Controls on foliar Al accumulation among populations of the tropical shrub *Melastoma malabathricum* L. (Melastomataceae)

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ABSTRACT

Al accumulation is a common trait expressed in at least 60 plant families and particularly prevalent in tropical woody plants. However, the functional significance and genetic or physiological controls on Al accumulation are currently unknown. We tested the hypothesis that differential expression of Al accumulation among wild populations of the Al-accumulating tropical shrub *M. malabathricum* is associated with habitat-related variation in total and exchangeable soil Al concentrations. Mature leaves and seeds were sampled from 20 populations of *M. malabathricum* growing in six habitats across Peninsular Malaysia, and soil was collected from each site. The seeds were grown in hydroponic solutions comprising 50% Hoagland's solution amended with Al in the form of 1.0 mM $AlCl_3$ to test the hypothesis that differential expression of foliar Al accumulation is an inherited trait. Foliar Al concentrations varied significantly among populations, but were not consistently different among plants growing in different habitats and showed no relationship to total or exchangeable Al concentrations in soils collected at the 20 sites. Mean foliar Al concentration in wild plants was positively correlated with foliar Ca concentrations, and with total soil N, Ca and Mg concentrations, across the 20 populations, and Al addition increased foliar concentrations of P, Ca, Mg and K in seedlings. The differential expression of Al accumulation in *M. malabathricum* populations is uncoupled to local variation in soil Al concentrations, but may be sensitive to local soil-related variation in the availability of other macro-nutrients, in particular N, Ca and Mg. Further research on the factors controlling Al uptake should focus on the plasticity of this trait within populations of Al accumulators and interactions with micro-habitat variation in the availability of the macro-nutrient cations.

Key words: Aluminium accumulation, populations, functional trait, *Melastoma malabathricum*, Peninsular Malaysia

INTRODUCTION

Soil Al toxicity is a major constraint to global crop production, but many wild plants tolerate or even accumulate high tissue Al concentrations (Chenery, 1948; Jansen, 2002; Metali et al., 2012). The Al accumulation trait has been identified in 60 largely eudicot families and is particularly frequent in the Rubiaceae, Symplocaceae, Icacinaceae, Theaceae, Anisophyllaceae and Melastomataceae (Chenery, 1948; Jansen, 2002; 2003). Plants expressing this trait were originally defined as those possessing foliar Al concentrations greater than 1.0 mg Al g^{-1} dry mass (Chenery, 1948), but recent research has suggested that this threshold may vary among biomes (Metali et al., 2012). Al accumulators are most abundant and speciose in tropical forests and savannas where soils typically possess higher Al availability than in temperate vegetation (Haridasan & Araújo, 1988; Jansen, 2002; Osaki et al., 2003), and a higher threshold value of foliar Al concentration is required for statistical separation of Al accumulator from non-accumulator plants (Metali et al., 2012). However, despite the high frequency of the Al accumulator trait, particularly in tropical floras, there is limited understanding of its functional significance for individual plants or ecosystem processes.

Variation among plants in leaf element concentrations is determined by phylogenetic history, soil conditions, climate and physiological constraints on elemental uptake (Zhang et al., 2012; Hao et al., 2014; Metali et al., 2014). Al accumulation is a phylogenetically constrained trait (Metali et al., 2012) and one study has suggested that 49.5% of the variation in foliar Al concentration is explained at and above family level (Watanabe et al., 2007). Therefore, although plants acquire nutrients and other elements from their native soil environments, the concentrations expressed in tissues for a common soil may vary markedly among species and higher taxa (Broadley et al., 2007; Hao et al., 2014: Rascio & Navari-Izzo, 2011; Russell et al., 2017). Similarly, in comparisons across species, foliar concentrations of Al may correlate either positively or negatively with that of other elements, and outcomes vary among studies (Masunaga et al., 1998a; 1998b; Haridasan et al., 1982; 1988; Metali et al., 2014). For trees growing in lowland dipterocarp forests in Indonesia, Brunei and Peninsular Malaysia, foliar Al concentrations were positively correlated with concentrations of Ca and Mg (Masunga et al., 1998b; Metali et al., 2014), while foliar P and Al concentrations were positively correlated in Indonesia and negatively correlated in Brunei and Peninsular Malaysia. These differences among species within and among sites suggest that finer-scale contrasts and common garden experiments are required to uncouple the genetic, physiological and environmental controls on Al accumulation. A long-term study of four tropical tree species growing individually on plots in Costa Rica has shown that the sole Al-accumulator in the sample, *Vochysia guatamalensis*, increasd surface soil pH and accumulated higher cation biomass stocks than non-Al accumulator species (Russell et al., 2017). This study proposes that pH-related reduction in the dispersion of soil colloids leads to release of occluded cations in the rhizosphere of the Al accumulator. This interpretation provides a mechanism for positive correlations between Al and other cations in the tissues of Al accumulators.

Experiments on the effects of Al on elemental concentrations in Al accumulator plants grown in hydroponic solution culture partially support the conclusions of field surveys (Watanabe et al., 1998; Watanabe & Osaki, 2001; Jansen, 2002; Watanabe et al., 2007; Fung et al., 2008; Tolrà et al., 2011; Hajiboland et al., 2013; Zeng et al., 2013). Al addition stimulates the uptake of Ca and Mg (as well as K and Mn) in tea plants (*Camelia sinensis*, Konishi et al., 1985; Fung et al., 2008) and foliar Al concentrations are positively associated with tissue concentrations of Ca and Mg (as well as N, P and K) in the tropical shrub *M. malabathricum* (Watanabe et al., 1997; Watanabe & Osaki, 2001; Watanabe et al. 2008). The significance of these physiological links between Al accumulation and the concentrations of key nutrients limiting plant growth and ecosystem productivity requires further investigation under field conditions.

A common approach to understanding the role of environmental factors and adaptation in trait expression is to compare populations of the same species growing in different environments, on the basis that these plants share a recent evolutionary history and genetic background. To our knowledge, this approach has not been adopted previously for understanding Al accumulation, although it has been used in research on the factors involved in the accumulation of other metals in plant tissues (Lombi, 2000; Assunção et al., 2003; Escarré et al., 2013). For example, this research has identified that soil Zn concentrations contribute to variation in foliar Zn concentrations among populations of *Thlaspi caerulescens* (Escarré et al., 2013), and that uptake of Zn may inhibit concentrations of Ni in the leaf (Assunção et al., 2008). *Thlaspi caeurulescens* populations from sites with higher Zn and Cd concentrations have higher foliar Zn concentrations and are more tolerant to Zn when grown hydroponically than populations from sites with lower soil Zn concentrations (Assunção et al., 2008; Escarre et al., 2000; 2013).

In this study, we tested the hypothesis that foliar Al concentrations among populations of the Al accumulator plant *M. malabathricum* are positively associated with Al concentrations in local soils. Support for this hypothesis would suggest that differential expression of the Al accumulation trait may be associated directly with tolerance to high soil Al concentrations. We also collected seeds from these individuals and grew their progeny under uniform conditions to determine whether foliar Al concentrations express heritable variation. We used these data to test the following specific hypotheses.

1. Populations of *M. malabathricum* express variation in foliar Al and nutrient concentrations that reflect differences in the concentrations of these elements in local soils. We interpret support for this hypothesis as indicative of a contribution of environmental variation to foliar element concentrations.

2. Under common environmental conditions for plant growth, expression of differences in foliar Al accumulation among seedlings of *M. malabathricum* is correlated with foliar Al concentrations of wild plants from their source populations. We interpret support for this hypothesis as indicative of a genetic contribution to foliar element concentrations.
3. In common with studies across taxa within sites, variation in foliar Al concentration among *M. malabathricum* populations growing in the wild is positively associated with that of other elements, particularly Ca and Mg.
4. Similar correlations between foliar Al and major nutrient elements are expressed in seedlings of *M. malabathricum* in a common growing environment. We interpret support for this hypothesis as indicative of a role for physiological constraints independently of the soil environment in driving variation in foliar element concentrations.

MATERIALS AND METHODS

Study species

The study species was the tropical shrub *M. malabathricum* L. (Melastomataceae) which is a known Al accumulator plant (Chenery, 1948; Jansen, 2003; Watanabe et al., 2005). *M. malabathricum* is a pioneer species that occurs from islands in the Indian Ocean to South and South-East Asia, China, Taiwan, Australia, and the South Pacific Ocean, and is found in a range of natural vegetation types, as well as wasteland, secondary forest and roadsides (Jansen et al., 2002; Watanabe et al., 2005). In some countries, including Malaysia, *M. malabathricum* is reported to be useful for medicinal purposes (Sharma et al., 2001; Joffry et al., 2012).

Plant and soil sampling

Leaves and fruits of *M. malabathricum* L. were collected from 20 populations distributed across seven distinct habitats within Peninsular Malaysia in December 2013 and January 2014 (Figure S1, Table S1). These habitats represented lowland riverine vegetation (two populations), coastal beach vegetation (two populations), lowland swamp forest (one population), hill dipterocarp forest (one population), coastal hill dipterocarp forest (one population), lowland dipterocarp forest (12 populations) and heath forest (one population) (Table S1). The sample sites were distributed across an elevation range of 2 to 450 m a.s.l. and represent almost all the natural habitats occupied by *M. malabathricum* within Peninsular Malaysia. A total of 10-12 fruits from at least three individuals were collected per population and pooled to create a bulk sample for each population. The seeds were extracted from the partly opened fleshy fruits in distilled water, rinsed with distilled water several times and then filtered and left to air-dry in an air-conditioned laboratory at the Universiti Sultan Zainal Abidin, Malaysia. In addition, four undamaged mature leaves from fully exposed locations were collected from the same individuals that had been sampled for fruits and pooled per individual to form 3-6 samples per population. Finally, three soil samples from 0-15 cm depth were collected using a soil auger 1 – 2 m from each *M. malabathricum* individual that had been sampled for fruits and leaves. The leaf and soil samples were air-dried for 24 hours starting on the day of collection and then transported, with the seeds, to the University of Aberdeen, U.K., for analysis.

Chemical analysis of field collected plants and soils

The *M. malabathricum* leaf and soil samples were re-dried to constant mass in an oven at 60°C for at least 30 minutes. The leaf samples were then ground to a fine powder, digested with 4.8% sulfuric acid and analysed to determine their concentrations of nutrients and Al. Four blanks and four reference samples of hay (CRM, BCR-129, EU) were included in each set of digests. Foliar N and P concentrations were determined colorimetrically using a flow injection auto-analyser (FOSS FIA star 5000 Analyser and OSS TECATOR 5027 Sampler for the auto sampler, USA). The concentration of K in the digest was determined using a flame emission spectrophotometer (Perkin Elmer AAnalyst 100, Norwalk, USA) and those of Al, Ca and Mg were measured using an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 100, Norwalk, USA) following dilution of the acid-digested samples with $LaCl_3$ (H_2SO_4: $LaCl_3$ in the ratio of 1:1). The mean and variance of foliar nutrient concentrations (mg g^{-1} dry mass of leaf tissue) were calculated per population based on three replicates of the bulked samples. The recovery of elements based on comparison with the reference materials was 95 to 99 % in all cases.

Roots, small stones, and litter were removed by hand from the re-dried soil samples and they were then sieved through a 2 mm mesh to remove aggregates. The pH of the soil was determined using a soil: distilled water ratio of 1:2.5 (Conklin, 2005). Exchangeable Ca, Mg and K concentrations were determined in 2.5% acetic acid extracts, and Al concentrations were determined in 2.5% ammonium acetate extracts (Conklin, 2005) using the instruments described above. Total concentrations of K, Ca, Mg and Al were determined using an atomic absorption spectrophotometer (AAS Perkin Elmer concentrations AAnalyst 100, Norwalk, USA) following sulfuric acid digestion (Conklin, 2005). Total N and P concentrations in the digests were determined colorimetrically using a flow injection auto-analyzer (FOSS FIA star 5000 Analyser and OSS TECATOR 5027 Sampler for the auto sampler, USA).

Foliar Al and nutrient concentrations in seedlings grown under uniform conditions

In a preliminary trial, seeds of two populations (Sidim and Ara Kuda, Table S1) failed to germinate and these populations were excluded from the experiment described below. For the remaining 18 populations, seeds were soaked in 5% bleach solution for three minutes and then rinsed three times for at least three minutes with sterilized distilled water. Three seeds from a single population were then sown together on the surface of Daishin agar (0.5 g agar/100ml with 50% Hoagland's nutrient solution) in sterilized 0.5µL Eppendorf tubes (see Table S2 for the composition of the Hoagland's solution). The bottom 2 mm of the Eppendorf tubes had been removed to enable growth of the *M. malabathricum* roots into a nutrient solution. Each population was represented by 24 Eppendorf tubes (72 seeds per population), yielding a total of 432 Eppendorf tubes for the entire experiment. A pilot experiment had shown that germination percentage of *M. malabathricum* seeds was higher when they were irrigated with 50% Hoagland solution (70-80% germination) than with either 25% (60-70%) or 10% (60-70%) Hoagland solution (M. Khairil, unpublished data). Therefore, the 432 tubes were suspended in groups of six in sterilized boxes (dimensions 12 x 8 x 7 cm) containing 50% Hoagland nutrient solution with each box containing three tubes of each of two populations. The boxes were divided equally between two growth chambers both set to deliver a temperature of 27°C and 12/12 h light/dark photoperiod with irradiance of 200-250 μmol m^{-2} s^{-1}.

The pH of the nutrient solutions was checked daily and adjusted to 4.0 following Watanabe & Osaki, (2002) using 0.1M NaOH or 0.1M HCl, and the nutrient solutions were renewed weekly throughout the growing period. The seeds germinated after 7-10 days, and 14 days after sowing the seedlings were thinned to one per Eppendorf tube by randomly selecting excess surviving individuals for removal. Twenty-eight days after sowing, half the containers were randomly selected to receive Al (Al+ treatment) in the form of 1.0 mM Al3+ added as AlCl3 to the 50% Hoagland's solution, subject to the constraint that half the replicate individuals of each population in each growth chamber received the Al+ treatment. The other replicates received no Al addition to the 50% Hoagland's solution (Al-) treatment, yielding 18 individuals in six boxes per population in each treatment distributed equally between the two growth chambers. Use of the chemical speciation programe GEOCHEM-EZ (Shaff et al. 2010) suggested that approximately 75% of the Al in the 50% Hoaglands solution amended with 1 mM $AlCl_3$ would be precipitated (7.3%) or complexed (5.7%) with sulfate, or complexed with phosphate (60.9%), yielding an estimated concentration of free Al^{3+} in the solution of 0.25 mM. Boxes were re-randomised weekly within each growth chamber. Three replicate individuals per container were harvested 28 days after sowing, dried to constant mass and weighed (harvest 1), and all remaining seedlings were harvested after 56 days, dried and weighed (harvest 2) (Figure S2).

To determine elemental concentrations in plant leaves, a fragment of 0.5-1.0 cm^2 of the leaf was removed from the margin for three randomly selected individuals per population x treatment combination at harvest 2. This material was cut in transverse section, washed in deionized water and placed in a 50µml Teflon tube. The samples were then dried in an oven at 88°C for 20-22 hours. The dried samples were digested using 70% nitric acid and analyzed by inductively-coupled plasma mass spectrometry (NexION 300D, ICP Mass Spectrometer, PerkinElmer, USA). This analysis yielded foliar concentrations of Al, Ca, Mg, K, and P as well as 15 other elements.

Statistical analysis

Analysis of variance was used to determine the significance of differences in foliar concentrations of N, P, K, Ca, Mg, and Al among the 20 source populations collected in the wild and seedlings of the 18 populations that were grown in solution culture. The major trends across the multivariate data-set of 12 soil chemical properties were summarised using a Principal Components Analysis (PCA) on centred and standardised data. In this exploratory analysis the first PC axis was associated with variation in concentrations of total Ca, total Mg, total K and exchangeable Ca and Mg. The strength of the associations among population means of the foliar element concentrations and among these means and soil chemical properties was determined using Pearson correlations. All analyses were conducted using R version 3.3.1 (R Development Core Team 2016).

RESULTS

Foliar Al and nutrient concentrations among wild populations of M. malabathricum and relationship to soil nutrient concentrations

Mean (± standard error) foliar Al concentrations varied significantly ($F = 3.86$, $p < 0.001$), from 4.1 ± 1.3 to 15.5 ± 2.3 Al mg g^{-1}, among wild plants from the 20 populations of *M. malabathricum* (Figure 1, Table S3). Foliar concentrations of N, P, K and Mg were also significantly different among populations, but differences in foliar Ca concentrations were non-significant. Among the 20 populations there were significant positive correlations between mean foliar concentrations of N and P ($r = 0.721$, $p < 0.001$) and Al and Ca ($r = 0.373$, $p < 0.05$, Table S4).

Mean values of all measured chemical properties apart from exchangeable K varied significantly among soils taken from the 20 sites (Table S5). The first axis of a principal components analysis of the soils data explained 29.3% of variation and the first five PC axes cumulatively explained 80.6% of the variance (Figure 2, Table S6). The first PC axis was positively associated ($p < 0.05$) with variation in total Ca, total Mg, total K, and exchangeable Ca and exchangeable Mg (Figure 2). The second PC axis was positively associated with variation in concentrations of total N and exchangeable Al, and negatively associated with extractable P concentration ($p < 0.05$). Mean foliar Al concentration per population was positively correlated with concentrations of total and exchangeable Ca, and total Mg in soil (Table 1), and the corresponding correlation with site scores along the first PC axis was marginally non-significant ($P = 0.06$). Population mean foliar Al concentrations were also correlated with total N concentrations in soil, but there was no evidence of an association with total or exchangeable Al concentrations or site scores along soil PC axes 2 to 4 (Table 1).

Foliar Al and nutrient concentrations in seedlings grown in solution culture and comparisons with wild plants

Mean foliar Al concentration varied significantly ($F = 16.33$, $P < 0.001$), from 2.8 ± 0.5 to 10.5 ±2.8 mg g^{-1}, among seedlings derived from 18 populations when they were grown in hydroponic solution culture with addition of 1.0 mM Al^{3+} (Figure 1, Table S7). Foliar concentrations of P, K, Ca, Mg, and 11 out of 15 other elements also differed significantly among populations for seedlings grown in hydroponic solutions (Table S7), but there were no significant correlations in mean foliar concentrations of Al, P, K, Ca and Mg between the 18 populations sampled in the wild and their progeny grown in solution culture (Figure 1, Table S8).

When grown in the presence of Al in solution culture foliar concentrations of Al, P, K, Ca and Mg were positively associated and generally inter-correlated (Table 2). However, with the exception of a strong positive correlation between foliar Ca and Mg concentrations, these associations among foliar element concentrations were absent when Al was excluded from the composition of the nutrient solution. More generally, a PCA of foliar element concentrations of seedlings grown in the presence of Al displayed a first axis explaining 46.7% of variance that reflected tight coordination among 11 elements including Al, P and the macronutrient base cations (Figure 3, Table S9). A similar PCA of foliar element concentrations for seedlings grown in the absence of Al had a much less dominant first axis, which explained 31.9% of variance and reflected variation in six elements but not the macro-nutrient cations K, Ca or Mg (Figure 4, Table S10). When grown in nutrient solutions, Al addition increased the foliar concentrations of P, K, Ca and Mg (Table 3) as well as those of 14 other elements (Table S11).

DISCUSSION

Variation in Al accumulation among M. malabathricum *populations*

Foliar Al concentrations in populations of *M. malabathricum* sampled from the wild varied in the range 4.1 ± 1.3 mg g^{-1} (Population 9 growing in coastal vegetation) to 15.5 ± 2.3 mg g^{-1} (Population 2 growing in lowland dipterocarp forest). These values lie above the thresholds of 1 mg g^{-1} (Chenery 1948) and 2.3 – 3.5 mg g^{-1} (Metali et al. 2012) identified by previous authors for recognition of Al accumulators and confirms that the Al accumulation trait was differentially expressed across the populations sampled in this study. Previous studies of foliar Al concentration in *M. malabathricum* report values in the range 2.0 – 10 mg g^{-1} (Metali, 2010; Watanabe & Osaki, 2001; Maejima et al., 2014) and our results suggest that part of this variation may reflect differences in the geographical origin of the material sampled. Al uptake by *M. malabathricum* is facilitated by root mucilage, a gelatinous high molecular weight compound rich in polysaccharides (Watanabe et al., 2008). Al is then complexed with citrate for tranport from roots to shoots, where it is transformed to an Al-oxalate complex and sequestered in the cell walls and vacuoles of upper epidermal and mesophyll cells within leaves (Watanabe et al., 1998; Watanabe & Osaki, 2001; Watanabe et al., 2005). Variation in the expression of these physiological processes may contribute to the differential accumulation of Al among populations of *M. malabathricum*, but this remains unexplored.

Variation in foliar Al concentrations was decoupled from soil Al concentrations among populations of *M. malabathricum*. This lack of association occurred despite a 14-fold and 8-fold range of variation in topsoil concentrations of total and exchangeable Al concentrations across the 20 sites respectively. It is clear that differences in soil Al concentrations are not an important contributor to the significant variation in mean foliar Al concentrations among these 20 populations, which supports related research on intraspecific variation in tissue Al concentrations in other tropical woody plants (Haridasan & Araujo 1988, Watanabe et al., 2008, Metali et al., 2014). However this general outcome contrasts with evidence suggesting that variation in foliar Al concentrations among species, and the frequency of Al accumulators within a community, are strongly related to among-site variation in soil Al concentrations (Masunaga et al., 1998a; 1998b; Metali et al., 2014). This highlights a contrast in the explanatory factors underlying within vs between species variation in plant tissue element concentrations, and strengthens the emerging conclusion that Al accumulation is a fundamental plant trait (Jansen, 2002; Watanabe et al., 2007; Metali et al. 2012, 2014).

Variation in foliar Al concentration among *M. malabathricum* populations was strongly correlated with concentrations of total and exchangeable Ca and total Mg in soil, and weakly correlated with the first axis of a PCA of soil chemical properties that was driven by increasing values of these nutrient cations. An association between foliar Al concentrations and soil Ca and Mg concentrations was not detected in studies comparing across species (Masunaga et al., 1998a; Metali et al., 2014), but positive correlations among foliar concentrations of Al and either or both of these cations has been demonstrated for other Al accumulator species (Masunaga et al., 1998a, Metali et al., 2014). The mechanisms underlying this association is unknown, but our results support the hypotheses that interactions in the rhizosphere of Al accumulators jointly influence the solubilizaton of these elements from the soil mineral fraction (Haridasan & Araújo, 1988; Osaki et al., 1998), or pH-related increases in release of cations occluded in soil colloids (Russell et al., 2017). The capacity of Al accumulators to manipulate their soil environment reflects an adaptation to the low availability of these nutrients in heavily leached tropical forest soils (Burslem et al., 1995; John et al., 2007; Katabuchi et al., 2012, Russell et al., 2017).

Concentrations of Al and other elements

Concentrations of foliar Al and Ca were positively correlated among 20 populations of *M. malabathricum* sampled across Peninsular Malaysia. To our knowledge there has been no equivalent study comparing foliar Al and Ca concentrations among populations of a single species, but an analogous positive relationship between foliar concentrations of these elements has been detected in comparisons among Al accumulating tree species in Sumatra, Indonesia, and in Brunei (Masunaga et al., 1998, Metali et al., 2014). These positive relationships between tissue Al and Ca concentrations are contrary to the general finding that Al inhibits Ca uptake in non-Al accumulating plants (Rengel, 1998; Naik, et al., 2009; Famoso et al., 2010). The mechanism whereby Al accumulators reverse the inhibitory effect of Al on Ca uptake is currently unknown, but our research suggests that this mechanism operates within as well as between species.

A positive correlation between mean foliar Al and Ca concentrations again emerged among seedlings derived from 18 of the *M. malabathricum* populations when their seedlings were grown in a uniform environment in the presence of Al, and there were, in addition, positive correlations of foliar Al with foliar P, K and Mg concentrations. The association between foliar Al and Ca concentrations for seedlings growing in a uniform nutrient solution across multiple populations suggests that there is an underlying physiological basis to this relationship, which may contribute to the pattern that consistently emerges among populations and species of Al accumulator plants sampled in the wild (Masunaga et al., 1998a, Metali et al., 2014). This physiological mechanism may be linked to a common uptake pathway or allocation rule within plant tissues for these elements. This conclusion is supported by the apparent stimulation of uptake of K, Ca, Mg, Na, Ti, Cr, Fe, Co, Zn, As, Se and Sr in response to Al addition in our experiments on *M. malabathricum*, and stimulation of P, Ca, K and Mg in other experiments on this species (Osaki et al., 1998; Watanabe et al., 2001; 2005; 2008; Metali, 2010) and of P, Fe, K, and Mg in tea, *Camelia sinensis*, which is also an Al accumulator (Carr et al., 2003; Fung et al., 2008; Hajiboland et al., 2013).

Relationship between foliar Al concentrations in wild plants and their progeny

There was no evidence that foliar Al concentrations were correlated between adult wild plants growing in the field and progeny derived from the same populations when grown in a common environment with Al addition. This experiment therefore provides no support for the hypothesis that genetic differentiation among populations contributes to the variation in foliar Al accumulation, which contrasts with local adaptation to differential Zn tolerance among populations of *Thlaspi caeurulescens* (Assunção et al., 2008; Escarre et al., 2000; 2013). However in our research on *M. malabathricum* it is possible that the atypical growing conditions of seedlings in solution culture, including the absence of arbuscular mycorrhizas and other rhizosphere microorganisms, as well as the substantial differences in the maturity of plants sampled from the two settings and the different analytical techniques, obscured the expression of genotypic effects. Further research is required to confirm the absence of a genetic basis for differentiation in foliar Al concentration.

CONCLUSIONS

Differential expression of Al accumulation in *M. malabathricum* populations is uncoupled to local variation in soil Al concentrations, but is sensitive to local soil-related variation in the soil concentrations of the macro-nutrient cations, in particular Ca and Mg. For seedlings grown in a uniform environment, foliar Al concentration is tightly coupled to that of the major nutrient cations as well as numerous other elements, and a positive relationship with foliar Ca concentrations emerges for plants sampled in the wild. These patterns support other research suggesting that Al accumulation may function in enhancing uptake of these limiting elements from soils (Russell et al., 2017).

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TABLE 1. Pearson correlation coefficients comparing mean foliar Al concentration and soil chemical properties for 20 populations of *M. malabathricum* growing in the wild. The significance of these values is indicated as follow: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Soil Variable	Pearson correlation
Total Al	0.049
Total K	0.404
Total N	**0.538***
Total P	0.265
Total Ca	**0.575****
Total Mg	**0.623****
Extractable P	0.276
Exchangeable Al	-0.175
Exchangeable K	-0.098
Exchangeable Ca	**0.645****
Exchangeable Mg	0.132
pH	0.321
PC axes	
Soil PC1	0.432
Soil PC2	-0.087
Soil PC3	-0.085
Soil PC4	-0.260

TABLE 2. Pearson correlation coefficients comparing mean foliar Al and nutrient concentrations of seedlings derived from 18 populations of *M. malabthricum* grown with (values below the diagonal) or without Al addition (above the diagonal) in nutrient solutions. Correlation coefficients along the diagonal compare elemental concentrations between the two treatments. The significance of these values is indicated as follow: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Element	Al	P	K	Ca	Mg
Al	0.244	0.149	0.402	0.156	0.446
P	0.442	0.127	0.398	0.423	0.179
K	**0.667*** **	0.182	**0.739*** **	0.143	0.188
Ca	**0.771*** **	**0.817*** **	0.363	0.127	**0.496***
Mg	**0.759*** **	**0.835*** **	**0.475***	**0.935*** **	0.322

TABLE 3. Mean foliar concentrations (mg g^{-1}) of P, K, Ca and Mg, and F and P values for the Al treatment effect following two way analyses of variance, for *M. malabthricum* seedlings grown hydroponically either with or without addition of 1.0 mM $AlCl_3$ to the nutrient solutions.

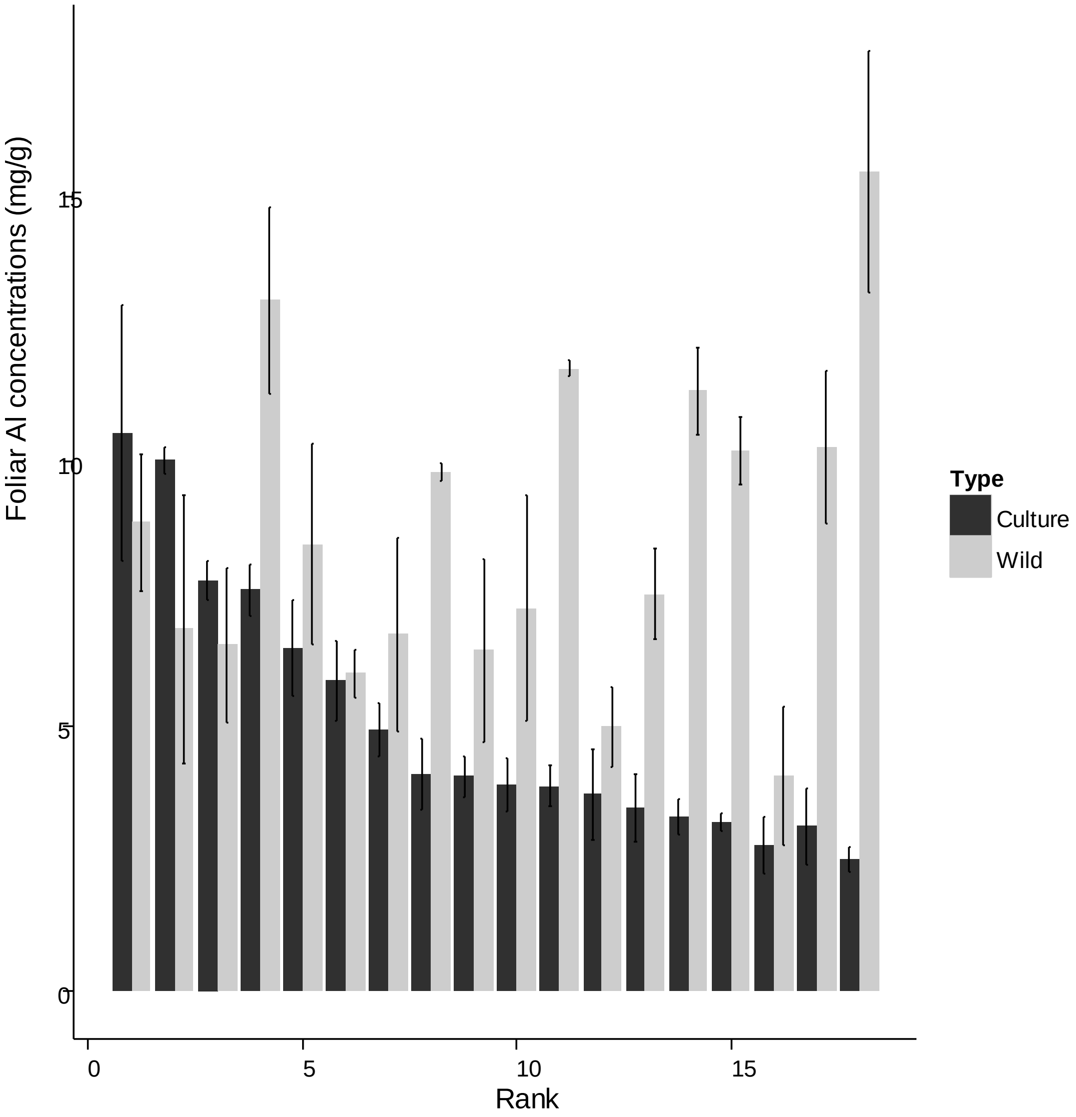
Variable	Treatment	N	Mean(±SE)	F Value	P Value
P	With Al	18	4.92 ± 0.21	3.111	0.002
	Without Al	18	4.58 ± 0.38		
K	With Al	18	11.09 ± 1.58	389.7	<0.001
	Without Al	18	10.57 ± 1.86		
Ca	With Al	18	3.67 ± 0.59	4.501	<0.001
	Without Al	18	3.23 ± 0.98		
Mg	With Al	18	11.65 ± 0.17	4.501	<0.001
	Without Al	18	10.63 ± 0.29		

Fig. 1. Mean (± SEM) foliar Al concentration of *M. malabathricum* seedlings grown in hydroponic solution culture (black bars) and wild plants (grey bars) derived from 18 populations growing across Peninsular Malaysia. The populations are ranked from highest to lowest foliar Al concentrations in the seedling cohorts.

Fig. 2. Biplot of scores for principal component axes (PC) 1 and 2 from principal component analysis (PCA) of 12 top-soil chemistry variables (soil pH and nutrient concentrations) of 20 *M. malabthricum* populations. PC1 and PC2 accounted for 29.3% and 19.0% of the total variation respectively. The arrows show the loadings of each variable on the first two principal component axes.

Fig. 3. Biplot of scores for principal component axes (PC) 1 and 2 from principal component analysis (PCA) of concentrations of 20 elements in leaves of seedlings derived from 18 *M. malabthricum* populations and grown in the presence of Al (1.0 mM Al^{3+}) in nutrient solutions. PC1 and PC2 accounted for 46.7% and 16.5% of the total variation respectively. The arrows show the loadings of each element on the first two principal component axes.

Fig. 4. Biplot of scores for principal component axes (PC) 1 and 2 from principal component analysis (PCA) of concentrations of 20 elements in leaves of seedlings derived from 18 *M. malabthricum* populations and grown without Al (0.0 mM Al^{3+}) in nutrient solutions. PC1 and PC2 accounted for 31.94% and 17.6% of the total variation respectively. The arrows show the loadings of each variable on the first two principal component axes.



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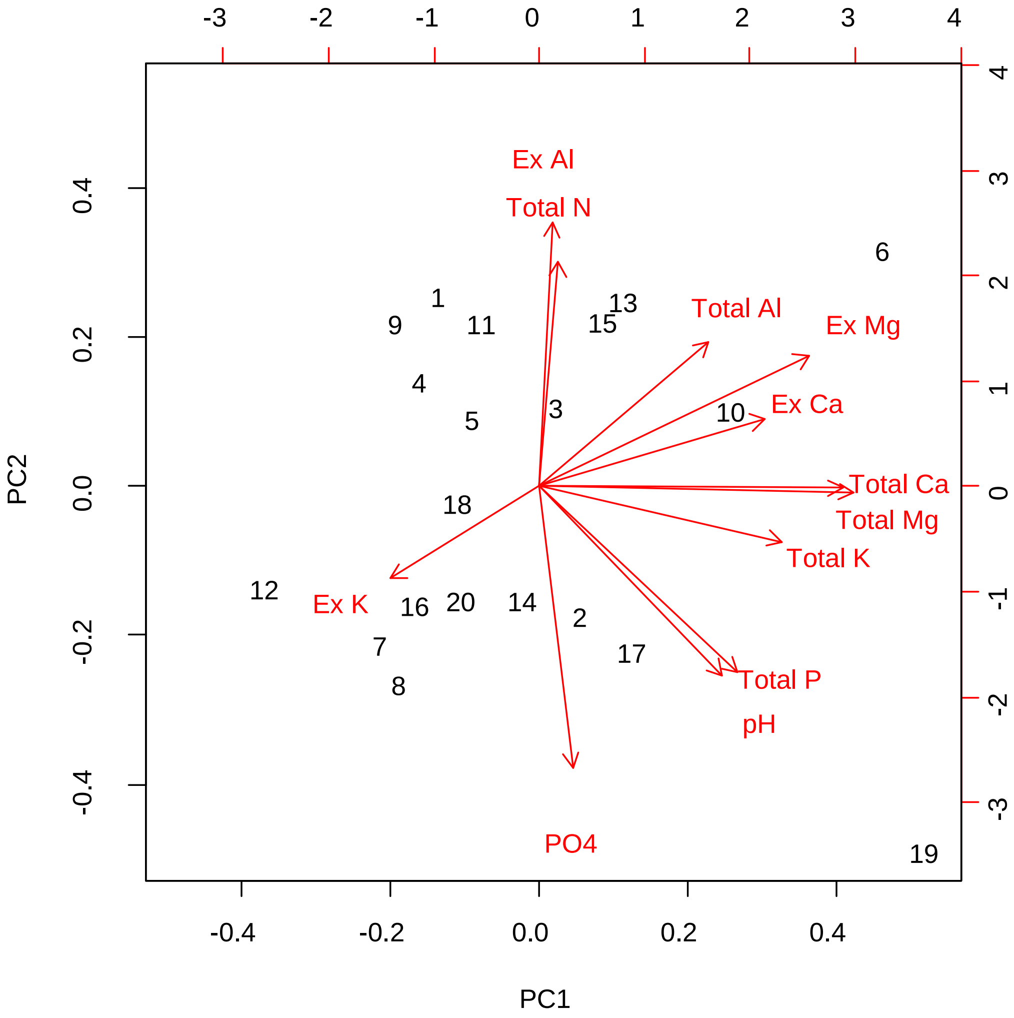

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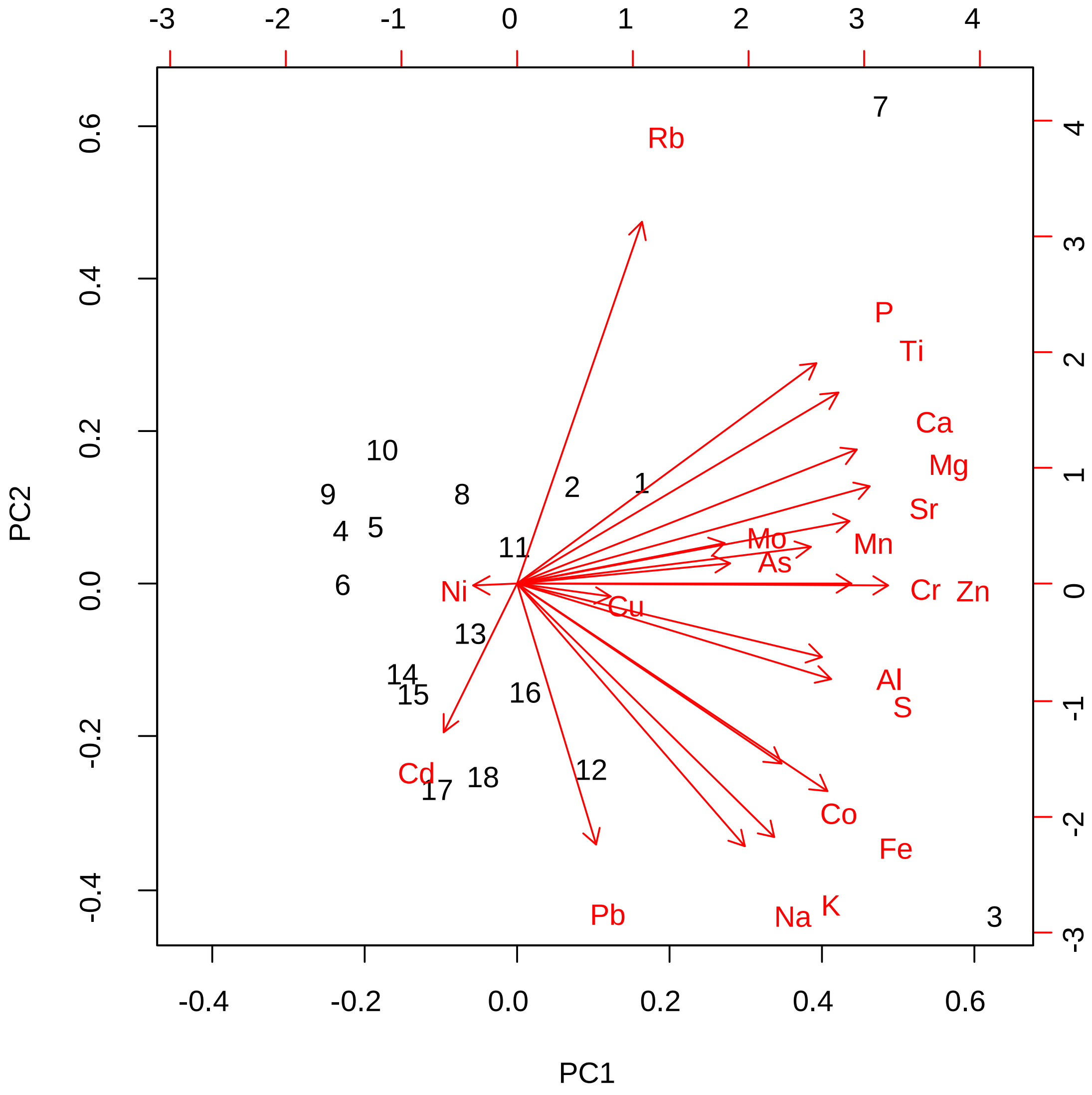

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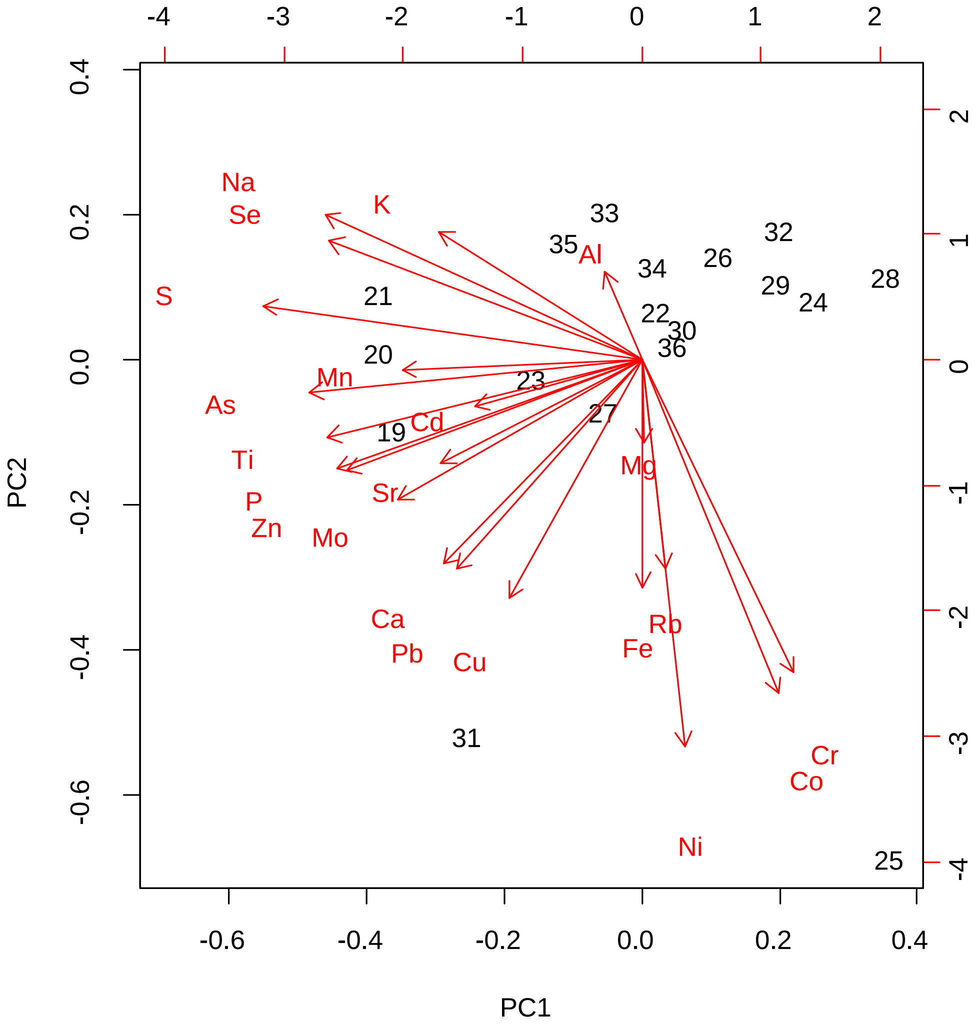

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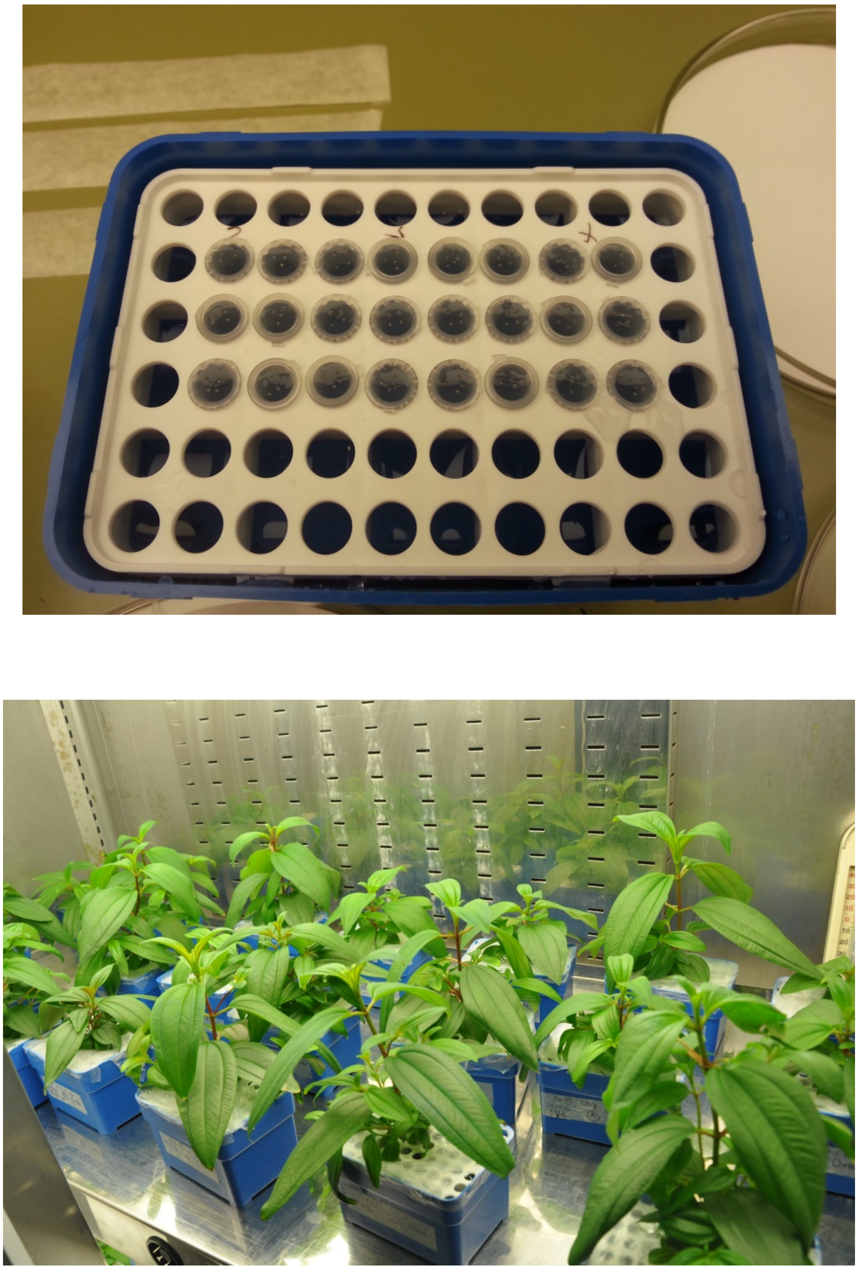

Figure S2: The hydroponic set up for (a) germination of *Melastoma malabathricum* seeds on the surface of Daishin agar in Eppendorf tubes and (b) growth of seedlings to 56 days after sowing.

TABLE S1. The location, habitat type and elevation (El) of the 20 populations of *M. malabathricum* sampled for this study.

Pop	Location	State	Habitat/Forest types	El (m a.s.l.)
1	Bangi Forest Reserve	Selangor	Lowland dipterocarp	70
2	Gombak	Selangor	Lowland dipterocarp	31
3	Genting Highlands	Pahang	Hill dipterocarp	450
4	Chemerong Forest Reserve	Terengganu	Lowland dipterocarp	54
5	Bukit Bauk Forest Reserve	Terengganu	Coastal hill dipterocarp	32
6	Mata Ayer Forest Reserve	Perlis	Lowland dipterocarp	25
7	Perlis National Park	Perlis	Lowland dipterocarp	65
8	Jambu Bongkok Forest Reserve	Terengganu	Heath	4
9	Keluang	Terengganu	Coastal	5
10	Ledang	Johor	Lowland dipterocarp	31
11	Sungai Linggi	Malacca	Riverine	7
12	Muar	Johor	Lowland dipterocarp	12
13	Pasoh	Negeri Sembilan	Lowland dipterocarp	15
14	Rantau	Negeri Sembilan	Riverine	31
15	Seri Kaya	Kelantan	Lowland dipterocarp	15
16	Tapah	Perak	Swamp	58
17	Teratak Batu	Kelantan	Lowland dipterocarp	26
18	Telok Kemang	Negeri Sembilan	Coastal	2
19	Ara Kuda	Penang	Lowland dipterocarp	34
20	Sidim	Kedah	Lowland dipterocarp	42

TABLE S2. Composition of Hoagland's stock solution and quantities required to creates solutions of 10%, 25% and 50% strength.

Nutrients	Stock (g/L)	Stock (M)	Element
Macronutrients			
KNO_3	101.10	1.00	N/K
$Ca(NO_3)_2.4H_2O$	236.15	1.00	Ca
$NH_4H_2PO_4$	115.03	1.00	P
$MgSO_4.7H_2O$	246.47	1.00	Mg/S
$AlCl_3$	133.34	1.00	Al
Micronutrients (1 x)	Stock (g/L)	Stock (mM)	Element
KCl	0.075	1.00	Cl
H_3BO_3	1.546	25.00	B
$MnSO_4.4H_2O$	0.446	2.00	Mn
$ZnSO_4.7H_2O$	0.575	2.00	Zn
$CuSO_4.5H_2O$	0.025	0.10	Cu
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.124	0.10	Mo
Other	Stock (g/L)	Stock (mM)	Element
Fe(Na)EDTA	7.342	20	Fe/Na
Stock solutions needed for 1 L of nutrient solution:			
Strength	10 %	25 %	50 %
Stocks:	ml	ml	ml
KNO_3	0.6	1.5	3.0
$Ca(NO_3)_2.4H_2O$	0.4	1.0	2.0
$NH_4H_2PO_4$	0.2	0.5	1.0
$MgSO_4.7H_2O$	0.1	0.25	0.5
Fe(Na)EDTA	0.2	0.5	1.0
1 X Micronutrients	0.2	0.5	1.0
$AlCl_3$ (1.0 mM Al)	1.0	1.0	1.0

TABLE S3. Mean (± SEM, n =3) foliar concentrations of N, P, K, Ca, Mg and Al (mg g^{-1}) in 20 populations of *M. malabathricum* sampled in Peninsular Malaysia. See Table S1 for the corresponding locations and habitats of the populations. F and P values from analysis of variance report the statistical significance of differences among populations in foliar element concentrations. The significance of these values is indicated as follow: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Pop	Al	P	K	Ca	Mg	N
1	9.8±0.2	1.0±0.1	8.5±0.5	17.1±2.1	1.1±0.2	21.6±1.6
2	15.5±2.3	1.0±0.2	7.5±0.7	20.9±1.7	1.0±0.1	23.1±1.4
3	6.8±2.5	0.9±0.2	5.3±0.4	17.4±4.6	0.7±0.2	18.1±3.4
4	5.0±0.8	0.7±0.1	8.1±1.2	14.6±0.7	1.6±0.2	15.6±0.3
5	7.5±0.8	1.0±0.1	8.1±1.9	18.9±2.7	1.7±0.3	17.0±0.9
6	10.3±1.5	1.1±0.1	7.1±1.3	20.1±2.5	1.7±0.2	18.5±0.4
7	6.0±2.1	0.8±0.0	7.1±0.3	20.4±1.0	1.5±0.3	15.3±1.3
8	6.4±1.7	0.9±0.1	8.5±0.3	15.8±1.0	1.8±0.5	17.7±0.3
9	4.1±1.3	1.4±0.2	10.4±0.3	11.9±0.4	2.0±0.1	19.9±2.2
10	10.2±0.7	0.6±0.1	5.1±2.6	16.2±2.3	1.8±0.3	17.2±1.1
11	7.2±2.1	0.9±0.1	7.7±0.6	12.0±1.9	1.3±0.3	18.0±0.8
12	6.6±1.5	1.1±0.2	6.2±1.1	17.5±2.1	2.0±0.2	16.4±2.3
13	11.3±0.4	1.4±0.1	8.9±1.1	16.4±0.5	1.1±0.1	22.6±1.3
14	11.2±0.8	1.5±0.2	7.7±0.4	19.3±2.1	1.3±0.2	20.9±2.1
15	6.0±0.5	1.5±0.2	5.6±0.6	19.6±1.5	2.7±0.5	25.6±3.8
16	6.8±1.8	1.1±0.2	6.1±0.4	16.3±0.3	0.8±0.1	17.7±1.0
17	8.4±1.9	1.3±0.2	8.8±0.6	18.8±3.6	1.4±0.4	21.9±1.4
18	13.0±1.8	0.7±0.0	6.5±0.3	16.3±1.4	1.5±0.3	16.3±0.8
19	9.6±0.7	0.9±0.1	7.6±1.3	18.8±1.9	1.0±0.1	14.6±1.3
20	6.2±1.0	1.0±0.0	8.3±1.6	19.5±4.3	1.3±0.2	18.4±0.6
F value	**3.86**	**3.92**	**2.23**	1.25	**3.42**	**3.09**
P value	0.000***	0.000***	0.016*	0.269	0.001***	0.001***

TABLE S4. Pearson correlation coefficients testing the significance of associations among mean foliar concentrations of N, P, K, Ca, Mg and Al (mg g^{-1}) for 20 populations of *M. malabathricum* populations sampled in Peninsular Malaysia. The significance of these values is indicated as follow: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

	Al	Ca	K	Mg	N
Ca	0.373*				
K	-0.122	-0.289			
Mg	-0.358	-0.069	-0.040		
N	-0.044	0.128	0.323	0.204	
P	0.264	0.193	0.159	0.150	0.721***

TABLE S5. Mean (± SEM) values of pH, total N, P, K, Ca, Mg and Al (mg g^{-1}) and exchangeable K, Ca, Mg and Al (mg g^{-1}) in top-soil samples collected from 20 sites across Peninsular Malaysia where *M. malabathricum* populations were sampled.

Num	Code	Total Al	Total Ca	Total Mg	Total K	Total P	Total N	pH
1	HSB	9.40±4.1	0.04±0.01	1.8±1.01	1.8±0.11	0.10±0.011	0.81±0.001	4.13±0.11
2	GMB	7.71±2.9	0.23±0.14	1.4±-0.61	1.7±0.50	0.13±0.001	0.84±0.002	5.03±0.31
3	GT	21.1±1.3	0.22±0.13	3.4±0.10	0.8±0.06	0.31±0.001	0.93±0.160	4.70±0.10
4	HLC	13.4±4.2	0.08±0.01	0.5±0.11	1.7±0.60	0.11±0.002	0.75±0.101	4.63±0.10
5	BBK	14.1±0.8	0.22±0.16	1.4±0.50	1.5±0.04	0.10±0.001	0.86±0.03	4.33±0.10
6	HSMA	10.9±0.3	0.23±0.04	6.9±0.21	4.0±0.27	0.20±0.001	1.6±0.10	4.67±0.20
7	HSP	3.50±0.1	0.09±0.01	0.4±0.11	0.5±0.04	0.08±0.001	1.6±0.050	5.49±0.21
8	JB	7.70±1.8	0.08±0.02	1.7±0.30	2.3±0.55	0.23±0.001	0.42±0.501	4.40±0.10
9	K	13.1±4.1	0.12±0.02	0.7±0.31	0.9±0.30	0.20±0.001	2.0±0.180	4.36±0.11
10	L	16.8±1.7	0.35±0.22	2.3±0.30	2.4±0.40	0.17±0.001	0.9±0.040	5.27±0.10
11	SL	11.4±1.5	0.12±0.01	2.3±0.60	1.6±0.40	0.31±0.002	1.4±0.301	4.12±0.30
12	M	4.61±1.2	0.04±0.002	0.81±0.21	2.4±0.14	0.07±0.001	0.3±0.020	3.87±0.20
13	P	18.5±1.8	0.36±0.14	0.86±0.11	1.9±0.08	0.17±0.003	1.4±0.081	4.10±0.05
14	RT	11.8±2.3	0.11±0.01	2.3±1.10	1.8±0.70	0.23±0.001	0.6±0.200	4.75±0.25
15	SK	32.7±1.9	0.22±0.11	2.4±1.26	0.6±0.01	0.2±0.0001	0.6±0.101	4.52±0.04
16	TP	2.4±0.05	0.12±0.02	0.72±0.01	0.8±0.001	0.12±0.002	0.34±0.060	4.76±0.18
17	TB	8.80±0.8	0.20±0.02	4.9±1.01	1.8±0.40	0.1±0.0001	0.6±0.080	5.13±0.09
18	TKM	8.60±0.1	0.09±0.01	1.6±0.12	1.9±0.12	0.14±0.001	0.9±0.201	4.44±0.07
19	AK	18.07±2.2	0.39±0.10	3.95±0.36	4.86±1.00	0.26±0.004	1.85±0.3	5.38±0.09
20	SD	11.14±2.9	0.07±0.02	1.01±0.15	0.94±0.4	0.10±0.001	0.81±0.2	4.34±0.07
F value		**9.467**	**1.983**	**7.164**	**6.925**	**5.89**	**6.034**	**8.475**
P value		0.000***	0.034*	0.000***	0.000***	0.000***	0.000***	0.000*

TABLE S5. (cont)

Num	Code	Exch_Al	Exch_Ca	Exch_Mg	Exch_K	PO_4
1	HSB	0.40±0.12	0.24±0.19	0.020±0.005	0.11±0.020	0.005±0.007
2	GMB	0.13±0.02	0.34±0.13	0.030±0.003	0.11±0.005	0.061±0.007
3	GT	0.25±0.03	0.12±0.03	0.030±0.010	0.17±0.040	0.015±0.004
4	HLC	0.38±0.03	0.04±0.02	0.030±0.006	0.16±0.020	0.020±0.001
5	BBK	0.18±0.02	0.05±0.003	0.020±0.003	0.04±0.008	0.004±0.003
6	HSMA	0.31±0.05	0.25±0.06	0.130±0.005	0.11±0.011	0.010±0.004
7	HSP	0.1±0.001	0.11±0.02	0.020±0.001	0.30±0.220	0.050±0.004
8	JB	0.20±0.04	0.03±0.006	0.013±0.007	0.33±0.280	0.074±0.034
9	K	0.27±0.08	0.04±0.021	0.011±0.003	0.07±0.014	0.021±0.009
10	L	0.26±0.15	0.43±0.03	0.040±0.004	0.10±0.032	0.007±0.004
11	SL	0.31±0.12	0.14±0.03	0.040±0.001	0.20±0.026	0.011±0.001
12	M	0.15±0.003	0.02±0.003	0.010±0.001	0.60±0.560	0.009±0.001
13	P	0.23±0.07	0.45±0.211	0.030±0.007	0.16±0.020	0.014±0.004
14	RT	0.24±0.05	0.16±0.03	0.020±0.003	0.01±0.002	0.081±0.050
15	SK	0.27±0.05	0.15±0.08	0.050±0.020	0.05±0.007	0.021±0.020
16	TP	0.05±0.01	0.20±0.07	0.020±0.004	0.04±0.010	0.009±0.003
17	TB	0.15±0.01	0.21±0.01	0.040±0.002	0.14±0.020	0.067±0.030
18	TKM	0.27±0.04	0.11±0.02	0.030±0.004	0.11±0.008	0.058±0.020
19	AK	0.14±0.02	0.31±0.10	0.020±0.001	0.17±0.010	0.130±0.005
20	SD	0.28±0.02	0.07±0.01	0.010±0.001	0.09±0.010	0.020±0.002
	F value	2.10	2.54	14.68	0.714	2.633
	P value	0.024 *	0.007 *	0.000***	0.783	0.005 **

TABLE S6. Principal components analysis of soil chemical variables among soils associated with 20 populations of *M. malabathricum* sampled in Peninsular Malaysia. The significance of these values is indicated as follow: *, P < 0.05; **, P < 0.01; ***, P < 0.001

	PC1	PC2	PC3	PC4	PC5
Std. Deviation	1.874	1.510	1.246	1.148	1.006
Proportion of Variance	0.297	0.190	0.129	0.109	0.084
Cumulative Proportion	0.297	0.483	0.612	0.722	0.806
Loadings of soil chemical variables on PC axes					
Total Al	0.240	0.254	-0.242	0.526***	-0.128
Total P	0.280*	-0.328**	0.079	0.412***	-0.111
Total Ca	0.445***	-0.010	-0.266	-0.017	-0.377**
Total K	0.344**	-0.097	0.504***	-0.072	-0.175
Total N	0.027	0.395**	-0.080	-0.432***	0.199
Total Mg	0.430***	-0.010	0.311	-0.049	0.224
Extractable P	0.047	-0.495***	-0.017	0.096	0.436***
pH	0.259	-0.331**	-0.295*	-0.261	0.293*
Exchangeable Al	0.019	0.463***	0.220	0.370**	0.257
Exchangeable Ca	0.319**	0.120	-0.290*	-0.285*	-0.312**
Exchangeable Mg	0.382***	0.229	0.289*	-0.177	0.233
Exchangeable K	-0.210	-0.162	0.451***	-0.179	-0.463***

TABLE S7. Mean (± SEM) foliar concentrations (mg g^{-1}) of 20 foliar element concentrations for seedlings grown from 18 populations of *M. malabathricum* grown in solution culture with addition of 1.0 mM Al. Populations are ranked by foliar Al concentration. See Table S1 for linking the codes to the corresponding locations and habitats of these populations. F and P values from analysis of variance report the statistical significance of differences among populations in foliar element concentrations (***, $P < 0.001$).

Rank Al	Pop	Al	P	K	Ca	Mg	Na	S	Ti	Cr	Fe
1	7	10.5±2.8	9.7±3.1	8.2±2.4	26.2±7.5	7.4±2.3	0.08±0.04	7.63±2.14	7.81±2.63	1.94±0.57	70.9±20.8
2	3	10.1±2.4	6.1±0.7	29.5±8.7	17±2.9	5.3±1.1	0.44±0.10	13.74±1.51	6.12±0.49	1.77±0.06	156.8±28.9
3	18	7.8±0.5	2.9±0.2	18.6±4.0	12.2±0.8	3.8±0.2	0.24±0.06	5.9±0.68	1.55±0.15	1.18±0.24	70.14±5.6
4	12	7.7±0.4	4.9±0.3	21.8±2.2	13.2±1.1	4.8±0.4	0.2±0.03	8.88±1.23	3.53±0.26	1.2±0.08	87.06±8.99
5	17	6.5±0.9	4.1±0.5	22.2±1.0	9.1±0.5	3.1±0.2	0.26±0.05	5.44±0.10	2.28±0.18	1.28±0.24	74.21±7.38
6	15	5.8±0.7	3.1±0.6	5.2±0.9	8.2±2.0	2.3±0.5	0.23±0.06	6.1±0.89	2.54±0.5	1.36±0.30	50.06±20.8
7	16	5.1±0.5	4.8±0.4	19.3±0.7	9.4±0.7	3.4±0.3	0.25±0.01	7.83±1.02	3.64±0.4	1.11±0.05	106.25±16.85
8	2	4.5±0.2	5.4±0.6	12.4±4.4	11.4±0.8	3.6±0.3	0.11±0.01	8.94±0.14	4.35±0.50	1.27±0.08	56.32±4.04
9	11	4.2±0.5	5.0±0.6	7.7±2.7	10.2±0.8	3.8±0.6	0.12±0.04	9.86±1.67	3.26±0.32	1.32±0.16	54.21±8.34
10	1	4.1±0.7	7.3±0.9	10.3±2.3	12.8±1.2	4.6±0.5	0.17±0.01	9.14±0.92	5.05±0.45	1.35±0.1	62.6±6.5
11	8	4.1±0.4	6.1±0.6	10.5±5.5	11.4±0.8	3.2±0.2	0.15±0.02	8.41±0.83	3.88±0.51	1.09±0.07	44.98±3.64
12	13	3.8±0.4	4.4±0.6	5.4±0.3	10±1.0	2.7±0.3	0.16±0.03	8.56±1.08	2.83±0.47	1.34±0.06	54.23±7.4
13	4	3.7±0.8	3.3±0.4	5.3±2.2	9.6±0.2	2.5±0.1	0.04±0.03	4.92±1.66	2.82±0.14	0.71±0.06	44.56±12.9
14	5	3.4±0.6	4.9±0.5	8.9±4.8	9.5±0.9	3.0±0.1	0.09±0.02	6.09±0.79	3.66±0.37	0.89±0.1	33.6±3.27
15	14	3.3±0.3	3.3±0.3	4.93±0.4	9.4±0.7	2.5±0.2	0.1±0.04	5.27±0.81	2.57±0.17	1.25±0.07	43.25±4.4
16	10	3.2±0.2	4.9±0.6	2.5±0.3	9.2±0.5	3.7±0.3	0.03±0.01	5.44±0.51	3.48±0.37	1.04±0.09	35.62±2.4
17	6	3.1±0.7	4.4±0.4	3.2±0.2	10±1.0	3.3±0.2	0.04±0.02	3.6±0.30	3.5±0.29	0.82±0.07	31.67±2.05
18	9	2.8±0.5	4.1±0.6	3.6±0.01	11.1±1.4	3.1±0.5	0.03±0.01	4.34±0.71	2.53±0.22	0.84±0.06	36.9±4.1
F value		**16.33**	**3.11**	**10.79**	**4.05**	**3.47**	**6.83**	**4.99**	**3.75**	**2.19**	**6.27**
P value		0.000***	0.001***	0.000***	0.000***	0.002***	0.001***	0.000***	0.000***	0.019*	0.000***

(TABLE S7 Cont).

Rank Al	Pop	Co	Ni	Cu	Zn	As	Rb	Se	Mo	Cd	Pb
1	7	0.02±0.001	2.12±1.4	11.04±3.11	66.47±16.5	0.07±0.02	1.38±0.42	10.11±3.4	6.5±1.69	0.03±0.01	1.03±0.31
2	3	0.06±0.001	0.87±0.18	11.03±1.71	91.17±5.67	0.07±0.01	0.25±0.02	19.73±1.95	9.18±3.07	0.14±0.05	3.25±0.56
3	18	0.02±0.001	1.52±1.08	6.94±0.22	33.80±2.10	0.04±0.01	0.15±0.01	12.49±1.04	5.16±0.42	0.06±0.01	2.39±0.51
4	12	0.01±0.001	0.53±0.12	7.51±0.72	45.33±6.69	0.05±0.01	0.25±0.04	14.92±1.36	6.29±0.83	0.13±0.02	3.19±0.41
5	17	0.02±0.001	0.36±0.12	5.94±1.01	31.03±1.52	0.04±0.01	0.19±0.02	12.75±0.94	2.96±0.53	0.05±0.01	1.62±0.21
6	15	0.02±0.001	1.21±0.45	6.13±1.71	28.61±5.56	0.06±0.01	0.31±0.06	9.41±1.41	3.33±0.63	0.11±0.03	2.04±1.11
7	16	0.03±0.001	0.45±0.11	8.67±0.21	50.29±8.10	0.05±0.01	0.15±0.03	11.81±0.6	4.88±0.72	0.04±0.01	1.07±0.51
8	2	0.02±0.001	0.44±0.04	7.73±0.31	49.26±5.13	0.05±0.01	0.64±0.11	7.81±0.46	13.9±1.10	0.09±0.03	1.04±0.53
9	11	0.01±0.001	0.76±0.16	7.56±1.54	44.34±2.97	0.05±0.01	0.57±0.11	8.09±1.68	5.55±0.84	0.13±0.05	1.51±1.04
10	1	0.02±0.001	0.43±0.07	9.17±1.31	66.58±8.32	0.07±0.01	0.61±0.03	11.28±0.96	9.83±1.93	0.09±0.03	1.27±0.41
11	8	0.02±0.001	1.21±0.86	5.54±0.89	42.58±4.30	0.05±0.01	0.71±0.07	8.06±0.81	4.78±0.78	0.07±0.03	1.03±0.30
12	13	0.03±0.001	1.67±0.18	25.7±17.81	42.38±6.67	0.06±0.01	0.44±0.03	9.68±1.23	5.97±1.51	0.07±0.01	1.51±0.40
13	4	0.01±0.001	4.12±3.16	8.48±2.70	30.81±12.2	0.03±0.01	0.42±0.05	4.33±1.52	4.52±0.69	0.04±0.03	1.63±0.27
14	5	0.01±0.001	0.52±0.25	5.26±0.42	36.45±1.80	0.04±0.01	0.51±0.09	5.72±0.39	4.67±0.97	0.07±0.04	0.82±0.51
15	14	0.02±0.001	4.46±3.21	10.34±1.82	27.46±1.89	0.05±0.01	0.35±0.04	7.39±1.96	6.56±0.94	0.11±0.03	3.13±0.41
16	10	0.01±0.001	2.74±1.21	6.84±1.02	32.20±4.87	0.04±0.01	0.57±0.04	3.55±0.51	5.50±0.54	0.05±0.01	0.93±0.32
17	6	0.01±0.001	0.25±0.06	6.01±0.81	29.59±4.30	0.03±0.01	0.54±0.05	3.81±0.37	4.70±0.73	0.10±0.07	2.33±1.40
18	9	0.01±0.001	0.61±0.23	5.62±0.61	28.73±2.20	0.04±0.01	0.63±0.05	3.3±0.46	4.01±0.83	0.07±0.04	1.63±1.04
F value		**4.75**	1.24	1.43	**5.78**	**2.37**	**5.61**	**8.29**	**5.54**	1.16	1.59
P value		0.000***	0.273	0.164	0.000***	0.01*	0.000***	0.000***	0.000***	0.331	0.105

TABLE S8. Mean (± SEM) foliar concentrations (mg g^{-1}) of Al, P, K, Ca and Mg in seedlings derived from 18 populations *M. malabathricum* and grown in hydroponic solutions and in wild plants sampled from those populations, with values of the Pearson correlation coefficient and degree of significance.

Element	Mean (Hydroponic)	Mean (Wild)	Pearson corr.	P value
Al	5.0 ± 0.51	8.45±0.71	-0.205	0.308
P	4.97±0.38	1.05±0.06	-0.355	0.148
K	11.57±2.18	7.41±0.31	-0.352	0.151
Ca	11.84±1.04	17.2±0.61	0.223	0.373
Mg	3.75±0.30	1.50±0.11	-0.322	0.192

TABLE S9. Principal components analysis of 20 foliar element concentrations in seedlings derived from 18 populations of *M. malabathricum* in Peninsular Malaysia and grown with Al (1.0 mM $AlCl^{3+}$) in nutrient solutions.

Importance of components	PC1	PC2	PC3	PC4
Standard deviation	3.204	1.903	1.613	1.322
Proportion of Variance	0.466	0.164	0.118	0.079
Cumulative Proportion	0.466	0.631	0.749	0.829
Loadings of foliar concentration				
Al	0.272*	-0.073	0.190	0.288*
Ca	0.290**	0.214	0.131	0.191
K	0.272*	-0.335***	0.159	-0.063
Mg	0.292**	0.160	0.177	0.061
P	0.281*	0.300**	0.024	-0.062
Na	0.296**	-0.362***	-0.057	0.026
S	0.277**	-0.116	-0.197	-0.213
Ti	0.246	0.274*	0.061	-0.030
Cr	0.268*	0.026	-0.124	0.063
Fe	0.252*	-0.264*	0.095	0.045
Co	0.249	-0.201	0.063	-0.024
Ni	-0.030	0.001	-0.471***	0.439*
Cu	0.079	-0.008	-0.470***	0.382
Zn	0.296**	0.015	-0.028	-0.105
As	0.175	0.062	-0.376	-0.142
Rb	0.086	0.489***	0.027	0.027
Sr	0.257*	0.126	0.078	0.078
Mo	0.163	0.083	-0.170	-0.170
Cd	-0.052	-0.184	-0.179	-0.179
Pb	0.071	-0.324	0.040	0.040

Correlation between foliar concentrations with PC axes ***,** $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

TABLE S10. Principal components analysis of 20 foliar element concentrations in seedlings derived from 18 populations of *M. malabathricum* in Peninsular Malaysia and grown without Al (0.0 mM $AlCl^{3+}$) in nutrient solutions.

Importance of components	PC1	PC2	PC3	PC4	PC5
Standard deviation	2.710	2.015	1.645	1.563	1.342
Proportion of Variance	0.319	0.176	0.117	0.106	0.078
Cumulative Proportion	0.319	0.496	0.613	0.720	0.798
Loadings of foliar concentration					
Al	-0.033	0.101	0.468***	-0.099	-0.043
Ca	-0.180	-0.237*	0.157	-0.336*	-0.104
K	-0.184	0.148	0.383**	0.211	0.1843
Mg	0.002	-0.096	0.435***	-0.168	-0.354*
P	-0.278*	-0.127	0.057	0.138	-0.089
Na	-0.288**	0.168	0.093	0.201	0.197
S	-0.344***	0.063	-0.058	0.128	-0.077
Ti	-0.286**	-0.090	-0.127	0.175	-0.044
Cr	0.138	-0.365***	0.058	0.285	-0.059
Fe	0.001	-0.265*	0.422**	0.131	-0.005
Co	0.124	-0.388***	0.039	0.272	-0.056
Ni	0.039	-0.449***	-0.088	0.010	0.233
Cu	-0.120	-0.277*	-0.118	-0.258	0.365
Zn	-0.269*	-0.129	0.162	0.081	-0.136
As	-0.302***	-0.038	-0.121	0.083	0.232
Rb	0.021	-0.242	-0.079	0.414***	0.026
Sr	-0.183	-0.120	0.029	-0.345*	0.284
Mo	-0.222	-0.162	-0.168	-0.095	-0.257
Cd	-0.151	-0.053	-0.048	-0.020	-0.251
Pb	-0.168	-0.244	0.0132	-0.3278*	0.073

Correlation between foliar concentrations with PC axes *, $P < 0.05$; **, $P < 0.01$; ***, $P< 0.001$

TABLE S11. Mean square (MS), F Statistics and P values following two way analysis of variance (ANOVA) of 19 foliar concentrations between treatment (0mM and 1.0 mM $AlCl_3$) and population of *M.* seedlings grown hydroponically. The significance of these values is indicated as follow: *, P < 0.05; **, P < 0.01; ***, P < 0.001

	Foliar P				Foliar K			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	6.16	2.422	0.122	17	21.4	21.4	0.462
Treatment	1	7.912	3.111	0.0002***	1	389.7	389.7	0.0001***
Population: Treatment	17	6.092	2.395	0.004**	17	64.9	64.9	0.0782
Residuals	97	2.54			97	40.5	40.5	
	Foliar Ca				Foliar Mg			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	56.07	4.895	0.029*	17	9.893	8.439	0.004**
Treatment	1	51.56	4.501	0.0001***	1	5.287	4.501	<0.001 ***
Population: Treatment	17	40.95	3.575	0.0001***	17	3.065	2.615	0.00163 **
Residuals	97	11.46			97	1.172		
	Foliar Na				Foliar S			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	0.0023	0.484	0.4884	17	18.19	3.97	0.0490 *
Treatment	1	0.0580	11.977	<0.01 ***	1	39.07	8.535	<0.001 ***
Population: Treatment	17	0.010	2.094	0.0128 *	17	10.91	2.384	0.0041 **
Residuals	97	0.004			97	4.58		

(TABLE S11 continue)

			Foliar Ti			Foliar Cr		
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	4.606	2.886	0.0926	17	274.6	1.020	0.315
Treatment	1	6.425	4.025	<0.001 ***	1	245.7	0.913	0.561
Population: Treatment	17	4.207	2.636	0.0015 **	17	229.5	0.852	0.630
Residuals	97	1.596			97	269.2		

	Foliar Mn				Foliar Fe			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	586.5	1.086	0.3000	17	1376	0.890	0.34770
Treatment	1	1880.9	3.482	<0.001 ***	1	3557	2.302	<0.001 **
Population: Treatment	17	984.4	1.823	0.0356 *	17	1861	1.204	0.27598
Residuals	97	540.1			97	1545		

	Foliar Co				Foliar Ni			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	0.1911	1.156	0.285	17	50.75	0.451	0.5032
Treatment	1	0.1447	0.875	0.604	1	176.14	1.567	0.0884
Population: Treatment	17	0.1433	0.867	0.614	17	56.88	0.506	0.9444
Residuals	97	0.1653			97	112.42		

(TABLE S11 continue)

	Foliar Cu				Foliar Zn			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	154.34	1.674	0.1988	17	1235.3	5.759	0.0183 *
Treatment	1	257.43	2.791	0.0079 ***	1	1007.0	4.695	<0.001 ***
Population: Treatment	17	31.19	0.338	0.993	17	431.5	2.012	0.0175 *
Residuals	97	92.23			97	214.5		

	Foliar As				Foliar Se			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	0.0005	0.282	0.597	17	7.30	1.012	0.3169
Treatment	1	0.00136	6.435	<0.001 ***	1	102.94	14.267	<0.001 ***
Population: Treatment	17	0.00041	1.952	0.022 *	17	17.19	2.383	0.00411 **
Residuals	97	0.0002			97	7.21		

	Foliar Rb				Foliar Sr			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	3.309	109.503	< 0.001 ***	17	3.896	22.386	<0.001 ***
Treatment	1	0.194	6.423	<0.001 ***	1	0.657	3.777	<0.001***
Population: Treatment	17	0.136	4.488	<0.001 ***	17	0.492	2.830	<0.001 ***
Residuals	97	0.030			97	0.174		

(TABLE S11 continue)

	Foliar Mo				Foliar Cd			
Factors	df	MS	F Value	P Value	df	MS	F Value	P Value
Population	17	765.2	60.828	<0.001 ***	17	0.471	1.851	0.176
Treatment	1	95.5	7.594	<0.001 ***	1	0.471	1.849	0.032 *
Population: Treatment	17	17.1	1.360	0.174	17	0.458	1.799	0.038 *
Residuals	97	12.6			97	0.254		

	Foliar Pb			
Factors	df	MS	F Value	P Value
Population	17	119.46	1.52	0.219
Treatment	1	136.29	1.74	0.048 *
Population: Treatment	17	143.57	1.83	0.034 *
Residuals	97	78.35		