Soil conditions and evolution of aluminium resistance among cultivated and wild plant species on the Island of Madeira

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ABSTRACT

Soil samples collected from different soil formations throughout the Island of Madeira were analysed for pH, aluminium (Al) and organic matter content. On average, the Madeira soils appeared to be acid with a mean pH of 5.01, containing 0.79 cmol/kg of ionic Al and 3.02% organic matter, which may create favourable conditions for the development of Al resistance among plant species inhabiting the island. Six plant species were evaluated for their resistance to Al using the erichrome cyanine *R* tests: three agricultural species including wheat (*Triticum aestivum* L. and *Triticum turgidum* L.) and corn (*Zea mays* L.) and three wild species from the genus *Sinapidendron*. Profound differences in adaptation to local edaphic parameters (Al content, acidity, organic matter, altitude) between cultivated and wild species were observed. Comparison of the distribution patterns of the cultivated and wild species on the island may indicate that the anthropogenic activities played a pivotal role in the development of enhanced Al resistance among the cultivated species, while edaphic conditions seem to be a secondary factor.

Keywords: aluminium resistance; corn; *Sinapidendron*; Madeira; soil acidity; wheat

The presence of high amounts of naturally occurring metals in soil can limit plant distribution and colonization of local ecosystems (Bradshaw and McNeilly 1981, Gravin and Carver 2003). Low soil pH creates favourable conditions for the release of metals into the soil solution at concentrations that can be toxic to growing plants (Kidd and Proctor 2001). These metals in ionic form can affect the growth and reproductive fitness of many plant species (Bradshaw and McNeilly 1981). As a result of selective pressure, inter- and intra-species differences in response to toxic concentrations of metals are widely observed in the plant kingdom (Taylor 1995, Stanton et al. 2000). Speciation processes are particularly common in volcanic acid soils where high amounts of exchangeable metals may originate both from the natural level of metal or the intensification of the releasing processes into soil solution (Pintro et al. 1999).

The Portuguese Archipelago of Madeira is located in the Atlantic Ocean, between the latitudes 33°10'–32°20'N and longitudes 16°10'–17°20'W, 630 km west of the coast of North Africa, and consists of five islands: Madeira, Porto Santo, Deserta Grande, Deserta Pequena and Bugio. The Island of Madeira with an area of 728 km^2 (about 50 by 25 km) itself is the largest and highest of the islands, where Pico Ruivo (1861 m asl) and Pico Areeiro (1820 m asl) are the highest peaks. Soils on the island predominantly belong to andosols – highly porous, dark-coloured soils developed from parent material of volcanic origin, such as volcanic ash, tuff, and pumice that typically occur in wooded highland areas (Wada 1985). The volcanic origin determines the presence of high concentrations of several metals, including Al in both soil minerals and soil solution (Furtado et al. 1990). This characteristic allows for investigation of the development of different ecotypes among wild and agricultural plant species.

Over 1100 native plant species have been identified on the Archipelago of Madeira, including

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species of the evergreen forest, *Laurisilva*, with widespread distribution at altitudes between 150 and 1200 m in ecosystems where most of the soils are acid and rich in organic matter.

These chemical and physical characteristics of soils determine the distribution of native plant species showing narrow ecological tolerances. Press and Short (1994) suggested that these ecological tolerances are closely linked to altitude, with species confined to specific altitudinal bands.

One of the native plants is *Sinapidendron* sp., an endemic genus of the Archipelago of Madeira (Short 1994). The genus is represented by five species of small perennial shrubs, while four of them can be found on the Island of Madeira including *S. gymnocalyx* (Lowe) Rustan; *S. angustifolium* (DC.) Lowe; *S. frutescens* (Aiton) Lowe; *S. ruprestre* (Ait.) Lowe, and one – *S. semprevivifolium* Mnzs – on the Deserta Grande Island (Short 1994). The Madeiran *Sinapidendron* species can be differentiated by their morphology, rarity and ecosystem distribution. *S. gymnocalyx* is a glabrous shrub with glabrous to sparsely scarbid, ovate to suborbicular leaves, common in the cliffs of the North coast from sea level to 500 m asl, sporadically found at altitudes up to 1000 m asl. *S. angustifolium* is a glabrous slender shrub with acute and linear leaves, entire or rarely toothed. The distribution of this species is confined to the south coast of Madeira on the cliffs and near the sea slopes from the sea level to 200 m asl. *S. frutescens* is a slender lower adpressed-pubescent shrub with elliptic to elliptic-oblanceolate leaves. It can be found in cliffs and slopes along the north coast and in ravines of central peaks of the island up to 1800 m asl. *S. ruprestre* is a perennial herb with woody below and coarse oblong to ovate leaves irregularly dentate. Generally rare or very rare, it exists in ravines and mountains of the northern Madeira from 850 to 1500 m asl. Although these species belong to an endemic genus, they are closely related to several *Brassica* species and cultivated subspecies and varieties of *Brassica oleracea* L. Some of them, such as *S. gymnocalyx* are traditionally used by farmers as forage for cattle and cultivated on the borders of agricultural fields.

Over centuries, the flora of Madeira has been enriched through the introduction of numerous agricultural and ornamental species that were cultivated in the areas previously occupied by native forest. The first introductions of cultivated plants commenced in the 15th century along with the colonisation of the island, when a great variety of crop species and cultivars including wheat, maize, sugarcane or sweet potato was brought from different geographical regions of the world (Vieira 1984). A significant number of these species become spontaneous, while other maintained and cultivated by local farmers developed into landraces adapted to local edaphic and ecological conditions.

The Archipelago of Madeira could be a good location to search for new forms of metal resistant species because of the selective pressure of acid soils and widespread metal toxicity, especially Al. The Madeiran cultivated species exhibit an enhanced resistance to Al and could be used as a source of genes for Al resistance (Pinheiro de Carvalho et al. 2003), while no information is available regarding response to Al toxicity among wild species that could have agronomic importance. Aluminium toxicity is a main growth and yield-limiting factor on mineral soils with pH below 5.0 (Haug 1984, Foy 1992). Under acidic conditions, monomeric Al ions are released into soil solution from minerals and polycationic, non-toxic Al complexes that exist at neutral pH. Once in the soil solution, soluble Al ions can be uptaken by roots and adversely affect plant growth. The first visual symptom of Al toxicity is a drastic reduction of root growth (Foy et al. 1978). Measurements of root elongation under Al stress in hydroponic systems are often used to predict Al resistance of crops grown in the field. The use of eriochrome cyanine staining to visualize root regrowth after exposure to Al can facilitate the detection of this resistance (Aniol 1984, Slaski 1992).

The objective of this study was to analyse the range of resistance to Al among the Madeiran populations of three wild *Sinapidendron* species with distinct ecological distribution and to compare them with three cultivated species (two wheat species and one corn) with the aim of identifying the role of anthropogenic and edaphic parameters in the distribution of Al resistant genotypes across the island.

MATERIAL AND METHODS

Soil samples were collected from the surface 0–20 cm depth at 101 locations throughout the Island of Madeira. Simultaneously, seeds of 16 populations of *S. gymnocalyx*, *S. angustifollium* and *S. frutescens*, 45 cultivars of wheat (*Triticum aestivum* and *T. turgidum* L.) and 40 cultivars of corn (*Zea mays*) were collected at the same sampling sites.

Table 1. Distribution of Al between soluble forms and solid complexes in treatment solutions used to evaluate Al resistance among the Madeiran plant species. Calculations made using the GEOCHEM-PC version 2.0

Al forms	Al in treatment solution			
	25μM	50μM	100μM	200μM
Al as a free metal (%)	86.31	60.94	30.52	15.31
Al complexed with OH^- (%)	13.69	9.47	4.74	2.37
Al in solid form with OH^- (%)	0.00	29.59	64.74	82.31

Prior to chemical analyses soil was dried at 105°C for 24 hours. The pH, exchangeable Al and organic matter of soil samples were analyzed. Ten grams of soil was suspended in 25 ml of 0.01M KCl and shaken for 1 hour (Forster 1995) and then the pH was measured using the WTW 320 pH meter. Exchangeable Al was extracted from 5 g of soil using sodium acetate (Morgan reagent) and the Al content was measured according to the modified hydroxylamine acid method (Ross and Wang 1993) using the Shimadzu UV-2401 PC spectrophotometer at 585 nm wavelength and expressed in the SI units as cmol/kg of soil. Soil organic matter (OM) was determined using the modified wet oxidation Walkley-Black method (Jackson 1958) where the amount of total soil organic carbon was converted to OM using the Van Bemmelen factor (1.724).

Seeds of each cultivar of corn and wheat were surface sterilized in 5% sodium hypochlorite and germinated for 1–3 days at 25°C in Petri dishes. Seeds of *Sinapidendron* were surface sterilized in 15% sodium hypochlorite and subsequently washed in 70% ethanol and distilled water. After sterilization the seeds were transferred to the Murashige and Skoog (MS) (1962) solid media and germinated in a growth chamber at 20°C for 5 days. Sprouted seeds were placed on a raft floating on a surface of aerated full nutrient solution (Pinheiro de Carvalho et al. 2004) and grown for 3 days in a growth chamber at 23°C. For Al exposure, seedlings of all species were transferred for 72 h to fresh nutrient solution containing (in μM) 2900 NO_3^-, 300 NH_4^+, 1000 Ca^{2+}, and 300 Mg^{2+}, supplemented with Al in form of $AlCl_3 \cdot 6\ H_2O$. Al at concentrations 25 or 50μM was used for *Sinapidendron* treatment, while 100 or 200μM for wheat and corn. Al solution was replaced daily to minimize pH fluctuation and Al depletion. Al activities in nutrient solution were calculated using the program GEOCHEM-PC version 2.0. The program predicted that the free activity of Al^{3+} was $1.134.10^{-5}$M, $1.593.10^{-5}$M, $1.588.10^{-5}$M and $1.577.10^{-5}$M for the 25, 50, 100 and 200μM treatment solution, respectively. Treatment solutions differed drastically in the amounts of precipitated hydroxyl form of Al (Table 1).

After Al treatment the seedlings were transferred to an Al free nutrient solution for 2 days to determine the ability of roots to recover from Al stress. In all treatments, the pH of nutrient solutions was measured every 12 h and adjusted to 4.3 with 0.1N HCl, if necessary. At the end of the experiment, roots were stained for 10 min with a 0.1 solution (w/v) of eriochrome cyanine R250, which facilitated the visualization of root survival and regrowth (Slaski 1992). After staining, the roots were extensively rinsed under tap water to remove the excess dye. Root tips of plants that were able to continue their growth after exposure to Al remained white, while roots with irreversibly damaged apical meristems were dark purple, indicating the absence of regrowth even after the transfer to an Al free medium.

All experiments were run in four replicates for each experimental variant and the results represent the mean values of these experiments. Data were analyzed using SAS system for Windows version 10.0 (SAS Institute Cary, NC) software. A linear correlation analysis was used to examine the mean relationships between the examined factors using the CORREL procedure. Correlations were considered significant at *P* values indicated in the graphs.

RESULTS

The pH of topsoil sampled from fields planted with wheat, corn and *Sinapidendron* species on the Island of Madeira ranged from 3.83 to 7.97, with a mean soil pH_{KCl} value of 5.01. Noticeable differences between species were observed as the average pH was 6.79 and 4.73 for the wild and cultivated species, respectively. Normal-like frequency distribution of soil pH in cultivated species was skewed towards acid values and 65% of the sites exhibited

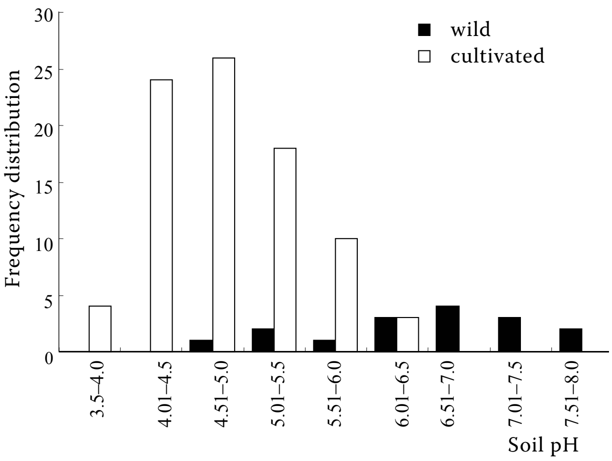

Figure 1. The frequency distribution of pH of topsoil sampled from the collection sites of wild *Sinapidendron* populations and from farmed plots seeded to the species cultivated on the Island of Madeira (wheat, corn)

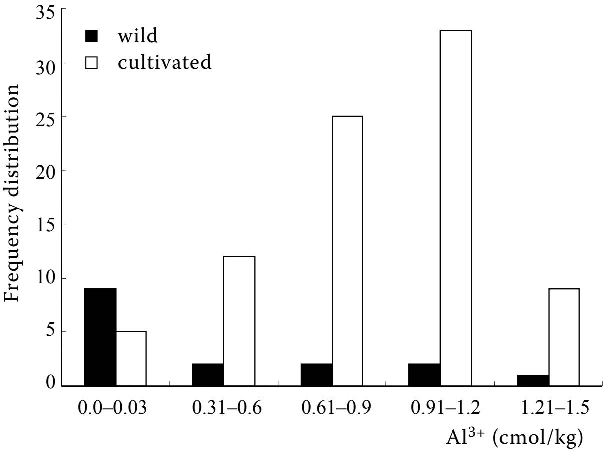

Figure 2. The frequency distribution of Al^{3+} concentration in topsoil sampled from the collection sites of wild *Sinapidendron* populations and from farmed plots seeded to the species cultivated on the Island of Madeira (wheat, corn)

pH below 5.0. An opposite trend was observed in the wild species where the neutral sites dominated and 75% of them had pH over 6.0 (Figure 1). Low mean pH of the Madeiran soils was accompanied by high amounts of exchangeable Al. On average, 0.79 cmol of ionic Al per kg of soil was found in the analysed topsoils. Again, profound differences between sites occupied by the wild and cultivated species were noticed as *Sinapidendron* populations were collected from sites containing on average 50% Al less (0.44 cmol/kg of soil) than the cultivated species (0.83 cmol/kg of soil). Fifty nine per cent of the sampled soils showed levels of exchangeable Al exceeding 0.8 cmol/kg of soil in cultivated species, while only 19% of the S*inapidendorn* sites surpassed this value (Figure 2).

Differences between the cultivated and wild species in soil pH and Al content revealed by the frequency distribution patterns were further validated by the analysis of reciprocal relationship of the two edaphic factors. A strong negative correlation (r = –0.349***) between soil ionic Al content and soil pH was found in the samples from the cultivated fields, while a positive correlation (r = 0.407*) was obtained in soils on which wild populations of *Sinapidendron* were identified (Figure 3).

Both species of the cultivated plants were characterized by a wide vertical distribution and they

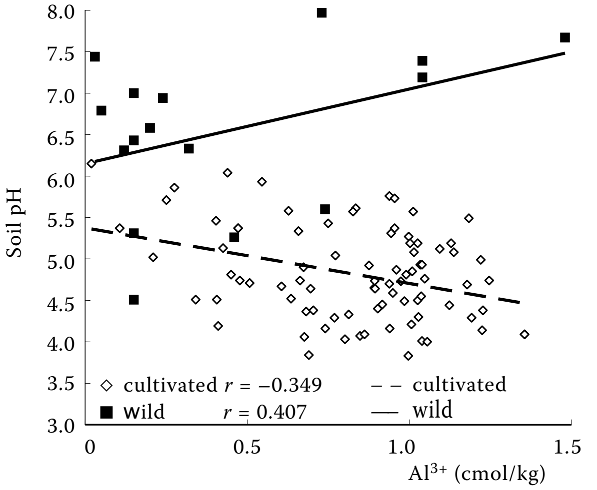

Figure 3. Relationship between Al^{3+} concentration and pH of topsoil sampled from the collection sites of wild *Sinapidendron* populations and from farmed plots seeded to the species cultivated on the Island of Madeira (wheat, corn)

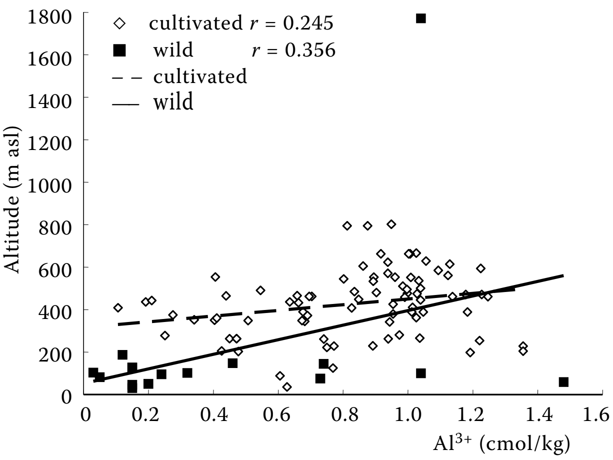

Figure 4. Relationship between altitude and Al^{3+} concentration in topsoil sampled from the collection sites of wild *Sinapidendron* populations and from farmed plots seeded to the species cultivated on the Island of Madeira (wheat, corn)

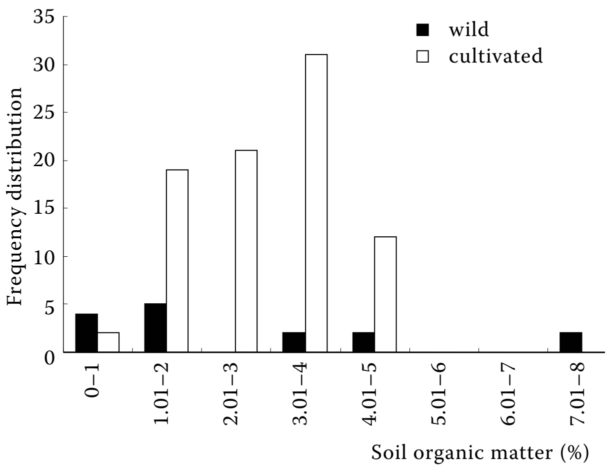

Figure 5. The frequency distribution of organic matter content in topsoil sampled from the collection sites of wild *Sinapidendron* populations and from farmed plots seeded to the species cultivated on the Island of Madeira (wheat, corn)

were grown on plots located at altitudes between 35 and 800 m asl. The wild species, however, were found on locations not exceeding 200 m asl, with one exception of *Sinapidendron frutescens* collected in the mountainous region at a high altitude of 1772 m asl (Figure 4). Interestingly, soil Al content increased along with elevation of the plot location. This relationship between the two edaphic factors was particularly obvious in the cultivated species (r = 0.245**), while the observed trend was not significant in the wild species (Figure 4).

We were unable to identify an obvious pattern of relationship between the altitude and Al resistance of the tested species of the respective sample locations (data not presented).

Content of soil organic matter is an important factor affecting Al toxicity. While the tested sites contained on average 3.02% of OM, noticeable differences between the species were observed. The normal-like frequency distribution ranging between 0.55 and 4.89% was found in the cultivated species whereas sites inhabited by *Sinapidendron* contained between 0.38 and 7.64% distributed irregularly across the range (Figure 5).

We expected that edaphic parameters including soil pH, ionic Al or organic matter could contribute to the development of Al resistance among both cultivated and wild plant species found on the island. To verify our hypothesis we evaluated the performance of hydroponically grown plants under Al stress using the eriochrome cyanine test. To achieve separation between cultivars or populations in response to Al we had to subject seedlings to the very stringent stress conditions of 72 h exposure at 100 and 200μM Al in the case of wheat and corn or 25 and 50μM Al in the case of *Sinapidendron*. The degree of response of both species of the Madeiran cultivated plants to Al stress imposed in nutrient solution was positively correlated with the amount of ionic Al in soils (r = 0.37***), while an opposite tendency (not significant) was detected in three populations of wild *Sinapidendron* (Figure 6).

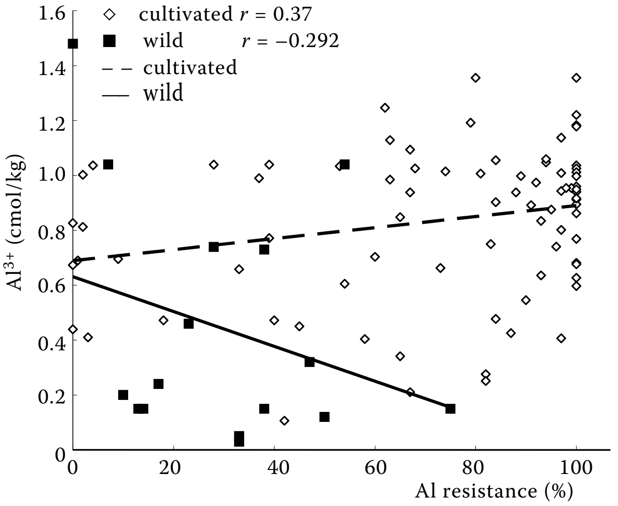

Figure 6. Relationship between soil ionic aluminium content and resistance to 25 or 100μM aluminium of wild *Sinapidendron* populations and the cultivated species (wheat, corn) from the Island of Madeira. The aluminium resistance was determined using the eriochrome cyanine staining method

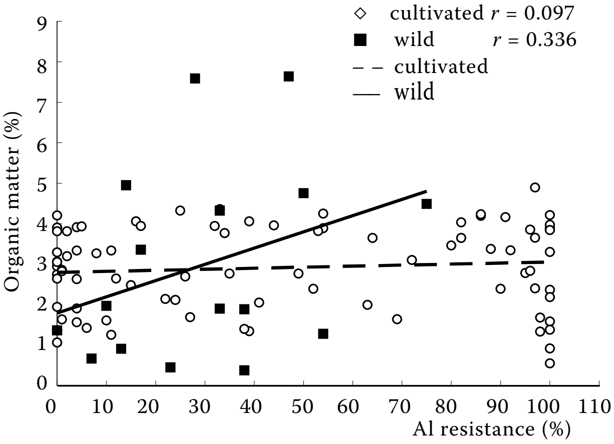

Figure 7. Relationship between the content of organic matter in topsoil and resistance to aluminium of wild *Sinapidendron* populations and the cultivated species (wheat, corn) from the Island of Madeira. The aluminium resistance was determined using the eriochrome cyanine staining method

Surprisingly, the level of Al resistance among all tested species did not correlate with soil pH (data not presented).

Relationship between soil OM and level of Al resistance was more convoluted (Figure 7). In cultivated species no association between Al resistance and content of OM could be identified, while a weak positive correlation was found in wild species (0.336*).

DISCUSSION

Enhanced resistance to Al appeared to be a common phenomenon among the plant species cultivated on the Island of Madeira (Pinheiro de Carvalho et al. 2003, 2004, dos Santos et al. 2001, 2005). The prevalence of acid soil on the Island creates conditions favourable for releasing Al to soil solution that could be one of the limiting factors determining the narrow plant ecological tolerances of the Madeiran flora (Press and Short 1994). To modulate the role of this factor and to achieve the separation between the cultivated species in response to Al we have subjected wheat and corn seedlings to very stringent stress conditions. Concentrations of 100 or 200μM Al are considered to be very toxic for wheat and corn (Carver and Ownby 1995, Pintro et al. 1996).

We hypothesise that a high number of Al resistant genotypes found among the cultivated species on the island may indicate a possible involvement of anthropogenic and environmental factors in the diversification processes and the development of new ecotypes highly adapted to local edaphic conditions. Both wheat and corn cultivars currently present on the island resulted from multiple introductions from different geographical regions during the course of several centuries (Silva and Meneses 1984). It is safe to assume that these cultivars differed in their ability to withstand Al stress and due to specific cultivation practices on the island (i.e. preservation of local seed stocks) (Vieira 1984) the initial artificially introduced variability has been maintained and/or enhanced throughout centuries. Subsequent human interventions have also greatly contributed to the present distribution of cultivars on the island. In the past, fields with predominantly stringent Al stress conditions were instinctively seeded to the cultivars or cultivars mixtures that would guarantee reasonable yield, while the under-performers were either discarded or confined to the fields displaying less stressful conditions (Pinheiro de Carvalho et al. 2003, 2004). Cultivars with high initial resistance subjected to stringent environmental conditions over extended period of time may have acclimated to the specific edaphic conditions existing on a given field and they have likely further enhanced their ability to withstand Al stress. The initial genetic variability of plant populations was previously identified as a critical parameter for the successful development of plant resistance to toxic levels of metals in soil (Bradshaw and McNeilly 1981, Aniol 1990).

In addition to anthropogenic factors, specific soil conditions present on the island may have had profound effects on the present distribution of both cultivated and wild species. As very acidic andosols dominate on the island (Madeira et al. 1994), low mean pH of the Madeiran soils (Figure 1) has resulted in the increased release of high amounts of exchangeable Al to the soil solution. Additionally, we observed that the Al content in soil increased with increasing altitude, most likely because of the lower buffering capacity of soils at earlier stages of development and lower content of organic matter. Our data seem to support a hypothesis that the Al content in soil solution was the major environmental factor involved in the development of Al resistant genotypes of cultivated plants (Figure 3). The most Al resistant genotypes as evaluated by the eriochrome cyanine test were collected from fields containing high amounts of Al^{3+}. Other tested edaphic parameters including soil pH or elevation were weakly associated with the Al resistance.

The wild endemic populations of *Sinapidendron* present on the island could provide some knowledge regarding the contribution of edaphic factors in acquiring and/or developing Al resistance in plant species having agronomic potential. In general, *Brassicaceae* resistance to the presence of Al is much lower than cereals and did not exceed the 50μM of ionic Al in the soil (Basu et al. 2001). Identification of genes controlling among *Brassicaceae* to improve stress resilience of *Brassica* species of agricultural importance such as rapeseed is a mainstream research goal in many laboratories (Huang et al. 2002). To screen wild populations of *Sinapidendron* for Al resistance in the laboratory we have applied stringent Al exposure conditions of 25 and 50μM Al in nutrient solution. The lower concentration of Al was more effective in obtaining separation between the collected ecotypes as 50μM Al resulted in irreversible damage of root apical meristems in majority of collected populations. This might indicate that with few exceptions the overall level of resistance among the wild populations was lower than in the cultivated species. However, identification of two ecotypes of *S. angustifolium* with the enhanced Al resistance should justify considering *Sinapidendron* as a species worth further attention. These exceptional ecotypes deserve more detailed molecular studies to confirm their suitability as candidates for putative donors of Al resistance genes in Brassicaceae breeding programs.

Interestingly, the analysis of the edaphic factors revealed different patterns of response of the wild *Sinapidendron* populations than those observed in the cultivated species. In general, *Sinapidendron* tended to inhabit sites characterized by less stressful soil conditions. Soil pH of the *Sinapidendron* sites was predominantly neutral as opposed to acidic or very acidic occupied by the cereals (Figure 1), while Al content on the *Sinapidendron* locations was lower than those in the cereals (Figure 2). Therefore, a negative correlation between soil Al content and Al resistance of the *Sinapidendron* populations measured in the laboratory was an expected result (Figure 6). Putting these observations together one can argue that the role of the edaphic factors in acquiring of Al resistance by the cultivated species was of lesser importance, while anthropogenic activities (previous introductions of Al resistant genotypes, selection towards resistance, cultivation practices) seem to be critical factors, assuming that an extrapolation between response of cereals and *Brassicaceae* to Al stress is a legitimate notion. Therefore, we tempt to hypothesise that soil pH and Al content may be primarily involved in maintaining the introduced resistance of the cultivated species.

Al toxicity in soils is often affected by organic matter content. In the presence of organic matter the toxicity of Al in soils was considerably reduced presumably due to the detoxifying effect of organic acids or via the formation of Al-organic complexes (Bessho and Bell 1992, Haynes and Mokolobate 2001). Analysis of effect of soil organic matter on Al resistance among the wild and cultivated species revealed an interesting phenomenon. Our data indicate that Al resistance was positively correlated with the level of organic matter in soil but only in the wild species, whereas no association between these two parameters could be found in the cultivated species (Figure 7). We found that the *Sinapidendron* populations collected from sites with extremely high levels of organic matter exhibited enhanced level of Al resistance as evaluated by the exposure to 50μM Al in hydroponic solution. Perhaps, this observation could be explained by the selective pressure of highly toxic Al polymer forms such as Al_{13} tridecamer (Hunter and Ross 1991, Kochian 1995) that might have been formed in soils with high organic matter colonized by the wild species. We incline to suggest that organic matter content played a secondary role in sustaining of Al resistance of the cultivated species, whereas it was a dominant factor affecting distribution of the wild species across the island. A detailed analysis of Al speciation in soil solution from both cultivated and natural sites characterized by various levels of organic matter would be necessary to validate this conclusion.

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