicas reconhecidamente envolvidas na resposta à toxidez do alumínio em outras espécies de plantas e leveduras. Para a maioria dos genes relacionados com alumínio em outras espécies foram identificados similares em cana-de-açúcar, tais como aqueles que codificam enzimas que combatem o stress oxidativo ou a infestação por patógenos, proteínas responsáveis pela exudação de ácidos orgânicos e pela transdução de sinais. O papel desses genes na tolerância ao alumínio é revisado. Devido ao alto grau de conservação do genoma entre espécies próximas de gramíneas como milho, cevada, sorgo e cana-de-açúcar, esses genes serão uma ferramenta valiosa para a melhor compreensão e manipulação da tolerância ao alumínio nestas espécies.

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