Universitat Autònoma de Barcelona

Local adaptation of wild populations of Arabidopsis thaliana to coastal and inland habitats in Catalonia

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Local adaptation of wild populations of *Arabidopsis thaliana* to coastal and inland habitats in Catalonia

A dissertation submitted by **Sílvia Busoms González**

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Single thesis jointly supervised by

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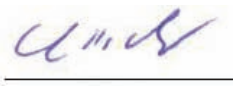

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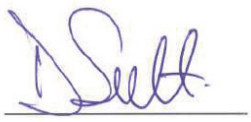

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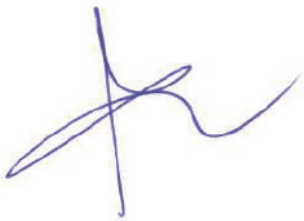

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"It is not the strongest of the species that survives,

nor the most intelligent that survives.

It is the one that is most adaptable to change"

Charles Darwin, 1809

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Abstract

The natural genetic variation among *A. thaliana* populations in Catalonia was used to identify local adaptation to coastal and inland habitats. A Species Distribution Model (SMD) was created to locate multiple small stands of *A. thaliana* (demes). Results using 425 genome-wide SNP markers under clustering and population analysis indicate a high percentage of shared alleles among demes. Multi-year field-based reciprocal transplant experiments were designed to identify fitness trade-offs between inland and coastal demes. Progenies from these demes performed better in their local/home environments. Similar results were obtained in greenhouse common garden experiments, confirming that soil is a driving factor for local adaptation. Plants from the coastal habitat outperformed those from inland when grown together under high salinity. It is concluded that *A. thaliana* is locally adapted to coastal environments, and this adaptation is driven, at least in part, by the elevated salinity of coastal soils.

Our results do not point to a single mechanism of salinity tolerance. *AtSOS1* and *AtHKT1;1* may cooperate increasing leaf Na^+ and its vacuolar storage achieving better osmotic adjustment. Crossings between coastal (salt tolerant) and inland (salt sensitive) plants suggest maternally inheritance of salt tolerance. Polymorphisms in both *AtHKT1;1* and *AtMOT1* may be of adaptive significance because the weak alleles were only detected in coastal demes. However, all results indicate that genetic variability in *AtHKT1;1* allele is not responsible for the salinity tolerance. We conclude that the weak allele of *AtHKT1;1* persists and coexists with plants bearing the strong allele thanks to early flowering and better tolerance of moderate salinity. Moreover, the weak allele of *AtMOT1* was more frequent and was detected nearer to the sea than the weak allele of *AtHKT1;1*. Results with *mot1* knockout mutants under NaCl treatments indicate that loss of function of *AtMOT1* may enhance tolerance to salt stress.

Resum

La variació genètica natural existent entre poblacions d' *Arabidopsis thaliana* a Catalunya s'ha utilitzat per identificar fenòmens d'adaptació local a hàbitats costaners i continentals. Es va crear un Model de Distribució d'Espècies (SMD) per localitzar petits rodals de poblacions biològiques d' *A. thaliana* (que anomenem 'demes'). Els resultats obtinguts de la utilització de 425 marcadors (SNP) sotmesos a anàlisis d'estructura de la població i d'agrupament indiquen un alt percentatge d'al·lels compartits entre 'demes'. Durant dos anys consecutius es va repetir un experiment de trasplantament recíproc en condicions naturals dissenyat per mesurar l'aptitud de les plantes procedents de 'demes' continentals i costaneres als dos tipus d'hàbitats. La progènie d'aquestes 'demes' va mostrar una millor aptitud quan creixia en els seu hàbitat d'origen. En experiments realitzats amb sòl procedent de la costa i de l'interior sota condicions controlades en cambra de cultiu es van obtenir uns resultats similars, confirmant-se que el tipus de sòl és un factor determinant per l'adaptació local. A més, les plantes procedents de 'demes' costaneres van mostrar una millor aptitud i tolerància sota condicions d'elevada salinitat. Es conclou que *A. thaliana* està adaptada als ambients costaners i aquesta adaptació és impulsada en gran part per l'elevada salinitat dels sòls costaners.

Tots els resultats apunten que les plantes costaneres utilitzen més d'un mecanisme per combatre els alts nivells de Na al sòl. És probable que *AtSOS1* i *AtHKT1;1* treballin conjuntament per traslladar el Na a les fulles i emmagatzemar-lo dins les vacuoles per aconseguir un millor ajust osmòtic. D'altra banda, creuaments entre plantes costaneres (tolerants a la sal) i plantes de l'interior (sensibles a la sal) apunten a una possible herència materna del caràcter "tolerància a la salinitat". Els polimorfismes detectats tant en *AtHKT1;1* com en *AtMOT1* poden tenir un significat adaptatiu ja que els al·lels febles d'ambdós només es van detectar en 'demes' costaneres. No obstant, tots els resultats indiquen que la variabilitat genètica d' *AtHKT1;1* no és responsable de la tolerància a la salinitat observada. Hem arribat a la conclusió que l'al·lel feble d' *AtHKT1;1* persisteix i conviu amb plantes que tenen l'al·lel fort gràcies a una floració primerenca i a una millor tolerància a la salinitat moderada. D'altra banda, l'al·lel feble d' *AtMOT1* és més freqüent i es va detectar en zones més properes al mar que l'al·lel feble d' *AtHKT1;1*. Els resultats d'estudis de salinitat amb mutants noquejats d' *AtMOT1* indiquen que la pèrdua de funció d' *AtMOT1* podria augmentar la tolerància a l'estrès salí.

Abbreviations

- **ACDC:** Atles Climàtic de Catalunya
- **ANOVA**: Analysis of variance
- **BLA:** Blanes
- **Chr:** Chromosome
- **dCAPS:** derived Cleaved Amplified Polymorphic Sequence
- **DNA**: Deoxyribonucleic acid
- $\mathbf{F_{ST}}$:Fixation index
- **Gencat**: Generalitat de Catalunya
- **GFP:** Green Fluorescent Protein
- **GIS**: Geographic information system
- **GLMM**: Poisson log-normal generalised linear mixed effects model
- **HSD**: Honest Significant Difference
- **ICC**: Institut Cartogràfic de Catalunya
- **ICP-MS**: Inductively Coupled Plasma Mass Spectrometry
- **LMM**: Linear Mixed effects Model
- **LSD:** Least Significant Difference
- **MCMC**: Markov Chain Monte Carlo
- **Moco:** Molybdenum cofactor
- **PCR**: Polymerase Chain Reaction
- **PVC**: Polyvinyl Chloride
- **qRT-PCR:** real-time Reverse Transcription Polymerase Chain Reaction
- **QTL**: Quantitative Trait Locus
- **SCF:** Santa Coloma de Farners
- **SDM:** Species Distribution Model
- **SNP:** Single Nucleotide Polymorphism
- **SSR:** Simple Sequence Repeat
- **RC:** Reducing Capacity
- **RIL**: Recombinant Inbred Line
- **RNA**: Ribonucleic acid
- **UTM:** Universal Transverse Mercator
- **WHC:** Water Holding Capacity

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1. Introduction

1. Introduction

1.1. General information about *Arabidopsis thaliana*

1.1.1. Taxonomic status

Arabidopsis thaliana (L.) Heyhn (2n = 10) is a small annual weed in the mustard family (Brassicaceae, formerly Cruciferae). Supported by molecular analyses, O'Kane & Al-Shehbaz (1997) revised the taxonomy of the genus *Arabidopsis* and assigned nine species and eight subspecies that can be distinguished by morphological characteristics such as fruit and seed shape.

Figure 1: Picture of *Arabidopsis thaliana* (L.) Heyhn

1.1.2. Geographic distribution, habitat and ecology

Arabidopsis thaliana is native to Europe and central Asia and is now naturalized in many places elsewhere in the world (O'Kaneand & Al-Shehbaz, 1997). Hoffmann (2002) described the biogeography of the species in detail and showed that too-low spring and autumn temperatures, and high temperatures with low precipitation in summer, limit its distribution range. *A. thaliana* has a wider climatic amplitude than other well-investigated species of the Brassicaceae, and it has an impressive latitudinal range from 68º N (North Scandinavia) to 0º (mountains of Tanzania and Kenya), which makes it suitable for analysing variation in adaptive traits.

Figure 2: Map of *A. thaliana* worldwide distribution.

Image credit: Ute Krämer and Klaus Hagemann (2015)

Arabidopsis thaliana occupies open or disturbed habitats often on sandy or loamy soils such as riverbanks, roadsides, rocky slopes, wasteland, and cultivated grounds. It has been found from sea level up to 4250 m (O'Kaneand & Al-Shehbaz, 2002). Its life history and occurrence in disturbed habitats indicate that the species is vulnerable to rapid colonization and extinction cycles and is a poor competitor in dense vegetation.

The ecology of *A. thaliana* was reviewed by Pigliucci (2002). This emphasized that flowering time and seed dormancy are key traits that determine the timing and length of the *A. thaliana* natural life cycle. In general, most accessions (understanding 'accession' as a unique identifier in a collection from a particular location (Alonso-Blanco & Koornneef, 2000)) from Northern Europe are late flowering and typical winter annuals; southern populations are both summer and winter annuals (Johanson *et al.*, 2000). In Western Europe, both early and late flowering types are found among the progeny of seeds harvested in early summer (Le Corre *et al.*,2002).

1.1.3. *Arabidopsis thaliana* as the model system

Arabidopsis thaliana has been adopted as a major model or reference plant especially suitable for genetic and molecular research. This has led to the establishment of a large research community with important biological and molecular resources available (Meinke *et al.*, 1998). The presence of the complete genomic sequence (The Arabidopsis Genome Initiative, 2000) and a huge collection of gene disruptions provide a research resource that is unique for higher plants (http://www.arabidopsis.org).

Arabidopsis thaliana shows a wide range of genetic and trait variations among wild collected accessions (Shindo *et al.*, 2007). To understand the significance of natural genetic variation in functional terms, it is necessary to both identify the traits of ecological relevance and determine their genetic basis. To achieve this, it is critical to first identify adapted populations in a plant species amenable to the rapid molecular genetic dissection of phenotype. With its excellent genomic tools, small genome size, and extensive collections of native populations along with the general availability of high-throughput whole-genome resequencing, *A. thaliana* is a tempting species to use for such studies (Bergelson & Roux, 2010). The use of natural genetic variation in *A. thaliana* has already proved to be a very powerful approach for the discovery of novel genes and alleles (reviewed in Alonso-Blanco *et al.*, 2009).

1.2. Identification and characterization of wild populations of plants

Identifying where species occur in the landscape is of primary importance for many conservation issues (Margules & Pressey, 2000). Unfortunately, information on geographic distribution is often incomplete or biased and such resources are generally unavailable or depend on trade-offs between allocation for prospective fieldwork or for direct protection (Le Lay *et al.*, 2010). Moreover, knowledge about a given species distribution is often limited by expert availability or is based due to geographically restricted information (Murray *et al.*, 2009). In this context, tools that help to quickly and efficiently find a given species in the field are highly valuable.

1.2.1. Species Distribution Modelling

Species Distribution Modelling (SDM) is a technique to interpolate biological survey data in space. From a conservation viewpoint, habitat modelling has become a very important and useful analytical tool for predicting species occurrences at the landscape level across unsampled areas (Pinzón & Spence, 2013). Predictive models are based on species presence at geo-referenced localities and environmental variables within an area of interest. Species data can be simple presence, presence–absence or abundance observations based on random or stratified field sampling, or observations obtained opportunistically, such as those in natural history collections. In order to achieve model prediction improvements, one possibility could be the use of readily available occurrence data, such as found in public or museum databases.

Environmental predictors can exert direct or indirect effects on species, arranged along a gradient from proximal to distal predictors (Austin, 2002), and are optimally chosen to reflect the three main types of influences on the species (modified from Guisan & Zimmermann, 2000; Huston, 2002): (i) limiting factors (or regulators), defined as factors controlling species eco-physiology (e.g. temperature, water, soil composition); (ii) disturbances, defined as all types of perturbations affecting environmental systems (natural or human-induced) and (iii) resources, defined as all compounds that can be assimilated by organisms (e.g. energy and water).

Nowadays, the quantification of the species–environment relationships represents the core of predictive geographical modelling in ecology. The models are generally based on various hypotheses as to how environmental factors control the distribution of species. These

approaches combine occurrence data with ecological/environmental variables (both biotic and abiotic) to create a model of the species requirements based on examined variables that are available for further extrapolation. The resulting model is then projected onto a map of the study region, showing the potential geographic distribution of the species (Peterson & Vieglais, 2001; Jones *et al.*, 2002).

Climate is often assumed to be the most determining ecological factor in plant species distribution, and it is widely accepted that its importance increases as the spatial resolution of occurrence data decreases (Thuiller *et al.*, 2004), consequently spatial distribution of plant species is often studied with regard to climatic factors alone (Coudun *et al.*, 2006). But in small scale studies where climatic differences are almost imperceptible, other limiting factors such as soil composition must be considered.

1.2.2. *Arabidopsis thaliana* population dynamics and trends

It is essential to have a detailed knowledge of the population genetic patterns of natural populations to properly design ecological studies. *Arabidopsis thaliana* exhibits a range-wide pattern of isolation by distance, which can also be evident at a regional scale. Even neighbouring stands of *A. thaliana* are often strongly differentiated, suggesting low inter-population migration rates and limited dispersal distances. Several studies have uncovered considerable variability among stands in genetic diversity and/or heterozygosity (Bomblies *et al.*, 2010). Picó *et al.* (2008) demonstrated that Iberian genetic diversity was geographically structured but inference of four spatially separated Iberian genetic clusters indicates a complex regional population dynamics.

A strong population structure exists both across the native range of *A. thaliana* (Beck *et al.*, 2008; Schmid *et al.*, 2006; Nordborg *et al.*, 2005; Platt *et al.*, 2010) and also regionally (Brennan *et al.*, 2014). Importantly, this structure has in large part remained undisturbed by human-induced long-distance migration, with human disturbance being swamped out by natural processes (Platt *et al.*, 2010). Such population structure supports the idea that even though *A. thaliana* is known as a human commensal that occupies disturbed habitats (Pigliucci, 2002), migration has not been sufficient to dilute local genotypes. This existing population structure, taken together with the extensive geographic range occupied by *A. thaliana* (Hoffmann, 2002), suggests that *A. thaliana* may have evolved populations locally adapted to

the prevailing local environmental condition, a common feature of plant populations (Leimu & Fischer, 2008).

1.2.3. *Arabidopsis thaliana* zonation. Description of Catalan coastal and inland habitats

The characteristic spatial distribution of plant species or zonation in sea-affected habitats was first considered as initial stages of succession (Odum, 1969; Doing, 1985). Another approach tends to analyse zonation from the point of putative effective environmental factors. Recent studies suggest that coastal zonation cannot be explained only on the basis of soil salinity. Nutrient imbalance or even toxicity of several ions should also be considered (Silvestri *et al.*, 2005).

It becomes clear from the above that the nature of the relationships in coastal or inland vegetation cannot be understood simply by means of statistical data analysis based on temporal and spatial distribution of individuals. The causal character of phenomena in the sequence: environmental factors » adaptive plant features » endogenous control mechanism needs to be determined. While relatively good information is available on the environmental factors, what is lacking here is the knowledge on the processes lying between the perception of a certain signal and the resulting putative adaptive feature. The need for this type of knowledge clearly shifts plant conservation biology from ecological studies towards physiological investigations (Ievinsh, 2006).

Catalonia is located along the north-eastern Spanish Mediterranean coast. The Catalonian coastline is 826.5 km long. It is composed of a large variability of coastal systems, from large flat areas with long sandy beaches to abrupt rocky shores with high cliffs. This coastal structure yields a highly diverse geomorphology associated with a highly diverse biological community. At least four attributes make the Catalan coastal areas different, and which largely determine their ecosystems: specific climate, geology, geography and the long term effects of humans.

Catalonia's coastal climate is characterized by dry and mild winters, rainy springs and autumns with some months of excess rainfall over evapotranspiration and warm and dry summers with moisture deficits that cause the drying out of soils and their annual vegetation (Xeric moisture regime). On Catalan coastal soils, sand grains are the most abundant size fraction (generally higher than 65%) and Cl^-, SO_4^{2-}, Na^+ and Mg^{2+} are the most abundant ions.

Arabidopsis thaliana was found only in the northern half of the Catalan coast in two specific areas: (1) Cap de Creus and the Gulf of Roses and (2) the Serra de Marina.

The peninsula of Cap de Creus is a steep and rocky hill, 672 meters high, which stands on the Mediterranean, forming a small mountainous peninsula. From a geological and morphological point of view, it is part of the foothills of the eastern Pyrenees, sinking into the sea by the massif of Cap de Creus on granitic terrains with laminar structure from the Ordovician (Paleozoic). The climate type is Mediterranean Maritime with a long period of summer drought. The average annual precipitation of this region is 500-600 mm and the average annual temperature varies between 15-17 ºC. It has a dominant vegetation and shrub thickets (*Quercus coccifera, Cistus ladanifer, Lavandula angustifolia*).

The Serra de Marina is set in the coastal mountains of the Selva Marítima, with a maximum elevation of 423 meters. The Catalan Coastal Range includes a range of hills not very high but they constitute a real barrier between the coast and the inland plain. The geomorphology of the area is characterized by large masses of granitic rocks. These granites, under certain conditions of erosion, are transformed into a sandy texture altered rock known by the name of "sauló". The average annual precipitation of this region is 550-700 mm and the average annual temperature varies between 14-16 ºC. The vegetation of these mountains is Mediterranean scrub (*Cistus sp., Erica sp.*) with oaks (*Quercus suber*) and pines (*P. halepensis, P. pinea*) (Gordi *et al.*, 1993).

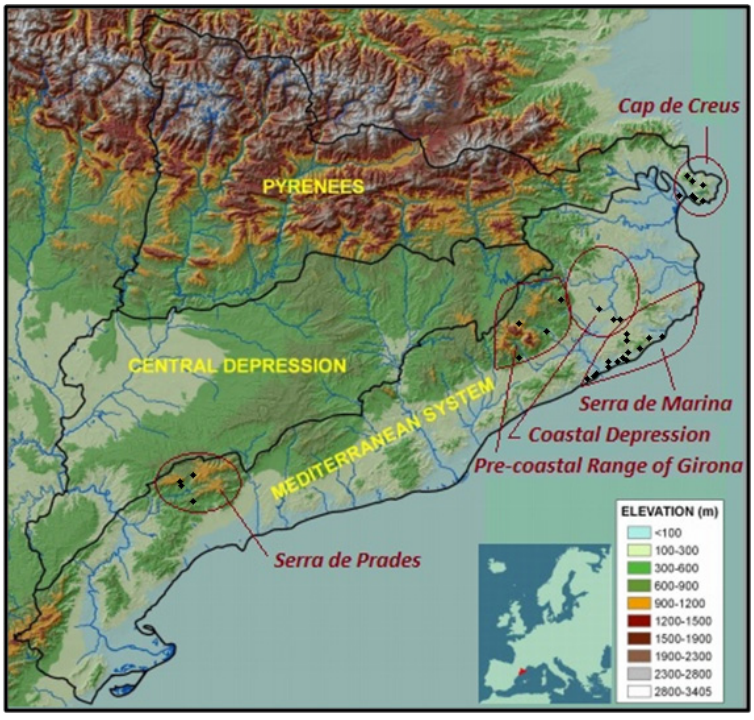


Figure 3: Elevation map of Catalonia with the three geo-climatic regions written in yellow and the outlined areas where *A. thaliana* was localized marked in garnet.

In both regions there have been periodic forest fires and reforestations. Traditionally, flat areas, lower reaches and deltas of rivers, as well as low mountains comprised a territory whose mild Mediterranean climate, diversity of natural environments, and agriculture and industrial activities encouraged its occupation by people. Besides the municipalities located on the coast, the slopes of these mountains are covered with a multitude of urban developments, especially those facing the sea, losing their original appearance and causing degradation and a high risk of disappearance of natural habitats (Sardá *et al.*, 2005).

The systems that characterize adjacent inland areas where *A. thaliana* was localized in Catalonia are the Catalan Coastal Depression and the Catalan Pre-Coastal Range.

The Catalan Coastal Depression is a natural depression between the Catalan Pre-Coastal Range and the Catalan Coastal Range which follows the Mediterranean Sea. The average annual precipitation of this region is 550-750 mm and the average annual temperature varies between 14.5- 16 ºC. The geological basement is formed by granitic batholiths and paleozoic metamorphic rocks. Much of the surface of the Catalan Coastal Depression is subject to severe land degradation, owing mainly to urban sprawl.

The Catalan Pre-Coastal Range is a system of mountain ranges running parallel to the Mediterranean Sea coast but at a distance of 30 to 60 km from the sea. The highest point is 1.706,7 m. Within the Pre-Coastal Range we can differentiate: the northern part, located in the province of Girona, with an average annual precipitation of 750 to 1.000 mm and an average annual temperature of 14-15 °C; and the southern part, constituted by the "Serra de Prades", with lower annual precipitation and temperature (600-800 mm; 12-14 °C). The geological basement is formed by plutonic rocks, emplaced in intrusive bodies between metamorphic rocks, mainly granodiorites.

Although the Mediterranean character of the two regions, with rainfall maxima in autumn, the contrasts in altitude and exposition (slope and orientation) form a complex mosaic of microclimates. The influence of the Mediterranean Sea is evident but this influence is less clear further inland. The ranges isolate the inland from the maritime influence of climate and give continental climatic characteristics to this region. In addition, major rivers have carved orthogonal valleys in the alignments of the relief. This has generated a sharp compartmentalization of the topography in depressions or valleys bound by mountains that have involved a large differentiation of the environmental variables (Arnau *et al.* 2004).

In the Pre-Coastal system, on siliceous or limestone without carbonate substrates, the areas below 700 meters are represented by Mediterranean plant associations with central-Europeans vegetal formations: Downy and African oak forests, hazelnut trees, grasslands and meadows. On granitic land with an oligotrophic nature (soil with few nutrients) the Cork (*Quercus suber*) is the dominant forest. In this area, natural populations of pine are usual (*Pinus pinea*) and also others from reforestation (*Pinus pinaster, Eucalyptus sp.*). In the lowest places, often flooded, there are reedbeds, meadows and unproductive grasslands. In the areas between 700 and 1200 meters there are Holm oak forests (*Quercus ilex*), poor in Mediterranean thermophilic species (absence of *Rhamnus alaternus*) but rich in herbaceous layer with numerous accompaniment trees and shrubs (*Pinus sylvestris, Sorbus aria, Erica arborea*) (Vilar *et al.*, 1992). Notably, in the "Serra de Prades" there are deteriorated masses of Pyrenean oak (*Quercus pyrenaica*) that have been colonized by the Scotch pine.

1.3. Local adaptation of *A. thaliana* to diverse native habitats

The analysis of natural variation in wild species has begun to elucidate the molecular bases of phenotypic differences related to plant adaptation to distinct natural environments and to determine the ecological and evolutionary processes that maintain this variation (Mitchell-Olds *et al.*, 2007). The model plant *A. thaliana* shows a wide range of genetic and trait variation among accessions collected in the field. In addition, because of the unparalleled availability of genomic resources, the potential of *A. thaliana* for studies of natural genetic variation is increasingly recognized (Buescher *et al.,* 2010; Weigel, 2012).

1.3.1. Genetic basis of local adaptation

When different *A. thaliana* accessions are grown together and compared under similar environmental conditions, genetic variation can be observed for many traits. To understand the significance of natural genetic variation in functional terms it is necessary to identify the traits of ecological relevance and determine the genetic basis of these traits. Furthermore, such an understanding would provide significant benefits to efforts directed to developing crop varieties that can maintain yields against a backdrop of changing global temperature and precipitation patterns (for review see Friesen & Wettberg, 2010).

Correlations of life history traits with climate (Montesinos *et al.*, 2009; Suter *et al.*, 2014; Wolfe and Tonsor, 2014; Debieu *et al.*, 2012), edaphic conditions and interspecific competition (Brachi *et al.*, 2013) in natural populations of *A. thaliana* suggest that these are strong selective agents driving adaption in local populations.

The differentiation of specific alleles in *A. thaliana* across environmental gradients for temperature-dependent vernalization (Li *et al.*, 2014), seed dormancy (Kronholm *et al.*, 2012), drought-induced proline accumulation (Kesari *et al.*, 2012), sodium accumulation associated with distance to the coast (Baxter *et al.*, 2010), and molybdenum accumulation associated with soil molybdenum (Poormohammad *et al.*, 2012) is starting to suggest specific alleles involved in adaptation. Further, studies *in silico* and in common garden field experiments have identified evidence for local adaptation on a genome-wide scale in *A. thaliana* (Hancock *et al.*, 2011; Fournier-Level *et al.*, 2011; Huber *et al.*, 2014; Lasky *et al.*, 2014). However, the final proof of the adaptive role of a given allelic variant will require testing the fitness effects of alternative alleles of the gene in the same genetic background in the field in the contrasting environments in which the alleles are hypothesised to be adaptive.

1.3.2.Quantification of local adaptation by reciprocal transplants

As a first step towards such proof reciprocal transplant experiments are usually performed to test if the two populations containing the contrasting alleles are locally adapted (Blanquart *et al.*, 2013). Conventionally, local adaptation is considered to exist when demes (local populations or small stands of plants) have higher fitness in their own habitat compared to demes from any other habitat, and this has been termed the 'local vs. foreign' criterion (Kawecki & Ebert, 2004). Ideally, to establish such local adaptation experimentally requires reciprocal transplant experiments in the field in which the fitness of genotypes from different demes are all directly compared by growing them together in each of the demes local habitats.

An approach to estimating local adaptation in a metapopulation (in our case coastal and inland metapopulations) is to calculate the difference between the average fitness of demes on their home sites (in sympatry) and fitness of demes when transplanted to other sites (in allopatry). This 'sympatric vs. allopatric' contrast has the appealing property of quantifying the extent to which the genotypic composition of individual populations matches their local environmental conditions (Blanquart *et al.*, 2013).

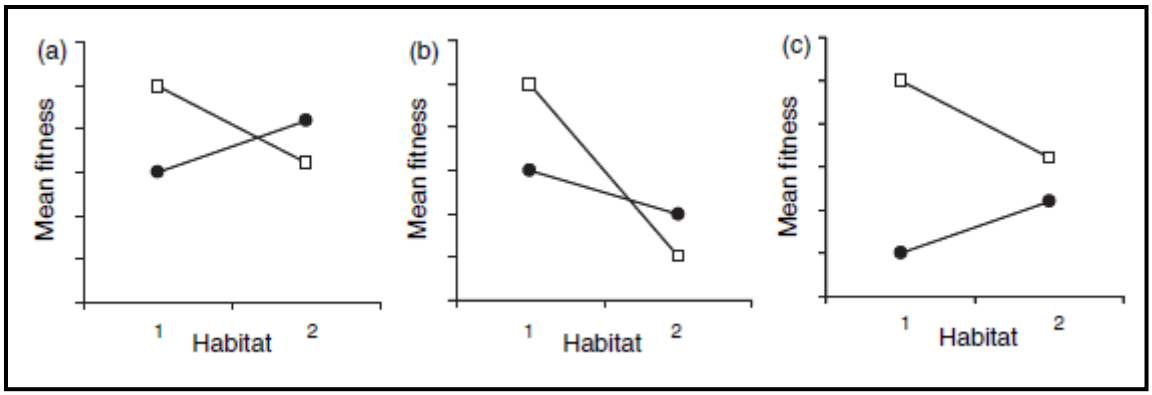


Figure 4: Hypothetical patterns of deme x habitat interaction for fitness. Squares: the average of demes originating from habitat 1; circles: the average of demes originating from habitat 2. The patterns in panels (a) and (b) satisfy the 'local vs. foreign' criterion. The patterns in panels (a) and (c) satisfy 'sympatric vs. allopatric' criterion (Kawecki & Ebert, 2004).

Such transplant experiments can be performed using demes taken from populations where gene flow is occurring between demes, or using isolated demes with no gene flow between demes. When demes within a population exchange genes the development of local adaptation is a balance between spatially varying selection and migration (Savolainen *et al.*, 2013). Under these conditions local adaptation if it evolves must be due to ongoing (or very recent) selection driven by differences in the demes habitats. In the absence of such selection any genetic differentiation would be lost by gene flow (Kawecki & Ebert, 2004). Therefore, under these conditions it should be possible to identify the agent of divergent selection giving rise to the local adaptation.

The adaptation against a backdrop of gene flow is thought to favour the evolution of fewer adaptive, more linked loci with larger effect size (Yeaman & Whitlock, 2011). Further, fitness trade-offs are likely to be due to trade-offs between individual alleles (Savolainen *et al.*, 2013). Such considerations suggest that adaptive traits between populations with gene flow may therefore be more tractable to genetic dissection. In contrast, isolated populations are expected to adapt independently to their environment, and trade-offs at the fitness level between populations may be due to different loci that influence the same trait in the different populations (Savolainen *et al.*, 2013) and be dominated by numerous small effect alleles (Orr, 1998).

To date there are only three published reciprocal transplant experiments performed to detect adaptation in the field using *A. thaliana*. Two of these experiments tested for local adaptation to shade (Callahan & Pigliucci, 2002) and dune *vs*. inland habitats (Arany *et al.*, 2005), and were

performed using demes likely to be undergoing gene flow, due to their close proximity (though this was not tested explicitly). Evidence for local adaptation to dune habitats was observed but not to shade. The third reciprocal transplant study investigated the adaptive differentiation between single reciprocally transplanted genotypes from northern Sweden and southern Italy; genotypes that are isolated genetically by distance (Ågren & Schemske, 2012). This study identified a clear local *vs*. foreign signal of adaptive differentiation over the five consecutive years of the experiment.

A Quantitative Trait Locus (QTL) analysis of this adaptive differentiation in the field using a biparental Recombinant Inbred Lines (RIL) created from the Swedish and Italian ecotypes identified 15 fitness QTLs, five of which showed small but significant fitness trade-offs with local alleles being favoured at each site (Ågren & Schemske, 2012). Furthermore, fitness QTL identified in the reciprocal transplant experiment were found to co-localise with QTL for freezing tolerance and flowering time identified in the same RILs grown under controlled growth conditions, suggesting that flowering time and cold tolerance contribute to the adaptive differentiation observed between the Swedish and Italian accessions (Oakley *et al.*, 2014; Dittmar *et al.*, 2014).

1.4. Soil-plant relationships

1.4.1. Soil properties and composition. Mineral nutrient uptake by plants

Soil is a reservoir of nutrients that contains essential elements for plant growth. All plants need 17 essential elements. According to quantitative requirements these can be divided into macronutrients, required in the mM range (C, H, O, N, P, S, K, Ca, Mg), and micronutrients required in the µM range (Fe, Mn, Zn, Cu, B, Mo, Cl, Ni). Besides these, some plants have specific demands: Na is a macronutrient in obligate halophytes and a micronutrient in C4 and CAM plants. Silicon is essential for certain algae and horsetails, it improves mechanical strength, photosynthesis and growth in grasses and plays a significant role in plants under stress; therefore it is considered as quasi-essential (Epstein & Bloom, 2005).

Along the path from the soil to the leaf, there are several control points and processes that allow plants to accumulate and exclude elements from their leaves. Plants do exhibit selectivity in that they preferentially take up some elements and exclude others. They can also take up nutrients in excess of their needs, termed luxury consumption, and store these in the

vacuole. However, there are feedback mechanisms that reduce ion uptake as internal concentrations increase, maintaining a balance between demand and acquisition (Gregory, 2006b).

Under natural conditions, the major factors affecting ion availability to plants are the ion's concentration in the soil solution, the degree of ion interaction with the soil's solid phases and the discrimination by the plant root during ion uptake (Bohn *et al.*, 2001). The relative quantity of a particular nutrient delivered to the plant will depend on the nutrient and soil properties such as the structure of the soil aggregates that can affect the size and distribution of soil pores and consequently the hydraulic conductivity. The other properties relevant for water and essential nutrient acquisition by plants are soil texture, water holding capacity (WHC), organic matter content and pH of the soil solution.

The ability of roots to obtain nutrients from the soil and to deliver these to the aerial tissues at a rate that matches the needs of growth is key to ensuring that the shoot has the resources it needs to function effectively. When roots fail to do this, plants show a range of visual symptoms indicative of nutrient deficiencies (e.g. smaller size, discolouration, infertility). In agricultural systems this results in smaller yields, while in natural ecosystems it may affect the ability of plants to compete effectively (Marschner, 1995; Tester & Leigh, 2001).

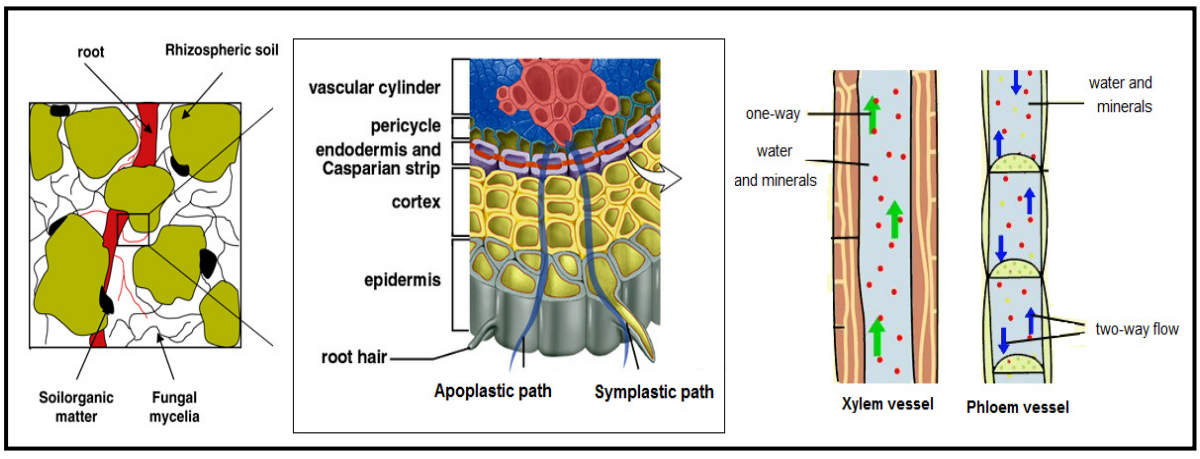


Figure 5: Scheme of soil-plant interaction, nutrient uptake by roots and mineral transport to leaves via xylem.

Roots, soil and microorganisms interact to determine the rhizosphere environment. Roots are in direct contact with only a very small part of the nutrients in the soil solution, so that nutrients must move from the bulk soil to the root surface. This movement occurs through the processes of mass flow (convection) and diffusion. Mass flow occurs as a result of

transpiration; dissolved ions are carried to the root surface in the hydraulic continuum formed by the soil–plant–atmosphere. Membranes of the root are highly selective about what enters the plant cells. Diffusion occurs when ions move along a concentration gradient established between the root surface and the bulk soil; ions diffuse towards the root if they are taken up faster than they are carried to the root surface by mass flow and away from the root if the converse pertains. A consequence of the differential mobility of ions in soil solution is that the zones of competition for different ions by a root system differ (Gregory, 2006b).

Many of the considerations pertaining to water transport across the root to the xylem, such as the role of the cell walls, membranes, exodermis and endodermis, also apply to the uptake and movement of nutrients, although there are some important differences. A small proportion of the nutrients measured in plants are absorbed passively into 'free spaces' within cell walls as a consequence of cation exchange with the negatively charged cell walls; anions are excluded from this space. Most uptake, however, is active and ion-specific against an electrical or concentration gradient (Marschner, 1995).

The transfer from apoplast to symplast (cytoplasm) across the plasmalemma is a particularly important part of the pathway for nutrient transport to the xylem, while transfer from symplast to cell vacuole across the tonoplast is important for the storage of ions in cells. Uptake across all membranes is driven by specific energy-driven carriers or through ion channels embedded in the membranes together with the aquaporins. Several sources of energy associated with ATP are used to transport ions across membranes against electrochemical gradient (Gregory, 2006b).

The principal *A. thaliana* nutrient transporters are summarized in Table 1:

Table 1. Top *A. thaliana* transporter families described for each nutrient.

Nutrient	Transporter families	Nutrient	Transporter families
K	*AKT, SKOR, HKT, KUP/HAK*	**B**	*BOR, NIP*
P	*PHT, PHF*	**Co**	*IREG/FPN*
Ca	*CAX*	**Mo**	*MOT*
N	*NRT*	**Cd**	*CDM, HMA*
Fe	*IRT, FPN, FRO, ZIP*	**Ni**	*IREG/FPN*
Na	*HKT1, SOS, NHX*	**Cu**	*ZIP, YSL, COPT*
Mg	*MRS*	**Se**	*SULTR*
S	*SULTR*	**Mn**	*NRAMP, MTP*
Zn	*MTP, HMA, ZIP*	**Rb**	*HAK*

In order to grow on soils that vary widely in chemical composition, plants have evolved mechanisms for regulating the elemental composition of their tissues to balance the mineral nutrient and trace element bioavailability in the soil with the requirements of the plant for growth and development. Some plants show specific adaptations to certain soils, and many efforts have been directed towards identification of the mechanisms permitting growth in these environments. The biodiversity that exists within a species can be used to investigate how regulatory mechanisms of individual elements interact and to identify genes important for these processes (Baxter *et al.*, 2012)

1.4.2. Ionomic studies

The composition of mineral nutrients and trace elements (i.e. the inorganic component of an organism) is now referred to as the ionome (Salt *et al.*, 2008). The plant ionome is a vector of tissue analytical data generally constrained to the dry or fresh matter content (Parent *et al.*, 2013b). The need for linking plant ionomes – often referred to as plant nutrient signatures (Willby *et al.*, 2001) or profiles (Tennakoon *et al.*, 2011) – with genetics (Conn & Gilliham, 2010) and adaptation to environmental factors (Chapin, 1989) elevated the study of mineral nutrition of plants as a central topic in ecology (Aerts & Chapin, 2000) and agronomy (Norton *et al.*, 2010).

Elemental uptake, distribution and storage processes involve multiple molecular components including transporters, channels, chelators and the genes that encode and regulate them. Processes that alter the physiological properties such as root architecture and transpiration can also affect elemental accumulation. Interestingly, many of these changes can affect multiple elements. For example, altering the Fe and P availability in the soil leads to reproducible and predictable alterations in five and six elements respectively in *A. thaliana* (Baxter *et al.*, 2008). However, the physiological and molecular drivers of the elemental response to many other elements and the rules governing relationships between them are far from clear (Baxter *et al.*, 2012). One method of elucidating these rules is to identify the genes controlling the accumulation of individual elements or groups of highly correlated elements.

By screening mutant populations for alterations in the ionome, three genes that alter the sphingolipid and Casparian strip pathways were identified which control root processes involved in mineral ion homeostasis and water relations (Chao *et al.,* 2011; Hosmani *et al.*, 2013; Kamiya *et al.*, 2015). Detailed analyses of the ionome in *A. thaliana* have shown

considerable variation for leaf mineral concentrations under various mineral/metal supply conditions (Salt *et al.*, 2008). *A. thaliana* QTL analyses have been focused on accumulation of specific minerals, including N (Loudet *et al.*, 2003); K (Harada & Leigh, 2006), Cu (Kobayashi *et al.*, 2008), Mo (Baxter *et al.*, 2008), Na (Rus *et al.*, 2006), S (Loudet *et al.*, 2007; Koprivova *et al.*, 2013; Chao *et al.*, 2014) and Zn (Pineau *et al.,* 2012).

A preliminary sampling of soil from 16 different sites in and around Tossa del Mar (Catalonia) has already revealed a 10-fold difference in top soil Na^+ in this region and *A. thaliana* accessions collected from the same region also show significant variation in their leaf Na^+ accumulation capacity (Bomblies unpublished data). One of the factors that cause alterations in the levels of any given element in a plant is the changes in the soil chemical environment. While the interaction of genotype and environment is clearly a critical driver of alterations in the ionome, most studies to date looking at relationships between elements have focused on identifying either genetic or physiological correlations in isolation (Baxter, 2009).

A complete set of elemental profiling data of *A. thaliana* from different accessions and studies are available at the iHUB website (http://www.ionomicshub.org) (Baxter *et al.*, 2007).

1.4.3. Characteristics of coastal soils and plant adaptations

In the semi-arid coastal regions, the presence of saline soils is a common characteristic due to the ingress of sea water through tidal waves, underground aquifers or through wind transport of salt spray. The concentration of salts in the air or in the rainwater decreases exponentially with increasing distance from the sea, becoming uniform at about 50 km from the shore (Yaalon, 1963). Saline soils are dominated by neutral soluble salts consisting of chlorides and sulphates of sodium, calcium and magnesium. Although Na^+ is generally the dominant soluble cation, the soil solution also contains appreciable quantities of divalent cations as Mg^{2+} and/or Ca^{2+} (Abrol *et al.*, 1988).

Salinity affects about 7% of the world's land area with the area increasing as a consequence of the clearing of native, perennial vegetation and the introduction of irrigation schemes without proper drainage. Soil salinity inhibits growth through effects of both Na^+ and Cl^- . If salinity is high and the plant's ability to exclude NaCl is poor, then Na^+ or Cl^- (or both) accumulate in transpiring leaves and eventually exceed the ability of cells to compartmentalize these ions in the vacuole. The ions then build up in either the cytoplasm inhibiting enzyme activity or the cell walls causing them to dehydrate and shrink (Munns, 2002).

Soils in coastal areas are usually deficient in major nutrients, highly saline and with low water availability due to the low soil water potential. Consequently, these habitat conditions are very harsh for plants. However, many of them have adapted and thrive in coastal environments through adaptations such us: (1) an increased thickness in the leaves to protect the plant from dehydration, exposure to the sun and salt spray; (2) the ability to delay germination in response to excessive salt spray, dehydration or other harsh environmental conditions; (3) the ability to produce very large seeds to increase the viability and vigour of seedlings; (4) the ability to roll the leaves, in response to heat, salt and lack of water; (5) occurrence of hairs on the leaves, which helps to avoid heat stress– common in plants found close to the shore; (6) wiry stiff leaves and stems which enable the plants to tolerate abrasion by salt-laden winds and sands; or (7) altered regulation of ion uptake and compartmentation.

Although plant species vary in their sensitivity and response to the decrease in water potential caused by drought, low temperature, or high salinity, it may be assumed that all plants have an altered capability for stress perception, signalling, and response (Bohnert *et al.*, 1995). The large variation in evolved stress responses within a species can be a powerful tool to identify ecologically important and adaptive traits. Quantitative genetics combined with appropriate phenotypic data can be one avenue for identifying genes important for coastal adaptation. In *A. thaliana* populations, natural variation for stress responses have been observed at different levels of integration and the genetic bases of those variations have been analysed using two strategies: classical linkage and association (LD) mapping (e.g. salinity stress: Shi *et al.*, 2000; drought, cold and salinity stress: Seki *et al.*, 2002; osmotic stress: Droillard *et al.*, 2002; nutritional stress: Hirai *et al.*, 2004). High-throughput accurate phenotyping is required to study the complex genetic architecture of physiological responses to abiotic factors (Trontin *et al.*, 2011).

1.5. Salt stress and tolerance in plants

Environmental stresses such as drought, high salinity, and low temperature are major factors that limit plant growth and productivity by disturbing the intracellular water balance (Yancey *et al.*, 1982). Studying the processes of tolerance to these stresses is of vital importance not only from the biological point of view, where there are plants capable of tolerating extreme concentrations of salts and long periods without water, but also from the viewpoint of agriculture (Pitman & Läuchli, 2002).

High concentrations of salts in the soil make it harder for roots to extract water, and high concentrations of salts within the plant can be toxic. Salts on the outside of roots have an immediate effect on cell growth and associated metabolism; toxic concentrations of salts take time to accumulate inside plants before they affect plant function. Because NaCl is the most soluble and widespread salt, it is not surprising that plants have evolved mechanisms to regulate its accumulation and to select against it in favour of other nutrients commonly present in low concentrations.

Arabidopsis thaliana accession Columbia (Col-0), when compared with halophytes (such as *Atriplex amnicola*) under similar conditions of light and humidity, can be considered a salt-sensitive species (Cramer, 2002). However, Rus *et al.* (2006), based on the elemental profiling of shoot tissue from 12 different *A. thaliana* accessions, show that two coastal populations of *A. thaliana* collected from Tossa del Mar (Ts-1) and Italy (Tsu-1) (Anastasio *et al.*, 2011) accumulate higher shoot levels of Na^+ than do Col-0 and other accessions. This supports the hypothesis that certain wild collected accessions of *A. thaliana* are behaving like halophytes, with coastal/saline soil associated accessions having the capacity to accumulate elevated leaf Na^+ potentially for osmotic balance.

1.5.1. Salt tolerance and natural adaptation to saline environments

Salt tolerance depends on the ability of the plant to control the transport of salt at five sites (Munns, 2002):

1. Selectivity of uptake by root cells. It is still unclear which cell types control the selectivity of ions from the soil solution.

Initial entry of Na^+ from soil solution into the root is via cation channels (Tester and Davenport, 2003), but roots of most plants exclude at least 95% of the Na^+ present in the soil solution and this is the main mechanism of tolerance (Munns, 2005). A failure in Na^+ exclusion manifests its toxic effect after days or weeks, depending on the species, and causes premature death of older leaves (Munns & James, 2003).

2. Loading of the xylem. There is evidence for a preferential loading of K^+ rather than Na^+ by the cells of the stele.

Mechanisms for Na^+ tolerance at the cellular level involve keeping Na^+ out of the cytoplasm, and sequestering it in the vacuole of the cell. Generally, Na^+ starts to inhibit most enzymes at a concentration above 100 mM. If Na^+ and Cl^- are sequestered in the vacuole of the cell, K^+ and organic solutes should accumulate in the cytoplasm and organelles to balance the osmotic potential of the ions in the vacuole (Kant & Kafkafi, 2002). Most Na^+ that is delivered to the shoot remains in the shoot, because for most plants, the movement of Na^+ from the shoot to the roots in the phloem can probably recirculate only a small proportion of the Na^+ (Munns & Tester, 2008). Intracellular compartmentation is by a vacuolar Na^+/H^+ antiporter, driven by a pH gradient across the tonoplast (Blumwald *et al.*, 2000).

3. Removal of salt from the xylem in the upper part of the roots, the stem, petiole or leaf sheaths. In many species, Na^+ is retained in the upper part of the root system and in the lower part of the shoot, indicating an exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles in stems and petioles.

4. Loading of the phloem. There is little retranslocation of Na^+ or Cl^- in the phloem, particularly in the more tolerant species.

5. Excretion through salt glands or bladders. Only halophytes have these specialised cell types.

Tolerance to salinity can also be increased with a greater tolerance to osmotic stress: Osmotic stress immediately reduces cell expansion in root tips and young leaves, and causes stomatal closure. A reduced response to osmotic stress would result in greater leaf growth and stomatal conductance, but the resulting increased leaf area would benefit only plants that have sufficient soil water (Munns & Tester, 2008).

Ion transporters (molecular level):

Under saline conditions, absorption of Na^+ and Cl^- competes with the uptake of nutritional elements such as K , N, P, and Ca by plants, causing nutritional disorders that result in yield quantity and quality reduction (Grattan & Grieve, 1998). Several researchers indicated that increased NaCl concentrations in the root zone of plants causes accumulation of Na^+ and Cl^- in shoot tissues and decline in Ca^{2+}, K^+ and Mg^{2+} levels in a number of plants (Bayuelo-Jimenez *et al.*, 2003; Khan *et al.*, 2000a; Perez-Alfocea *et al.*, 1996). The movement of cations and anions from soil solution to the cytoplasm is controlled by permeability of cell membranes which contain protein transporters that facilitate the passage of ions (Jiménez-Casas, 2009).

Na^+ enters roots passively, via voltage independent (or weakly voltage-dependent) non-selective cation channels that are strongly influenced by Ca^{2+} (Amtmann & Sanders, 1999) and possibly via other Na^+ transporters such as some members of the high-affinity K^+ transporter (HKT) family (Haro *et al.*,2005). These cation channels could allow entry of large amounts of Na^+ from a highly saline soil if not adequately regulated (Amtmann & Sanders, 1999). Na^+ can be effluxed from the cytoplasm through Na^+/H^+ antiporters, driven by the pH gradient across the plasmalemma (Blumwald, 2000). These transport processes all work together to control the rate of net uptake of Na^+ by a cell (Munns, 2002). High affinity Na^+ influx is also mediated by some members of the HKT transporter family in low salt roots (Horie *et al.*, 2007), but this is repressed by moderate concentrations of Na^+ and so is unlikely to be relevant to salinity tolerance.

Molecular genetic analysis using the *A. thaliana* Salt Overly Sensitive (*sos)* mutants revealed that a plasma membrane Na^+ /H^+ antiporter, *SOS1*, plays a crucial role in Na^+ extrusion (Zhu, 2002). Besides this, SOS proteins seem to be involved in cell signalling under saline stress conditions (Ji *et al.*, 2013). The most abundant vacuolar Na^+ /H^+ antiporter, *NHX1*, functions in the sequestration of Na^+ into the vacuole (Yamaguchi & Blumwald, 2005). Many plants accumulate organic osmolytes such as proline, betaine, polyols, and soluble sugars for osmotic adjustment (Yokotani *et al.*, 2009).

An important recent discovery is the observation that the chloroplast protein CEST induces tolerance to multiple environmental stresses such as salt, drought or high-temperature stress (Yokotani *et al.*, 2009). The chloroplastic isoform of aldehyde dehydrogenase is involved in the detoxification of damaged lipids or proteins and also plays a protective role in tolerance to environmental stress (Sunkar *et al.*, 2003; Kotchoni *et al.*, 2006). In *A. thaliana*, more than 2000 distinct proteins are estimated to be located in chloroplasts (Leister, 2003). Interestingly, some of these genes are predicted to be associated with environmental stress responses (Ishikawa *et al.*, 2003; Myouga *et al.*, 2006).

1.5.2. Whole plant Na^+ homeostasis in *A. thaliana*

Different Na^+ transporters are implicated in the regulation of intracellular and *in planta* Na^+ homeostasis (Apse & Blumwald, 2007; Flowers & Collmer, 2008; Munns & Tester, 2008; Horie *et al.*, 2009). *NHX* and *SOS1* families mediate Na^+ sequestration in organelles and efflux across

the plasma membrane, respectively (reviewed in Apse & Blumwald, 2007; Horie *et al.*, 2009). *Atsos* mutants have been shown to be hypersensitive to Na^+ stress, showing severe growth retardation and high shoot and xylem Na^+ concentrations which ultimately leads to the death of the plant. There are numerous studies showing that overexpression of *AtNHX1* and *AtAVP1* leads to increased salinity tolerance by detoxifying the cytoplasm by pumping and accumulating Na^+ in the vacuole (Jha *et al*., 2010).

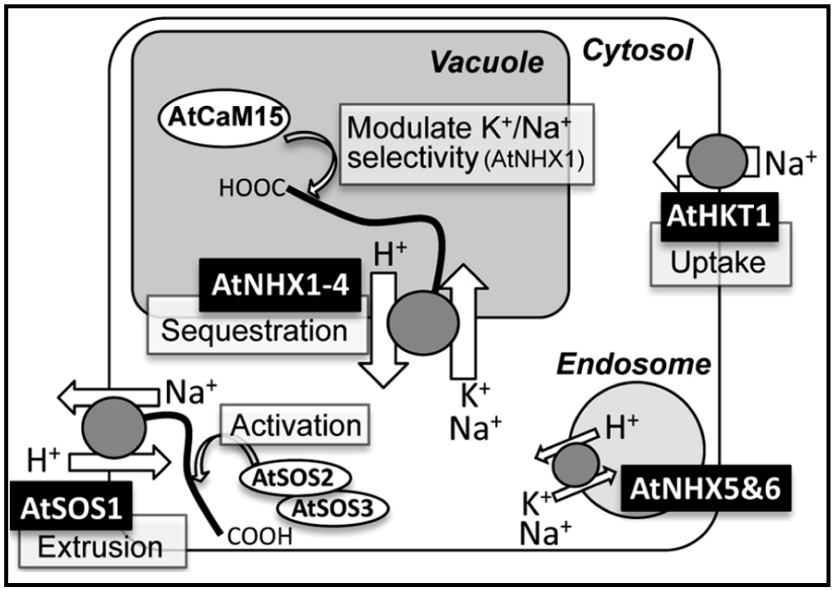


Figure 6: Schematic representation of subcellular localizations, functions and regulations of *NHX, SOS* and *HKT* Na^+ transporters in *A. thaliana* (Yamaguchi *et al.*, 2013).

HKT (high affinity K^+ transporter) family of Na^+ transporters are implicated in Na^+ unloading from xylem vessels (Ren *et al.*, 2005; Sunarpi *et al.*, 2005; Davenport *et al.*, 2007; Horie *et al.*, 2009; Moller *et al.*, 2009). Class I proteins are Na^+ selective uniporters (Gassman *et al.*, 1996; Schachtman and Schroeder, 1994; Uozumi *et al.*, 2000; Ren *et al.*, 2005; Platten *et al.*, 2006; Horie *et al.*, 2009) and are typified by *A. thaliana HKT1;1*. *AtHKT1;1* is encoded by a single gene and has been implicated in Na^+ exclusion from leaves and leaf K^+ homeostasis (Rus et al; 2001; 2004; 2006; Maser *et al.*, 2002; Nublat *et al.*, 2001). *AtHKT1;1* expression occurs in root and shoot vasculature (Maser *et al.*, 2002; Berthomieu *et al.* 2003) and *AtHKT1;1* protein localizes to the plasma membrane of xylem parenchyma cells (Sunarpi *et al.*, 2005; Horie *et al.*, 2009).

The *hkt1* null mutation results in an alteration of the Na^+ distribution in the plant, with higher levels of Na^+ accumulation in shoots and lower accumulation in roots compared to wild-type plants. Loss of *AtHKT1;1* expression in the null *hkt1* mutant also confers sensitivity to high levels of NaCl (Berthomieu *et al.* 2003; Rus *et al.*, 2004; Sunarpi *et al.*, 2005). Conversely, a

constitutive overexpression of *AtHKT1;1* in all tissues also results in increased Na^+ accumulation in the shoot and increased NaCl sensitivity, probably due to both increased unidirectional Na^+ influx and its increased transfer from the root to shoot. The apparent paradox that both overexpression and knock out of this transporter lead to the same phenotype, hints to the fact that *AtHKT1;1* may be under a very precise and sensitive whole plant regulation (Moller *et al.*, 2009).

1.5.3. Natural variation of *AtHKT1;1*

Using genome-wide association studies (GWAs) and deficiency complementation, Baxter *et al.* (2010) determined that different alleles of the Na^+-transporter *HKT1* control leaf Na^+ across a broad range of concentrations in *A. thaliana.* Elemental profiling of shoot tissue collected from different *A. thaliana* accessions revealed that the Ts-1 accession from Tossa del Mar, Spain accumulates higher levels of Na^+ than Col-0 and others in soil with low levels of NaCl under controlled growth conditions (Rus *et al.*, 2006).

To establish the differences present in the *AtHKT1;1* allele of Ts-1 accession, the nucleotide sequence was determined for the *AtHKT1;1* locus. The sequence region was comprised of 5,456 bp upstream (363 bp downstream of the previous gene At4g10300) of *AtHKT1;1* translational start codon that includes the promoter to 386 bp downstream of the *AtHKT1;1* stop codon. Several polymorphisms were identified both in the coding region and in the upstream and downstream regions of the *AtHKT1;1* gene:

In the coding region, no nonsense mutation was identified and 19 single nucleotide polymorphisms (SNPs) were identified, seven of which result in the amino acid changes R31, K10N, V66L, C134Y, E385G, V453L and F477L. None of the amino acid changes affect the first serine residue at position 68 in the P-loop A that has been implicated in the Na^+ specificity of the transporter.

In the upstream region, which includes the promoter of *AtHKT1*, four major polymorphisms were identified. The most upstream polymorphism affects two repeated units of 681 and 673 bp, linked by a 34-bp sequence. The second major polymorphism identified in this region, about 3.100 bp upstream of the start codon, is an insertion of 13 bp in the sequence of Ts-1, resulting in the duplication of a 10-bp sequence (AATGTGTTAT) in Ts-1 that is present only once in Col-0. Similarly, 1.210 bp upstream of the start codon, a 14-bp insertion results in a duplicated 10-bp sequence (TCATTGCAAA) in Ts-1. The last major polymorphism in the

promoter region is localized 148 to 98 bp upstream of the translational start codon. Compared to Col-0, these sequence polymorphisms found in Ts-1 abolish the first of the two putative CAAT boxes suggested by Uozumi *et al.* (2000).

In the downstream region, however, are the major differences in the sequence. Several SNPs, as well as deletions in Ts-1, result in a sequence of 246 and 140 bp shorter than the 386-bp sequence found in Col-0.

Given the clear relationship between reduced *HKT1* mRNA in Ts-1, the hypofunctionality of $HKT1^{Ts\text{-}1}$ and the lack of any frameshift mutations in the *HKT1* coding sequence of these alleles, one reasonable explanation of the reduced functionality is that a DNA polymorphism in the promoter is responsible for reduced expression of *HKT1*. However, sequencing and haplotype analyses have not revealed any obvious candidate for QNTs in the *HKT1* promoter (Brazelton and Salt, unpublished).

Accessions with hypofunctional *HKT1* alleles appear to be primarily distributed in coastal regions or in regions with known exposed paleological marine environments (Baxter *et al.*, 2010). Besides, apart from different geographical origins they also differ phenotypically, and the DNA polymorphism analysis showed a large genetic distinction (Rus *et al.*, 2006). Such specific distribution patterns raise the possibility that certain properties of the environment are driving the observed allelic distribution.

1.6. Importance of Molybdenum in plants

Molybdenum (Mo) is an important micronutrient for plants being incorporated into molybdopterin, an essential cofactor for four enzymes: (*1*) nitrate reductase catalyses the first and rate-limiting step in nitrate assimilation, (*2*) peroxisomal sulphite oxidase that detoxifies excessive sulphite, (*3*) Aldehyde oxidase catalyses the last step of abscisic acid biosynthesis, and (*4*) xanthine dehydrogenase is essential for purine degradation and stress response (Schwarz and Mendel, 2006).

In soils, the molybdate anion is the only form of Mo that is available for plants and bacteria (Stiefel, 2002). In all organisms, Mo has to be complexed by a pterin compound, thereby forming the molybdenum cofactor (Moco), in order to gain biological activity. Biologically, Mo belongs to the group of trace elements, i.e. the organism needs it only in minute amounts.

However, deficiency in Mo is lethal for the organism. Uptake of Mo as molybdate anion requires specific uptake systems to scavenge molybdate in the presence of competing anions (Mendel, 2007).

The availability of molybdenum for plant growth is strongly dependent on the soil pH, the concentration of adsorbing oxides (e.g. Fe oxides), the extent of water drainage, and the concentrations and type of organic compounds found in the soil colloids. In alkaline soils, molybdenum becomes more soluble and is accessible to plants mainly in its anionic form as MoO_4^-. In contrast, in acidic soils (pH < 5.5) molybdenum availability decreases as the adsorption of anions to soil oxides increases (Reddy *et al.*, 1997). Soil moisture also influences MoO_4^- availability where poorly drained wet soils (e.g. peat marshes, swampy organic matter rich soils) tend to accumulate MoO_4^- to high levels (Kubota *et al.*, 1963). In contrast, well-drained sandy soils have been shown to leach significant amounts of applied molybdenum (Jones & Belling, 1967). However, the retention of molybdenum in sandy soils is very much pH dependent as acidic sands release negligible amounts of molybdenum in the leachate (Riley *et al.*, 1987).

The importance of molybdenum for plant growth is disproportionate in respect to the absolute amounts required by most plants. Apart from Cu, Mo is the least abundant essential micronutrient found in most plant tissues and is often set as the base from which all other nutrients are compared and measured (Kaiser *et al.*, 2005). When plants are grown under molybdenum deficiency, a number of varied phenotypes develop that hinder plant growth. Most of these phenotypes are associated with reduced activity of molybdoenzymes. In contrast, toxicity by molybdate is extremely rare and characterized by relatively mild symptoms such as yellowish leaves (Bergmann, 1992) or reduced seedling growth and increased anthocyanin concentrations (Kumchai *et al.*, 2013).

1.6.1. Whole plant Mo homeostasis in *A. thaliana*

Until recently, it was thought that molybdate transport was carried out by anion transport systems able to transport molybdate non-specifically. Physiological evidence for the presence of specific molybdate transporters in plants (Llamas *et al.*, 2000) was confirmed by the identification of *MOT1* in *Chlamydomonas* (Tejada-Jiménez *et al.*, 2007) and *A. thaliana* (Tomatzu *et al.*, 2007; Baxter *et al.,* 2008). In *A. thaliana MOT1* appears to be located at the mitochondrial membrane, it is found throughout the plant body and seems to be crucial in ensuring an

efficient uptake of molybdate from soil and in the maintenance of its intracellular level (Tomatzu *et al.*, 2007; Baxter *et al.*, 2008). The accumulation of any other element, including Na, Mg, P, S, K, Ca, Mn, Fe, Co, Ni, Cu, Zn and Cd, was altered by more than 20% in two independent experiments. This evidence is consistent with *MOT1* being a specific molybdate transporter (Baxter *et al.*, 2008).

Tomatzu *et al.* (2007) determined that *AtMOT1* was expressed in roots and shoots of *A. thaliana* plants. However, localization studies done by Baxter *et al.* (2008) with GFP fusion on the C-terminal end of *AtMOT1* demonstrated the localization at the mitochondria, rather than the plasma membrane as previously suggested by Tomatzu. Furthermore, a series of reciprocal grafting experiments combining shoots and roots of Ler-0 and Col-0 accessions identified *AtMOT1* to be primarily active in the roots. It is hypothesized that *AtMOT1* regulates the whole plant Mo accumulation at the level of the mitochondria in the roots.

AtMOT1 transcription is activated in the presence of molybdate in shoots but not in roots. Once inside the cells Mo is incorporated to molybdopterin (MPT) in the last step of Moco biosynthesis catalysed by CNX1 through an intermediate of adenylatedmolybdate. *CNX1* from *A. thaliana* binds to the cytoskeleton. This could be an adequate localisation to interact with an integral membrane protein like a molybdate transporter facilitating a coordinated Moco biosynthesis (Llamas *et al.*, 2006). Taking into account that Mo export mechanisms are unknown, the sequestration of Mo whether in the form of Moco or chelated as an organic complex with anthocyanin or malic acid would explain why plants having extremely low requirements for this element are fairly tolerant to Mo (Tejada-Jiménez *et al.*, 2009).

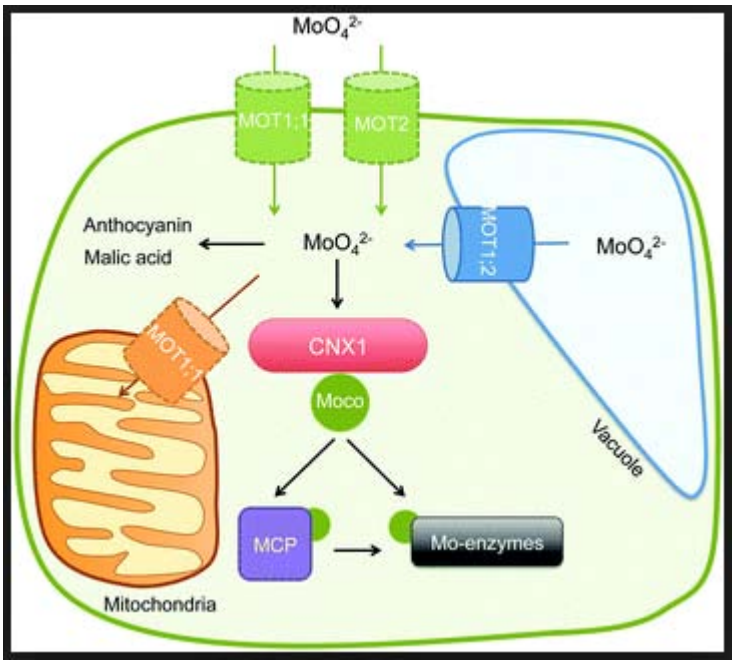


Figure 7: Organization of the Mo homeostasis process in a single plant cell (Tejada-Jiménez *et al.*, 2013)

Plants have long been assumed to take up molybdate through sulfate transporters, as the two anions are chemical analogues and may compete for the binding site of the same transporters (Dudev & Lim, 2004; Alhendawi *et al.*, 2005). Indeed, there are reports that sulphate (Alhendawi *et al.*, 2005) as well as phosphate (Heuwinkel *et al.*, 1992) starvation of plants enhances Mo uptake up to 10-fold which means that sulphate transporters and phosphate transporters might co-transport molybdate anions (Mendel, 2007). However, Baxter *et al.* (2008) detected no change in the shoot content of S in *mot1-1* mutant. Given that characterized members of the sulphate transporter superfamily are SO_4^{-2} / H^+ co-transporters, Baxter *et al.* (2008) speculate that *AtMOT1* is transporting MoO_4^{-2} from the acidic mitochondrial intermembrane space to either the cytoplasm or the matrix.

1.6.2. Natural variation on *AtMOT1*

As described by Baxter *et al.* (2008), to establish the differences present in the *AtMOT1* allele of Van-0 and Ler-0 ecotypes, *AtMOT1* was sequenced including 1 kb upstream and 200 bp downstream of the coding sequence. They found 15 single nucleotide polymorphisms (SNPs) in the 1 kb upstream region, two SNPs that change two amino acids (168T and V30L) in the coding region, and a 53 bp deletion 27 nucleotides upstream from the translation start site including a TATA box. The significantly reduced expression of *AtMOT1* in these low Mo accessions suggests that the 5' deletion may be the causal polymorphism driving low shoot Mo content.

Reciprocal grafting experiments demonstrate that the roots of Ler-0 are responsible for the low Mo accumulation in leaves, and GUS localization demonstrates that *AtMOT1* is expressed strongly in the roots. *AtMOT1* contains an N-terminal mitochondrial targeting sequence, and expression of *AtMOT1* C-terminally tagged with GFP in protoplasts, and transgenic plants, establishes the mitochondrial localization of this protein (Baxter *et al.*, 2010).

Loss-of-function alleles of *AtMOT1* show an 80 % reduction of Mo in roots, shoots and seeds and this reduced Mo accumulation causes an inability to grow on acid soils (Poormohammad *et al.*, 2012), in which the solubility of molybdate is expected to be reduced. Addition of supplemental molybdate to the growth media was also found to complement the growth defect, supporting the conclusion that this growth defect is related to an impaired ability to accumulate Mo from these acids soils in which molybdate is poorly available to plants (Poormohammad *et al.*, 2012).

2. Objectives

2. Objectives

The overall aim of this study is to take advantage of the natural genetic variation that occurs between populations of the genetic model plant *Arabidopsis thaliana* to identify cases of local adaptation that drive genetic differentiation. However, this global aim can be itemized into seven sections:

A. Identification of coastal and adjacent inland populations of *A. thaliana* in the northeast of Catalonia.

The global distribution of *A. thaliana* and its unprofitableness as a crop or for human use has meant that the register of citations of wild populations of this plant in Catalonia were sparse. Faced with this problem and the need to locate a large number of new populations of *A. thaliana*, we decided to build a potential distribution map of *A. thaliana* in the northeast of Catalonia using a Species Distribution Model (SDM) created using GIS tools.

B. Determination of allele sharing between coastal and inland demes.

Once located a large number of wild *A. thaliana* populations in the northeast of Catalonia, we found that about half of them were localized around the coast in places influenced by the sea (coastal demes) and the other half lived in inland habitats (inland demes). Through the use of SNP markers, we looked for genetic differences between the two metapopulations and the rate of allele sharing was evaluated to determine if genotypes were differentiated by geography.

C. Find evidences for coastal and inland local adaptation.

Climatology and soil composition are very important in plant development and it is known that the sea can substantially influence these environmental factors. To estimate if *A. thaliana* coastal and inland demes are adapted to their own habitat, we designed a reciprocal transplant experiment at one representative site of the coastal environment (Blanes) and one representative site of the inland environment (Santa Coloma de Farners). As a reasonable

proxy for the fitness of each plant, growth (as rosette diameter) was measured weekly and we counted the number of siliques produced as an estimate of female reproductive success.

D. Establish the agent of divergent selection between coastal and inland habitats.

Having demonstrated the adaptation of our plants to their original habitat, it was necessary to find out which factors are responsible for the observed differentiation. Climatic variables, soil physical properties and composition have been analysed over the years in all sites where *A. thaliana* was localized. In addition, to study which nutrients were more or less absorbed and accumulated by plants, the leaf ionome of *A. thaliana* plants collected from each site was also analysed each year.

E. Determine which physiological and genetic traits contribute to salinity tolerance.

One of the elements that showed large differences both in the concentration in the soil between locations and in the accumulation inside the plants was Na. Although the studied coastal areas are not extremely saline, Na levels are sufficiently high to affect a non-halophytic plant such as *A. thaliana*. Therefore, to corroborate if our coastal plants have some mechanism that makes them more tolerant to salinity we conducted several hydroponic and irrigation experiments with different concentration of NaCl.

F. Study the natural variation of *AtHKT1;1* and *AtMOT1* in our plants and their effects.

We have cited that allelic variation at *AtHKT1;1* and *AtMOT1* is associated with the landscape distribution of native populations of *A. thaliana* growing on potentially saline impacted soils throughout Europe. To help develop further evidence linking allelic diversity in *AtHKT1;1* and *AtMOT1* with local adaptation and landscape distribution of *A. thaliana* containing these alleles, we decided to perform high density tissue recollection, genotyping and soil sampling at all inland and coastal target sites.

G. Explain the possible origin and benefits of having the weak alleles of *AtHKT1;1* and *AtMOT1.*

Ts-1-like and Van-0-like hypofunctional alleles (weak alleles) of *AtHKT1;1* and *AtMOT1* were detected only in plants from our coastally collected demes. To test the hypothesis that weak alleles of *AtHKT1;1* and *AtMOT1* could be correlated with improved *A. thaliana* fitness on soils with elevated Na, we performed hydroponic and irrigation experiments with NaCl treatments and we also conducted a 2-year reciprocal transplant on a coastal (BLA) and inland (SCF) common garden. Such studies will tie our laboratory based molecular mechanistic studies with the ecological function of these alternative alleles in the environment, helping to close the loop between natural genetic polymorphisms and a mechanistic understanding of their role in local adaptation.

3. Material and Methods

3. Material and Methods

3.1. Species Distribution Model and sampling design

The SDM was created using various geographic information system tools and the software MiraMon 7.0 (Complete SIG and Remote Sensing software; Pons, 1994-2011). The inputs for the model were (1) Universal Transverse Mercator (UTM) coordinates of 36 known occurrences of *A. thaliana* in Catalonia (excluding the Pyrenees and the Balearic Islands) obtained from the *Anthos* database (http://www.anthos.es) and a field study made by us in 2007 and (2) 20 cartographic layers of environmental variables obtained from *Gencat* (http://www.gencat.cat) and *WordClime* (http://www.worldclim.org) databases (Table 2).

Table 2: Summary of 20 ecological variables tested to perform the distribution map of *A. thaliana* in NE of Catalonia. Factors in bold had a significant influence.

Variable	Source	Scale/Grid resolution	% of selected classes *(*)*
Altimetry (hypsometry, m)	**Gencat (ICC)**	**5 x 5 m**	**< 50 %**
Soil covers 2007(MCSC-3v2)	Gencat (CREAF)	2 x 2 m	> 50 %
Land uses 2002(LANDSAT-TM)	**Gencat**	**30 x 30 m**	**< 50 %**
Geological base 2006	**Gencat (ICC)**	**1 : 50.000**	**< 50 %**
Habitats of Catalonia 2005	Gencat (ICC, UB)	1 : 50.000	> 50 %
Annual temperature range (ºC)	Gencat (ACDC)	1 x 1 km	> 50 %
Potential Evapotranspiration	Gencat (ACDC)	1 x 1 km	> 50 %
Climatic *water deficit* (CWD)	Gencat (ACDC)	1 x 1 km	> 50 %
Daily global radiation, mean annual	Gencat (ACDC)	1 x 1 km	> 50 %
Average annual precipitation (mm)	Gencat (ACDC)	1 x 1 km	> 50 %
Average monthly precipitation (mm)	Gencat (ACDC)	1 x 1 km	> 50 %
Seasonal precipitation (mm)	Gencat (ACDC)	1 x 1 km	> 50 %
Average annual temperature (ºC)	Gencat (ACDC)	1 x 1 km	> 50 %
Average monthly temperature (ºC)	Gencat (ACDC)	1 x 1 km	> 50 %
Mean diurnal range	WorldClim	30 asec (1 km)	> 50 %
Isothermality	WorldClim	30 asec (1 km)	> 50 %
Maximum temperature of warmest month (ºC)	WorldClim	30 asec (1 km)	> 50 %
Minimum temperature of coldest month (ºC)	WorldClim	30 asec (1 km)	> 50 %
Precipitation of wettest month (mm)	WorldClim	30 asec (1 km)	> 50 %
Precipitation of driest month (mm)	WorldClim	30 asec (1 km)	> 50 %

(*) Number of classes where the known occurrences of *A. thaliana* are situated regarding the total number of classes of the corresponding map.

Geographically referenced sites where *A. thaliana* had been previously located were introduced into the model as points in a vector file (Map A1). This file was overlaid with 20 cartographic layers (maps of ecological variables) to evaluate the spatial overlap between the locations of the existing *A. thaliana* demes and the chosen ecological variable. Each ecological variable has multiple classes (for example, the variable land uses has 20 classes [1, sandy; 2, soil with spares vegetation; 3, rainfed fruit trees, etc.]). If the locations for existing *A. thaliana* demes were classified into less than 50% of the classes for a particular ecological variable, then this variable could be a conditioning factor for the location of *A. thaliana*. For example, the 36 known occurrences of *A. thaliana* used in the model were found at locations classified into only six of 20 land uses classes; therefore, this variable was considered useful. The three ecological variables that fulfilled this criteria (altimetry (Map A2), land uses (Map A3), and geology (Map A4)) were reclassified into a binary system and used to derive the polygons that define the predicted distribution of *A. thaliana* in this region (Jones *et al.*, 2002). Polygons with an area of less than 2 km^2 were discarded to help control for errors introduced by combining raster and vector data types (Gardiner, 2002).

During the *A. thaliana* growing seasons (February–May) of 2012, 2013 and 2014, the accessible zones of the polygons were explored by transect walks and visually inspected for the presence of *A. thaliana*. Geographical coordinates of demes of *A. thaliana* plants were taken using a handheld global positioning system unit (Garmin eTrexVenture) when they were observed. The persistence or disappearance of the identified deme was evaluated each year, and the number of individuals in each deme was counted (Table A1). To evaluate the efficacy of the method, we selected 24 random points outside the polygons in which the SDM predicted *A. thaliana* would occur using a grid of 5 x 5 km (Map A5). These areas were also searched for *A. thaliana* over the same periods as the sites predicted to contain *A. thaliana*.

3.2. Collection of plant and soil material

Twenty-six demes of *A. thaliana* were selected from two different regions of Catalonia. A 'deme' is defined as a small group or stand of *A. thaliana* plants growing in relatively homogeneous ecological conditions and separated from other groups by at least 35 m). Thirteen demes were selected from the littoral region (less than 3 km from the sea) which constitutes the local coastal population and 13 demes from adjacent inland areas (between 3 and 34 km from the sea) which constitutes the local inland population (Table A2). In May 2011,

seeds of each deme were collected directly in the field from multiple plants and pooled. Seeds were stored in packets over silica gel in a sealed box until used. Before sowing seeds were surface sterilised by soaking in 70% (v/v) ethanol for 1 min, suspended in 30% (v/v) commercial Clorox bleach and 1 drop of Tween-20 for 15 min and rinsed 5 times in sterile 18 MΩmilli-Q water. Seeds were kept at 4 ºC for 48 hours in the dark to synchronize germination.

For analysis of the elemental composition of soils, during the first week of March and May of 2013, 2014 and 2015 we collected three soil samples (approximately 50 grams of soil from the first 10 cm depth) at each site. 20 g of soil was kept intact in the fridge to analyze the physical properties and the rest was air-dried under laboratory conditions, passed through a 2-mm sieve and stored in a fresh, dry place.

For analysis of the elemental composition of plant tissue, during the first week of March 2013, 2014 and 2015, between 2 and 10 whole plants from each site were collected and transported to the laboratory in plastic pots (Table A2).

3.3. DNA isolation, marker genotyping and knockout lines cultivation

DNA was extracted from leaf tissue that had been frozen at 280 ºC using a Biosprint 96 DNA plant kit on a Biosprint 96 robotic workstation (Qiagen). SNP assays were designed as described by Warthmann *et al.* (2007) and Clark *et al.* (2007). Single progeny of 98 individuals was genotyped using 425 genome-wide SNP markers (Table A2). Of these markers, 401 were polymorphic, and 356 had a minor allele frequency greater than 5% (Figure 8). The overall level of heterozygosity was low at 0.2%, with only 82 heterozygotes identified across all markers and individuals. Genotyping was performed by Bomblies and Weigel and analyzed by Douglas (Busoms *et al.*, 2015).

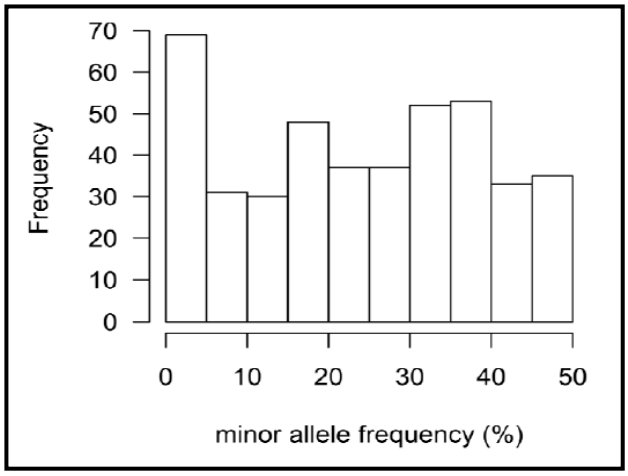


Figure 8: Distribution of the minor allele frequency of 425 genome-wide single nucleotide polymorphism (SNP) markers across 98 *A. thaliana* individuals.

3.4. Field-based reciprocal common garden experiments

To detect local adaptation between plants from coastal and inland habitats we designed a reciprocal transplant experiment with common gardens laid out in the Marimurtra Botanic Garden, Blanes (BLA) (41º 40' 37,64"; 2º 48' 3,86"), a representative coastal environment, and in the grounds of the School of Forestry and Agricultural Training, Santa Coloma de Farners (SCF) (41º 50' 41,04", 2º 40' 36,13"), a representative inland environment. We had previously identified demes of *A. thaliana* growing at both these sites. The same common garden design was reproduced at both sites. The common garden was 2 x 6 m in the native soil at each site, and each garden was covered with a shading mesh that reduced 70 % light incidence on sunny days and 50 % on cloudy days.

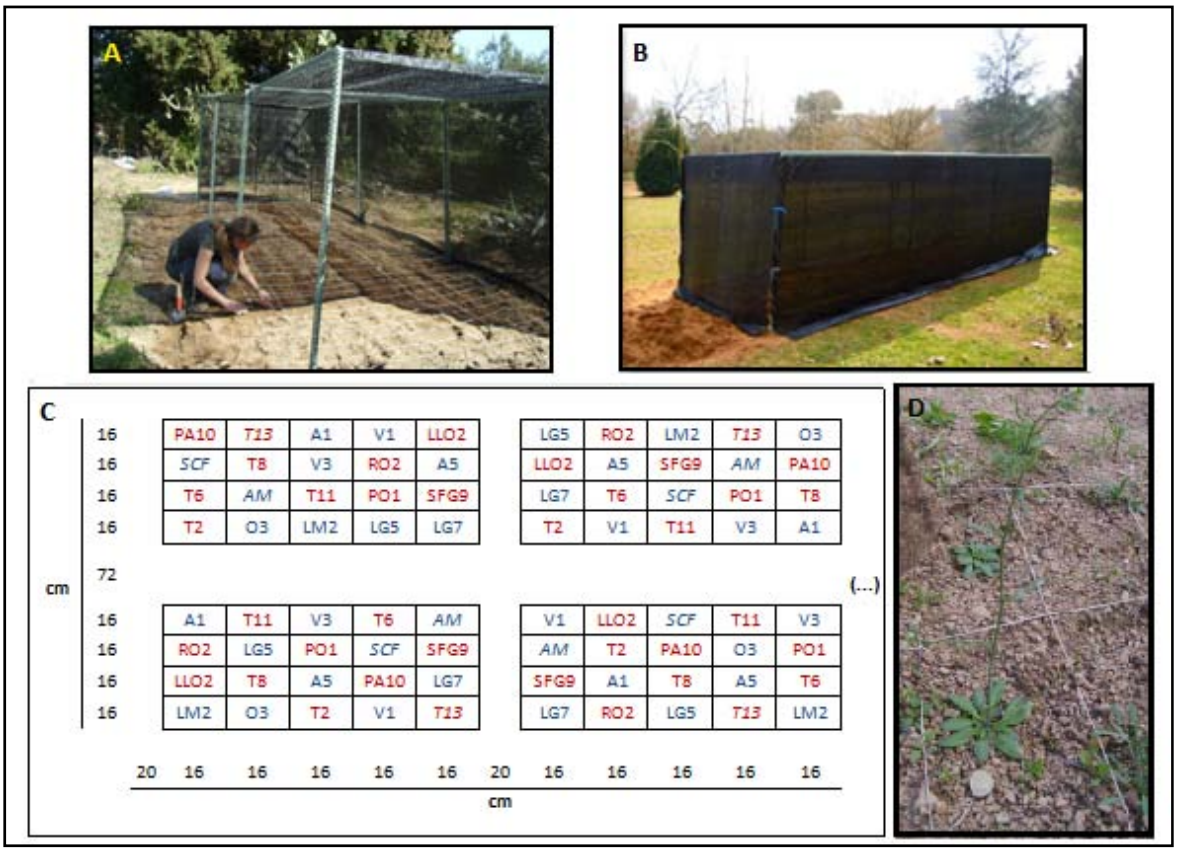


Figure 9. Pictures of common garden plots used in the reciprocal transplant experiments in BLA **(A, D)** and SCF **(B). (C)** Sketch of the random distribution of demes inside plots (coastal demes in red, inland demes in blue).

In April and May of 2012 and 2013, we harvested seeds from a pool of plants of each deme in the wild. These seeds were sowed on potting mix soil in a growth chamber (GROW 360/HR) with 16 h light / 8 h dark photoperiod, an irradiance of 80 $mmol \cdot m^{-2} \cdot s^{-1}$, a day / night

temperature of 21 ºC /18 ºC and irrigation of 0,5-strength Hoagland solution once a week, to obtain an enough amount of seeds for the field experiment planned for the following year.

In March 2013 and 2014 100 seeds (10 in each square) of 10 coastal and 10 inland demes (Table A2) were sown at both sites with individual genotypes planted into 16 x 16 cm squares (Figure 9), obtaining 10 plots of 64 x 80 cm with 20 demes distributed randomly (in each replicated plot each deme had a different position). Two weeks after germination, we left 2 plants in each square. We studied the fitness of 10 plants for each deme at each site and the other 10 plants of each deme per site were harvested on April of 2013 and 2014 to analyse their leaf ionome. Rosette diameter was measured every week during 2 month and the number of siliques was counted at maturity. During the 3 months of the field experiments minimum and maximum temperatures, precipitation and soil properties and composition were monitored.

3.5. Controlled environment common garden experiments

To determine if soil type had an effect on fitness and could be a potential factor responsible for local adaptation 10 seeds of each deme were sowed individually in 4 x 4 x 8 cm pots in soil excavated from BLA or SCF. Soil was collected in September 2013, at 5 m distance from where the garden was located and a depth of less than 20 cm. Plants were grown in a growth chamber (GROW 360/HR) with 8 h light / 16 h dark photoperiod, an irradiance of 80 $mmol \cdot m^{-2} \cdot s^{-1}$ and a day / night temperature of 21 ºC /18 ºC. Plants were watered as needed without fertilization. Rosette diameter was measured every week over 8 weeks. After that, the photoperiod was increased 2 hours every 3 days to reach 16 h of light with a constant temperature of 22 ºC to induce flowering.

3.6. Ecological characterization of coastal and inland regions

3.6.1. Climatic studies

To identify climate variability between regions, we obtained maps of annual precipitation (mm), monthly precipitation (mm), annual mean temperature (ºC), annual evapotranspiration (L) and annual water deficit (L) from the Digital Climatic Atlas of Catalonia (http://www.opengis.uab.cat/acdc/). Temperature data were obtained from 160 weather

stations over a series of 15 years; precipitation data from 257 stations over 20 years and solar radiation from 46 stations over 4 years. Values of each variable at the geographic location for each of the demes used in the study were extracted using MiraMon 7.0 software (Pons, 1994-2011).

3.6.2. Soil studies

For soil analysis, we performed three independent soil analyses per site. pH, water-holding capacity, and texture were measured using fresh soil following the methods described by Carter & Gregorich (2006). Organic matter and carbonate content were analyzed following the procedures described by Black *et al.* (1965). Sulphate content was determined following the work by Rehm & Caldwell (1968). Chloride was measured using a Chloride Ion-Selective Electrode (CRISON).

To characterize the elemental composition of soil, analyses were performed on the 2-mm fraction samples. Five grams of soil was dried for 42 h at 60°C in 50-mL Falcon tubes. Extraction method (adapted from Soltanpour & Schwab, 1977) consisted of a digestion with 20 mL of 1 M NH_4HCO_3, 0,005 M diaminetriaminepentaacetic acid, and 5 mL of pure water during 1 h of shaking on the rotary shaker at low speed. Each sample was gravity filtered through qualitative filter papers until obtaining approximately 5 mL of filtrate, which was transferred into Pyrex tubes; 0,7 mM trace grade c. HNO_3 was added and digested at 115°C for 4,5 h. Each sample was diluted to 6,0 mL with 18 MV of water and analyzed for As, B, Ca, Cd, Co, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Rb, S, Se, Sr and Zn content (ppb) on an Elan DRCe ICP-MS (PerkinElmer Sciex). National Institute of Standards and Technology traceable calibration standards (ULTRAScientific) were used for the calibration.

3.7. Elemental analysis

Plants from the field or the laboratory were sampled by removing 2–3 leaves (1–5 mg dry weight) and washed with 18 MΩ water before being placed in Pyrex digestion tubes. Sampled plant material was dried for 42 hr at 60 ºC, and weighed before open-air digestion in Pyrex tubes using 0,7 mL concentrated HNO_3 (Mallinckrodt AR select grade) at 110 ºC for 5 h. Each sample was diluted to 6.0 mL with 18 MΩ water and analyzed for As, B, Ca, Cd, Co, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Rb, S, Se, Sr and Zn content (ppm) on an Elan DRCe ICP-MS

(PerkinElmer Sciex). NIST traceable calibration standards (ULTRAScientific, North Kingstown RI) were used for the calibration.

3.8. Salinity tolerance assays

3.8.1. Irrigation experiments

Figure 10: *A. thaliana* plant growing in potting mix soil irrigated with nutrient solution.

To assess the fitness response of plants from coastal and inland habitats to elevated salinity 4 plants from 8 coastal and 8 inland demes (Table A2) were cultivated individually in circular pots (10 cm diameter) with potting mix soil. Seeds were sowed on wet soil and the pots covered with PVC film until the seedlings had germinated. Pots with germinated seedlings were placed in a growth chamber (Conviron CMP5090) with 8 h light / 16 h dark photoperiod, an irradiance of 80 $mmol \cdot m^{-2} \cdot s^{-1}$ and a constant temperature of 22 ºC. Plants were watered with 0,5-strength Hoagland solution every 2-3 days. After 2 weeks, all plants were irrigated every 2 – 3 days with 0,5-strength Hoagland solution containing 0, 50 or 100 mM NaCl. After two weeks of treatment the photoperiod was increased 2 h every 3 days until it reached 16 h light / 8 h dark to induce flowering.

3.8.2. Hydroponics experiments

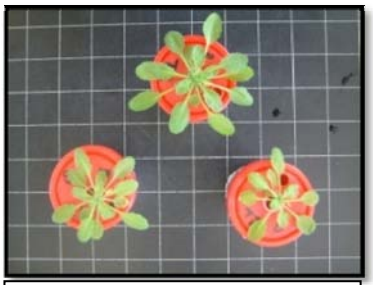


Figure 11: *A. thaliana* plants growing hydroponically in individual pots filled with nutrient solution.

For the hydroponic experiment 20 seeds from 8 coastal and 8 inland demes (Table A2) were sown in 1,5 mL eppendorf tubs filled with vermiculite and distilled water and placed in a growth chamber with 8 h light / 16 h dark photoperiod, irradiance of 80 $mmol \cdot m^{-2} \cdot s^{-1}$ and a constant temperature of 22 ºC. After emergence of the cotyledons the bottom 0,5 - 0,7 cm of each tube was removed to allow roots to grow into 0,5-strength Hoagland solution. When roots of the seedling were 2-3 cm long and the rosette diameter of the seedling was approximately 1,5 cm, six plants from each deme were transferred to individually hydroponic circular containers (50 mL) filled with 0,5-strength Hoagland solution (pH 6,0). The hydroponic solution was changed every third day to maintain a consistent concentration of nutrients in the solution. Salinity treatment was initiated 14 days after transplantation by the

addition of 0, 50 or 100 mM NaCl to the solution. To examine the growth effects of elevated salinity we measured rosette diameter every 2–3 days. These measurements were performed for 18 days. To test for differential accumulation of Na^+ and K^+ in leaves, the entire rosette of each plant was harvested, fresh weight was recorded and samples were stored for ICP analysis.

The last experiment conducted was the crossing of plants from 3 salt-tolerant coastal demes (T2, LLO2 and PO1) with plants from 3 salt-sensitive inland demes (LG5, A1 and V3). We made a total of 18 crossings (9 pairs of crossings) using the same deme as a male and female (e.g. T2 x LG5 and LG5 x T2). Parent plants chosen from coastal demes had *AtFPN2* allele like Ts-1 (CS155) (Morrissey *et al.,* 2009) and plants chosen from inland demes had *AtFPN2* allele like Col-0 (CS6000). One F1 plant from each crossing was genotyped for *AtFPN2* to corroborate that they were heterozygous for this marker.

Seeds from the crossings were harvested and sowed together with parental seeds in 1.5 mL Eppendorf tubs filled with vermiculite and distilled water and placed in a growth chamber with 8 h light / 16 h dark photoperiod, irradiance of 80 $mmol \cdot m^{-2} \cdot s^{-1}$ and a constant temperature of 22 ºC . After emergence of the cotyledons the bottom 0,5 - 0,7 cm of each tube was removed to allow roots to grow into 0,5-strength Hoagland solution. After two weeks, three plants from each crossing and three parental seedlings were transferred to individual hydroponic circular containers (50 mL) filled with 0,5-strength Hoagland solution (pH 6,0). The hydroponic solution was changed every third day to maintain a consistent concentration of nutrients in the solution.

Salinity treatment was initiated 14 days after transplantation by the addition of 0, 50 or 100 mM NaCl to the solution. After two weeks of treatment the photoperiod was increased 2 h every 3 days until it reached 16 h light / 8 h dark to induce flowering. To examine the fitness and growth effects of elevated salinity we measured rosette diameter every 2–3 days and counted the number of siliques at maturity.

3.9. Genotyping (PCR) and expression (qRT-PCR) of *AtSOS1*, *AtHKT1;1, AtMOT1* and *AtFPN2*

DNA was extracted from leaf tissue that had been frozen at -80 ºC. A dCAPS (derived Cleaved Amplified Polymorphic Sequence) marker was developed based on the C/T SNP on *AtHKT1;1* at Chr4:6392276 with forward primer 5'-AAGAGACGGTGATGGCAGAG-3' and reverse primer 5'-GAGGGCGAAATCTTCACCTCCT-3'. Two base pairs mismatch (underlined) was made in the

reverse primer generating a *XhoI* site in the strong allele of *AtHKT1;1* (C at the SNP of Chr4: 6392276; CTCGAG) but not in the weak allele of *AtHKT1;1* (T at the SNP of Chr4: 6392276; CTTGAG).

An SSR marker (Simple Sequence Repeat) was developed based on the deletion in the promoter of *AtMOT1* in Van-0 plants (CS1584) (Baxter *et al.*, 2008) with forward primer 5'-CTCCGGTTATCGCGTTGTAT-3' and reverse primer 5'-ACTGTCGCCATCAAGGTTTT-3'.

AtFPN2 was sequenced in Ts-1 and found to contain an adenine inserted after base pair 1228 of the genomic sequence. This frame shift produces a stop codon 117 amino acids earlier than in Col-0, resulting in truncation of *AtFPN2* (Morrissey *et al.*, 2009). An SSR marker was developed based on this insertion in Ts-1 plants (CS1552) with forward primer 5'-ACATTTGCAGCTTGGGCTAC-3' and reverse primer 5'- CTCCGGTTCTGAGAGGTGAG-3'.

DNA was extracted using 50 mM TRIS (pH 9) and 5 mM EDTA (pH 8). After heating at 95°C for 5 min, 4µl of extract was directly used as a template for PCR. 10 µl PCR reactions contained 2µl 5X Green GoTaq® reaction buffer (Promega), 0.8 µl 25mM $MgCl_2$, 0.8 µl 2.5 mM dNTPs, 0.4 µl 10 mM forward and reverse primer and 0.3 µl home made Taq polymerase. A total 45 cycles PCR was performed with 30 sec at 94°C, 15 sec annealing at 60°C followed by 30 sec extension at 72°C. PCR product was then digested with *Xho*I overnight and separated on 3% agarose gel.

Total RNA was extracted from each one of the samples using the Qiagen RNeasy Plant Mini Kit (http://www.qiagen.com), and DNase digestion was performed during the extraction procedure according to the manufacturer's instructions. Two micrograms of total RNA was used as a template to synthesize first-strand cDNA with random hexamers using SuperScript II Reverse Transcriptase (Invitrogen Life Technologies, http://www.invitrogen.com). Quantitative real-time PCR was performed with the first strand cDNA as a template on a sequence detector system (ABI Prism 7000, Applied Bio-systems) with Maxima SYBR Green qPCR Master Mixes (Thermo Scientific).

For normalization across samples, the expression of the *PP2A* gene (At2g37620) was used with the following primers: PP2A-F, 5'-TAACGTGGCCAAAATGATGC-3' and PP2A-R, 5'-GTTCTCCACAACCGCTTGGT-3'. For *ATSOS1* (At2g01980) transcript quantification the following primers were used: SOS-RTF, 5'-CTGCTTGCTACATTTCTGCTG-3' and SOS-RTR 5'-TGCTTCCTCTCCTTCCTTTTC-3' For At*HKT1* (At4g10310) transcript quantification the following primers were used: HKT-RTF, 5'-TGG GATCTTATAATTCGGACAGTT C-3' and HKT-RTR, 5'-GATAAGACCCTCGCGATAATCAGT-3'.

For each sample, the average value from triplicate real-time PCRs was used to evaluate the transcript abundance. Data was analyzed using the SDS software (Applied Bio-systems version 1,0), following the method of Livak & Schmittgen (2001). Ct values were determined based on efficiency of amplification. The mean Ct values were normalized against the corresponding *ACTIN 1* gene and Ct values calculated as ($Ct_{AtHKT1;1/MOT1}$- Ct_{Actin1}). The expression of *HKT1 and MOT1* was calculated as the $2^{-\Delta Ct}$ method. The final standard error was estimated by evaluating the $2^{-\Delta Ct}$ term using $2^{-\Delta Ct}$ plus standard deviation and $2^{-\Delta Ct}$ minus the standard deviation (Livak & Schmittgen, 2001).

3.10. *AtHKT1;1* and *AtMOT1* studies

3.10.1. Natural habitat sampling

From *A. thaliana* plants collected every year (since 2013 to 2015) in each site, half of the plant was earmarked to ionomic analysis and the other half was used to extract DNA and genotype *AtHKT1* and *AtMOT1* genes of each individual.

Trying to explain the distribution and persistence of these allelic variants, once obtained genotyping results we selected one coastal deme containing plants with the weak (Ts-1 like) and the strong (Col-0 like) allele of *AtHKT1;1* (**T13**); one coastal deme containing plants with the weak (Van-0 like) and the strong (Col-0 like) allele of *AtMOT1* (**T9**) and one coastal deme with plants homozygous for the strong allele of *AtHKT1;1* and *AtMOT1* (**T1**). In these three locations we collected soil samples twice a month (since February to June) to see if there is much variability in elements content between sites over time. As T13, T9 and T1 are sites from Tossa de Mar we obtained monthly precipitation data from the weather station of the town (http://www.meteo.cat) to corroborate if the variability detected in soil elements content is correlated with rainfall.

3.10.2. Field-based reciprocal common garden experiments

After genotyping the plants from 2012 and 2013 collection, we selected two demes containing plants with the weak (Ts-1 like) and the strong (Col-0 like) allele of *AtHKT1;1* (named T13 and JBB) and two demes containing plants with the weak (Van-0 like) and the strong (Col-0 like) allele of *AtMOT1* (named T9 and LLO2). Seed from genotyped plants were sowed on potting mix soil in a growth chamber (GROW 360/HR) with 16 h light / 8 h dark photoperiod, an

irradiance of 80 mmol·m^{-2}·s^{-1}, a day / night temperature of 21 ºC /18 ºC and irrigation of 0,5-strength Hoagland solution once a week, to obtain an enough amount of seeds for the field experiment planned for the following year.

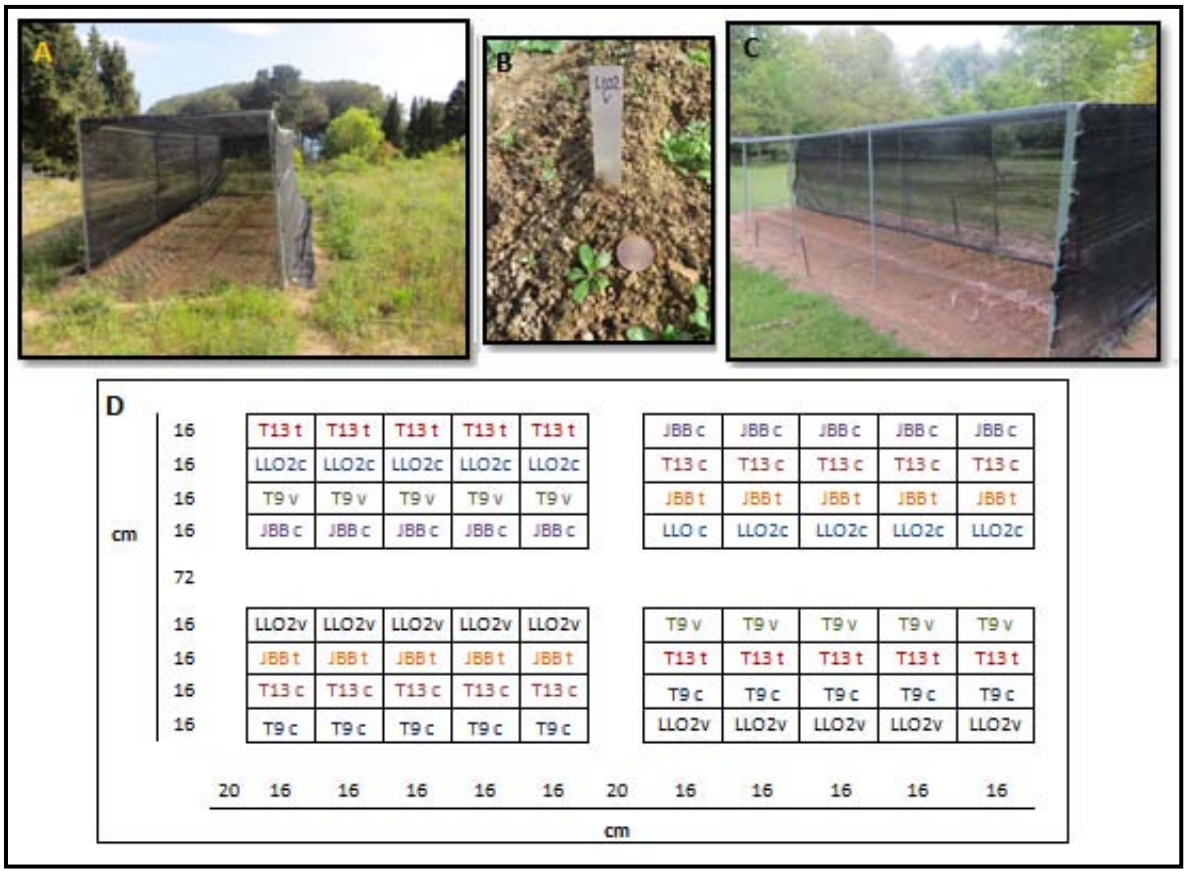


Figure 12. Pictures of common garden plots used in the reciprocal transplant experiments in BLA **(A)** and SCF **(B, C). (D)** Sketch of plant type distribution inside plots.

In March 2014 and 2015, 100 seeds (10 in each square) of each deme/*AtHKT1;1* genotype combination (T13c, T13t, JBBc, JBBt, T9c, T9v, LLO2c, LLO2v) were sown at BLA (coastal) and SCF (inland) common gardens into 16 x 16 cm squares (Figure 12). Two weeks after germination, we left 2 plants in each square. We studied the fitness of 10 plants for each deme/*AtHKT1;1* genotype combination at each site and the other 10 plants were harvested on April of 2014 and 2015 to analyse their leaf ionome. Rosette diameter was measured every week during 2 month and the number of siliques was counted at maturity. To measure flowering time, we annotated the day when the first flower of each plant appeared. During the 3 months of the field experiments, minimum and maximum temperatures, precipitation and soil properties and composition were monitored.

3.10.3. Salinity tolerance assays

The same four demes selected for the field common garden experiment were used to perform irrigation and hydroponics experiment with 0, 50 and 100 mM NaCl to test the salinity tolerance, analyse leaf ionome and quantify expression of *AtHKT1;1* on roots.

For the irrigation experiment, 15 plants of each deme/*AtHKT1;1* genotype combination (T13c, T13t, JBBt, JBBt) were cultivated individually in square pots of 5 x 5 x 10 cm with potting mix soil. Seeds were sowed on wet soil and the pots covered with PVC film until the seedlings had germinated. Pots with germinated seedlings were placed in a growth chamber (Conviron CMP5090) with 8 h light / 16 h dark photoperiod, an irradiance of 80 $mmol \cdot m^{-2} \cdot s^{-1}$ and a constant temperature of 22 ºC. Plants were watered with 0,5-strength Hoagland solution every 2-3 days. After 2 weeks 5 plants of each accession were irrigated once a week with 0.5-strength Hoagland solution containing 0, 50 or 100 mM NaCl. After two weeks of treatment the photoperiod was increased 2 h every 3 days until it reached 16 h light / 8 h dark to induce flowering. Rosette diameter was measured every 3-4 days during 3 weeks and the number of siliques produced was counted at maturation.

For hydroponics experiment, plants of each deme/*AtHKT1;1* genotype combination including Col-0 and *AtMOT1* knockout mutants were sown in 1,5 mL eppendorf tubs filled with vermiculite and distilled water and placed in a growth chamber with 8 h light / 16 h dark photoperiod, irradiance of 80 $mmol \cdot m^{-2} \cdot s^{-1}$ and a constant temperature of 22 ºC. After emergence of the cotyledons the bottom 0,5 - 0,7 cm of each tube was removed to allow roots to grow into 0.5-strength Hoagland solution. When roots of the seedling were 2-3 cm long and the rosette diameter of the seedling was approximately 1,5 cm, 60 (20 per treatment) plants of T13c, T13t, JBBc and JBBt; 48 (16 per treatment) plants of LLO2c, LLO2v, T9c, T9v; 12 (4 per treatment) plants of Col-0 and 12 (4 per treatment) plants of *AtMOT1* mutant were transferred to individually hydroponic circular containers (50 mL) filled with 0,5-strength Hoagland solution (pH 6,0). The hydroponic solution was changed every third day to maintain a consistent concentration of nutrients in the solution. Salinity treatment was initiated 14 days after transplantation by the addition of 0, 50 or 100 mM NaCl to the solution.

To examine the growth effects of elevated salinity we measured rosette diameter every 3–4 days. Leaves and roots from each plant were weighed at the time of harvest. To test for differential accumulation of ions in aerial tissue, 2 leaves of each plant was harvested, dried at 60 ºC during 2 days and stored for ICP analysis. Roots of each plant were put on Eppendorf

tubes, frozen with liquid nitrogen immediately after being cut and stored in the freezer at -80 ºC for subsequent RNA extraction.

In the case of Col-0 and *AtMOT1* mutant plants, ferric-reducing capacity was measured before harvest. Intact plants were pre-treated for 30 min in 1,5 mL of solution A with the following composition (mM) : 2 $Ca(NO_3)_2$; 0,75 K_2SO_4 ; 0,65 $MgSO_4$; 0,5 KH_2PO_4. Then transferred for 1 h to a similar solution that also contained 100 µM Fe 3^+ EDTA and 300 lm ferrozine, pH 5,0 (assay solution). The ferric-reducing capacity was determined by measuring the concentration of Fe^{2+}-ferrozine complex formed, via absorbance measurements at 562 nm in a spectrophotometer (Romera *et al.*, 1999). To calculate reduction capacity (enzyme activity) we used the following formula:

$$\textbf{RC}\ (\text{nmol Fe}^{2+} \cdot \text{g}^{-1}\ \text{root f. Wt h}^{-1}) = \frac{V(L) \times OD_{562nm}(cm^{-1})}{\varepsilon.Coef \times time(h) \times P_{root.F.Wt}}$$

3.11. Statistical analyses

To determine whether plants shared alleles across the coastal and inland habitats we used the software STRUCTURE 2.3.3 (Pritchard *et al.*, 2000). The number of genetic clusters (*K*) was set from 1 to 12 and 10 runs were performed for each *K* with 1.000.000 MCMC iterations (an initial 1.000.000 iterations were discarded as burn-in) using the admixture ancestry model with correlated allele frequencies. In addition, the analysis was repeated with the same parameters but also including *a priori* coastal and inland location as prior information (LOC-PRIOR setting) to identify any further structure not detected using the standard model (Hubisz *et al.*, 2009). STRUCTURE HARVESTER 0.6.94 (Earl *et al.*, 2012) was used to collate the results and infer the best supported *K* using the ΔK statistic (Evanno *et al.*, 2005). These analysis were conducted by Busoms and Douglass (Busoms *et al.*, 2015)

As an alternative, to obtain a cladogram, a progressive aligment of 425 SNPs from 40 coastal and 58 inland plants was performed in Clustal X2 software (Larkin *et al.*, 2007). Pairwise genetic distance between individuals and between demes was calculated using the Maximum Likelihood statistical method and Jukes and Cantor substitution model in MEGA 6.0 (Tamura *et al.*, 2013). F_{ST} was calculated following the methods of Weir & Cockerham (1984). Analysis performed by Busoms and Bomblies (Busoms *et al.*, 2015).

To assess the 'home vs. away' and 'local vs. foreign' criteria of local adaptation in the reciprocal transplant experiments we used a Poisson log-normal generalised linear mixed effects model (GLMM) with a log link to model silique number. The models included transplant site and origin of deme and their interaction as fixed effects and deme identity as a random effect. Orthogonal contrasts were constructed to test both criteria and P values associated with these contrasts were adjusted using Tukey's all-pair comparisons to control for type 1 error. We also tested for the effect of local adaptation on the number of siliques produced by *A. thaliana* using a modified version of sympatric vs. allopatric (SA) test described in Blanquart *et al.* (2013). Briefly, we used a Poisson log-normal general linear mixed model (GLMM) with a log link and included transplant site, deme and a sympatric vs. allopatric indicator variable as fixed effects and a deme x habitat interaction as a random effect to account for potential non-independence between individual plants within demes in each habitat. Analysis performed by Douglas (Busoms *et al.*, 2015).

We used a linear mixed effects model (LMM) to investigate changes in growth (measured as rosette diameter) in the reciprocal transplant experiments. The models included a three way interaction between time, transplant site and origin of deme as fixed effects and included a time varying random effect of individual plants nested within deme. Initial model validation detected heterogeneity of variance associated with an increase in variance over time. We incorporated a variance covariate into the model as the exponent of day to account for this heterogeneity. To assess changes in growth in the NaCl experiments we used a LMM with a three-way interaction between time, NaCl treatment and deme origin as fixed effects and used the same random effects and variance covariate structure as for the reciprocal transplant experiment described above. The rosette diameter was square root transformed and time was included as a second order polynomial term to account for non-linear patterns in growth and also centred at day 0 in all models.

Differences in Na^+, K^+ and the ratio Na^+/K^+ leaf concentration in the hydroponic experiment were assessed using a LMM with a two-way interaction between NaCl treatment and origin of deme as fixed affects and a deme random effect. The significance of fixed effects parameters in all models was assessed using likelihood ratio tests comparing nested models that had been fitted using maximum likelihood. Final models were refitted using restricted maximum likelihood prior to interpretation. All statistical analyses were conducted using the R statistical environment (R core Team, 2013). GLMMs were fitted using the lme4 package (version 1.1-7; Bates *et al.*, 2014) and LMMs fitted using the nlme package (version 3.1-118; Pinheiro *et al.*, 2014). Analysis performed by Douglas (Busoms *et al.*, 2015).

One-way ANOVA was used to test for significant differences between means of fitness, elemental contents of soil and leaf material and gene expression. Repeated Measures ANOVA was used to test statistical differences between means of growth over time. To test for correlations between soil properties and distance to the sea a Bivariate Fit was conducted. To perform multiple comparisons of group means we used Tukey HSD and Dunnett's test. Tukey HSD was used to compare all pairs of means because it protects the significance tests of all combinations of pairs. Dunnett's test was used to compare a set of means against the mean of a control group. The LSDs that it produces are between the Student's *t* and Tukey-Kramer LSDs, because they are sized to refrain from an intermediate number of comparisons (Proust, 2015). All analyses were preformed in JMP 12.0 (SAS, Cary, NC, USA).

4. Results

4. Results

4.1. Identification of coastal and adjacent inland populations of *A. thaliana* using a SDM

To help systematically identify coastal and adjacent inland demes of *A. thaliana* in Catalonia, Spain, we generated a Species Distribution Model (SDM), also known as an environmental or ecological niche model (Guisan & Zimmermann, 2000). These models represent an empirical method to draw statistical inferences about the environmental factors that control the distribution of species, with factors such as climate, soil and abiotic interactions being important (Coudun *et al.*, 2006; Meier *et al.*, 2010b). Results of these models are projected onto a map of the study area to show the potential geographic distribution of the species in order to predict suitable habitats and discover new populations (Williams *et al.*, 2009).

Twenty geographically referenced ecological and environmental variables (Table 2) across Catalonia were analysed in combination with the occurrence data of 36 *A. thaliana* demes identified in the region during a survey in spring 2007 (Map A1). Of the 20 variables analysed altitude, geology and land uses (Maps A2, A3 and A4) were identified as predictive for the occurrence of *A. thaliana*. The SDM was created by identifying the overlap area of these predictive variables to generate a map of locations suitable for the occurrence of *A. thaliana* (Figure 13). Using this approach, 26 spatial polygons, totalling an area of 517 km^2, were identified as potential areas where *A. thaliana* demes may occur across Catalonia. Twenty four sites outside of these predicted locations were also chosen randomly using a 5 x 5 km grid (Map A5).

In 2011 all locations predicted to contain *A. thaliana* from the SDM, and those locations chosen randomly outside the SDM where surveyed. During this survey 46 *A. thaliana* demes were located in 16 of the 26 areas predicted to contain *A. thaliana* by the SDM, of which 22 demes were coastal and 24 inland (Figure 13 and 14). Twenty six of these demes were newly identified, and only 20 of the original 36 demes identified in 2007, and used to build the SDM, were re-identified (Table A1).

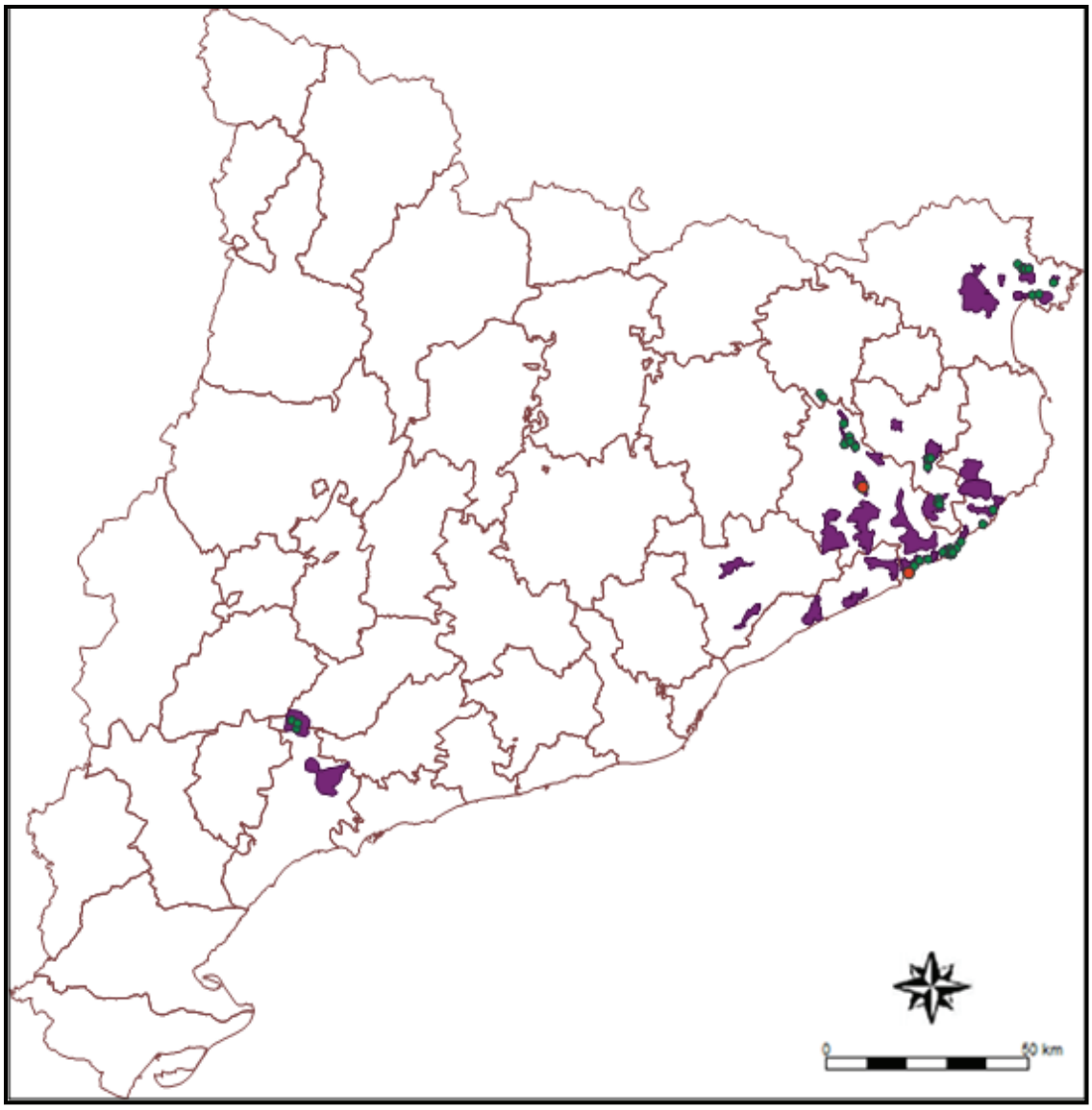


Figure 13: Map of potential geographic distribution of *A. thaliana* (purple polygons) and confirmed populations (green points) including the two sites chosen to perform reciprocal transplant experiments at Blanes and Santa Coloma de Farners (orange points).

In 2012 a further survey was performed which identified seven new demes, and found that nine demes identified in 2011 had disappeared due to human disturbance (five demes) or of unknown causes (four demes) (Table A1). In total, 44 demes detected in 2012 were sampled, 20 from coastal and 24 in adjacent inland areas (Figure 14). The majority of the demes are in degraded areas that are strongly influenced by human activity and therefore at continuous risk of extinction.

With the fragile nature of many of these demes in mind we selected 26 representative demes that we considered to have an elevated chance of persisting due to characteristics of the habitat and large population size. In support of the usefulness of the SDM we note that in the

2011 survey no *A. thaliana* were found in the 24 locations selected to be outside the SDM (Figure 13), and only three demes were found outside the SDM in the 2012 survey.

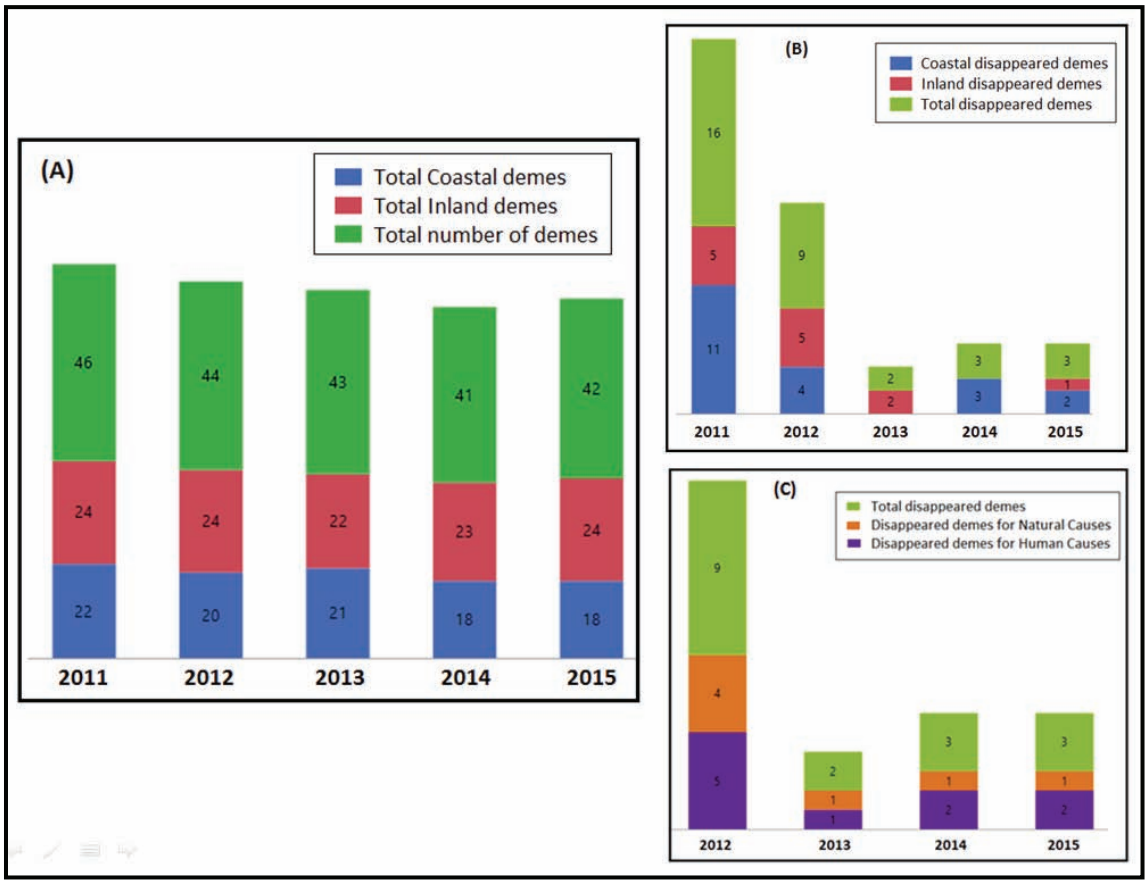


Figure 14: (A) Total number of populations found in each survey since 2011 to 2015 (green bars) and classification between coastal (< 3 Km from the sea, blue bars) and inland (> 3 km from the sea, red bars). **(B)** Total number of populations that disappeared in each year starting from our 2007 base-line survey (green bars) and their provenance (coastal, blue bars; inland, red bars). **(C)** Total number of populations that disappeared since 2012 to 2015 (green bars) from our survey in 2011 and possible cause of disappearance (human causes, orange bars; natural causes, purple bars).

In 2012 we selected these 26 populations for further investigation. However, our *A. thaliana* surveys and sampling continued every spring until 2015. Figure 14 shows that the total number of *A. thaliana* demes identified each year is quite stable. Even though each year two or three demes are lost (usually coastal populations due to human causes) (Figure 14 B and C), we were able to find a similar number of new demes or demes that had disappeared, but later re-established themselves from the soil seed bank. If the seed bank remains intact, seeds shed at least three-years previously can remain viable (Falahati-Anbaran *et al.*, 2014; Lundemo *et al.*, 2009; Pico *et al.*, 2012).

4.2. Extent of allele sharing between coastal and inland demes

The evolution of locally adapted populations represents a balance between the strength of divergent selection related to environmental differences and the extent of gene flow between demes in different environments. The development of genetically-based differentiation in fitness-related traits between demes that are connected by gene flow therefore represents strong evidence of ongoing (or very recent) locally-adaptive natural selection. To evaluate the extent of gene flow between inland and coastal *A. thaliana* demes within the study area in collaboration with Kirsten Bomblies (Harvard University) we genotyped 86 individual plants from 28 coastal and inland demes at 425 genome-wide single nucleotide polymorphisms (SNPs), as previously used by Bomblies *et al.* (2010). Of these SNP markers 401 were polymorphic and 356 had a minor allele frequency > 5 % in this study. Overall, these SNP markers had a level of heterozygosity of 0.2 % across all markers and individuals. We used the STRUCTURE software package (version 2.2.3) to investigate population structure in collaboration with Alex Douglas (University of Aberdeen).

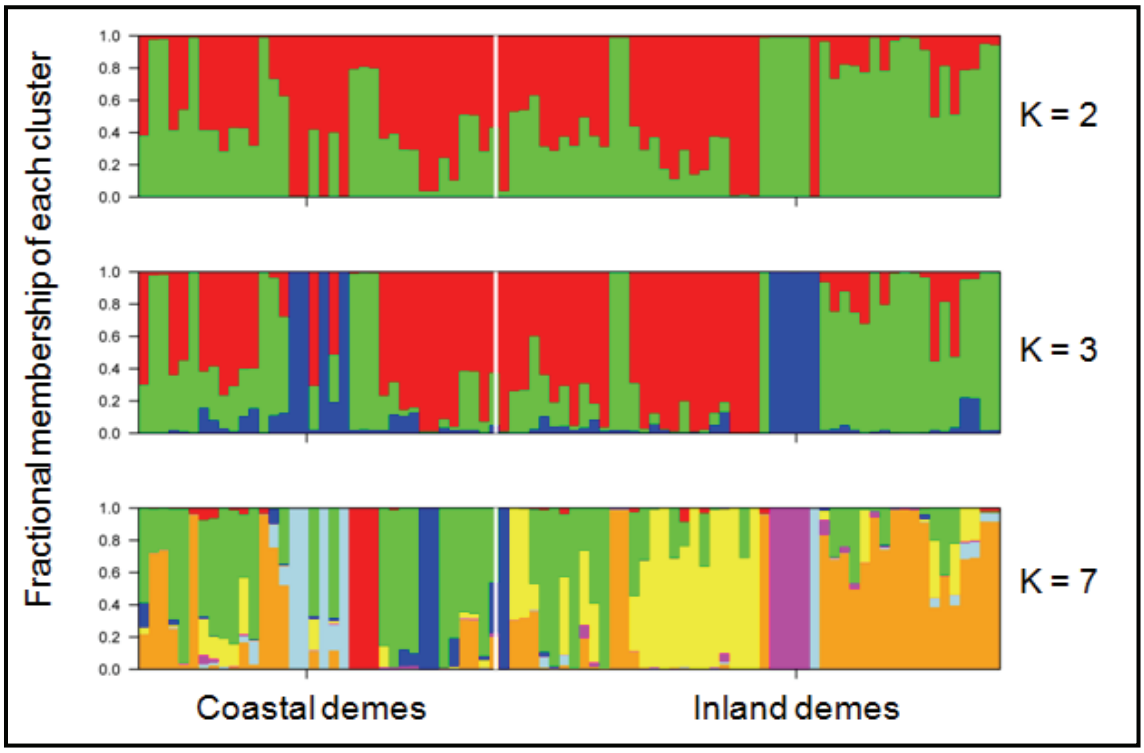


Figure 15: Estimation of genetic structure within the Catalonian *A. thaliana* population. Each vertical bar represents an individual plant genotyped at 425 genome-wide SNP markers, and each bar is divided into *K* coloured sections that indicate the fractional membership of an individual in *K* clusters based on its genotype. The figure of each *K* is based on the analysis with highest probability for that value of *K*. Vertical white line divides demes of coastal and inland origins. See Table A2 for details of which demes were genotypes.

Based on the ΔK statistic, the best supported number of *a posteriori* genetic clusters was *K* = 7 for the standard admixture model (Figure 15, Table 3). However, this stratification is not related to the distribution of coastal and inland demes since when individuals are clustered into two subgroups (*K* = 2), based on shared alleles, these groups do not stratify between demes of inland and coastal origin (Figure 15). The lack of clear population structure delineating coastal verses inland habitats shows that there is not a genome-wide signature associated with these habitats. For *K* = 3 clusters we do observe that the inland demes appear to cluster into two groups (Figure 15). However, this subgroup structure within the inland demes is not related to their distance from the coast. Analysis using the same parameters but also including a priori sampling location (inland or coastal) as prior information (LOC-PRIOR setting) resulted in the same pattern of clusters.

Table 3: The mean logarithmic likelihood of 10 runs at each *K* and the corresponding *ΔK* statistic (peak value in bold)

K	LnP(K)	ΔK
1	-34817.11	-
2	-33102.57	2.78
3	-31280.89	1.95
4	-30105.85	0.09
5	-28905.22	0.48
6	-27477.83	3.40
7	-26551.27	**4.14**
8	-25712.01	0.64
9	-25102.48	0.91
10	-24176.93	0.21
11	-23098.28	1.44
12	-22277.81	-

STRUCTURE assumes free outcrossing of individuals, and so due to the primarily selfing nature of *A. thaliana* research we assessed its robustness by also performing an analysis using the InStruct software package (Gao *et al.*, 2007), which is more appropriate in our case since it does not assume full outcrossing or Hardy-Weinberg equilibrium. This analysis produced near identical outputs to that obtained with STRUCTURE (Figure 15), and both analyses show that there is no clear stratification of the population between demes of inland and coastal origins.

An alternative approach to the identification of population structure is the use of nonparametric clustering analysis by genotype, an approach that makes no assumptions about the demography or natural history of the underlying populations (Bomblies *et al.*, 2010). Using such nonparametric clustering neither did we find any evidence of local genetic stratification between the coastal and inland demes (Figure 16). In this nonparametric clustering the tips of clusters sometimes grouped by geography, however the deeper nodes of the clusters did not. Such a pattern of population structure is similar to that previously observed for genotypic variation in local populations of *A. thaliana* from the Tübingen area in Germany (Bomblies *et al.*, 2010), and suggests that localized differentiation can occur despite clear differentiation by geography observed at larger continental and regional scales for *A. thaliana* across its native Eurasian habitat (Beck *et al.*, 2008; Schmid *et al.*, 2006, Nordborg *et al.*, 2005; Platt *et al.*, 2010; Brennan *et al.*, 2014; Cao *et al.*, 2011; Long *et al.*, 2013; Picó *et al.*, 2008).

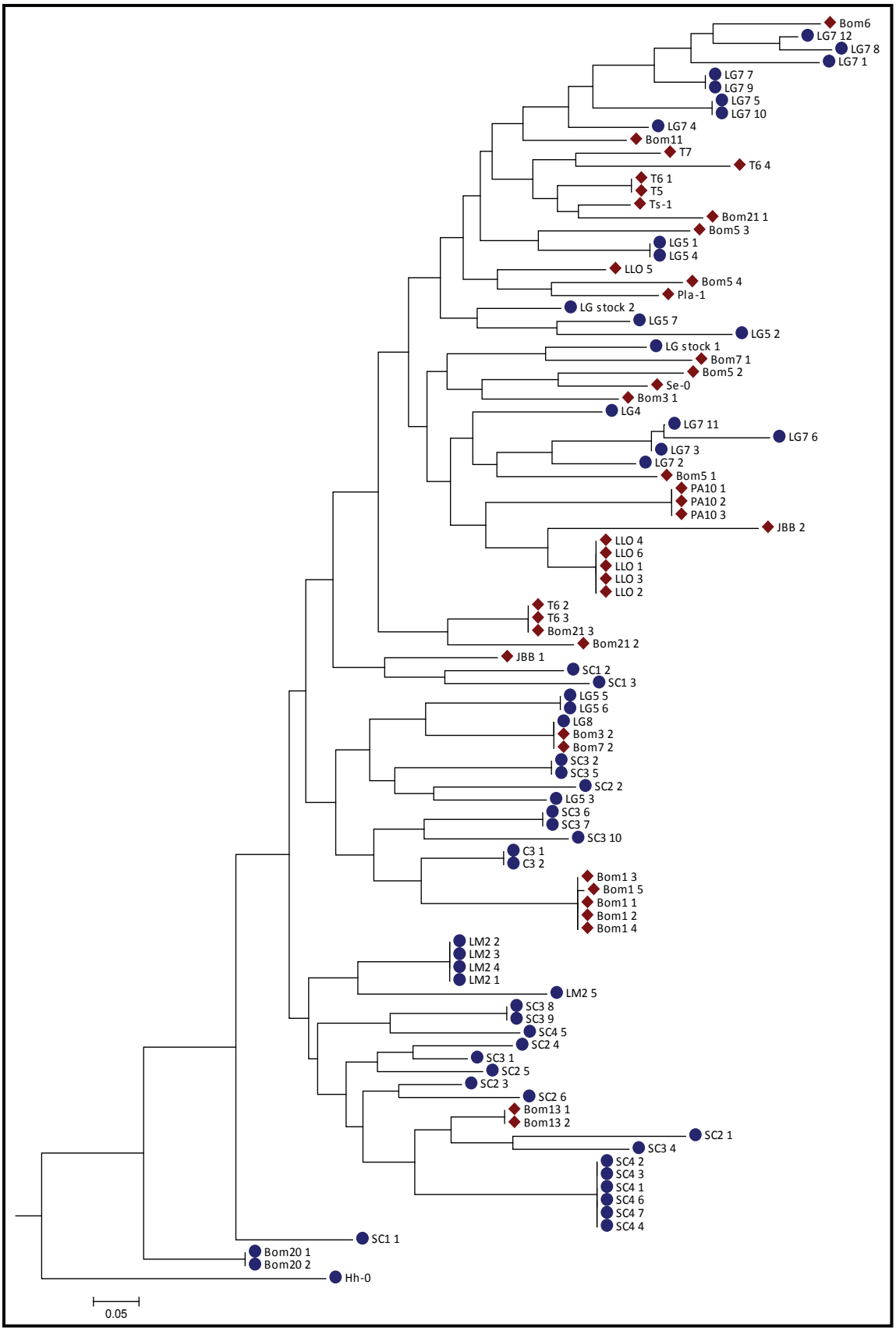


Figure 16: Clustering of individual plants from coastal and inland demes by genotype. Cladogram of 98 plants from 17 coastal (40 plants, red rhombus) and 13 inland (58 plants, blue circles) demes using 425 SNP markers aligned in ClustalX2 and performed using *MEGA 6.0*.

Furthermore, in collaboration with Kirsten Bomblies (Harvard University) we observed that the average fixation index (F_{ST}) (Holsinger & Weir, 2009) calculated across all 425 SNPs between individuals from coastal and inland habitats was found to be 0,04. This low average F_{ST} value of 0,04 between our coastal and inland demes is consistent with both types of clustering analyses indicating that there is little, if any, genotypic differentiation by geography between our sampled habitats. Even though the density of markers is too low to conduct any type of association mapping analysis, two markers were outliers with Fst > 0,25 (Loc 107, Chr1: 27634939 (Fst= 0,386) and Loc 180, Chr2: 16908054 (Fst= 0,428)) so we decided to make a list of candidate genes covering region within 200 kbp on either side of each marker (Table A3).

4.3. Evidence for coastal and inland local adaptation

One of the best methods to detect local adaptation is to perform reciprocal transplant experiments in the field (Kawecki & Ebert, 2004) testing the mean fitness of plants in their 'home' and 'away' habitats. Following this approach, in 2013 and 2014 we performed reciprocal transplant experiments at the Marimurtra Botanic Garden, Blanes (41º 40' 37,64" N, 2º 48' 3,86" E), a site representative of the coastal environment, and at the Forestry School of Santa Coloma de Farners (41º50'41,04" N, 2º40'36,13" E) a representative inland site (Figure 17) 23,52 km from Blanes. Both sites are within the area of our previous collections of *A. thaliana* demes and fall within our SDM. In both years we grew together 10 plants from 10 coastal demes, and 10 plants from 10 inland demes in common gardens in the field in the natural substrate at each location. These experiments were started in the field in March to mimic the natural spring flush of reproductive plants that occurs in the localised region of our study (Montesinos *et al.*, 2009).

As a reasonable proxy for the fitness of each plant we counted the number of fruits (siliques) produced, and also indirectly by monitoring growth as rosette diameter weekly throughout the plants development. For across site comparisons, in both 2013 and 2014, coastal plants performed better in their home environment when fitness was assessed as silique number (Figure 17 A and B; 2013: *z* value = 4,03, P < 0,001 and 2014: *z* value = 19,77, P < 0,001), whereas inland plants performed better in their home environment in 2013 (Figure 17 A; *z* value = 6.91, P < 0,001) but not in 2014 (Figure 17 B; *z* value = 0,53, P = 0,947). For within site comparisons, coastal plants outperformed inland plants in silique production when both were grown together in the coastal environment in 2013 (Figure 17 A; *z* value = 6,27, P < 0,001) and 2014 (Figure 17 B; *z* value = 8,04, P < 0,001), whereas inland plants displayed a significant

fitness advantage compared to coastal plants, assessed as silique production, when both where grown inland in 2014 (Figure 17 B; *z* value = 4,36, P < 0,001), but not in 2013 (Figure 17 A; *z* value = 1,50 P = 0,403).

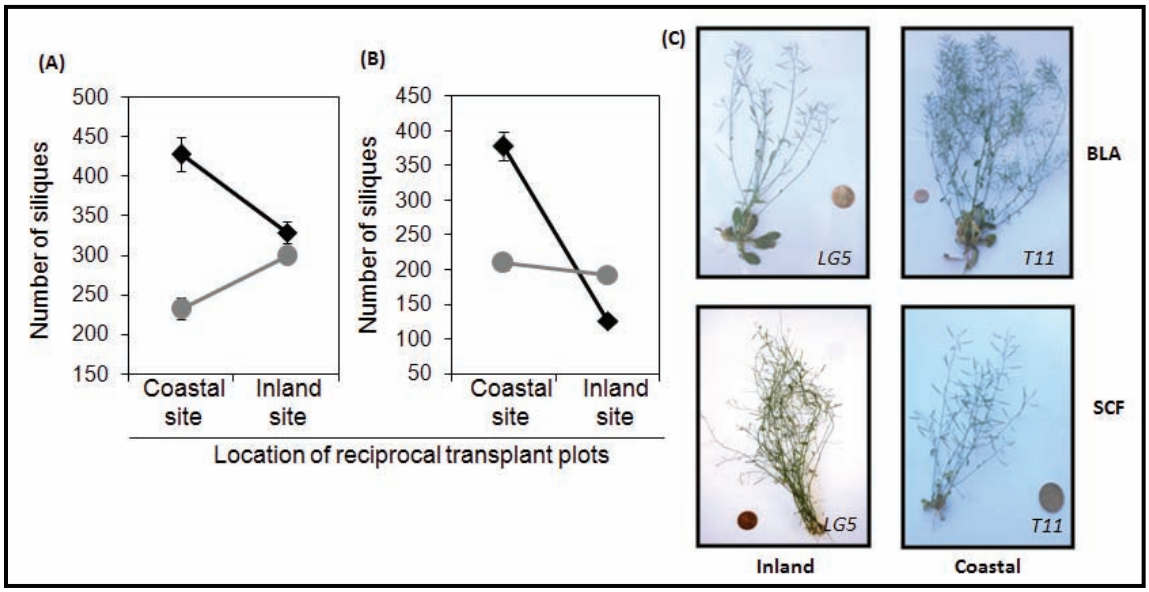


Figure 17: Mean fitness of *A. thaliana* plants from coastal and inland habitats measured as silique number in both field common garden sites. Plants from coastal (black diamonds) and inland (grey circles) demes cultivated in the coastal common garden in Blanes (BLA) and the inland common garden in Santa Coloma de Farners (SCF) in 2013 **(A)** and 2014 **(B)**. **(C)** Pictures of two plants from the same coastal deme (T11) and two plants from the same inland deme (LG5) growing in SCF and BLA common garden sites when they were harvested for counting the number of siliques.

As an alternative approach for the estimation of local adaptation in reciprocal transplant experiments we also followed the statistical approach recommended by Blanquart *et al.* (2013). Briefly, a generalised linear mixed model was used to statistically control for habitat and deme effects whilst estimating the difference in number of siliques produced by plants grown sympatrically (in their home location) to those grown allopatrically (not in their home location). Using this method we observed a significant increase in the number of siliques produced by plants grown sympatrically compared to those grown allopatrically in our 2013 (*L* ratio: 26,29, df = 1, P < 0,001) and 2014 (*L* ratio: 79,79, df = 1, P < 0,001) field experiments (Figure 17 A and B).

Although growth is not as good a proxy for fitness as silique production, growth can provide further evidence of a plants performance in a particular environment. We therefore measured the growth of all plants in our reciprocal transplant experiments as rosette diameter weekly during our field experiments in both 2013 and 2014. This growth data supports our conclusion

of local adaptation, consistent with the silique production data. In both 2013 and 2014 we observed a significant three-way interaction between time, transplant site location and origin of deme (2013: *L* ratio = 51,34, df = 2, P < 0,001; 2014: *L* ratio = 28,24, df = 2, P < 0,001). 'Local' plants grew larger than 'foreign' plants at both our coastal and inland sites (Figure 18), with the exception of the inland site in 2013, in which plants from both coastal and inland demes grew similarly (Figure 18). The pattern of plant growth in our reciprocal transplant experiments is therefore fully consistent with our conclusion from the silique production data that coastal and inland plants are locally adapted.

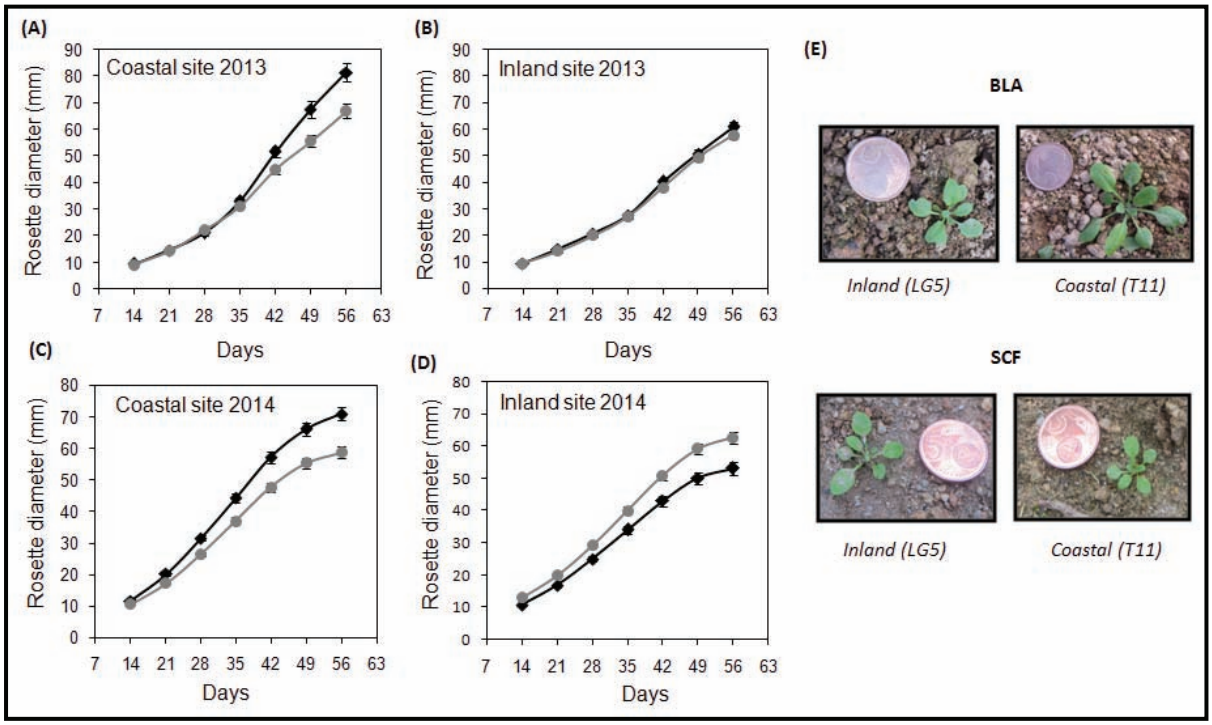


Figure 18: Mean fitness of *A. thaliana* plants from coastal and inland habitats measured as growth (rosette diameter) in both field common garden sites. Plants from coastal (black diamonds) and inland (grey circles) demes cultivated in the coastal common garden in Blanes **(A and C)** and the inland common garden in Santa Coloma de Farners **(B and D)** in 2013 **(A and B)** and 2014 **(C and D)**. Data represents the mean ± SE (n = 10 plants per deme and 10 demes from coastal and inland habitats, see Table A2 for details). **(E)** Pictures of two plants from the same coastal deme (T11) and two plants from the same inland deme (LG5) growing in SCF and BLA common garden sites after 35 days from sowing.

4.4. The agent of divergent selection between coastal and inland habitats

4.4.1. Controlled-environment common garden assay

Field tests are ambiguous as to the cause of differential survival of different genotypes. Thus, we tested if the environmental factor driving differential fitness is the soil at the two field common garden locations. We excavated soil from both the Blanes (coastal) and Santa Coloma

de Farners (inland) field sites in 2013 and used this soil to grow 10 plants from 10 coastal and 10 inland demes under controlled environmental conditions (Table A2). Fitness of the individual plants was assessed as silique production and indirectly as growth (measured as rosette diameter).

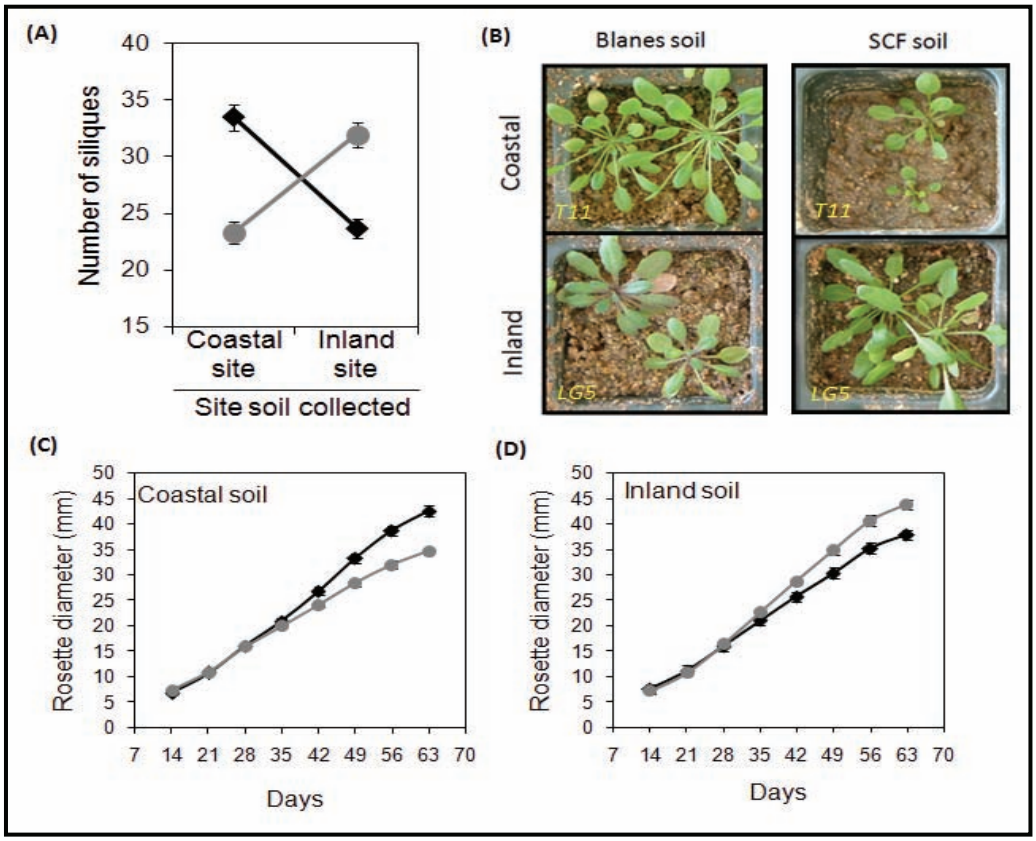


Figure 19: (A) Plants from coastal (black diamonds) and inland (grey circles) demes cultivated in a controlled-environment common garden in soil excavated from the sites used for the coastal and inland common gardens in Blanes **(C)** and the inland common garden in Santa Coloma de Farners **(D). (B)** Pictures of plants from the same coastal deme (T11) and plants from the same inland deme (LG5) growing in soil from SCF and BLA after 35 days from sowing.

We observed a clear signal of local adaptation between plants from coastal and inland habitats when fitness was measured as silique production. Both coastal and inland demes outperformed 'foreign' demes when grown together in their local soil (Figure 19 A; coastal: *z* value = 5,07, P < 0,001, inland: *z* value = 3,052, P < 0,01), and coastal and inland demes performed best when growing in their home soil (Figure 19 A; coastal: *z* value = 6,959, P < 0,001, inland: *z* value = 6,906, P < 0,001).

Using the statistical approach recommended by Blanquart *et al.*, (2013) we observed a significant increase in the number of siliques produced by demes grown sympatrically

compared to those grown allopatrically whilst controlling for sources of variation associated with soil and deme effects (*L* ratio: 55,62, df =1, P < 0,001). From this we conclude that differences in the physical and/or chemical properties of the coastal and inland soils are driving the divergent selection, which in turn gives rise to local adaptation. Assessment of plant performance as growth also revealed that relative to the source of the soil, plants from 'local' demes always grow better than plants from 'foreign' demes as reflected in a significant three-way interaction between time, soil source and origin of deme (Figure 19 C and D; *L* ratio = 91,98, df = 2, P < 0,001). These growth data are fully consistent with our conclusion from the silique data that differences in the soil are responsible for the local adaptation we observe.

4.4.2. *Arabidopsis thaliana* natural habitat studies

Climate is often assumed to be the most determinant ecological factor in plant species distribution. However, as suspected after verifying that no climatic factor had enough power to be included in the SDM created to locate *A. thaliana* populations, no differences between regions were detected in any of the bioclimatic variables analysed (Table A4). Rainfall tends to be higher in inland (Figure 20). However, differences between monthly precipitation data were not statistically significant (*F* ratio = 2,82 , df = 1, P = 0,0935). Nevertheless, clear differences were detected among years (*F* ratio = 30,2, df = 2, p < 0,001) and months (F ratio = 43,6, df = 5, p < 0,001). It is important to consider this fact because depending on the year and the time that the samples are collected soil composition can be quite different due to lixiviation effects.

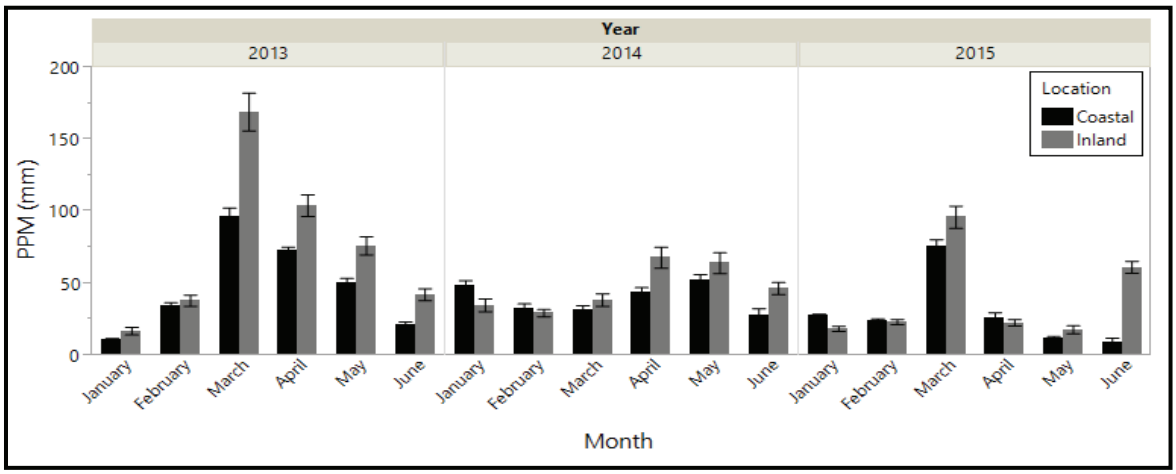


Figure 20: Mean ± Standard Error of monthly precipitation (PPM, mm) of 13 coastal sites (black bars) and 13 inland sites (grey bars) of *A. thaliana* demes from January to June of 2013, 2014 and 2015.

Coastal and inland soils in our study region do not differ in their geological base, given that all the sites were located on gravel, granidiorite or granitic rocks that tend to create siliceous

soils. However, we detected relevant differences in properties of soils collected in 2013, 2014 and 2015 from the sites of 26 *A. thaliana* demes within the study area. Coastal soils are sandier, have a lower water holding capacity than inland soils, and show a clear cline in water holding capacity being lowest closest to the sea and increasing inland (Figure 21A).

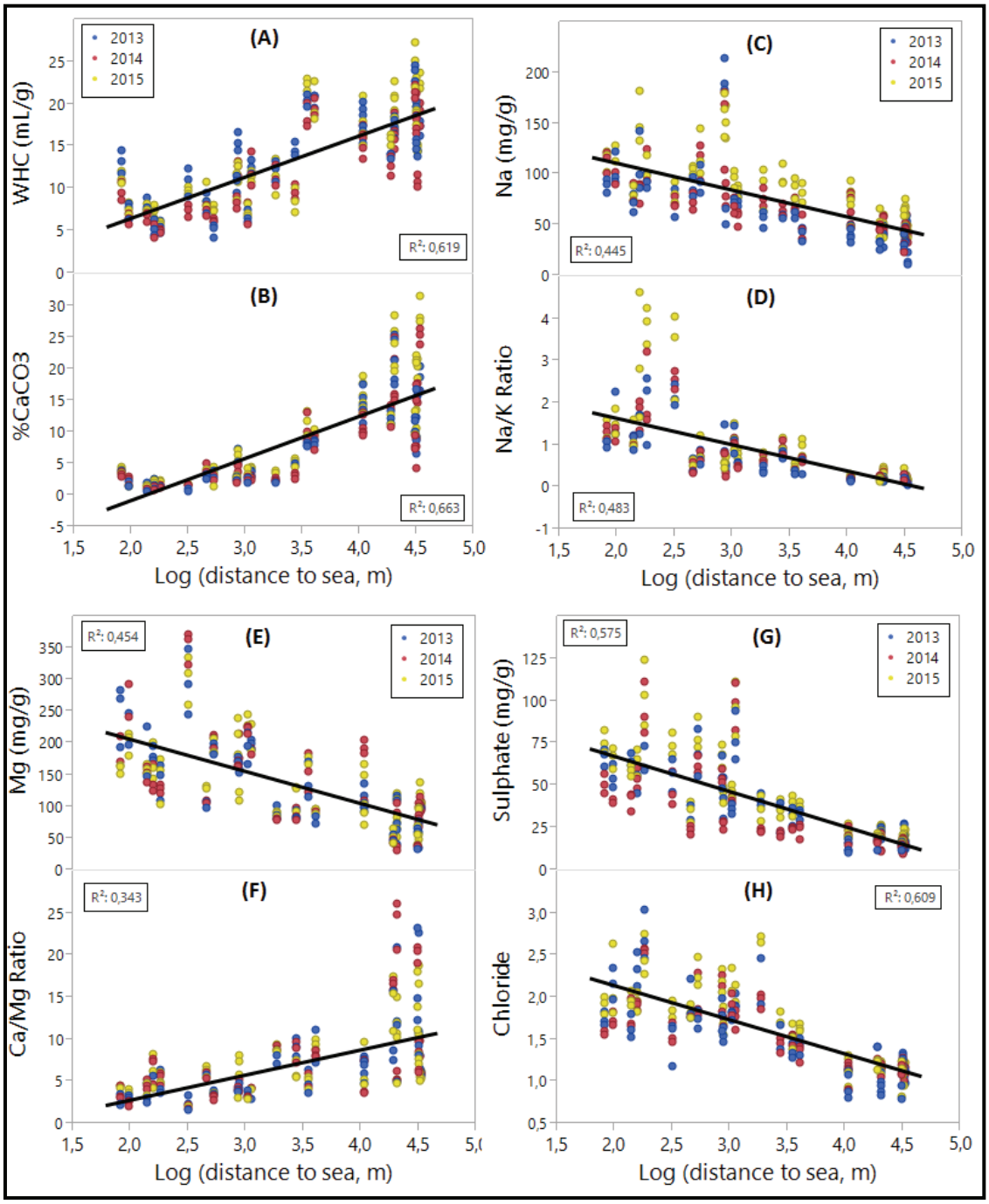


Figure 21: Clines of **(A)** Water Holding Capacity (WHC, mL/g); **(B)** Carbonate content ($CaCO_3$, %); **(C)** Na ($mg \cdot g^{-1}$); **(D)** Na/K Ratio; **(E)** Mg ($mg \cdot g^{-1}$); **(F)** Ca/Mg Ratio; **(G)** Sulphate ($mg \cdot g^{-1}$); **(H)** Chloride ($mg \cdot g^{-1}$) from soil samples collected in March of 2013, 2014 and 2015 and their relationship with distance to the sea (logarithm of meters to sea). Data includes three samples of soil per site and year (Table A2).

Carbonate content was low overall, but somewhat higher in the inland soils (Figure 21B), probably because they were located a short distance from limestone formations. Although coastal soils have a lower pH and less organic matter on the matrix, no significant clines exist for either soil pH or organic matter (R^2 < 3, Table A5). The concentration of Na, Mg, chloride and sulphate are significantly elevated (*F* ratio > 10, df = 1, P < 0.003, Table A5) in coastal soils compared to inland soils (Table A5), with clines exiting for all four solutes, highest closest to the sea and declining inland (Figure 21 C, E, G, H). This is expected, as these are the major inorganic solutes in seawater (Pilson, 1998).

Both Na and Mg are required by plants but can be toxic at high concentrations. Furthermore, both Na and Mg also compete for uptake with the essential plant nutrients K and Ca (Alam, 1999). We therefore also assessed the ratio of Na/K and Ca/Mg in the soils. The concentration of K is significantly elevated in inland soils compare to coastal soils (Table A5). The Na/K ratio was found to be significantly different between coastal and inland habitats (Table A5) and negatively correlated with distance to the sea (Figure 21D), with soils closest to the sea having the highest Na/K ratio. For Ca/Mg the ratio was also significantly different between coastal and inland soils in all years of analysis (Table A5), and found to be lowest in soils closest to the sea and increasing inland (Figure 21F). No significant difference between coastal and inland soil were observed for Ca (Table A5).

No significant clines were found for any other mineral nutrient but we detected that coastal soils are richer in Mo, Se and Sr and inland soils have higher concentrations of As, Cd, Co, Fe, Ni and Rb (Table A5).

To determine if the varying mineral contents of the coastal and inland soils impact the mineral nutrient homeostasis of *A. thaliana* in these habitats, we collected leaf material from the 13 inland and 13 coastal selected demes in March of 2013, 2014 and 2015 (Table A2). Elemental concentrations in leaves of the sampled plants were analysed by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) by John Danku (University of Aberdeen), and were found to generally reflect the concentration of these elements in the soils.

In both 2013 and 2014 leaves of plants collected from coastal demes contained significantly higher (*F* ratio > 10 , df = 1, P < 0.001) concentrations of Na^+ and Mg^{2+} and Na^+/K^+ ratio than leaves from plants collected from inland demes over years (Figure 22, Table A6). In contrast, Ca^{2+} and K^+ concentration in leaves of plants from coastal demes was significantly reduced (*F* ratio > 10 , df = 1, P < 0.001) compared to inland demes (Figure 22, Table A6). This is likely due to competition for uptake of Ca^{2+} with the elevated Mg^{2+} in the coastal soils, leading to

significantly increased Ca^{2+}/Mg^{2+} ratios in plants growing in inland habitats (Figure 22, Table A6).

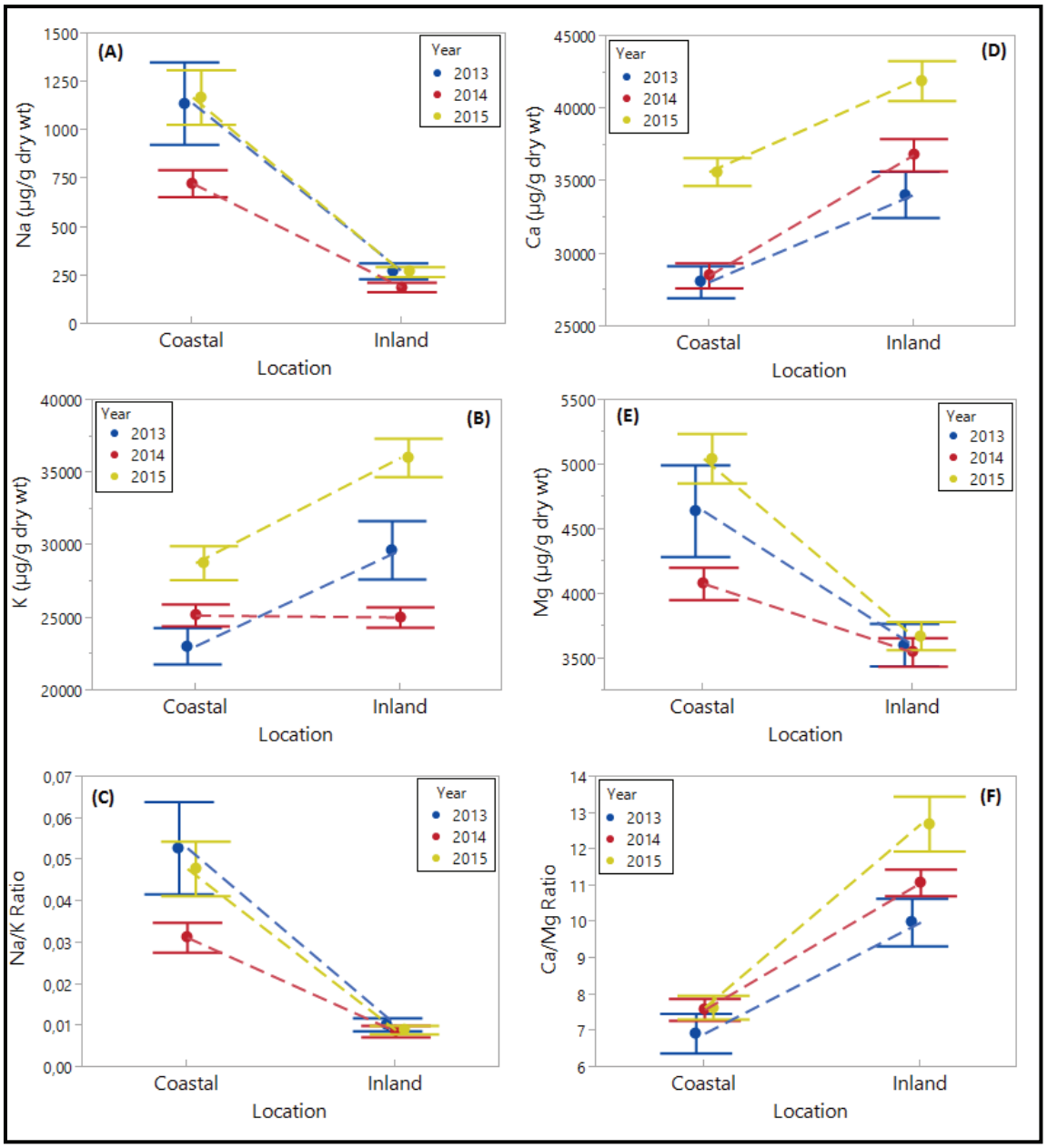


Figure 22: Differentiation in mineral nutrient content of 13 coastal and 13 inland *A. thaliana* demes growing in their native habitats. Leaf concentrations of **(A)** Na^+, **(B)** K^+, **(D)** Ca^{2+}, **(E)** Mg^{2+} (µg· g^{-1}, dry weight) and **(C)** Na^+/K^+ and **(F)** Ca^{2+}/Mg^{2+} ratios from tissue collected in the field in March 2013 (blue circles), March 2014 (red circles) and March 2015 (yellow circles). Data represents the mean ± SE (n = 26 coastal and 26 inland plants in 2013; 104 coastal and 104 inland plants in 2014 and 65 coastal and 65 inland plants in 2015, see Table A2 for details).

Of the other nutrients analysed, we detected that coastal plants tend to accumulate more Mn and Rb in their leaves and less Co than inland plants (Table A6). Clearly, variation in the mineral

content of coastal and inland soils impacts the mineral nutrient homeostasis of *A. thaliana* growing in these different habitats in a consistent manner over multiple years. The largest difference is in Na^+ concentration, which varies consistently across habitats by several percent (Figure 22, Table A6). Such observations make soil salinity a strong candidate as an agent driving divergent selection between adjacent coastal and inland *A. thaliana* populations in Catalonia.

4.4.3. Reciprocal transplant experiment

In the month of April in 2013 and 2014, 10 plants from each deme cultivated in the reciprocal field experiment were harvested for *AtHKT1;1* and *AtMOT1* genotyping and analysis of leaf ionome. Rus *et al.* (2006) and Baxter *et al.* (2008) demonstrated that plants with the Ts-1 *AtHKT1;1* allele type accumulate high concentrations of Na^+, and plants with the Van-0 *AtMOT1* allele type contain low concentrations of Mo in their leaves. Since the number of Ts-1-like or Van-0-like plants was quite low (2013: 21 of 200 plants in BLA and 19 of 200 plants in SCF; 2014: 16 of 200 plants in BLA and 17 of 200 plants in SCF) we decided to exclude these plants for this study because they could distort the global values of coastal demes (Table A8).

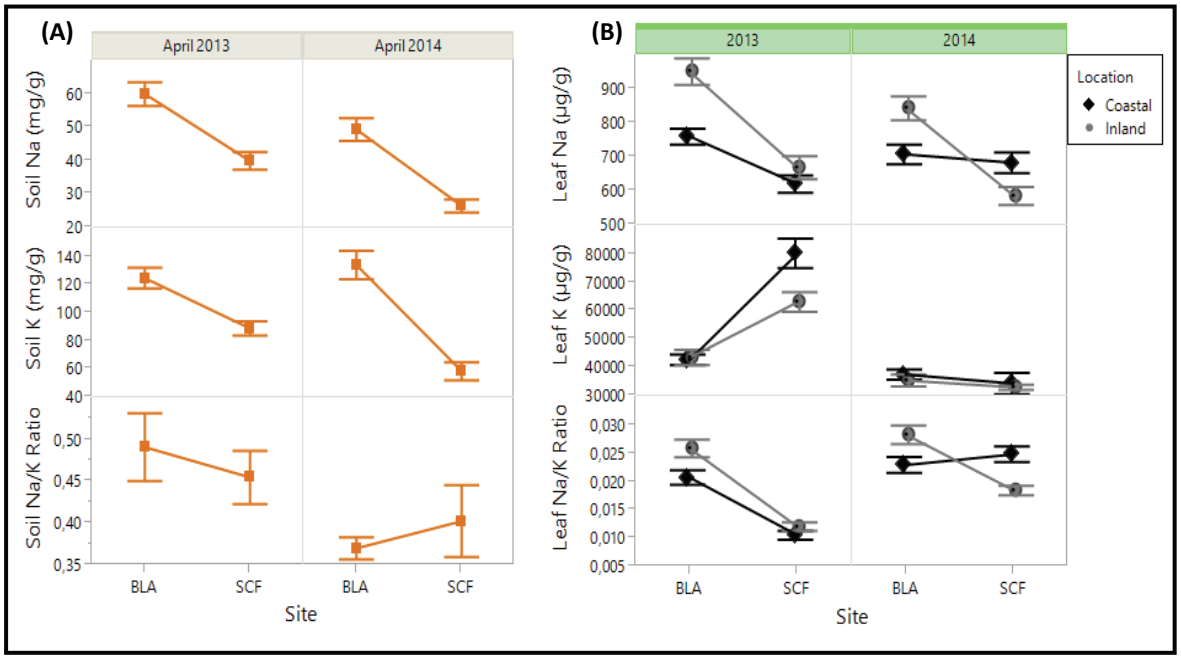


Figure 23: (A) Differentiation in Na and K content and Na/K ratio of 8 soil samples from coastal common garden (BLA) and inland common garden (SCF) collected in April of 2013 and 2014. **(B)** Differentiation in leaf Na^+ and K^+ content and Na^+/K^+ ratio of 10 coastal (black rhombus) and 10 inland (grey circles) *A. thaliana* demes growing in BLA and SCF common gardens collected in April of 2013 and 2014.

We collected soil samples from each common garden site the same day that plants were harvested to compare the availability of each nutrient with the content found within the plant, and to determine if plants from the coastal and inland habitats show differences in the ionome. In the two years of analysis, and consistent with results from coastal soils analysis, we found that soil from BLA common garden field has significantly high concentrations of Na, K, Mg, Co, Cu, P and Zn than soil from the SCF common garden. In contrast, soil from the SCF field site has a higher Ca / Mg ratio and higher Se content (Figure 23A, Table A7).

Despite the differences detected in soil from the sites of the coastal and inland common gardens, when we looked into leaf ionome variability between plants from coastal or inland demes cultivated at the same common garden, we only found significant differences in both years (F value > 5, P < 0,05) in Na^+ and Na^+/K^+ ratio at the BLA field site (Table A8). Figure 23B shows that plants from both coastal and inland demes accumulated more Na^+ in BLA than in SCF field (because Na content in the soil was higher in BLA than in SCF). However, coastal plants had decreased accumulation of Na^+, and lower Na^+/K^+ ratio in leaves than inland ones when grown at BLA. This could suggest that plants from coastal demes have a mechanism of Na^+ exclusion to help them tolerate high levels of salinity.

4.5. Salinity tolerance as a possible trait under divergent selection

To test the hypothesis that soil salinity is the agent driving divergent selection between adjacent coastal and inland *A. thaliana* populations, we performed common garden experiments with plants grown in artificial soil or hydroponically. The hydroponic experiments served to ensure a uniform NaCl treatment, and to reduce surface contamination confounding the analysis of the tissue content of Na^+. The soil experiments reproduced a more ecologically relevant condition and allowed measurement of silique production as an estimate of fitness.

For assaying salinity tolerance in soil, 4 plants from 8 coastal and 8 inland demes were grown in an artificial potting mix for 18 days, at which time we began to irrigate with 0,5-Hoagland nutrient solution containing 0, 50 or 100 mM NaCl once a week. The effect of NaCl treatment on the number of siliques was different for coastal and inland demes, with a significant interaction between treatment and origin of deme (Figure 24 A; *L* ratio = 79,97, df =2, P < 0,001). Coastal and inland plants produced similar numbers of siliques without NaCl treatment (Figure 24 A; *z* value = 1,17, P = 0,815), but coastal plants produced significantly more siliques than inland plants when treated with 50 mM (Figure 24 A; *z* value = 3,09, P = 0,017) or 100 mM

(Figure 24 A; *z* value = 4,0, P < 0,001) NaCl. This fitness benefit for coastal plants was also reflected in their increased ability to maintain growth after NaCl treatment (Figure 24 B).

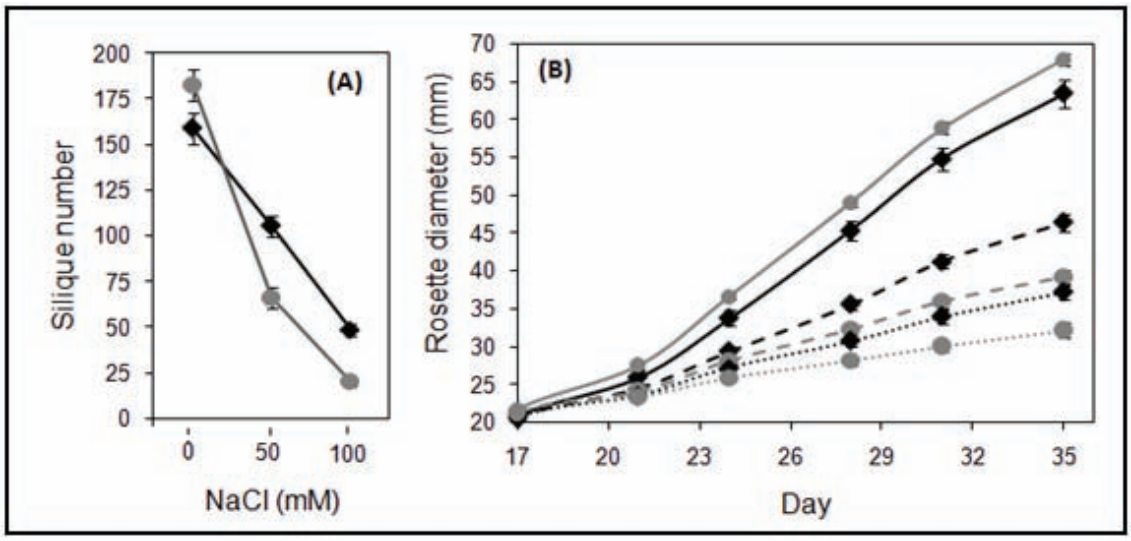


Figure 24: Effects of NaCl treatments on growth and fitness of coastal and inland *A. thaliana* demes. (**A**) Plants were grown in potting mix and irrigated with 0, 50 or 100 mM NaCl in 0,5-Hoagland nutrient solution and total silique number counted at maturity from coastal (black diamonds) and inland (grey circles) demes. (**B**) Rosette diameter of plants these plants was also measured during growth of plants from coastal (black diamonds) and inland (grey circles) demes exposed to 0 mM (solid lines), 50 mM (dashed lines) or 100 mM (dotted lines) NaCl. Data represents the mean ± SE (n = 3 plants per deme and treatment from 8 coastal and 8 inland demes).

We observed a significant three-way interaction between time, NaCl treatment and origin of deme (*L* ratio = 96,017, df = 4, P < 0,001). Inland plants grew more robustly than coastal plants in the absence of NaCl. Treatment with NaCl reduced the growth of coastal plants less than that of inland plants. Similar results were also observed for plants exposed to NaCl in hydroponic solution (Figure 25 A).

From these results we conclude that salinity tolerance is under divergent selection across the coastal and inland habitats we studied, and this selection, driven by soil salinity, is responsible at least in part for the local adaptation we observe. However, the question remains as to the mechanism of the enhanced salinity tolerance in the coastal populations. A first step in addressing this question was to distinguish between the hypotheses that salinity tolerance of coastal demes is due to either exclusion of Na^+ from the plant, or accumulation of Na^+ and internal tolerance to Na^+. These are two well established mechanisms of salinity tolerance in other plant systems (Munns & Tester, 2008). Using tissue collected from plants grown in our hydroponic salinity tolerance experiment (Figure 25) we measured the concentration of Na^+ and K^+ in leaf tissue after three weeks of NaCl treatment.

The accumulation of Na^+ and the Na^+/K^+ ratio in coastal and inland plants was dependent on the concentration of NaCl the plants were treated with (Figure 25 C and E) and we observe a significant interaction between NaCl treatment and origin of deme for both leaf Na^+ and the ratio of Na^+/K^+ (Figure 25 C; Na^+: *L* ratio = 11,70, df = 2, P = 0,002; Figure 25 E; Na^+/K^+: *L* ratio = 6,67, df = 2, P = 0,035). In contrast, only NaCl treatment had a significant effect on the ability of plants to accumulate K^+ (Figure 25 D; *L* ratio = 33,62; df = 2, P < 0,001) but with no effect of origin of deme (Figure 25 D; *L* ratio = 2,36, df = 1, P = 0,124).

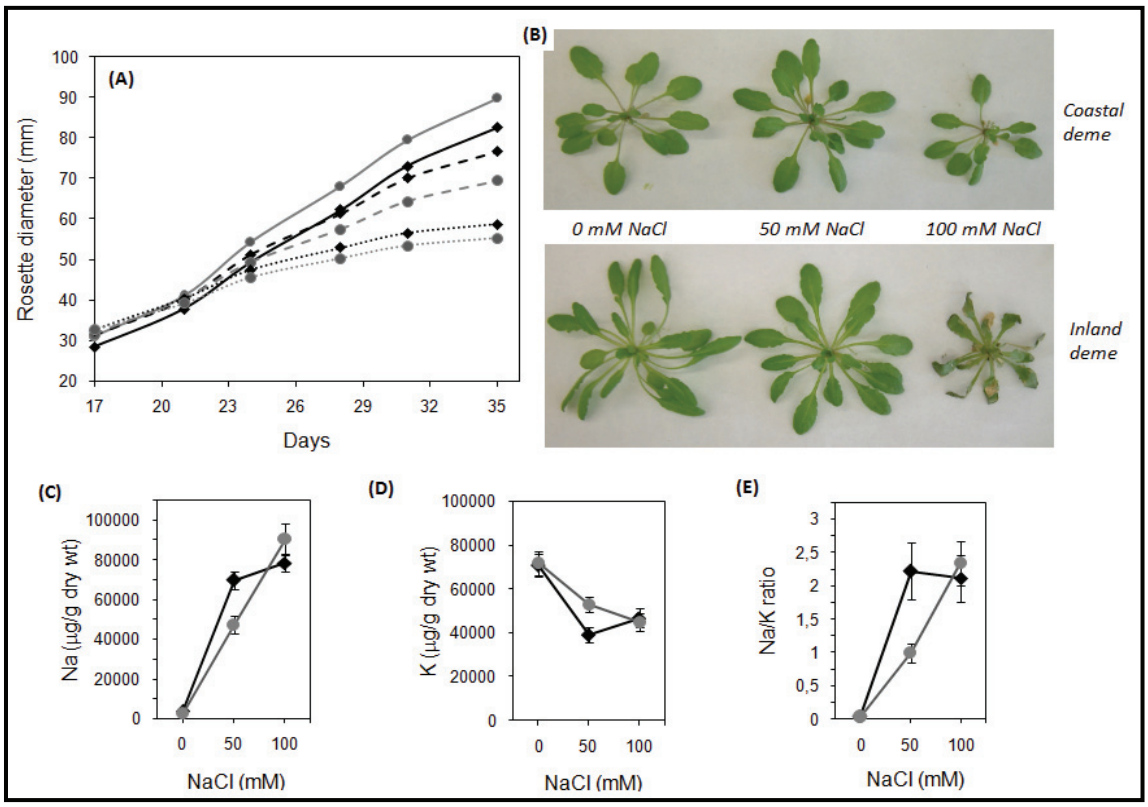


Figure 25: (A) Growth (measured as rosette diameter, mm) of plants from coastal (black diamonds) and inland (grey circles) demes after exposed to either 0 mM (solid lines), 50 mM (dashed lines) or 100 mM (dotted lines) NaCl in the hydroponic solution. **(B)** Pictures of plants from the same coastal or inland deme at the time of harvest after being treated with 0, 50 and 100 mM of NaCl. Plants from coastal (black diamonds) and inland (grey circles) demes were also grown hydroponically and exposed to different concentrations of NaCl in the hydroponic growth solution and the concentration of Na^+ **(C)**, K^+ **(D)**, and the Na^+/K^+ ratio (**E**) in leaves determined. Data represents the mean ± SE (n = 3 plants per deme and treatment from 8 coastal and 8 inland habitats, see Table A2 for details).

Plants from coastal and inland demes did not differ in their accumulation of Na^+ or K^+ under control conditions with no added NaCl to the hydroponic solution (Figure 25 C and D; Na^+: *z* value = -0,15 , P = 0,999, K^+: *z* value = -0,04 , P = 0,999). However, after exposure to 50 mM NaCl in the hydroponic solution, coastal plants accumulated more Na^+ than inland plants (Figure 25 C; *z* value = 3,10 , P = 0,025), resulting in an overall increase in the Na^+/K^+ ratio

(Figure 25 E; *z* value = 2,85, P = 0,046). However, such a difference were seen neither for Na^+ (Figure 25 C; *z* value = 1,61; P = 0,577) nor K^+ (Figure 25 D; *z* value = 0,53; P = 0,994) after exposure to 100 mM NaCl in the hydroponic solution, potentially reflecting that growth was severely compromised in this condition for both coastal and inland plants, although coastal plants were slightly less affected than inland plants and accumulated less Na^+ (Figure 25).

When we harvested plants cultivated hydroponically we dried a portion for analysis of the leaf ionome and roots were snap-frozen with liquid nitrogen for RNA extraction. Two coastal demes (T11 and PA10) and two inland demes (A1 and LG5) were chosen and we took 3 plants from each deme and treatment to measure the expression of *AtSOS1* and *AtHKT1;1* in roots.

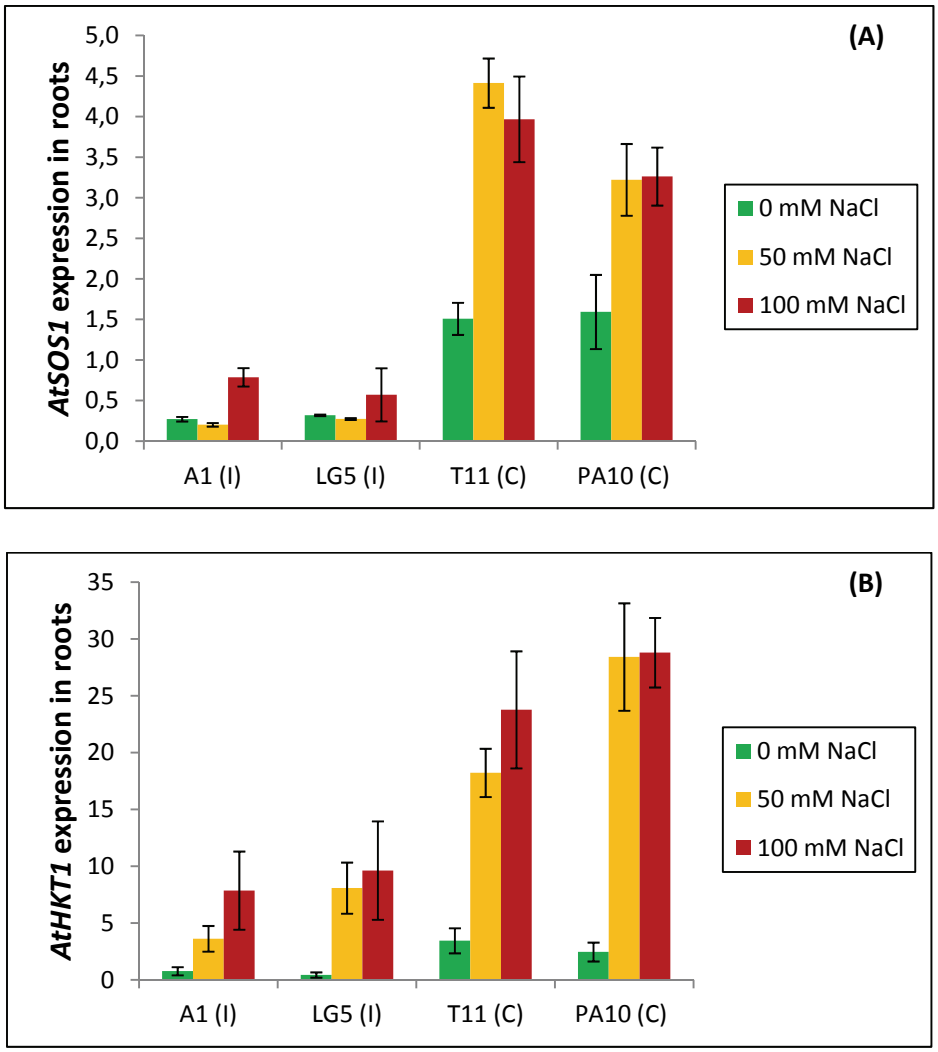


Figure 26: Expression profile of *AtSOS1* **(A)** and *AtHKT1;1* **(B)** in the roots of plants from two coastal (T11 and PA10) and two inland demes (A1 and LG5) grown for 5 weeks in hydroponics treated with 0 mM (green bars), 50 mM (orange bars) or 100 mM NaCl (red bars) for 18 days. Data represents the mean ± SE (n = 3 plants per deme and treatment)

We observed that *AtSOS1* expression was always higher in coastal demes, even under control conditions (0 mM NaCl treatment) (Figure 26 A). However, we detected the largest differences between demes from different origins when NaCl was added to the solution. 50 mM NaCl was enough to increase by more than twice the expression of *AtSOS1* in plants from coastal demes. In the 100 mM treatment, *AtSOS1* expression in inland demes also increased but not to the extent observed in plants from coastal demes (Figure 26 A).

Concerning *AtHKT1;1*, we detected low expression of this gene in all demes under control conditions ($2^{-\Delta Ct} < 5$, Figure 26 B). However, when we added 50 mM NaCl to the 0,5-Hoagland solution, expression of *AtHKT1;1* increased in all demes but, as in the case for *AtSOS1*, the change was greater in plants from coastal demes (Figure 26 B). In the 100 mM treatment the expression of *AtHKT1;1* was similar to that observed in the 50 mM NaCl treatment. This indicates that 50 mM of NaCl is sufficient to activate *AtHKT1;1* expression, and Figure 26B shows maximum expression levels observed in our plants.

The final experiment conducted to evaluate if coastal plants have the ability to transfer salinity tolerance to their progeny consisted in crossing three salt-sensitive inland demes (A1, LG5, V3) with three salt-tolerant coastal demes (T2, LLO2, PO1). We made a total of 18 crossings using the same deme as a male and female. Three plants from each crossing and three parental plants were cultivated on hydroponic solution treated with 0, 50 and 100 mM NaCl.

Under control conditions, F1 progeny from crossings and parental plants grew and produced a similar number of seeds (Figure 27, Table A9). However, when NaCl was added to the nutrient solution, we observed that the progeny from *Inland x Coastal* (male x female) crossings has a similar ability to maintain growth after treatment than coastal plants (Figure 27A) and even produced more seeds than their parents under both treatments (Figure 27B, Table A9). Conversely, progeny from *Coastal x Inland* (male x female) crossings displayed a similar NaCl tolerance to the salt-sensitive inland plants, producing a significantly lower number of seeds compared to plants from coastal demes (Par C) or plants from *Inland x Coastal* (male x female) crossings (Figure 27, Table A9).

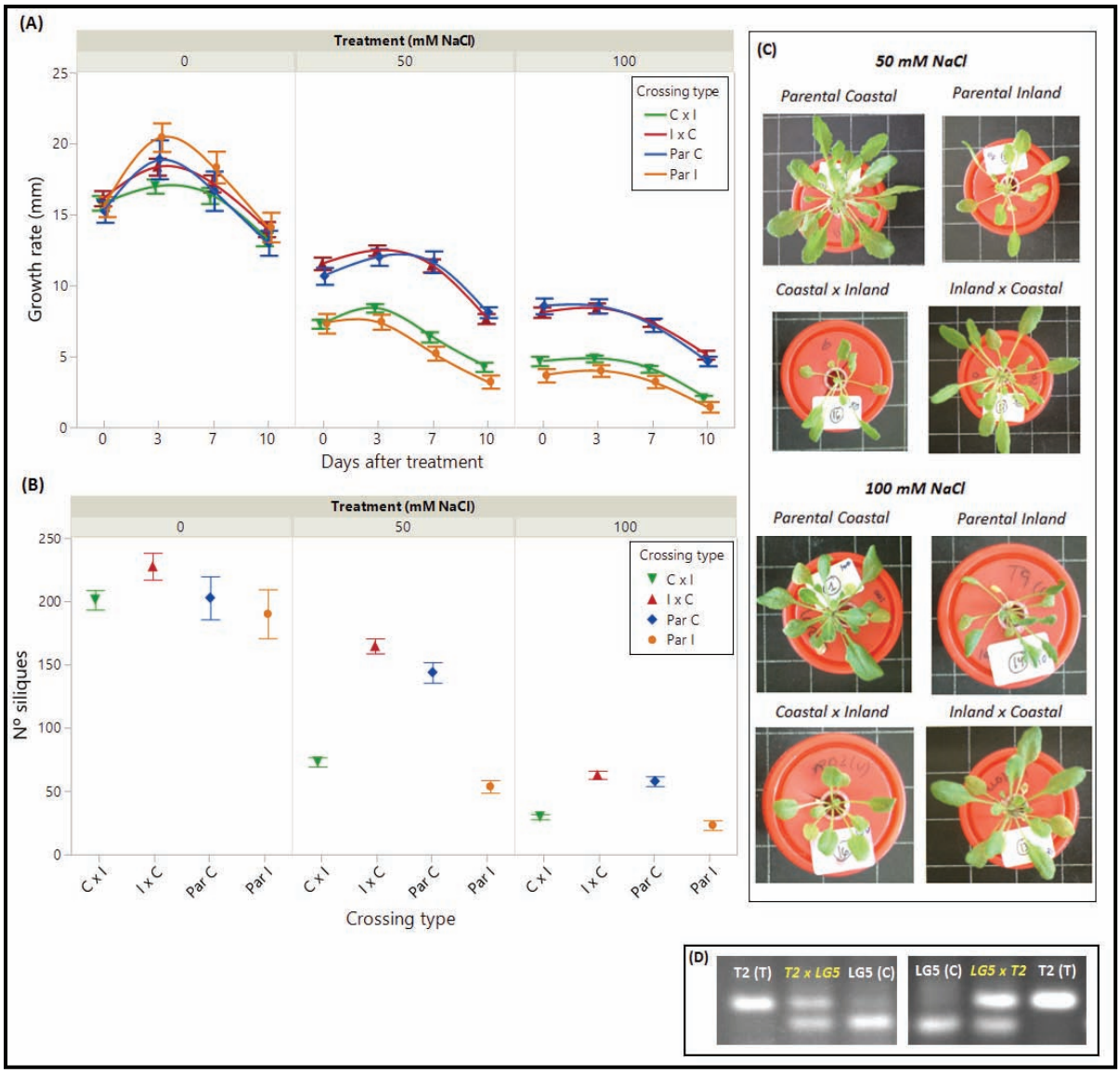


Figure 27: (A) Growth rate (rosette diameter increase, mm) and **(B)** fitness (nº of siliques produced) of 3 plants from 3 coastal demes (Parental Coastal, Par C), 3 plants from 3 inland demes (Parental Inland, Par I), 3 plants from 9 *Inland (male) x Coastal (female)* crossings (I x C) and 3 plants form 9 *Coastal (male) x Inland (female)* crossings (C x I) after being treated with 0, 50 and 100 mM of NaCl on hydroponic conditions. **(C)** Pictures example of each parental and crossing type under 50 and 100 mM NaCl. **(D)** Picture of gels obtained from PCR of *AtFPN2* performed for the screening of F1 plants obtained from the crossing *T2 (male, coastal, T) x LG5 (female, inland, C)* and *LG5 (male, inland, C) x T2 (female, coastal, T)*.

This result leads us to two hypotheses: salinity tolerance is only transmitted when coastal plants are the female parental, suggesting genetic imprinting phenomena, or the alternative explanation is that the F1 plants are not actually crosses but rather the maternal genotype. Before making the crossings, we ensured that plants from coastal demes had the *AtFPN2* allele like Ts-1 and plants from inland demes had the *AtFPN2* allele like Col-0 in order to use *AtFPN2* as a marker to check if F1 plants were hybrids. We used seeds from a single silique but only

one plant from each crossing was genotyped. Despite the genotyped plants were hybrids (Figure 27 D), *A. thaliana* flower contains multiple ovules (Maheshwari, 1950) that can be fertilized independently by different pollen (Drews & Koltunow, 2011). The possibility that a single silique could contain seeds derived from both cross fertilization (hybrid) and self fertilization (non-hybrid) events is feasible. To exclude this second possibility is necessary the testing and genotyping of F2 seeds in future studies.

4.6. Role of *AtHKT1;1* weak allele

4.6.1. Localization and relations with climatic and soil characteristics

Using the SNP at Chr4:6392276 as a genetic marker for the type of *AtHKT1;1* allele (Baxter *et al.*, 2010) we could identify 30 demes with plants having the 'strong allele' (accessions with a cytosine (C) at the SNP, *Col-0-like,* normal gene expression) and 6 demes with a mixture of plants homozygous for either the strong allele or the 'weak allele' of *AtHKT1;1* (accessions with a thymine (T) at the SNP, *Ts-1-like,* reduced gene expression) (Table A2).

The weak allele was only observed in coastal populations. Importantly, 12 of the 18 demes identified to contain only the strong allele also occurred on the coast. This type of coastal distribution of the weak allele of *AtHKT1;1* on a local scale is consistent with that reported by Baxter *et al.* (2010) on a population-wide scale. Surprisingly, the weak allele of *AtHKT1;1* was only present in coastal populations which also contained the strong allele (MIX). Demes only containing plants homozygous for the weak allele have not so far been identified. Further, we have also not identified any hybrid plant containing both the strong and weak alleles. Demes containing plants with the weak allele grow in an intermediate zone back from the coast at 0,5 km and with a soil Na of between 50 - 150 mg/g (Figure 28 B).

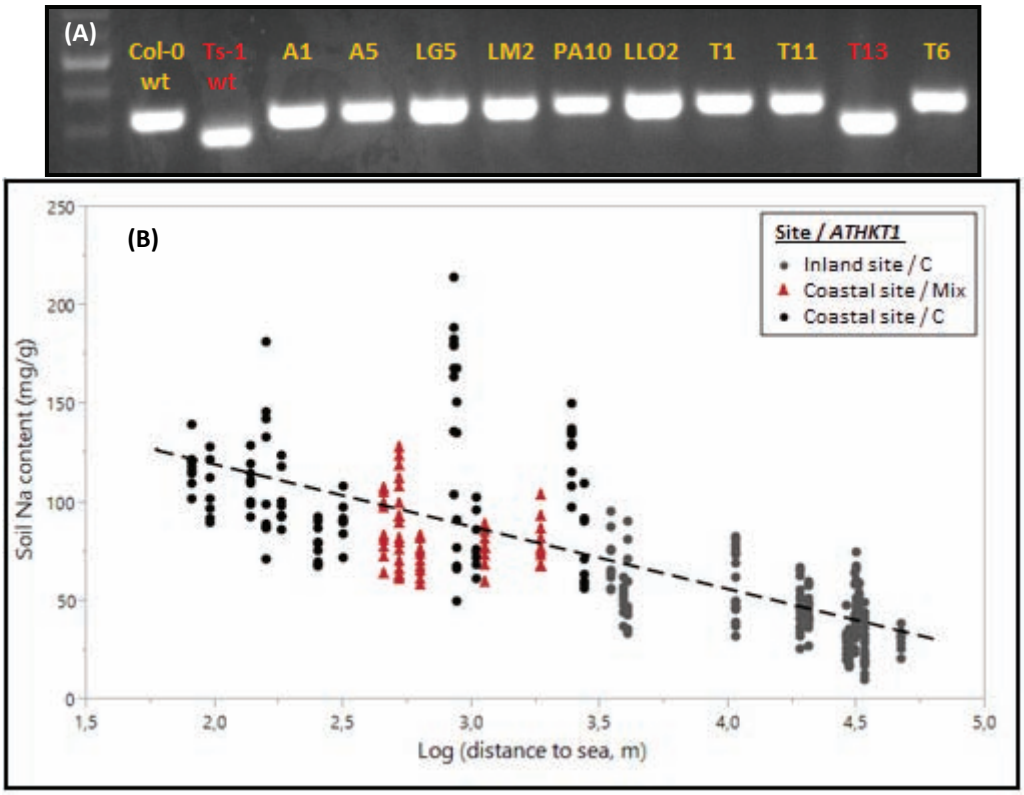


Figure 28: (A) Picture of a gel obtained from a PCR performed to classify plants according to *AtHKT1;1* allele type. **(B)** Na concentration of 3 samples of soil from 18 coastal and 18 inland sites collected on 2013, 2014 and 2015 and its relationship with the distance to the coast and the presence of *AtHKT1;1* weak allele. Black circles: costal sites with plant homozygous for the strong allele of *AtHKT1;1* (C); grey circles: inland sites with plants homozygous for the strong allele of *AtHKT1;1* (C); red triangles: coastal sites with plant with the weak allele and plants with the strong allele of *AtHKT1;1* (Mix).

On average, coastal demes growing on soils with elevated Na are more tolerant to elevated salinity than inland demes. However, the majority of the coastal demes lack the weak allele of *AtHKT1;1* and plants with the weak allele are not located at sites with the highest concentration of Na in the soil (Figure 28 B). The This strongly suggests that the weak allele of *AtHKT1;1* is not responsible for either the local adaptation of coastal demes or their enhanced ability to tolerate elevated salinity that were observe in our common garden experiments.

In order to explain the observed distribution of *AtHKT1;1* weak allele, we incorporated the genotypic data into a reanalysis of soil and leaf ionomic data from the samples collected in the field in 2013, 2014 and 2015. However, given that the weak allele is present only at the coast, we have compared each element concentration in the soil between coastal sites (12 sites containing only plants homozygous for the strong allele (C) and 6 sites having a mixture of plants homozygous for either the weak (T) or strong (C) allele of *AtHKT1;1*). Figure 29 shows that sites containing plants with the weak allele have several characteristics in common: on

average for all years of analysis, soils from these sites contain higher concentrations of As, Co, Cu, K, Mo, Ni, Rb, Zn and Ca^{2+}/Mg^{2+} ratio and, unexpectedly, they have lower levels of Na^{+}, Na^{+}/K^{+} ratio, Mg^{2+} and Se than soils from locations homozygous for strong allele (Table A10).

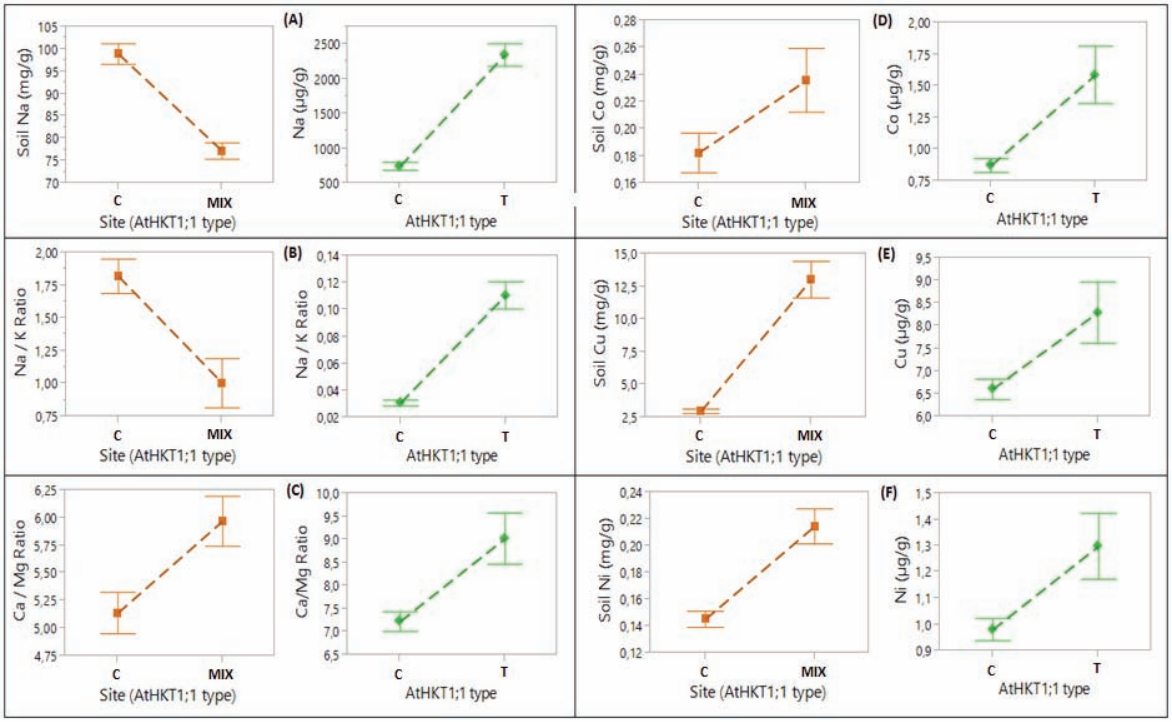


Figure 29: Na content **(A),** Na/K Ratio **(B)** , Ca/Mg ratio **(C)**, Co **(D),** Cu **(E)** and Ni **(F)** content in soil and leaves. Left panels, soil samples taken from 12 coastal sites with plants homozygous for the strong allele (C) and 6 coastal sites containing plants with the strong and plants with the weak allele of *AtHKT1;1* (MIX). Right panels, *A. thaliana* leaves (µg/g dry weight) from 173 plants with the strong allele (C) and 45 plants with the weak allele of *AtHKT1;1* (T) growing at the same sites samples for soil. Samples collected in March of 2013, 2014 and 2015.

The first notable observation in Figure 28B is that all demes with the weak allele were located on the coast but not in the demes closest to the sea. In contrast, leaf ionomic data confirmed that the presence of weak allele of *AtHKT1;1* (T) increase substantially the Na^{+} concentration in leaves despite the lower availability of Na in the soil (Figure 29 A). The same occurs for Na^{+} / K^{+} ratio (Figure 29 B) despite the absence of significant differences between the concentrations of K^{+} in leaves from plants containing the weak or the strong allele (Table A11). In the case of the Ca/Mg ratio and Co, Cu and Ni content, the higher concentrations in leaves are consistent with the elevated concentrations in the soil from their native sites (Figure 29 C, D, E and F). Finally, although no significant differences were detected in Ca, Li and Sr concentrations in soils, we found that plants with weak allele accumulate more Ca, Li and Sr in their leaves (Table A11). The elevated Ca/Mg ratio detected in Ts-1-like plants is due to an increase of Ca in leaves instead of lower concentrations of Mg in these plants (Table A11).

The weak allele of *AtHKT1;1* was associated with low levels of soil Na (Baxter *et al.*, 2010), but the fact that plants with the weak allele always occur in demes that also contain plants with the strong allele raises the hypothesis that demes mixed for the *AtHKT1;1* allele perhaps show elevated temporal fluctuations in soil Na. Soil Na concentration in these locations can vary greatly depending on wind and weather conditions. To test this hypothesis, we selected one coastal site with plants homozygous for the strong allele (T1, Tossa de Mar, located at 0,1 km from the sea) and one coastal site containing plants with strong or weak allele of *AtHKT1;1* (T13, Tossa de Mar, located at 0,53 km from the sea) and situated at less than 1 km from T1 site. We collected two soil samples twice a month from February to May of 2014 and 2015 to analyse the variability of Na and Na/K ratio during the *A. thaliana* growing season, and the relationship with monthly precipitation (data obtained from the weather station of Tossa de Mar).

Figure 30 shows that Na and Na/K ratio levels varied more in T13 site (Na (mg/g): σ^2 = 1171,13; Na/K ratio: σ^2 = 0,85) than in T1 site (Na (mg/g): σ^2 = 130,66 ; Na/K ratio: σ^2 = 0,12) over the months of both 2014 and 2015 (Table A12). Besides, Na/K ratio also fluctuates strongly at the site with the mixed alleles (T13) over the years. In 2014 Na/K ratio shows a peak in March-April at around 4, but, during the same period of the 2015, Na/K ratio drops to a minimum of approximately 0,1. This is a range of 0,1 – 4 over two consecutive years where as at the non-mixed site (T1) the Na/K ratio remains constant at 1 – 1,5 throughout the growing season in 2014 and 2015 (Figure 30 B and C). Moreover, one can observe that this variability is related to the amount of rainfall, since in the months with more precipitation Na and Na/K ratio levels at the T13 site clearly declined (Figure 30D).

The average soil salinity is usually higher in T1 but in May of 2015 we observed that Na^+ levels at T13 were higher, reaching concentrations of approximately 200 mg/g (Table A12). If soil Na levels increase suddenly, as in the case of T13, the presence of the weak allele (T) could be detrimental as plants no not have the ability to stop Na^+ accumulation, which reach toxic levels in the plant. This could explain the presence of the two types of *AtHKT1;1* alleles in the six locations tested.

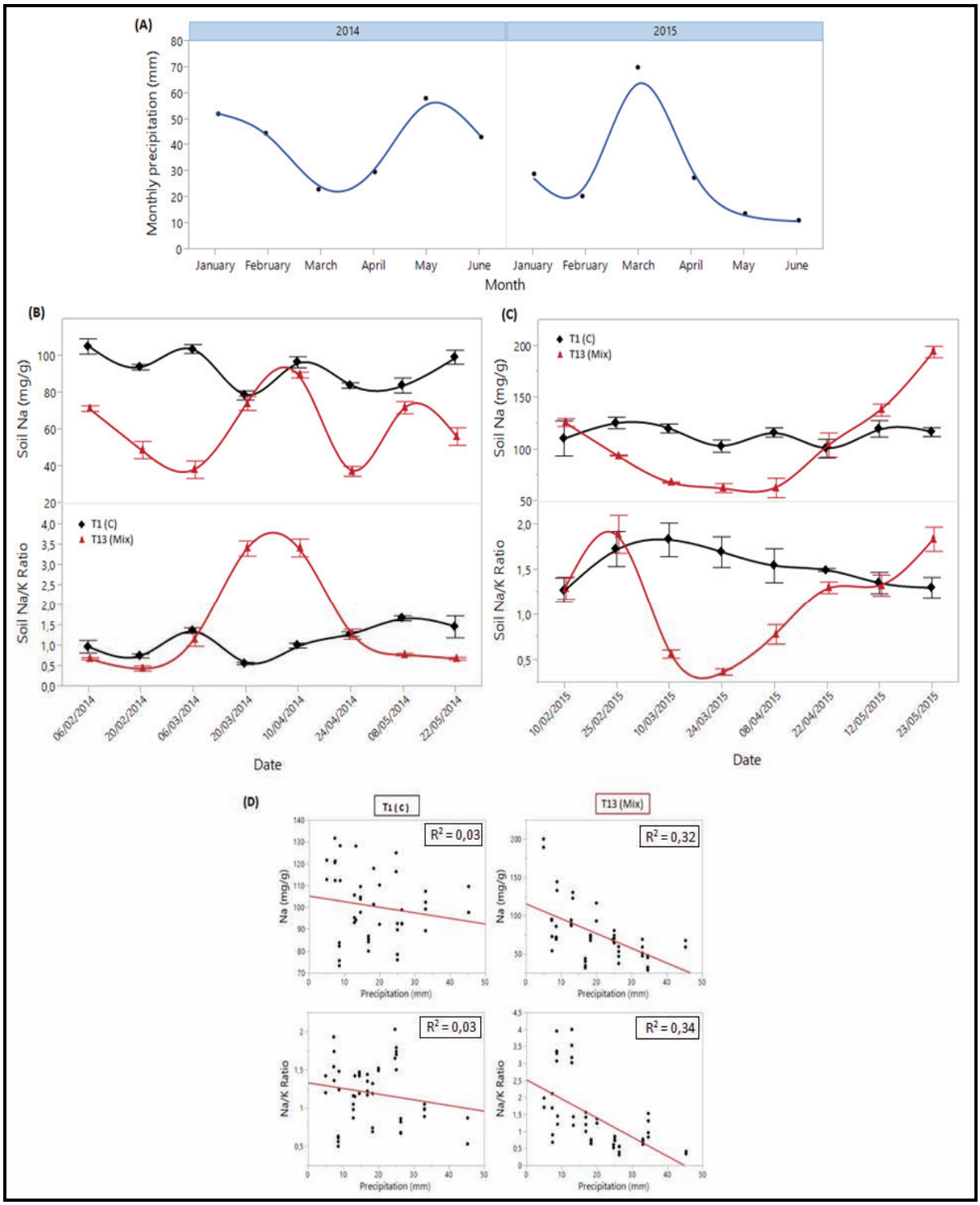


Figure 30: (A) Total monthly precipitation (mm) of Tossa de Mar from January to June of 2014 and 2015. Soil Na content (mg/g) and Na/K ratio of two independent samples from T1 (site with plants homozygous for the strong allele of *AtHKT1;1* (C) and T13 (site containing plants with the strong or weak allele of *AtHKT1;1* (Mix)) collected twice a month from February to May of 2014 **(B)** and 2015 **(C)**. **(D)** Correlation between Soil Na content (mg/g) or Na/K ratio and precipitation (mm) in T1 and T13 sites (data of 2014 and 2015 together).

4.6.2. Influence on salinity tolerance

4.6.2.1. Field-based common garden cultivation

T13 and JBB demes were selected because they have plants with strong (Col-0-like, C) and weak (Ts-1-like, T) allele of *AtHKT1;1* growing together at the same site. After the genotyping of plants collected from their natural habitat in 2013 and 2014, we stored seeds of each *AtHKT1;1* allele type plant (combinations called: T13c, T13t, JBBc, JBBt). Seeds were sown in potting mix soil and grown in controlled environmental conditions to obtain sufficient seeds for the field experiments planned in the following years. Therefore, in 2014 and 2015 we repeated the same cultivation at the coastal field common garden in Blanes (BLA) and the inland field common garden in Santa Coloma de Farners (SCF). In both years we grew 20 plants from each deme/*AtHKT1;1* genotype combination at each common garden. We monitored growth (rosette diameter) and counted the number of siliques produced by 10 plants of each deme/*AtHKT1;1* genotype combination at each field site. In April of both years we harvested the 10 remaining plants to analyse the leaf ionome and to quantify *AtHKT1;1* expression in roots.

Figure 31A shows that at the SCF inland field plants grew equally well and produced similar number of siliques independently of the type of *AtHKT1;1* allele they contained. However, at the BLA coastal field, Col-0-like plants for *AtHKT1;1* allele outperformed Ts-1-like plants in both years (Table A13). The same pattern was also observed for fitness. Silique production was higher in plants with the strong allele of *AtHKT1;1* when they were cultivated at the BLA coastal field but this difference is not observed at the SCF inland field site (Figure 31 B, Table A14).

Another indicator that plants with the weak allele of *AtHKT1;1* growing at the BLA coastal field were stressed was that they flowered earlier than Col-0-like plants cultivated at the same field common garden (Figure 31C, Table A14). At the SCF inland field, although all plants grew and produced less seeds than at BLA coastal field, they tended to flower later than plants at BLA coastal field likely due to the weather conditions (lower temperature and higher humidity). However, no difference in flowerin time was observed for plants with the weak allele of *AtHKT1;1* (Figure 31C, Table A14).

Even though the plants with the weak allele did worse than those with the strong allele when grown at the BLA coastal field, if we compare the fitness of coastal Ts-1-like plants with the fitness of plants from inland demes also cultivated at BLA in 2014, coastal plants with the weak

allele of *AtHKT1;1* still had a higher fitness than inland plants (Silique number ± SD in BLA: Coastal (C)= 396,3 ± 112,5 / Coastal (T) = 310 ± 88,2 / Inland (C) = 224 ± 75,5).

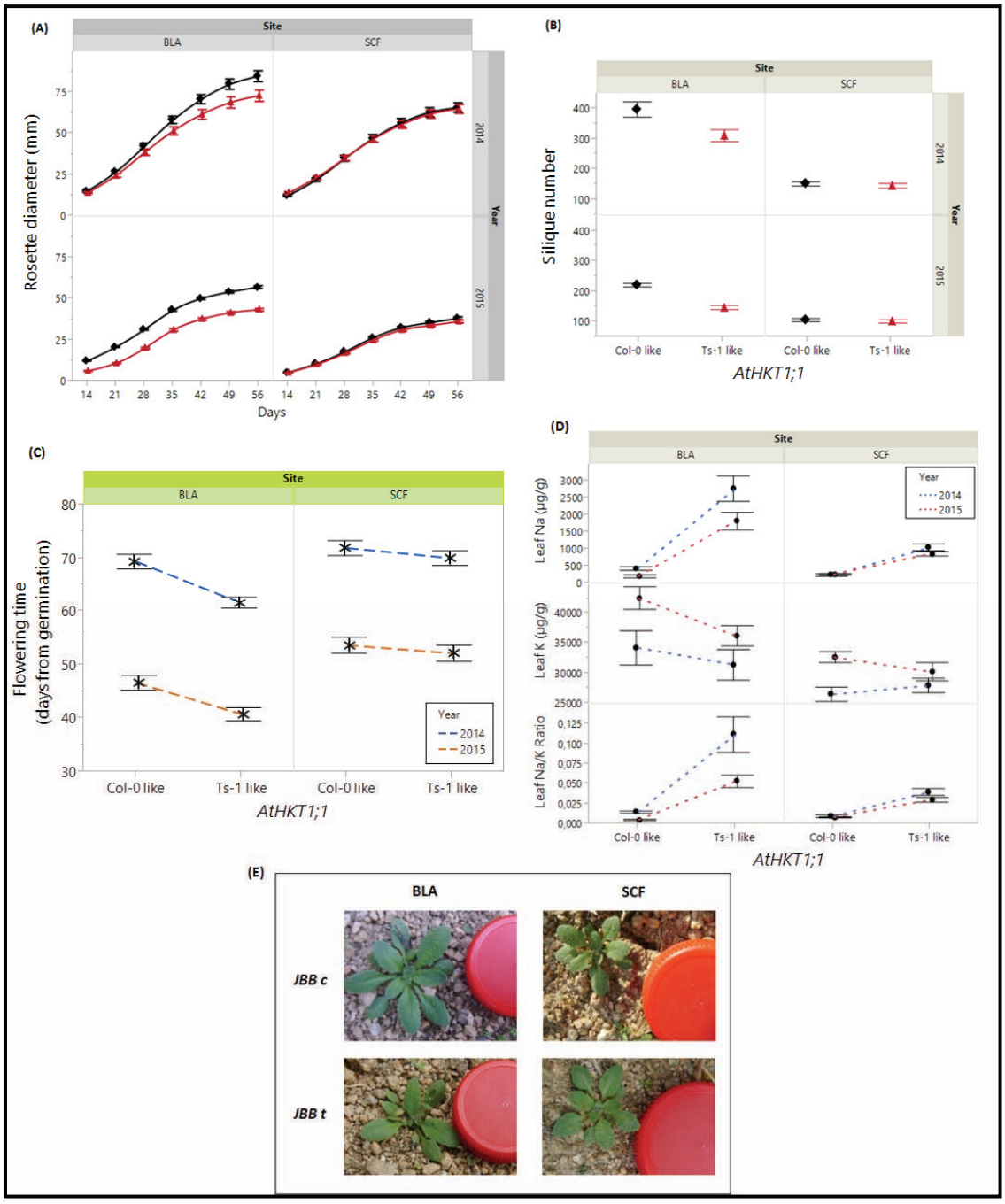


Figure 31: Mean fitness of *A. thaliana* plants measured as growth (rosette diameter) **(A)** and silique number **(B)**. Plants with the strong allele (black diamonds) and plants with the weak allele of *AtHKT1;1* (red triangle) from T13 and JBB demes cultivated at the BLA coastal and SCF inland common gardens in 2014 and 2015. Data represents the mean ± SE (n = 20 plants per *AtHKT1;1* allele type per field site). Flowering time (measured as days from germination when the first flower appeared), **(E)** leaf Na^+ and K^+ content (µg/g) and Na^+/K^+ ratio **(D)** from 20 plants with the strong allele (Col-0 like) and 20 plants with the weak allele of *AtHKT1;1* (Ts-1 like) cultivated on each common garden in 2014 (blue lines) and 2015 (orange lines). **(E)** Pictures of plants from JBB deme with the strong (JBBc) or the weak (JBBt) allele of *AtHKT1;1* growing at the SCF and BLA common gardens after 30 days from sowing.

Mineral content in the soil of BLA and SCF field sites was measured twice a month during the period of the common garden experiment in both 2014 and 2015. As expected, Na concentrations were always higher in BLA soil than in SCF, despite variation likely associated with different rainfall patterns. On average, in 2014 Na content was 53,89 ± 14,54 mg/g at BLA and 29,94 ± 5,90 mg/g at SCF. In 2015, a drier year than 2014, the mean Na soil concentration was increased to 86,39 ± 22,31 mg/g in BLA and 51,61 ± 10,34 mg/g in SCF.

The differences in growth and reproductive fitness between plants with a weak or strong allele of *AtHKT1;1* cultivated at the BLA field site were significantly larger in 2015 than in 2014 (Tables A13 and A14), supporting the hypothesis that higher levels of Na^+ in the soil are harmful for Ts-1-like plants. This is confirmed by the fact that plants with the weak allele of *AtHKT1;1* cultivated at the BLA field site accumulated high levels of Na^+ in their leaves that can become toxic to plants such as *A. thaliana* (Figure 31D). It is important to mention that among all the elements analysed in leaf ionome of plants cultivated at the BLA and SCF common gardens, only Na^+ and the Na^+/K^+ ratio were significantly different between plants with the weak or the strong allele of *AtHKT1;1* for both years of the analysis (Table A15).

In 2015, three intact plants of each deme/*AtHKT1;1* genotype from each of the field common garden with their rizospheric soil unaltered were transported to the laboratory in plastic pots. Next day, leaves were harvested and dried for ionomic analysis and roots were snap-frozen with liquid nitrogen for RNA extraction and analysis of *AtHKT1;1* expression in roots. Figure 32 shows that plants having the weak or strong allele of *AtHKT1;1* followed the same pattern regardless of the deme where they come from (T13 or JBB). As described by Rus *et al.* (2006), Ts-1-like plants have reduced expression of *AtHKT1;1* and consequently a reduced functionality of the gene in both sites, revealed by the elevated Na accumulated in the leaves of these plants (Figure 31D). The expression of *AtHKT1;1* in Col-0-like plants was higher in BLA than in SCF (Figure 32) confirming that coastal conditions with elevated soil Na trigger the expression of the Na-transporter *AtHKT1;1.* This result also confirms that plants cultivated at the BLA common garden are experiencing the physiological effects of elevated soil salinity supporting our use of BLA field site as a representative coastal site.

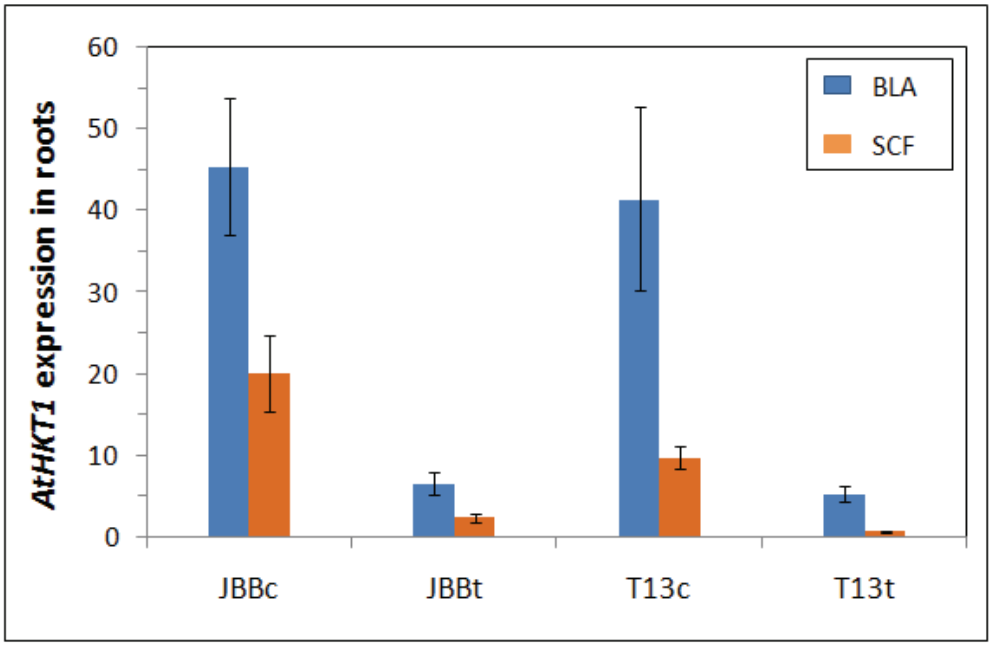


Figure 32: Expression profile of *AtHKT1;1* in the roots of three plants from T13c, T13t, JBBc and JBBt grown in BLA coastal common garden (blue bars) and SCF inland common garden (orange bars) in 2015. Data represents the mean ± SE (n = 3 plants per accession and common garden site).

4.6.2.2. Irrigation and hydroponic experiments with NaCl treatments

The next step taken to evaluate the salinity tolerance of our plants with the weak allele of *AtHKT1;1* was to grow 5 plants of each deme/*AtHKT1;1* genotype combination (T13c, T13t; JBBc, JBBt) in environmentally controlled conditions in potting mix soil irrigated with 0.5-Hoagland nutrient solution, with 0, 50 or 100 mM added NaCl applied 2 weeks after germination.

When the treatment started, all plants had a similar rosette diameter of approximately 35 cm. However, after 3 weeks of NaCl irrigation, differences in rosette diameter were quite pronounced. Growth of Col-0-like plants exposed to 50 mM NaCl was halved and plants exposed to 100 mM of NaCl showed almost no growth (Figure 33 A, Table A16). Ts-1-like plants were not affected by the 50 mM NaCl treatment, with growth similar to control plants unexposed to elevated NaCl. However, the growth of plants with the weak allele exposed to 100 mM NaCl treatment also declined sharply (Figure 33 A, Table A16).

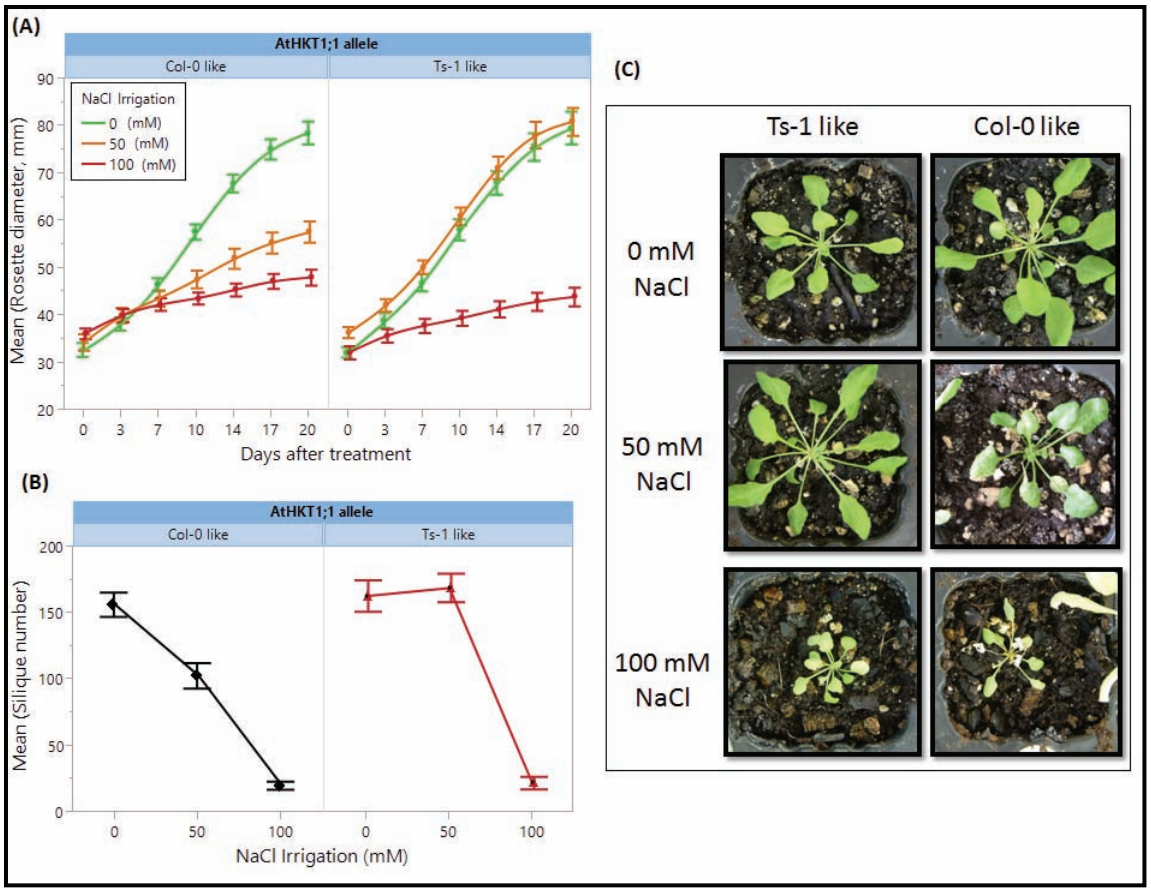


Figure 33: Growth (measured as rosette diameter, mm) **(A)** and fitness (Silique number produced) **(B)** of plants with the strong allele (Col-0 like) and plants with the weak allele of *AtHKT1;1* (Ts-1 like) from T13 and JBB demes cultivated in potting mix soil under environmental controlled conditions and irrigated with 0,5-Hoagland solution with 0 mM (green lines), 50 mM (orange lines) or 100 mM (red lines) NaCl once a week. **(C)** Pictures of plants from the same accession (T13c / T13t) under irrigation with 0, 50 or 100 mM NaCl 14 days from the beginning of treatment. Data represents the mean ± SE (n = 10 plants per *AtHKT1;1* allele type and treatment).

Figure 33 B corroborates the lack of detectable differences in fitness between plants with the weak or strong allele of *AtHKT1;1* cultivated under control conditions (0 mM NaCl) or after being irrigated with 100 mM NaCl, due to the high toxicity of this treatment in all plants. On the other hand, as observed for growth, under 50 mM NaCl irrigation the silique production was lower than under control conditions only in plants with the strong allele of *AtHKT1;1*, but not in those with the weak allele (Figure 33 B, Table A17). These results suggest that having the weak allele of *AtHKT1;1* can be beneficial in intermediate salinity conditions.

To complement the experiment in which we irrigated plants growing in soil with NaCl, we also conducted an hydroponic experiment using the same combination of demes/*AtHKT1;1* genotypes and NaCl treatments. This allowed us to measure growth and rosette fresh weight, and harvest leaves to analyze the ionome, and roots to quantify *AtHKT1;1* expression.

As in the previous irrigation experiment (Figure 33), plants containing the weak allele of *AtHKT1;1* grew similarly well in both control conditions with no added NaCl and after the addition of 50 mM NaCl (Figure 34A). However, after treatment with 50 mM NaCl plants containing the strong allele of *AtHKT1;1* showed a significant reduction in growth (Figure 34A). Further, exposure to 100 mM NaCl treatment caused severe growth retardation in all plants regardless of *AtHKT1;1* genotype (Figure 34, Table A18).

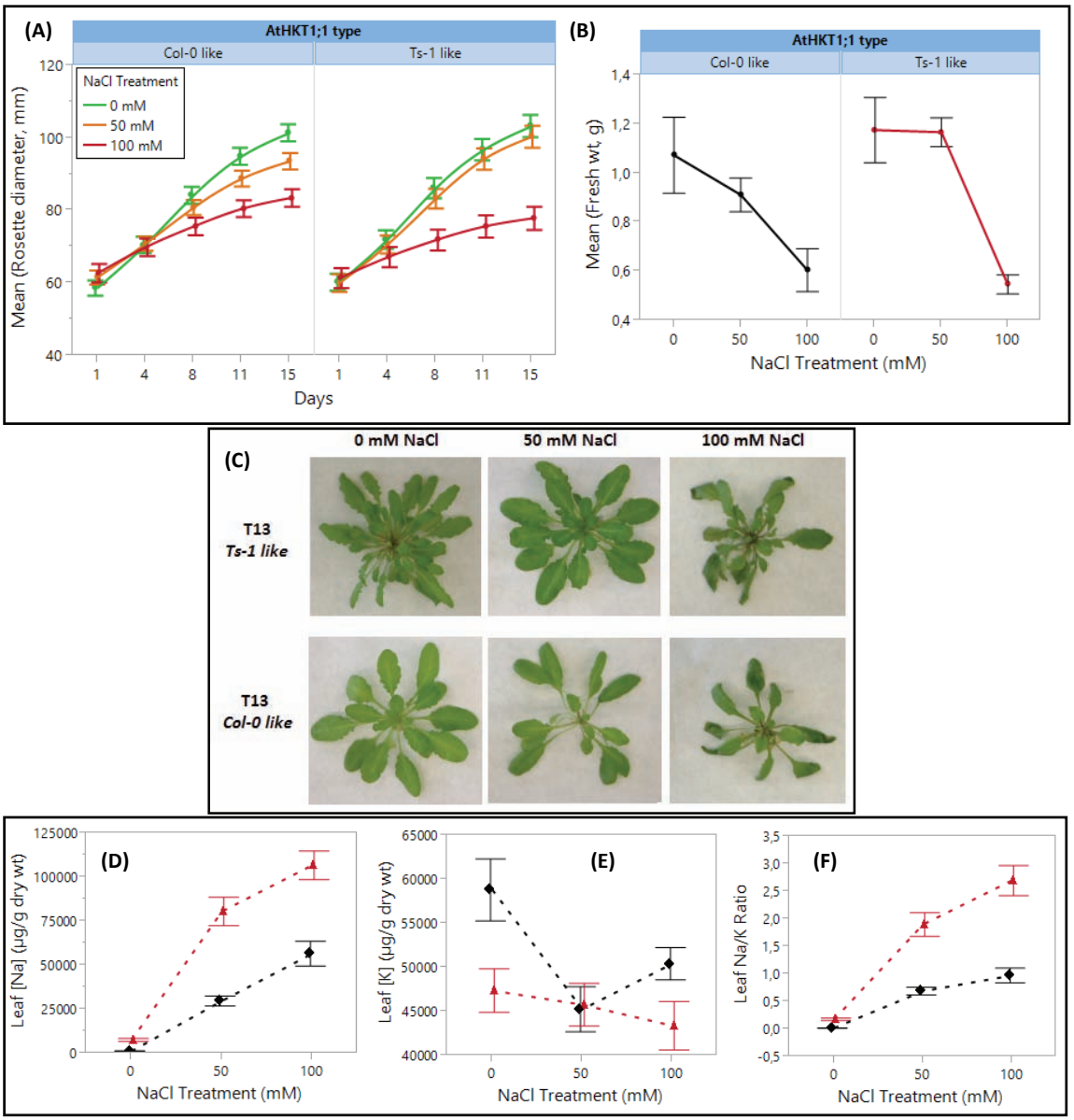


Figure 34: Mean ± SE of growth (rosette diameter, mm) **(A)** and rosette fresh weight (g) **(B)** of plants with the strong allele (Col-0 like) and plants with the weak allele of *AtHKT1;1* (Ts-1 like) from T13 and JBB demes after being exposed to either 0 mM (green lines), 50 mM (orange lines) or 100 mM (red lines) NaCl in the hydroponic solution during 2 weeks. **(C)** Pictures of T13c and T13t plants grown in hydroponics with 0, 50 and 100 mM NaCl. Leaf Na^+ **(D)** and K^+ **(E)** content (µg/g) and Na^+/K^+ ratio (**F**) of plants with the strong allele (black diamonds) and plants with the weak allele of *AtHKT1;1* (red triangles) after two weeks of exposure to 0, 50 and 100 mM NaCl in the hydroponic solution. Data represents the mean ± SE (n = 20 plants per *AtHKT1;1* allele type and treatment).

After analysing rosette fresh weight of plants with each *AtHKT1;1* allele (Figure 34 B, Table A19) we confirmed that this variable is a good proxy for fitness as it reflected the same results obtained through rosette diameter measurements, and was also correlated with the silique number measured in the soil irrigation experiment (Figure 33 B).

As we observed in the cultivation of T13c, T13, JBBc and JBBt in the BLA coastal and SCF inland common gardens, the only plant mineral nutrients analysed in leaves that varied significantly between plants having weak (T) or strong (C) allele of *AtHTK1;1*, regardless of the NaCl treatment applied, were Na^+, K^+ and Na^+/K^+ ratio (Table A20). Obviously, even though Na^+ and Na^+/K^+ ratio increased in both types of plants when NaCl was added to the growth solution, the content of Na^+ and Na^+/K^+ ratio was always higher in Ts-1-like plants (Figure 34 D and F).

In previous experiments, we did not find relevant differences in the accumulation of K^+ in leaves but, in this case, we found that in control conditions and when we treated the plants with 100 mM NaCl, leaf K^+ content in plants with strong allele of *AtHKT1;1* was considerably higher (Figure 34 E, Table A20). Curiously, in the 50 mM NaCl treatment, leaf K^+ content in Col-0-like plants was lower than K^+ content of the same plants treated with 100 mM of NaCl, reaching similar values to those of Ts-1-like plants (Figure 34 E, Table A20).

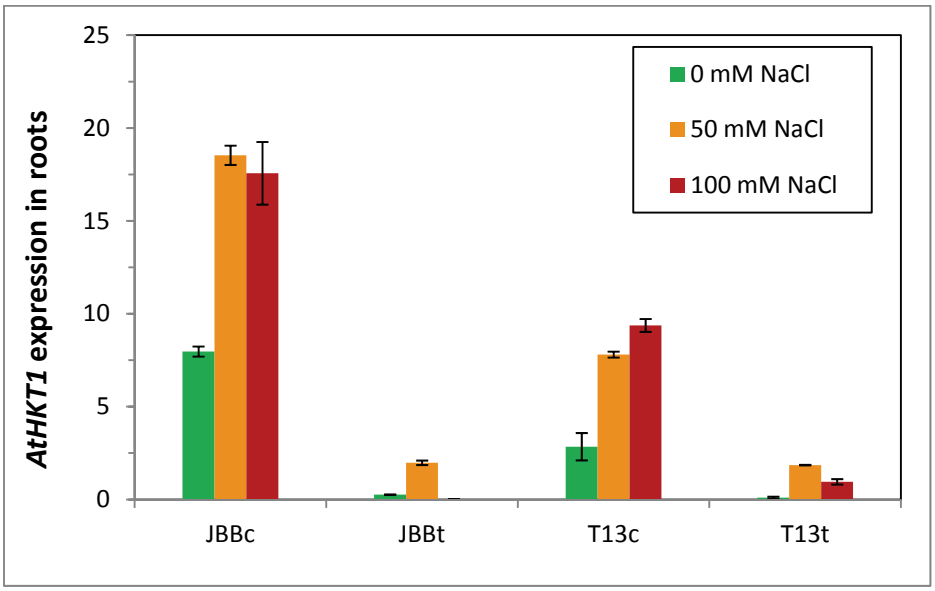


Figure 35: Expression profile of *AtHKT1;1* in the roots of three plants from T13c, T13t, JBBc and JBBt after being exposed to either 0 mM (green bars), 50 mM (orange bars) or 100 mM (red bars) NaCl in the hydroponic solution during 2 weeks. Data represents the Mean ± SE (n = 3 plants per demes/*AtHKT1;1* genotype and treatment)

Further, plants containing the Ts-1-like plants had low expression of *AtHKT1;1* regardless of the NaCl treatment (Figure 35). Whereas, expression of *AtHKT1;1* in roots from plants containing the strong allele of *AtHKT1;1* showed expression under control conditions (0 mM added NaCl), and a strong induction of expression after the addition of 50 mM NaCl (Figure 35). Addition of NaCl to 100 mM caused no further increase in *AtHKT1;1* expression. The low expression of *AtHKT1;1* in plants with the weak allele was also observed in roots of plants grown in both the field coastal and inland common gardens (Figure 26 B).

4.7. Role of *AtMOT1* weak allele

4.7.1. Localization and relations with climatic and soil characteristics

To determine if our *A. thaliana* plants had a similar deletion in the promoter as the Van-0 and Let-0 like alleles of *AtMOT1* (Baxter *et al.,* 2008), we developed an SSR marker and genotyped plants from 36 demes. We identified 29 demes containing plants with the strong allele (like Col-0 like, C) and 7 demes containing plants with the weak allele (Van-0 like, V) of *AtMOT1*. As we found with *AtHKT1;1*, demes containing plants with the weak allele were all from coastal sites but in this case we found 3 demes where all the individuals had the weak allele and 4 demes with a mixture of plants containing either the weak or strong allele (Table A2). There is only one deme (RO2) containing plants with the weak allele of *AtHKT1;1* and other plants with the weak allele of *AtMOT1.*

Distribution of the weak allele of *AtMOT1* seems not to be related with Mo content in the soil since the presence of these plants is not correlated with a high or low Mo concentration in their native soil (Figure 36 B). However, Figure 36B shows that demes containing only plants with the weak allele of *AtMOT1* are located closer to the sea than demes having a mixture of plants with either the strong or weak allele of *AtMOT1*. These 4 mixed demes are located at a distance between 0,3 and 2 km from the sea. Both mixed demes for *AtHKT1;1* allele and mixed demes for *AtMOT1* are located at an average distance of 1 km from the sea in places) where we found that levels of certain elements were highly variables (Figure 30 and 38). This distribution may suggest that the *AtMOT1* gene could be related with coastal adaptation and salinity tolerance.

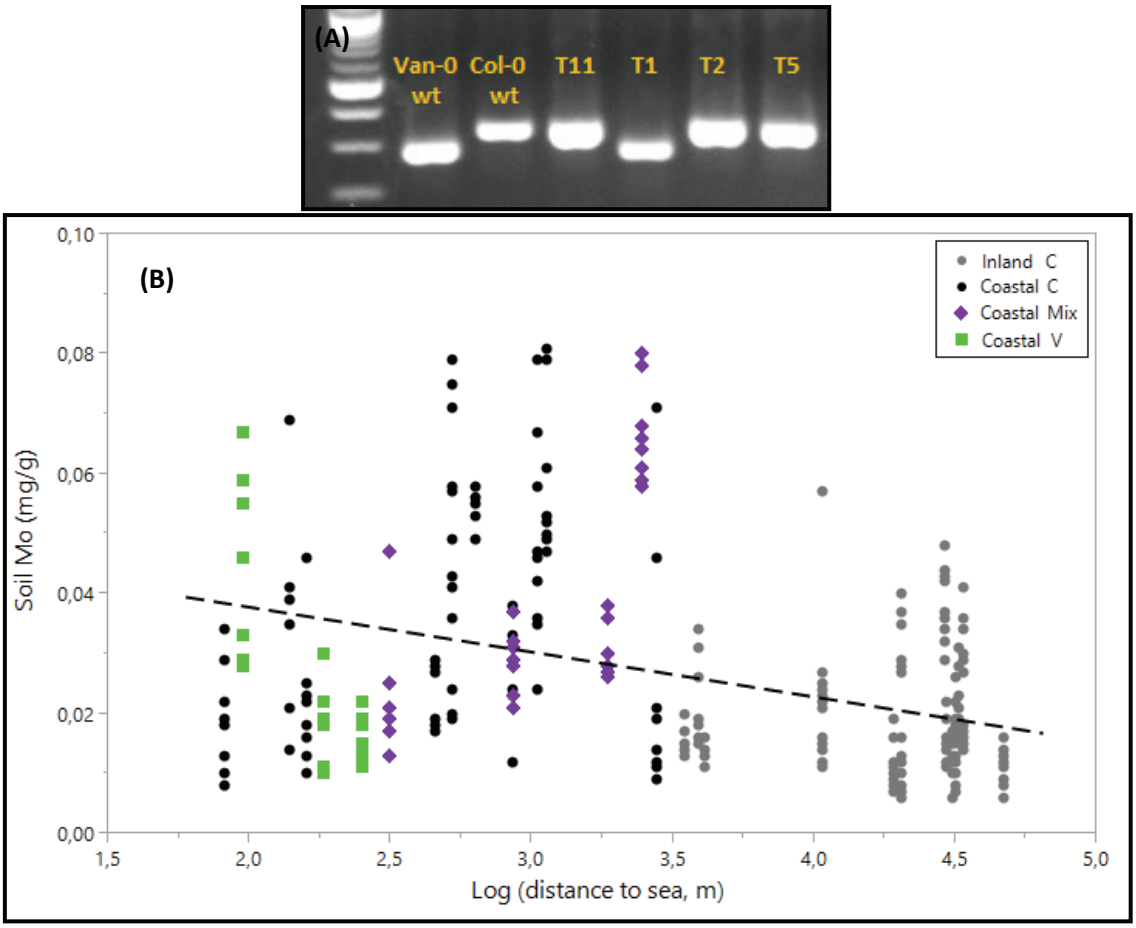


Figure 36: (A) Picture of a gel obtained from a PCR performed to classify plants according to *AtMOT1* allele type. **(B)** Mo concentration of 3 samples of soil from 18 coastal and 18 inland sites collected on 2013, 2014 and 2015 and its relationship with the distance to the coast (logarithm of meters from the sea) and the presence of *AtMOT1* weak (V) or strong (C) allele. Grey circles: inland sites; black circles: costal sites with plant homozygous for the strong allele; green squares: coastal sites with plants homozygous for the weak allele; purple diamonds: coastal sites having plants with the weak and plants with the strong allele of *AtMOT1* (Mix).

Following the steps taken in the *AtHKT1;1* study, we incorporated the *AtMOT1* genotypic data of 18 coastal demes into a reanalysis of soil and leaf ionomic data from field material collected in 2013, 2014 and 2015. Soils from sites containing all or some plants with the weak allele of *AtMOT1* have significantly higher Na^+/K^+ ratio, higher content of As, Co, Se, Sr and lower content of K, Cu, P, Rb and Zn (Table A21).

Except for P, it seems that the lower content of K, Cu, Rb and Zn detected in soils from sites having plants with the weak allele of *AtMOT1* does not interfere with the homeostasis of these mineral nutrients because no significant differences were found in the content of K, Cu, Rb or Zn in leaves from plants having either the strong or weak allele of *AtMOT1* (Table A21 and A22). On the other hand, we detected that the elevated concentrations of As, Co, Se and Sr in

soils from sites containing plants with the weak allele does result in high concentrations of these elements in the leaves of these plants (Figure 37 D, E, F and G).

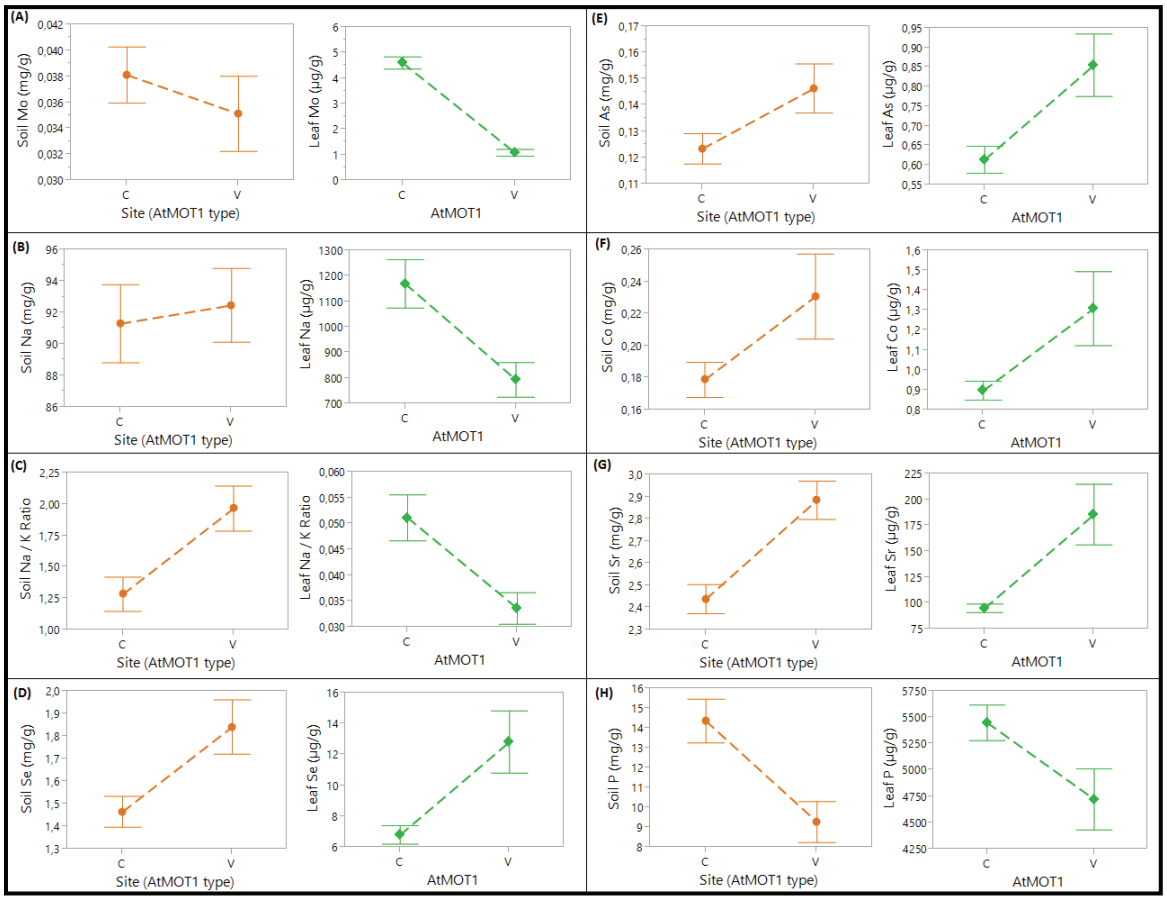


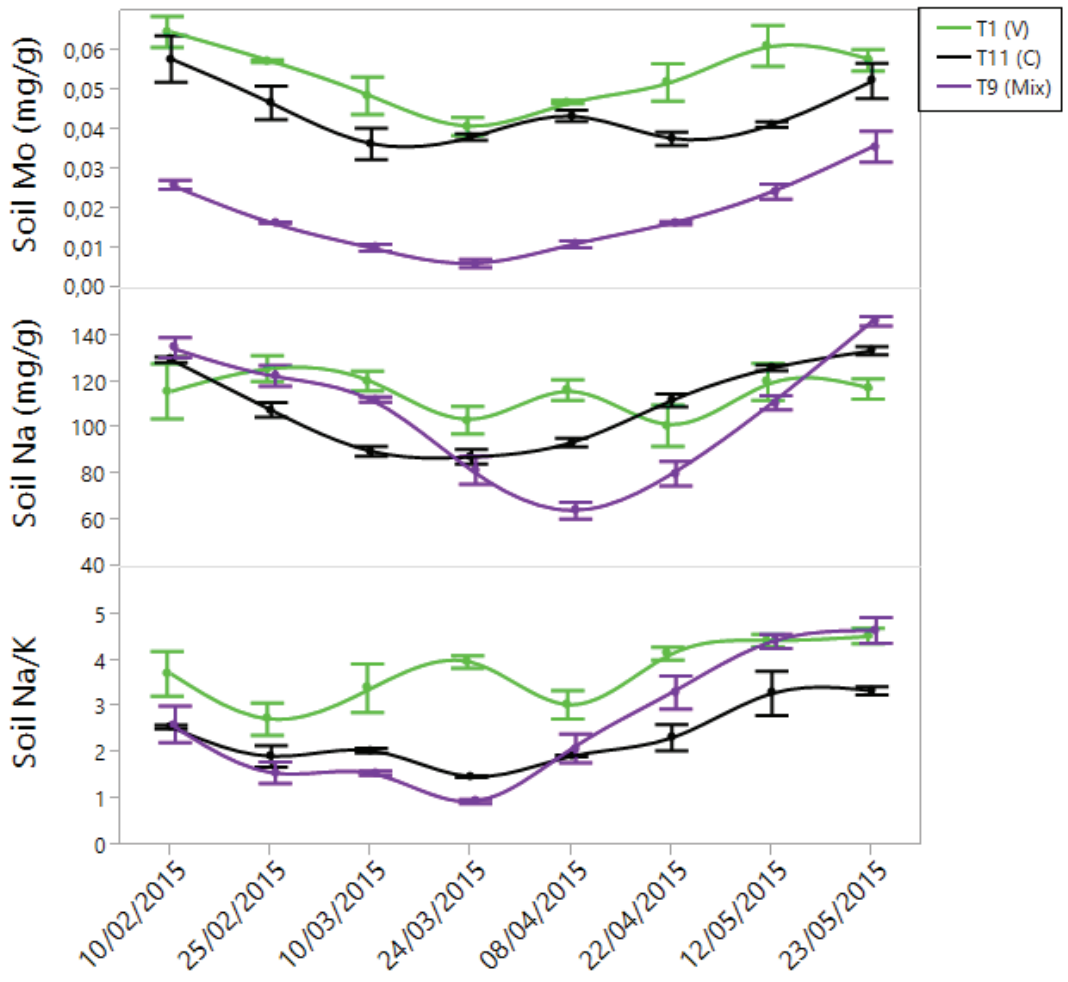
Figure 37: Mo content **(A)** Na content **(B),** Na^+/K^+ Ratio **(C)** , Se **(D)**, As **(E),** Co **(F),** Sr **(G)** and P **(H)** content in: soil (mg/g) from 11 coastal sites having plants homozygous for the strong allele (C) and 7 coastal sites containing plants (homozygous or mixture) with the weak allele of *AtMOT1* (V) and *A. thaliana* leaves (µg/g dry weight) from 152 plants with the strong allele (C) and 66 plants with the weak allele of *AtMOT1* (V). Samples collected on March of 2013, 2014 and 2015.

Consistent with Baxter *et al.* (2008), independent of soil Mo concentrations, Van-0-like plants accumulate less Mo in their leaves than Col-0-like plants (Figure 37 A). Besides, we also detected that Na^+ levels and Na^+/K^+ ratio in leaves from Van-0-like plants are always lower than in leaves from Col-0-like plants, independently of Na^+ content and Na^+/K^+ ratio in the soil (Figure 37 B and C, Table A21 and A22).

To verify the hypothesis that soil composition is more variable in the coastal locations where we found demes with a mixture of the weak and strong allele of *AtMOT1* growing together, we selected one coastal deme with all plants typus Col-0 (T11, C), one coastal deme having all plants typus Van-0 (T1, V) and one coastal deme having a mixture of plants with either

AtMOT1 alleles (T9, mix). Two soil samples were collected twice a month from February to May of 2015 and we analysed Na and Mo content (mg/g) and Na/K ratio.

(A)



(B)

	Soil element	Mean	Variance	Min Value	Max Value	Diff (Max-Min)
T11 (C)	Mo (g/g)	44,381	0,067	32,6	63,9	31,3
	Na (mg/g)	110,22	389	84,66	135,48	50,82
	Na/K ratio	2,37	0,47	1,48	3,78	2,3
T1 (V)	Mo (g/g)	53,75	0,073	38,61	68,72	30,11
	Na (mg/g)	115,21	459,65	92,21	131,62	39,41
	Na/K ratio	3,76	0,50	2,39	4,71	2,32
T9 (Mix)	Mo (g/g)	18,4	0,093	5,22	39,73	34,51
	Na (mg/g)	106,87	790,18	60,86	148,55	87,69
	Na/K ratio	2,65	1,82	0,91	4,94	4,03

Figure 38: Soil Na, Mo (mg/g) and Na/K ratio **(A)** and mean, variance, minimum, maximum and total difference between values of Na (mg/g), Mo (g/g) and Na/K ratio **(B)** of two independent samples collected twice a month from February to May of 2015 from T1 (deme with plants homozygous for the weak allele of *AtMOT1*, green lines), T11 (deme with plants homozygous for the strong allele of *AtMOT1*, black lines) and T9 (deme containing a mixture of plants with the strong or weak allele of *AtMOT1*, purple lines).

Figure 38 shows that, effectively, the site where soil Na, Mo and Na/K ratio was more variable during the *A. thaliana* growing season was T9, the deme containing plants with the weak and plants with the strong allele of *AtMOT1*. This fact is more appreciable in the case of Na because at the T1 and T11 sites Na content remained between 85 - 135 mg/g but at T9 site Na content decreased to 60 mg/g in April (the month with more precipitation) and reached the 150 mg/g Na at the end of May. Another important finding was that Mo in the soil from T9 - the deme with mixed alleles- is clearly lower over the whole season compared to Mo concentrations in soils from T1 or T11.

4.7.2. Influence on salinity tolerance

4.7.2.1. Field-based common garden cultivation

After observing the distribution of plants with the weak allele of *AtMOT1* in our region and the relationship with sodium levels in soil and within the plant, we decided to select two mixed demes (LLO2c, LLO2v; T9c, T9v) to cultivate them in the coastal common garden of Blanes (BLA) and in the inland common garden of Santa Coloma de Farners (SCF) to evaluate the effect on fitness of the weak or strong allele of *AtMOT1* in field-based conditions. In late February of 2014 and 2015, 100 seeds of each accession (obtained the previous year from genotyped plants) were sown in the field at each site. Two weeks post-germination we removed some individuals to leave 20 plants of each accession per site: 10 plants to monitor growth and silique number and 10 plants to harvest them in April for leaf ionome analysis.

Both Figure 39 A and B demonstrate that Van-0-like plants for *AtMOT1* outperformed fitness of Col-0-like plants when they were cultivated at the BLA coastal common garden. At the SCF inland common garden, however, we could not detect significant differences of growth or silique production between plants with either allele in either 2014 or 2015 (Figure 39 A and B, Tables A23 and A24). Col-0-like plants flowered earlier than Van-0-like plants in the BLA coastal common garden in 2014 and 2015 (Figure 39C, Table A24), indicating a higher fitness of plants with the weak allele of *AtMOT1* in a coastal habitat.

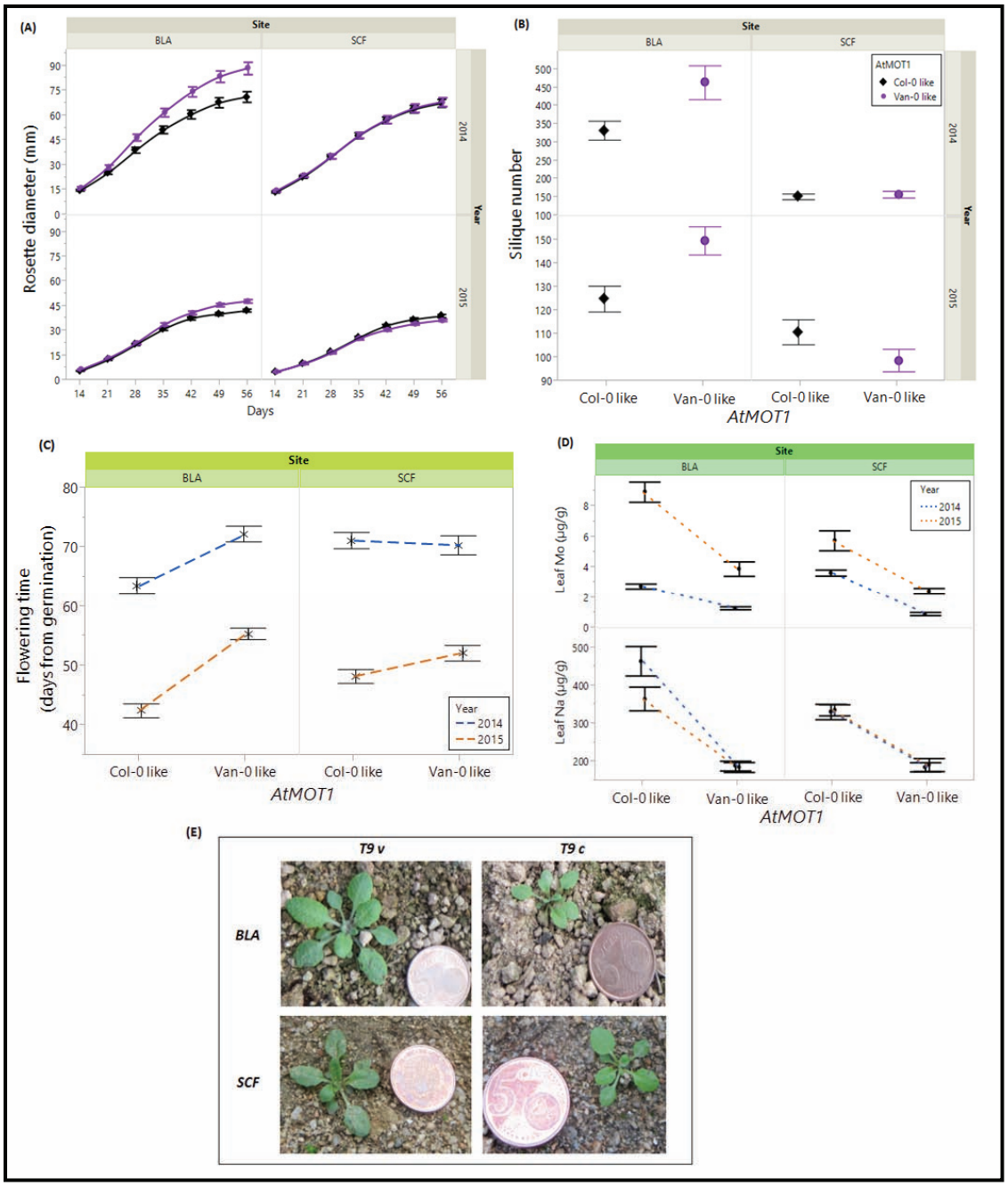


Figure 39: Mean ± SE of fitness of *A. thaliana* plants measured as growth (rosette diameter) **(A)** and silique number **(B)**. Plants with the strong allele (black diamonds) and plants with the weak allele of *AtMOT1* (purple circles) from T9 and LLO2 demes cultivated at the BLA coastal and the SCF inland common gardens in 2014 and 2015. Data represents the mean ± SE (n = 20 plants per *AtMOT1* allele type). Flowering time (measured as days from germination when the first flower appeared) **(C)** and leaf Mo and Na^+ content (µg/g) **(D)** from 20 plants with the strong allele (Col-0 like) and 20 plants with the weak allele of *AtMOT1* (Van-0 like) cultivated on each field common garden in 2014 (blue lines) and 2015 (orange lines). **(E)** Pictures of plants from T9 deme with the strong (T9c) or weak (T9v) allele of *AtMOT1* growing at the SCF and BLA common gardens after 20 days from sowing.

Taken together the results of leaf ionome analyses from the 2014 and 2015 field-based common gardens, significant differences between plants with the weak or strong allele of

AtMOT1 were only detected for Mo and Na content and Na/K ratio (Table A25). As shown in Figure 39D, Van-0-like plants always accumulate less Mo and Na in both coastal (BLA) and inland (SCF) common garden. These results suggest that, in addition to a loss-of-function of *AtMOT1* allele that results in minimal transport of Mo to the leaves, these plants also have a mechanism that allows them to exclude Na more efficiently. Furthermore, we can also affirm that this low Mo concentration in Van-0-like plants does not adversely affect their normal development as these plants had better growth and reproduction than Col-0-like plants when they were cultivated together at the BLA coastal common garden (Figure 39 A and B).

4.7.2.2. Hydroponic experiments with NaCl treatments

To confirm that plants with the weak allele of *AtMOT1* could withstand increased Na^+ whilst maintaining a low accumulation of Mo and Na in their leaves, we performed a hydroponic experiment with the same accessions cultivated in the field common gardens. 32 plants of LLO2c, LLO2v, T9c and T9v were grown hydroponically with 0,5-Hoagland solution and 8 plants of each were treated with 0, 50 and 100 mM NaCl over 3 weeks. After this period, plants were harvested, roots and leaves were weighed and the leaves were analysed for the ionome.

Without NaCl supply, plants containing the weak or strong allele of *AtMOT1* grew similarly. After treatment with 50 mM and 100 mM NaCl for 3 seeks growth of both types of plants was reduced. However, plants containing the weak allele of *AtMOT1* grew significantly (Exact F = 7,5 ; p = 0,01) better than plants with the strong allele after treatment with 100 mM NaCl (Figure 40 A, Table A26). This enhanced salinity tolerance of plants with the weak allele of *AtMOT1* was reflected in both the average fresh weight of leaves and roots. After treatment with 100 NaCl, Van-0-like plants for *AtMOT1* had a larger shoot and root biomass (Figure 40 B, Table A27).

The reduction of more than 50% of shoot Mo content in all treatments confirms the loss-of-function of *AtMOT1* allele in our Van-0-like plants (Figure 40 C). In this experiment, under control conditions we could not detect differences in the accumulation of Na^+ or in the Na^+/K^+ ratio between plants with either the weak or strong allele of *AtMOT1*. However, when NaCl was added to the nutrient solution, the accumulation of Na^+ in Van-0-like plants was clearly lower (*F* ratio > 4 ; p < 0,05), particularly in the 100 mM NaCl treatment (Figure 40 C and D, Table A28).

We also found significant differences (*F* ratio > 4 ; p< 0,05) for other elements. Calcium, and consequently the Ca/Mg ratio, was higher in Col-0-like plants for *AtMOT1* in all treatments, but the difference was more remarkable in the 100 mM NaCl treatment (Figure 40 D). In the case of Mn and Co, we found that plants with the strong allele of *AtMOT1* accumulated more Mn and Co in shoots in the 0 mM and 50 mM treatments relative to plants with the weak allele, but no difference was observed in the 100 mM treatment (Table A28).

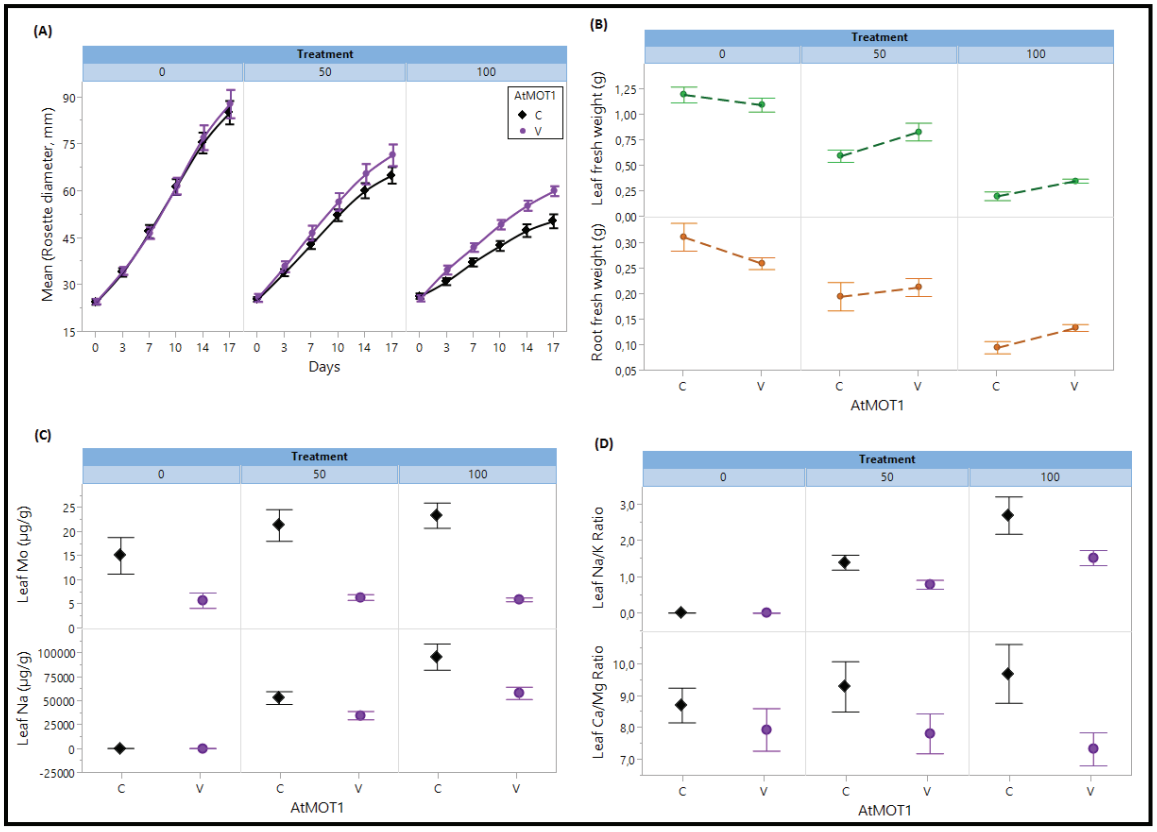


Figure 40: Mean ± SE of growth (rosette diameter, mm) **(A)**, roots and rosette fresh weight (g) **(B)** of plants with the strong allele of *AtMOT1* (C, black line) and plants with the weak allele of *AtMOT1* (V, purple line) after being exposed to either 0, 50 or 100 mM NaCl in the hydroponic solution during 3 weeks. Leaf Mo and Na^+ content (µg/g) **(C)** and leaf Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios **(D)** of Col-0-like plants (black diamonds) and Van-0-like plants (purple circles) after 3 weeks of exposure to 0, 50 and 100 mM NaCl in the hydroponic solution. Data represents the mean ± SE (n = 16 plants per *AtMOT1* allele type and treatment).

To evaluate if the loss-of-function of *AtMOT1* may increase salinity tolerance we repeated the hydroponic experiment using T-DNA insertion knockout mutants of *AtMOT1* in the Col-0 background. Col-0 and *mot1* mutant plants grew similarly after treatment with 0 and 50 mM NaCl (Figure 41 A), with Col-0 wild-type plants had a slightly larger leaf and root biomass than *mot1* mutant plants (Figure 41 B). However, when plants were treated with 100 mM NaCl in the nutrient solution the growth solution Col-0 wild type plants showed a severe reduction in

growth, whereas the *mot1* mutant continued to grow and increase its size and shoot fresh weight (Figure 41 A and B, Tables A29 and A30).

Root fresh weight does not seem to be a good indicator in this case since we could not detect differences between Col-0 and the *mot1* mutant in any of the treatments (Figure 41 B; Table A30). As it is known that Mo plays a critical role in reductive and catalytic processes, we measured the root ferric cheated reductase activity (RC) of both Col-0 wild-type and the *mot1* mutant after the three weeks of NaCl treatment.

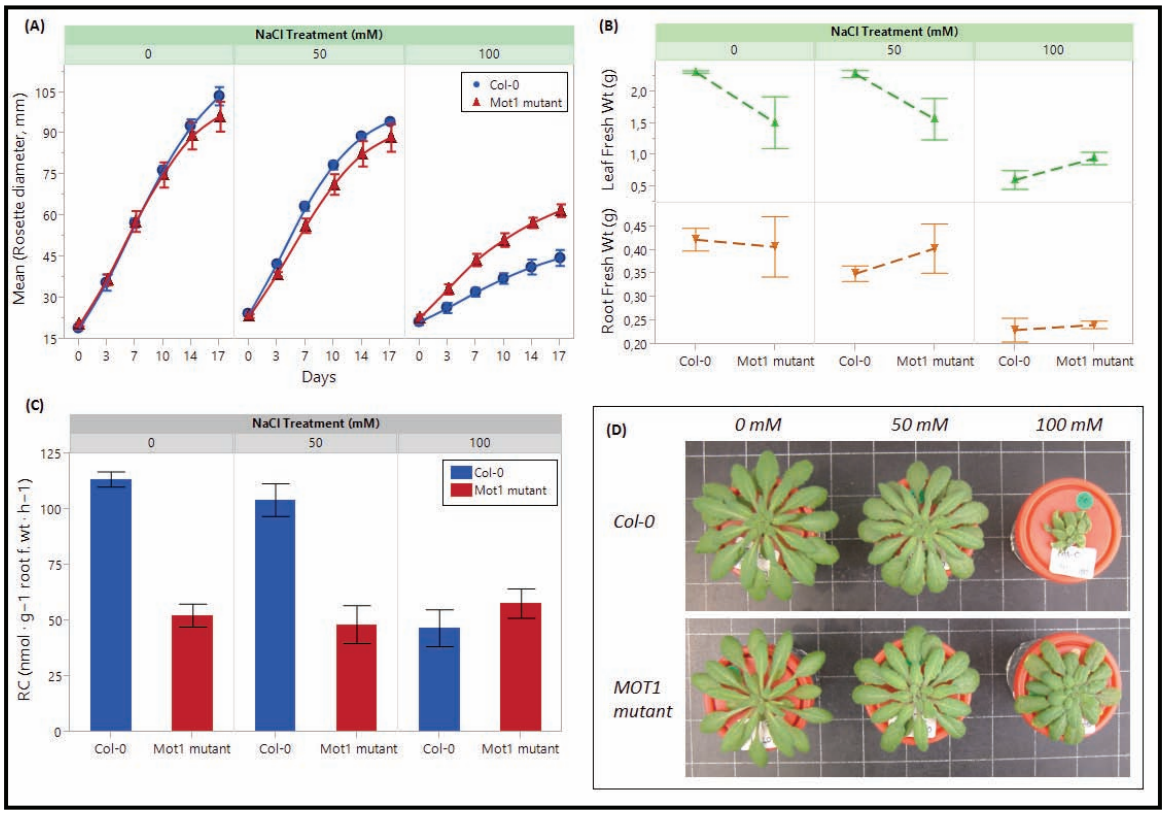


Figure 41: Mean ± SE of growth (rosette diameter, mm) **(A)**, roots and leaves fresh weight (g) **(B)** and and root ferric reducing capacity (RC, nmol $Fe^{2+} \cdot g^{-1}$ root fresh Wt · h^{-1}) **(C)** of Col-0 plants and *mot1* mutant plants after being exposed to either 0, 50 or 100 mM NaCl during 3 weeks. **(D)** Pictures of Col-0 and *mot1* mutant plants grown in hydroponics with 0, 50 and 100 mM NaCl before harvest.

As expected, ferric reducing capacity of *AtMOT1* knockout mutant plants was lower in control conditions and 50 mM NaCl treatment (*F* ratio > 20; p < 0,05). However, Figure 41C demonstrates that mutant plants had the capacity to maintain almost the same RC under all NaCl treatments. On the other hand, Col-0 plants had more RC under control conditions and when they were treated with 50 mM NaCl, but, when Na levels were increased to 100 mM, their RC was halved, indicating once again their low tolerance to salinity (Figure 41C, Table A30).

5. Discussion

5. Discussion

Localization and genetic study of coastal and inland A. thaliana populations

The annual plant *A. thaliana* has been widely used in studies of plant genetic diversity, adaptation and biogeography (Nordborg *et al.*, 2005; Schmid *et al.*, 2006; Montesinos *et al.*, 2009; Wolfe & Tonsor, 2014). The rapid Holocene expansion of *A. thaliana* into northern Europe from Asian and Mediterranean refugia suggests a high potential to disperse and adapt to diverse environments (Falahati-Anbaran *et al.*, 2014). Identifying the geographic distribution of wild *A. thaliana* populations is a basic, yet crucial step that provides key information on which many subsequent analyses depend.

Specie Distribution Models (SDMs) are powerful tools to enhance field sampling. These models relate data about species presence/absence to environmental variables. The calibrated models can then be projected onto the entire study area, producing a map that predicts how suitable each mapping unit is for the target specie (Le Lay *et al.*, 2010). In our case, several geo-referenced variables describing climatic conditions and landscape features were analyzed to identify potential factors influencing the likelihood of *A. thaliana* occurring in the NE of Catalonia. Cells of each map were reclassified by Miramon software (Pons, 1994-2011) with regard to species presence. The model used indicated that probability of existence was correlated with altitude, land uses and geology. These factors determined the suitable habitats (potential polygons) for *A. thaliana* which were surveyed (Figure 13).

Geographical distributions of many plant species can be mainly explained using climatic variables as predictors of a logistic regression and this seems to be the case for most species when working on a large scale (Thuiller *et al.*, 2004). Other predictors, however, may improve the climatic models or even substitute for them in the case of lower spatial resolution (less than 50 km × 50 km grid), as shown for land use variables (Pearson *et al.*, 2004) or lithologic data (Gastón *et al.*, 2009). The fact that geology was the most discriminatory variable regarding the localization of *A. thaliana* in Catalonia predicts that soil properties and composition would play a crucial role in the distribution and development of wild populations of *A. thaliana* in our study area.

The identification of more than 40 demes of *A. thaliana* (most of them not cited in any botanical record or not geo-referenced in any study) inside the areas provided by the SDM, allows us to validate the efficiency and usefulness of the method developed.

Most of the demes identified, especially those in coastal areas, are small (composed of less than 50 individuals), isolated and they occur in degraded areas very exposed to human disturbances. Therefore these demes are at high risk of extinction. Nonetheless, most of them have survived from our base-line survey on 2007 through to our last survey in 2015. We also detected certain cases of recolonization (Figure 14). Natural populations of annual plants are often subjected to large between-year fluctuations in census sizes, especially in disturbed habitats, and may experience extensive metapopulation dynamics. Metapopulation processes, in which local populations are connected by migration, can result in rapid genetic turnover through extinction-recolonization events. These processes have profound demographic consequences and are often expected to reduce the effective population size and, in consequence the within-population genetic diversity as a result of founder effects and increased population differentiation, relative to more stable conditions (Falahati-Anbaran *et al.*, 2014).

A set of 425 SNP markers was used to characterize the extent of gene flow between inland and coastal *A. thaliana* demes within the study area (Figures 15 and 16). We did not find clear stratification of the population between demes of inland and coastal origins but the F_{st}, an indicator of allelic differentiation, showed peaks in two markers from chromosome 1 and 2. Even though the number of SNPs is too low to perform a statistically powerful association analysis, this information was used to make a list of 19 candidate genes that could be important for the genetic differentiation between regions (Table A3). Notably, one of the candidate genes was At1G73480, a gene already described by Taji *et al.* (2004) as a salt stress-inducible gene in microarray analyses comparing salt cress and *A. thaliana* plants treated with 250 mM NaCl for 2 hours.

Measure of the local adaptation to coastal and inland environments

Ten demes from the coast and ten demes from inland were selected to perform a field-based reciprocal transplant experimental in two representative sites of coastal and inland environments. The design allowed us to directly test for local adaptation of coastal and inland

populations of *A. thaliana* by comparing the mean fitness of multiple local and non-local demes across the two environments. Using this approach we identified clear signals of local adaptation across two consecutive growing seasons. We used silique number as an estimate of seed number to give a measure of fitness. Furthermore, measurements of plant growth in the same reciprocal transplant experiments were also consistent with the existence of local adaptation.

In a similar manner to that recently reported by Ågren & Schemske (2012) for *A. thaliana* reciprocal transplant experiments between Sweden and Italy, we observe mean fitness trade-offs for silique number and growth in the field in our 2014 experiment, with locally adapted plants in their native habitat showing higher fitness than transplanted plants from other habitats. In the field in 2013 instead of fitness trade-offs we observed conditional neutrality, with plants showing local adaptation at the coast but equal fitness inland (Figures 17 and 18). This is consistent with both trade-offs and conditional neutrality being part of local adaptation (Anderson *et al.*, 2013; Blanquart *et al.,* 2013).

Adaptation against a backdrop of migration is thought to favour the evolution of fewer loci with larger adaptive effect size (Yeaman & Whitlock, 2011), where fitness trade-offs are likely to be due to trade-offs between individual alleles (Savolainen *et al.,* 2013). Such considerations suggest that adaptive traits between populations connected by migration would be more tractable to genetic dissection. In contrast, isolated populations are expected to adapt independently to their environment, and trade-offs at the fitness level between populations may be due to different loci that influence the same trait in the different populations (Savolainen *et al.,* 2013) and be dominated by numerous small effect alleles (Orr, 1998).

Fitness trade-offs were again observed in a controlled-environment common garden experiment using soil excavated from inland and coastal sites (Figure 19). Such fitness trade-offs established that coastal and inland individuals are better adapted to their native soil. Further, the lack of conditional neutrality in the controlled-environment common garden suggests that the conditional neutrality observed in the field in 2013 was an artefact caused by a lack of statistical power to detect adaptation in multiple environments (Anderson *et al.*, 2011). Alternatively, soil conditions inland may vary significantly across years, driving the variable performance we observe in our inland common garden of plants from coastal and inland demes.

Agents responsible for the divergent selection between coastal and inland habitats

The Ågren & Schemske (2012) study showed that single *A. thaliana* genotypes from Sweden and Italy are locally adapted. Even though they crossed the parents to generate RILs to identify QTLs associated with adaptive traits, they were studying effectively only two alleles per locus. Our study extends this to a much finer geographic scale and it has more representative results because we used multiple independent genotypes from inland and coastal habitats. Environmental heterogeneity favours the evolution of adaptive phenotypic plasticity. A genotype that in each habitat produces the locally optimal phenotype would become fixed in all demes. Adaptive phenotypic plasticity would thus lead to adaptive phenotypic differentiation, but without underlying genetic differentiation (Kawecki & Ebert, 2004). Thus, is important to measure local adaptation in a metapopulation where multiple demes are sampled.

More similar in scale to our study, and sampling from a nearby region, altitudinal clines in spring heat and drought over several hundred km from the coast into the Pyrenees Mountains in north-eastern Spain were correlated with the fitness of locally collected populations of *A. thaliana* when assayed in controlled environmental chambers (Wolfe & Tonsor, 2014). In our study, no obvious climate factor discriminates coastal and inland regions. Instead, we found significant differences in soil from coastal and inland habitats with the sea influencing coastal soils through the deposition of the major solutes of seawater Na, Mg, Cl^- and SO_4^{2-} (Figures 20 and 21).

We showed with both multi years field-based and controlled-environment common garden experiments that the fitness of plants from coastal and inland demes are adapted to local soils, with clear mean fitness trade-offs between the two habitats. Soil conditions have particularly strong effects on plant growth and can be spatially heterogeneous, and these are sources of the divergent selection necessary for local adaptation. Previous studies have found plant populations adapted to local soils in particular aspects such as chemistry (Sambatti & Rice, 2006; Turner *et al*., 2010), nutrient availability (Pregitzer *et al.*, 2013) or soil communities (Johnson *et al.,* 2010; Lankau *et al*., 2011).

Many studies have correlated different soil conditions with changes in elemental concentrations (Buescher *et al.*, 2010). Soils that are deficient or excessive in specific nutrients, can sometimes be diagnosed by assaying the leaf ionome for a specific pattern of changes (Baxter *et al.*, 2008). We found that plants growing in the field on coastal soils accumulated high leaf concentrations of Na^+, Na^+/K^+ ratio and Mg^{2+} and lower Ca^{2+} compared to plants growing inland. This corresponds with the elevated Na in the soils and the elevated Mg competing for uptake with Ca (Brady *et al.*, 2005). In laboratory studies *A. thaliana* only shows growth inhibition at Ca^{2+}/Mg^{2+} ratios below 0,25 (Bradshaw, 2005). Our coastal soils have a mean Ca^{2+}/Mg^{2+} ratio of 4,9, therefore the higher Mg^{2+} concentration detected in coastal soils is less likely to be playing a role in the divergent selection we observe (Figures 21 and 22).

High Na^+/K^+ ratio in saline growth medium may impair the selectivity of the root cell membrane and result in passive accumulation of Na in the roots and shoots (Kramer et al., 1977). Under typical physiological conditions, plants maintain a low Na^+/K^+ ratio in their cytosol with relatively high K^+ (100–200 mM) and low Na^+ concentrations (1–10 mM) (Apse and Blumwald, 2007). Sodium toxicity is caused mainly by the similarity of the Na^+ and K^+ ions to plant transporters and enzymes (Silva & Gerós, 2009). The combination of voltage-independent gating and selectivity for K^+ over Na^+ of a particular class of root cation channels in *T. halophila* underlies the fact that this salt-tolerant specie takes up K^+ while restricting Na^+ absorption in saline environments (Volkov & Amtmann, 2006). However, in this study the Na^+/K^+ ratio was higher in leaves of *A. thaliana* coastal plants living in more saline soils (Figure 22). This is probably due to a higher capacity of these coastal plants to sequester Na^+ in the leaf vacuoles.

Elevated salinity is harmful to plants (Munns & Tester, 2008, Cabot *et al.*, 2014), and soils from the sites of our coastal demes had significantly higher NaCl concentrations than inland sites. In controlled environment common garden experiments, plants from coastal demes showed more tolerance to NaCl compared to those from inland demes. Importantly, we also observed differences in mean fitness between plants from coastal and inland habitats when grown with different salinity treatments, with coastal plants having higher growth at elevated salinity (Figures 24 and 25).

Mechanisms of salinity tolerance

The salinity tolerance of our coastal plants appears to not be based on Na^+ exclusion because leaf material from the field and hydroponic experiments with NaCl treatments showed that plants from coastal demes accumulate more Na^+ compared to those from inland demes (Figure 23 and 25). One plausible hypothesis for this is that the salinity tolerance of the coastal plants is driven by the uptake and vacuolar compartmentalisation of Na^+ facilitating osmotic adjustment.

Under salinity, Na^+ ion efflux occurs from the cytosol of root cells. Different Na^+ transporters are implicated in the regulation of intracellular and *in planta* Na^+ homeostasis (Horie *et al.*, 2009). Available evidence supports the notion that *AtHKT1;1* is responsible for Na^+ unloading from root xylem vessels which reduces Na^+ flux to the shoot (Sunarpi *et al.*, 2005; Rus *et al.*, 2006; Davenport *et al.*, 2007; Horie *et al.*, 2009; Moller *et al.*, 2009). Similarly, rice *OsHKT1;5* is implicated in Na^+ unloading in the root xylem and maintenance of shoot Na^+ homeostasis (Ren *et al.*, 2005; Horie *et al.*, 2009). Molecular genetic analysis using *A. thaliana Salt Overly Sensitive (sos)* mutants revealed that a plasma membrane Na^+/H^+ antiporter, *AtSOS1*, plays a crucial role in sodium extrusion (Zhu, 2002). These *sos1* mutants have been shown to be hypersensitive to Na^+ stress, showing severe growth retardation and high shoot and xylem Na^+ concentrations which ultimately lead to the death of the plant (Jha *et al.*, 2010). While initial studies of *AtSOS1* suggested a role in regulating xylem loading (Shi *et al.* 2002), further studies have demonstrated a role in Na^+ efflux from the root (Shabala *et al.* 2005).

In high-salt conditions, constitutive over-expression of *AtSOS1* in *A. thaliana* has been shown to reduce plant Na^+ accumulation by over 50%, with transgenic plants showing higher survival and growth rates than wild-type controls (Shi *et al.* 2003). However, in this study we found that the same plants up-regulated *AtHKT1;1* and *AtSOS1* under high-salt conditions and they accumulate more Na^+ in leaves after NaCl treatments (Figure 26). A functional link and possible interplay between *SOS* genes and *HKT1;1* has already been suggested (Rus *et al.,* 2004; Pardo *et al.*, 2006; Olías *et al.*, 2009). Single *sos3* and *hkt1* mutations promote the undue accumulation of Na^+ in roots and shoots, respectively, whereas a double *sos3 hkt1* mutant achieves a more balanced partition of Na^+ that is closer to the profile of wild-type plants (Rus *et al.*, 2004). It appears that the transport function of the *SOS* system and *HKT1* are co-ordinated tightly and together they achieve Na^+ (and K^+) homeostasis. Dysfunction of either

system alters long-distance transport and partitioning of Na^+, thereby resulting in salt-sensitive phenotypes (Pardo *et al.* 2006).

In a coordinated action of these transporters, *SOS1* might efflux Na^+ from the endodermis and/or stele in the root and *HKT1* might act in the stele of the root to recycle Na^+ back out of the xylem. However the question of how the up regulation of *SOS1* and *HKT1* lead to an increased accumulation of leaf Na^+ is still unresolved. The elevated leaf Na^+ may not be the key feature. It could be that increased expression of *SOS1* and *HKT1* leads to increased accumulation of Na^+ in epidermis and cortex of roots (as Na^+ is pumped out of the stele) where it is put into the vacuole and acts as an osmoticum to allow better water uptake in coastal soils with elevated salinity. Further studies on Na^+ distribution in root tissues are required to clarify this. Most of the Na might get into the root vacuole but also some Na could spill into the leaves where the Na^+/H^+ antiportactivity of *NHX1* could have an important role. Transgenic plants expressing various *NHX1* transporters showed increased salt tolerance (see a review: Yamaguchi & Blumwald 2005), supporting the essentiality of Na+ sequestration in salt tolerance (Pardo *et al.,* 2006; Horie *et al.,* 2012).

For the last several decades it has been widely accepted that a plants K^+ nutrition status is of great importance for NaCl tolerance (Tester & Davenport, 2003; Amtmann *et al.*, 2004). Some studies have even gone as far as to conclude that salt tolerance is driven more by a plants ability to retain K^+ under salt stress, than its ability to simply exclude Na^+ ions (Wang *et al.*, 2006; Shabala & Cuin, 2008). In both the hydroponic and soil irrigation experiments to test salinity tolerance, and the BLA and SCF common garden experiments, significant differences in leaf Na^+ content and Na^+/K^+ ratio between coastal and inland demes were detected, but levels of K^+ in leaves were highly variable without following a clear pattern (Figures 23 and 25). Coastal plants in our study do not appear to tolerate elevated Na^+ by maintaining the Na^+/K^+ ratios at the level observed in inland plants. Instead, coastal plants have a higher Na^+/K^+ ratio than inland plants. However in our study we are estimating the total levels of Na^+ and K^+ in the leaf, and earlier studies have conclude that is in the cytosol where the maintenance of the Na^+/K^+ ratio is critical (Maathuis & Amtmann, 1999; Chen *et al.*, 2007; Cuin *et al.*, 2008). We currently do not have an estimate of the Na^+/K^+ ratio in the cytosol of inland and coastal plants and this should be considered for future studies.

When plants from 3 salt-tolerant coastal demes were crossed with plants from 3 salt-sensitive inland demes, it was observed that only the progeny from those crossings where the female

parent was from the coast had a similar or enhanced salinity tolerance than coastal parents. However, when the female parent was from inland, the coastal adaptation to salinity was not visible (Figure 27). All plants used in this experiment were not genotyped being the possibility that not all crossing's progeny were hybrids. Although we cannot exclude that a single silique could contain seeds derived from both cross fertilization (hybrid) and self fertilization (non-hybrid) events the possibility seems rather unlikely. Therefore, our results point to "parent-of-origin" effects. Nonetheless, further studies should be performed genotyping all individual progeny plants.

Genomic imprinting, also referred to as uniparental dominance or parent-of-origin effect, is a well-studied phenomenon in both mammals and plants (for reviews, see Alleman & Doctor, 2000; Reik & Walter, 2001). Imprinting refers to a reversible epigenetic modification of loci resulting in differential expression of genes depending on the parent of origin. Unlike Mendelian inheritance, a given allele of an imprinted gene is phenotypically expressed in the F1 depending on whether it is transmitted through the male or female parent (Kollipara *et al.*, 2002). In plants, gene imprinting occurs in the endosperm during seed development (Huh *et al.*, 2008). At fertilization, one sperm fertilizes the haploid egg cell, which becomes the diploid embryo, and the other sperm fertilizes the diploid central cell, generating the triploid endosperm. In *A. thaliana*, the 5-methylcytosine DNA glycosylase DEMETER (DME) demethylates maternal alleles of imprinted genes in the central cell before fertilization, thus establishing methylation asymmetry between embryo and endosperm (Gehring *et al.*, 2009).

As the known imprinted gene FWA, HD-ZIP genes are expressed primarily in siliques (Nakamura *et al.*, 2006). Based on crossing experiments done by Gehring *et al.* (2009) it was found that HDG9 was expressed exclusively from the maternal allele and *HDG8* and *HDG3* were also imprinted. We are mentioning this because, from the analysis of the 425 SNPs analyzed in individuals from coastal and inland habitats, one of the genes detected closely linked to the markers with high F_{st} values was *HDG11*, a gene from the HD-ZIP family that could be involved in the maternal effect observed in our coastal plants.

Interactions between cytoplasmic (generally organelle) and nuclear genomes may be relatively common and could potentially have major fitness consequences. As cytoplasmic factors are generally uniparentally inherited, the cytoplasmic genome is inherited along with only one of the nuclear haplotypes, and therefore, coadaptation is expected to evolve through the interaction of these coinherited (usually maternally inherited) genomes (Wolf, 2009). That

organellar genomic variation can play a role in plant adaptation has been suggested by several lines of evidence, including cytoplasm capture, cytoplasm effects in local adaptation and positive selection in a chloroplast gene. There are two main reasons why organelle genomes have long been disregarded as potential contributors to adaptation. First, these genomes encode a mere 10–15% of the proteomes of their organelles (Sun *et al.*, 2014) and depend on nuclear-encoded factors for their maintenance, transmission and expression. Second, most proteins encoded in organelles are involved in the structure or assembly of multimeric complexes of the electron transport chains in mitochondria or chloroplasts (Budar & Roux, 2011). Cytoplasmic local adaptation has also been detected at the within-species level (Leinone *et al.*, 2010; Galloway & Fenster, 2001). These observations indicate that intraspecific variation in the cytoplasm can contribute to adaptive population differentiation.

It is currently difficult to estimate to what extent organellar genetic diversity determines phenotypic variation, and how much this variation is constrained by genetic and physiological interactions with nuclear gene products. However, given the organellar genomic variation data available for *A. thaliana*, we looked for some mitochondrial or chloroplastic gene described for their relation to salinity tolerance. Mitochondria have uncoupling proteins (UCPs) that uncouple electron transport from ATP synthesis. There is evidence that UCPs play a role in alleviating stress caused by the overproduction of reactive oxygen species (Nicholls & Rial, 1999; Begcy *et al.*, 2011). These studies demonstrated that higher levels of *AtUCP1* improved tolerance to drought and salt stress, and this protection was correlated with lower oxidative stress.

Proteomic studies have focused on the identification of chloroplast proteins associated with stress responses (Agrawal *et al.*, 2009; Taylor *et al.*, 2009). (1) *AtCEST* gene, for example, is a novel chloroplast protein involved in chloroplast development, growth, and stress tolerance. Under salt and heat stresses, *CEST*-overexpressing transgenic *A. thaliana* retained more chlorophyll than the wild-type. Over-expression of *CEST* improved tolerance to ParaQuat, suggesting that *CEST* plays a role in providing protection from photooxidative stress (Yokotani *et al.*, 2010). (2) Omidbakhshfard *et al.* (2012) showed that *PRPL11, ATAB2* and *PDF1B* genes coding for the plastidial translation machinery are rapidly altered by salt stress. These salt-responsive genes are known to encode key functions in chloroplast biology. (3) Investigating the subcellular localization of the *A. thaliana* TPP family members, Krasensky *et al.* (2014) identified *AtTPPD* as a chloroplast-localized enzyme. Plants deficient in *AtTPPD* were salt hypersensitive, whereas plants overexpressing *AtTPPD* were more tolerant to high salinity

stress. Elevated stress tolerance of *AtTPPD* overexpressors correlated with high starch levels and increased accumulation of soluble sugars suggesting a role for *AtTPPD* in regulating sugar metabolism under salinity conditions.

Identification and role of AtHKT1;1 weak allele

Two natural accessions of *A. thaliana*, Tsu-1 and Ts-1, exhibit increased Na^+ accumulation in the shoot, decreased *AtHKT1;1* gene expression and a multitude of DNA differences compared to Col-0 (Rus *et al.*, 2006). Giving support to the importance of some of the polymorphisms described by Rus *et al.* (2006) it has recently been found that variations in the promoter are very important for *AtHKT1;1* function and expression (Baek *et al.*, 2011). Despite the low expression of *AtHKT1;1* detected in these two accessions, this naturally evolved *AtHKT1;1* allele appeared to be associated with enhanced NaCl tolerance and emerged in coastal or saline impacted soils suggesting that this gene could be under some form of evolutionary selection (Rus *et al.*, 2006; Baxter *et al.*, 2010).

The promoter of Ts-1 described in the cited article was used to genotype all the demes investigated here. Plants with the weak allele of *AtHKT1;1* (Ts-1-like) were identified only in coastal demes and always mixed with plants having the strong allele of *AtHKT1;1* (Col-0-like) (Figure 28). These demes containing plants having the weak allele are not situated in the areas more exposed to sea influence but rather in locations with intermediate levels of soil Na^+ which tend to fluctuate more significantly over time. These results suggest that *AtHKT1;1* is not a crucial gene for the general increased level of salinity tolerance observed in our coastal demes, since most coastal demes did not contain the weak allele. This is also supported by the fact that when plants with the weak allele of *AtHKT1;1* were removed from the analysis of the field common garden experiments, we have continued observing the same signals of adaptation.

Experiments done in a controlled environment with plants having the weak or strong allele of *AtHKT1;1* from two of the six demes identified to contain the weak allele confirmed that having the weak allele is beneficial when salt levels are moderate (50 mM NaCl in the nutrient solution). Ts-1-like plants outperformed in growth and fitness Col-0-like plants under these conditions. Instead, when the content of Na^+ is above these levels, the accumulation of Na^+ in leaves can become toxic for them due to the non-stop of Na^+ influx toward the shoots

corroborated by the low expression of *AtHKT1;1* in the roots of Ts-1-like plants. Under the 100 mM NaCl treatment, therefore, plants with the weak allele showed less tolerance and worse fitness than plants with the strong allele of *AtHKT1;1* (Figures 33, 34 and 35).

When plants with the weak and strong allele of *AtHKT1;1* were cultivated at the field coastal site (BLA), even though soil Na levels were not extremely high, Col-0-like plants outperformed in growth and fitness Ts-1-like plants in both years of assay. However, plants with the weak allele still had a better fitness than plants from inland demes also cultivated at the BLA field site (Figure 31). This is further evidence that the *AtHKT1;1* weak allele is not related to coastal local adaptation. This raises the question as to: why this allelic variation still persists?

Comparisons during the four months of the *A. thaliana* growing season between one of the coastal sites where all plants have the strong allele (T1) and one of the coastal sites having a mixture of plants with weak and strong allele of *AtHKT1;1* (T13), confirmed that the variability in soil Na^+ content was much higher and more dependent on rainfall at T13 than at the T1 site (Figure 30). T1 site is located at just 100 m from the sea, very exposed to winds and soil Na levels remained above 80 mg/g during all the growing season of 2014 and 2015. On the other hand, T13 is a site located at 530 meters from the coast, more sheltered from sea, and with highly variables soil Na levels increasing especially when approaching the summer months due to the decrease in rainfall (Figure 30). We believe that the high constant levels of soil Na at the sites closest to the sea (as T1) are too saline for *A. thaliana* plants with the weak allele of *AtHKT1;1*. For this reason Ts-1-like plants always occur at more than 0,5 Km from the coast where Na concentrations in the soil are intermediate (Figure 28). This is supported by the role of *AtHKT1;1* in tolerance at intermediate salinity levels. The huge differences detected in growth and fitness when Ts-1 like and Col-0-like plants were treated with 50 mM NaCl (Figure 33 and 34) suggest that sites with intermediate levels of soil Na may be more appropriate for plants with the weak allele of *AtHKT1;1*.

Moreover, these intermediate sites always have a mixture of plants with the weak and strong allele of *AtHKT1;1* because soil conditions are more variable over time, sometime having a lower Na/K ratio and sometimes having a higher Na/K ratio (Figure 30). This alludes to a balancing selection maintaining the two alternative alleles in the same place. Balancing selection refers to a variety of selective regimes that maintain advantageous genetic diversity within populations (Delph & Kelly, 2014). The mechanisms include heterozygote advantage, negative frequency-dependent selection, spatial or temporal habitat heterogeneity,

antagonistic pleiotropy, and sexual antagonism, among others. While differing in details, these mechanisms share the feature that whether an allele is beneficial or detrimental is conditional in some way. An allele cannot be described as advantageous or deleterious, except in a particular context. A second common attribute of most balancing selection mechanisms is that selection favours an allele when it is rare (e.g. Clarke, 1979; Trotter & Spencer, 2007; Delph & Kelly, 2014).

There is abundant evidence that the direction of selection can vary among microsites within contiguous plant populations (e.g. Tonsor *et al.,* 1993). This kind of heterogeneity can maintain alternative alleles, although the conditions for this to happen depend on the relative scales of fitness heterogeneity *vs* seed and pollen migration. If seed/pollen dispersal is sufficiently restricted and localized selection regimes are sufficiently consistent across generations, microhabitat adaptation can occur within populations. In order to eliminate historical or random effects as being responsible for the pattern of variation, it is important to conduct reciprocal-transplant experiments (Ennos, 1983; Delph & Kelly, 2014). The fact that plants with the weak allele of *AtHKT1;1* showed a similar fitness to plants with the strong allele in SCF inland common garden but a lower growth and fitness in BLA coastal common garden (Figure 31) leads to two reflections. Firstly, that BLA common garden was located too close to the sea to be representative for the "intermediate places" where weak allele of *AtHKT1;1* is found. Secondly, having the weak allele is not advantageous neither deleterious in inland conditions so the no migration of this allele into inland is probably caused by the restricted seed/pollen dispersal.

However, in this reciprocal transplant experiment we also observed that plants with the weak allele of *AtHKT1;1* flowered earlier than plants with the strong allele when they were cultivated together in the BLA common garden. This event can be a symptom of stress, an adaptation to protect themselves from the increase of salinity or a combination of both (Figure 31). Stress-induced flowering is not only known to induce defence genes but also to interact with key floral transcription repressors (Yaish *et al.*, 2011). One example of this is that short day plants can be induced to flower under long days with added abiotic stress, such as salinity stress, through interactions with salicylic acid (Wada and Takeno, 2010). We conclude that the weak allele of *AtHKT1;1* remains in these mixed demes thanks to the ability to flower before the values of soil Na^+ reach harmful levels for them. The early flowering capacity of our plants with the weak allele of *AtHKT1;1* is an adaptive response -plastic response because it only occurs at the coast- which allows the plants to avoid the time of year when salinity is highest.

For short-lived annuals, earlier reproduction in the season is a commonly observed strategy of temporal avoidance of seasonally stressful environments (e.g. Griffith & Watson 2005; Verhoeven *et al.,* 2008; Brachi *et al.,* 2012). In populations where the environmental stresses build up during the growth phase (e.g. drought, soil salinity), the early reproduction may allow plants to reproduce before that the stress becomes deadly (Castro *et al.,* 2013).

In addition, we did not detected heterozygous plants for *AtHKT1;1* allele in these locations suggesting that there is no cross-pollination between the two types of plants. It is possible that early flowering may be a reproductive barrier for the outcrossing of the plants with the weak and the strong allele of *AtHKT1;1*. The evolution of early flowering could have 'trapped' (due to the reproductive barrier) the weak allele in these plants meaning that the weak allele is not adaptive. We should also consider that *AtHKT1;1* is genetically linked to *AtFRI*, the main flowering time gene. The weak allele of *AtHKT1;1* and the early flowering version of *AtFRI* may be on the same haplotype block. They are physically close and it is unlikely that they could be separated by recombination. In this case, the plants that have the early flowering allele of *AtFRI* would also always have the weak allele of *AtHKT1;1*. Then, it would look like that weak allele of *AtHKT1;1* is adaptive but in fact it would be avoidance by early flowering. This hypothesis does not explain that weak allele plants are more tolerant to intermediate levels of salinity before they flower but could be part of this complex story.

Identification and role of AtMOT1 weak allele

Molybdenum is an essential element for healthy plant growth. Molybdate, which is the predominant form available to plants, is required at very low levels where it is known to participate in various redox reactions in plants as part of the pterin complex Moco (Kaiser et al., 2005). Moco is involved in ABA biosynthesis through the isoform *AAO3* which catalyzes the oxidation of abscisic aldehyde to abscisic acid in the last cytosolic step of ABA synthesis (Bittner, 2014). ABA is the key regulator of responses to abiotic stresses such as drought and high-salt stress and may be important for coastal adaptation.

MOT1 belongs to the sulphate transporter superfamily and can specifically transport Mo. It is required for efficient uptake and translocation of Mo, as well as for normal growth under limited Mo supply (Lefebvre *et al.*, 2009). Recent studies identified a 53bp deletion in the promoter region of *MOT1* strongly associated with low *MOT1* expression and low shoot Mo content in Ler-0, Van-0 and across 92 other accessions of *A. thaliana* (Baxter *et al.*, 2008,

Tomatsu *et al.*, 2007). We used the promoter of Van-0 described by Baxter *et al.* (2008) to determine whether some of our plants also have this 53 bp deletion. In three coastal demes, located at less than 300 m to the sea, all the individuals were homozygous for the weak allele of *AtMOT1* (Van-0-like) and four coastal demes, located between 0,3 and 2 km from the sea, had a mixture of plants with either the weak allele and the strong allele of *AtMOT1* (Figure 36). The rest of coastal and all inland demes were homozygous for the strong allele of *AtMOT1* (Col-0-like). Once again, we were faced with a genetic variation that occurs only on the coast, despite involving a transporter that has not previously been associated with coastal environments.

Demes with a mixture of plants with either the weak and the strong allele of *AtMOT1* (MIX) were located at similar regions (similar distance to the coast and soil properties highly variables) than demes with a mixture of plants with the weak and the strong allele of *AtHKT1;1*. Mo levels in these MIX sites were always lower but increasing at the end of *A. thaliana* growing season (Figure 38). We also detected that Van-0 like plants flowered later than Col-0 like plants in coastal conditions (Figure 39) and no heterozygous plant for *AtMOT1* was found in any deme. These observations also point to balancing selection phenomena facilitated by differences in flowering time. On the other hand, iron availability is a crucial regulatory element for plant molybdenum metabolism (Bittner, 2014). Carbonate content in soil shows a positive cline into inland consequently iron availability in inland soils may be lower. It is possible that the weak allele of *AtMOT1* does not move into inland also for this reason.

Poormohammad *et al.*, (2012) investigated potential relationships between *AtMOT1* alleles and soil properties but they only found a correlation between the activity of *MOT1* in natural accessions of *A. thaliana* and the molybdenum availability (related to soil pH) in native soil. However, this correlation was not observed in a study made with European populations (Salt, unpublished). Our results from native soil analyses between coastal sites containing plants homozygous for the strong allele of *AtMOT1* and coastal sites containing plants homozygous for the weak allele of *AtMOT1* or both types mixed did not show any relation with soil pH or soil Mo concentrations but we detected significant differences in soil Se, As, Co, Sr, P and Na/K ratio. These differences were also observed in the leaf concentrations of the same elements in plants having the weak or the strong allele of *AtMOT1* and the analysis of leaf ionome confirmed that Van-0-like plants accumulate less Mo and also less Na than Col-0-like plants (Figure 37). These results suggest that *AtMOT1* could have an indirect role in the homeostasis

of other ions besides Mo despite the fact that Baxter *et al.* (2008) detected no change in the shoot content of Na, Mg, P, S, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, and Cd in *mot1-1* plants and other independent experiments.

The fact that Van-0-like plants had a higher fitness (higher growth, biomass and silique production) than Col-0-like plants when they were both cultivated at the BLA coastal common garden and when they were treated with NaCl in hydroponics and soil (Figure 39 and 40) is a signal of an adaptive advantage. However, these higher fitness was only observed in coastal conditions or when salt-stress was induced in the laboratory. At the SCF inland common garden or under control conditions (0 mM NaCl) we could not observe differences between the type of plants (Figure 39 and 40), suggesting a conditional neutrality in inland conditions as we observed for *AtHKT1;1*. In support of a role of the loss-of-function allele of *AtMOT1* in elevated salinity tolerance we also observed elevated salinity tolerance in the loss-of-function T-DNA insertion allele. This strongly suggests that in the same genetic background loss-of-function of *AtMOT1* can directly lead to increased salinity tolerance. However, a second independent allele or transgenic complementation is required to prove this.

As expected, Van-0-like plants always had lower leaf Mo concentrations than Col-0 like plants regardless the growth conditions; weather, native or foreign soils, or hydroponic treatments. However they never displayed symptoms of Mo deficiency (Figures 39 and 40). The overall reduction in Mo content might cause a reduction in molybdopterin, negatively impacting the activity of MoCo containing enzymes such as nitrate reductase. In absence of Mo, *mot1-1* mutant plants show yellow leaves and retarded growth and Col-0 plants show curled leaves. However, the shoot and root fresh weights of *mot1-1* and Col-0 plants do not show differences in control conditions (Ide *et al.*, 2011). Baxter *et al.* (2008) already observed no significant reduction in growth, N accumulation or nitrate reductase activity in *mot1-1* lines when grown with nitrate as the sole N source. Our hydroponic cultivation of knockout mutants of *AtMOT1* match this result since we did not detect significant differences in growth or fresh weight between *mot1* mutants and Col-0 plants under control conditions (Figure 41).

Surprisingly, Van-0-like plants accumulate less Na than Col-0-like plants under natural conditions in the field, and when they were treated with 50 and 100 mM NaCl in hydroponics, displaying an elevated salinity tolerance (Figures 39 and 40). Whilst more experiments with other mutant lines and more replicates should be performed, the fact that the *mot1* mutant plants outperformed Col-0 plants in growth and biomass in the 100 mM NaCl treatment, and

that Fe^{3+} reducing capacity of the mutant plants did not change in any of the treatments (Figure 41) supports the hypothesis that *AtMOT1* contributes to salt-stress tolerance.

Ide *et al*. (2011) detected *in A. thaliana* numerous Mo-responsive genes and genes related to Moco involved in transport, stress responses (PPC), signal transduction (*ABA3*) and in the metabolisms of nitrogen (*NR2*) , sulfur (*SULTR*), and phosphate, but also the levels of amino acids, sugars, organic acids, and purine metabolites were significantly altered, indicating that molybdate nutrition has global impact on plants. A link between *AtMOT1* and some gene involved in ABA regulation could explain the increase in salinity tolerance observed in *mot1* mutants.

Final considerations

The existence of *A. thaliana* populations locally adapted to contrasting coastal and inland habitats together with the study of the polymorphisms detected in these populations opens up the possibility of applying powerful genomic tools such as population-level whole genome scans (Turner *et al.*, 2010; Hollister *et al.*, 2012) to identify the molecular mechanism that underlies this adaptation. This information should help to resolve some of the outstanding theoretical questions related to local adaptation and clarify the potential role of the polymorphisms detected in *AtMOT1* and *AtHKT1;1* alleles.

6. Conclusions

6. Conclusions

The genetic diversity between and within *Arabidopsis thaliana* populations from Catalonia has proven to be high despite the small geographic scale (< 5.000 km^2) of this study. Combining this information with ecological, physiological and genetic approaches might well establish *A. thaliana* as a model organism for the study of the natural genetic variation associated with adaptation to local environments.

The results of this study highlight the importance of soil composition in the distribution and genetic differentiation of the species. The SDM created to locate new *A. thaliana* demes revealed that this specie is only found on substrates of silicic origin. Chemical analysis of native soils from the habitats where *A. thaliana* was found showed a negative cline for the major solutes in seawater (Na, Mg, chloride and sulphate) as we move away from the coast. We also observed significant differences between coastal and inland soils with a similar cline. Field-based reciprocal transplants and cultivations under controlled conditions with soil excavated from the coast and inland confirmed that *A. thaliana* plants are adapted to their native soil. Finally, we observed that the distribution on the landscape of the genetic variability detected at *AtHKT1* and *AtMOT1* was also conditioned by soil composition, especially by soil Na levels.

We conclude that the local adaptation we observe between adjacent coastal and inland populations is due to ongoing divergent selection driven by the differential salinity between coastal and inland soils. Although it has been demonstrated that coastal *A. thaliana* plants have a greater ability to tolerate salt stress, results from experiments with NaCl do not point to a single mechanism for salinity tolerance in our coastal plants. Coastal plants tend to accumulate more Na^+ in leaves than inland plants and *AtSOS1* and *AtHKT1* expression was higher in roots from coastal plants, suggesting that these Na transporters work together to elevate leaf Na^+, where it is likely compartmentalized in the vacuole for better osmotic adjustment of coastal plants. In addition, although further studies are required, the fact that salinity tolerance adaptation may be uniparental inherited (in our case maternally inherited) opens the door to the genomic imprinting world, a field that already described numerous genes involved in local adaptation and positive selection.

In contrast, it has been validated that the presence of the weak allele of *AtHKT1;1* (Ts-1-like plants) is not responsible in general for either the local adaptation of coastal demes or their enhanced ability to tolerate elevated salinity. The low frequency of plants with the weak allele; its location at more than 0,5 km of the coast; the lower fitness shown in the coastal common garden; and the breakdown of these plants when they were treated with 100 mM NaCl confirms this conclusion. However, the higher growth and fitness of Ts-1-like plants when they were exposed to moderate salinity (50 mM NaCl), their location in habitats with intermediate levels of salinity excepting the drier months at the end of the growing season, their capacity to flower earlier than Col-0-like plants in coastal conditions, and probably the balancing selection maintaining the two alternative alleles in the same place and favouring the "rare" allele, have allowed the presence and persistence of *AtHKT1;1* weak allele in mixed demes.

The other studied allele, *AtMOT1,* has shown surprising results in relation to adaptation to coastal conditions. Even though the presence of the weak allele of *AtMOT1* (Van-0 like plants) was not extended to all coastal demes, we found three demes with all plants homozygous for the weak allele situated at less than 0,3 km from the coastal line and four demes with a mixture of plants having the weak or the strong allele of *AtMOT1* in the intermediate coastal zone where mixed demes for *AtHKT1;1* were also detected. Field-based cultivations and NaCl experiments have confirmed that plants with weak allele of *AtMOT1* are more adapted to coastal conditions and elevated salinity than plants whit the strong allele. The *mot1* knockout mutants also outperformed in growth and biomass Col-0 plants in the 100 mM NaCl treatment. We hypothesize that loss of function of *AtMOT1* may be linked with some gene involved in ABA regulation that leads to elevated salinity tolerance. These results lead us to conclude that *AtMOT1* may be related with salt stress and it is a good candidate gene to be considered for future studies.

The answer of why the weak allele of *AtHKT1;1* and *AtMOT1* are not moving inland is still unknown. In the reciprocal transplants performed in 2014 and 2015 plants, we did not observe differences of fitness between plants having the weak or the strong allele at the inland common garden. Therefore, having the weak allele of *AtHKT1;1* or *AtMOT1* is not harmful in inland conditions. The most plausible hypothesis that explains the no migration of these alleles into inland is probably the restriction of seed/pollen dispersal.

7. Literature cited

7. Literature cited

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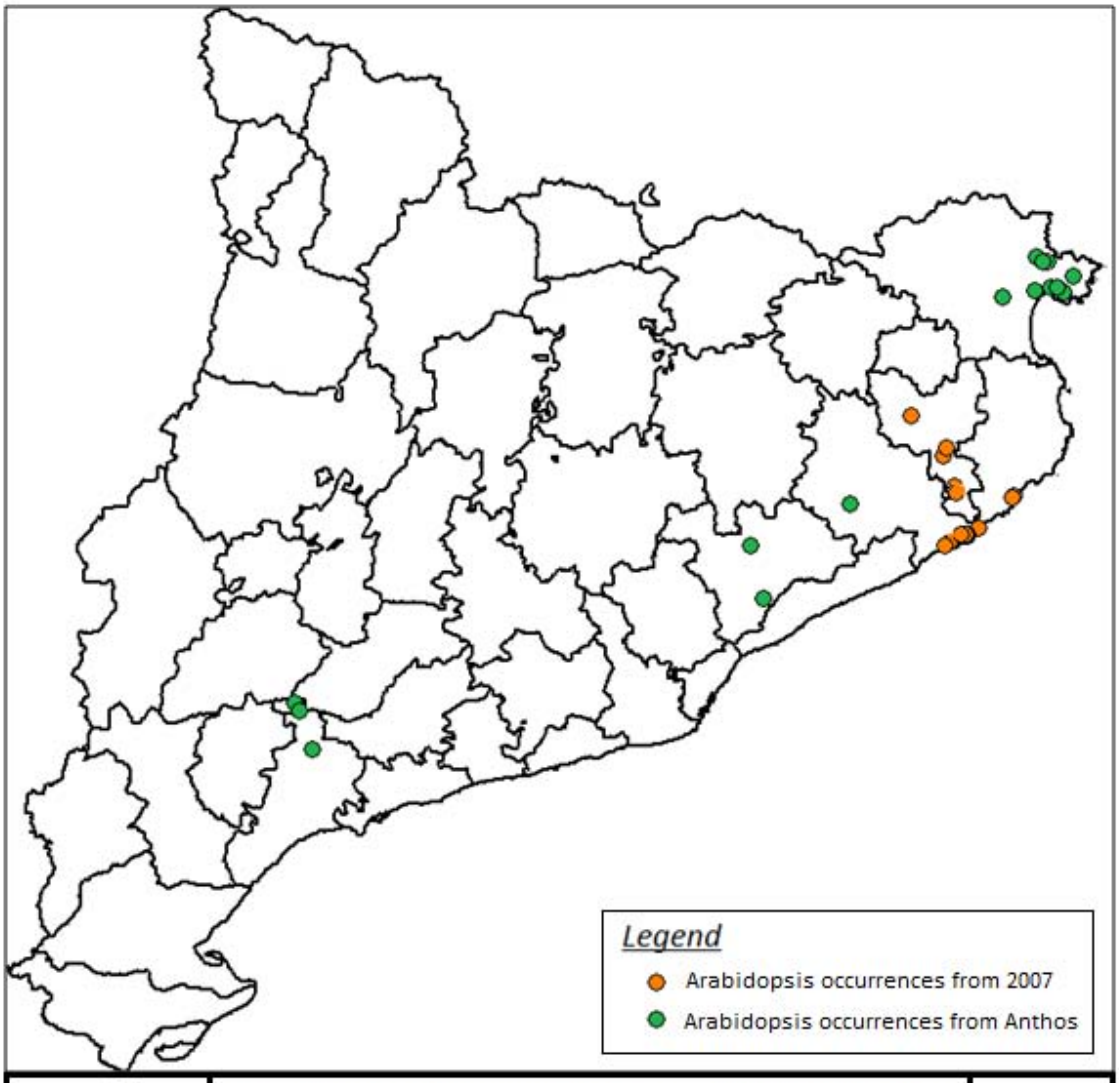
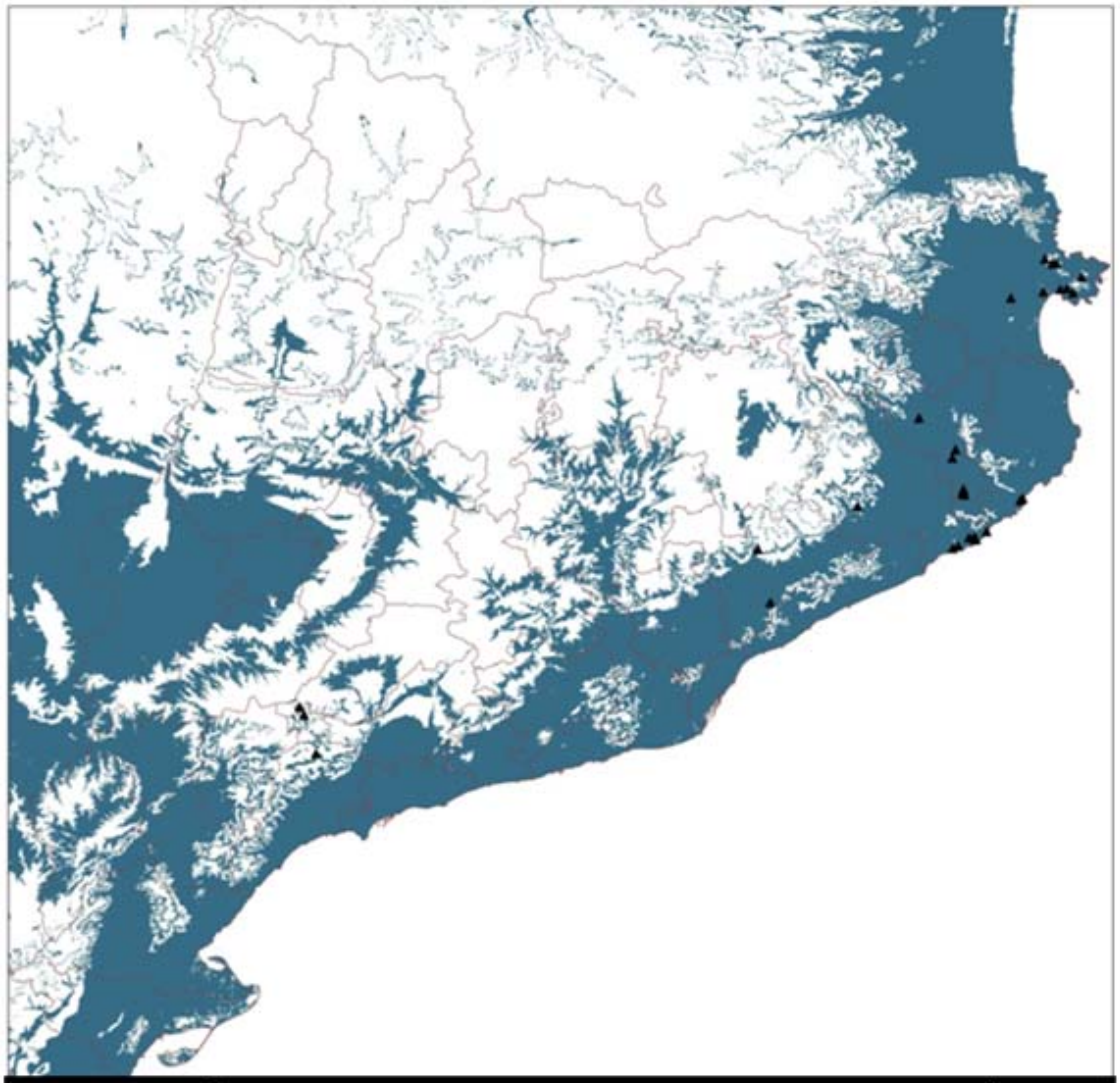
8. Annex

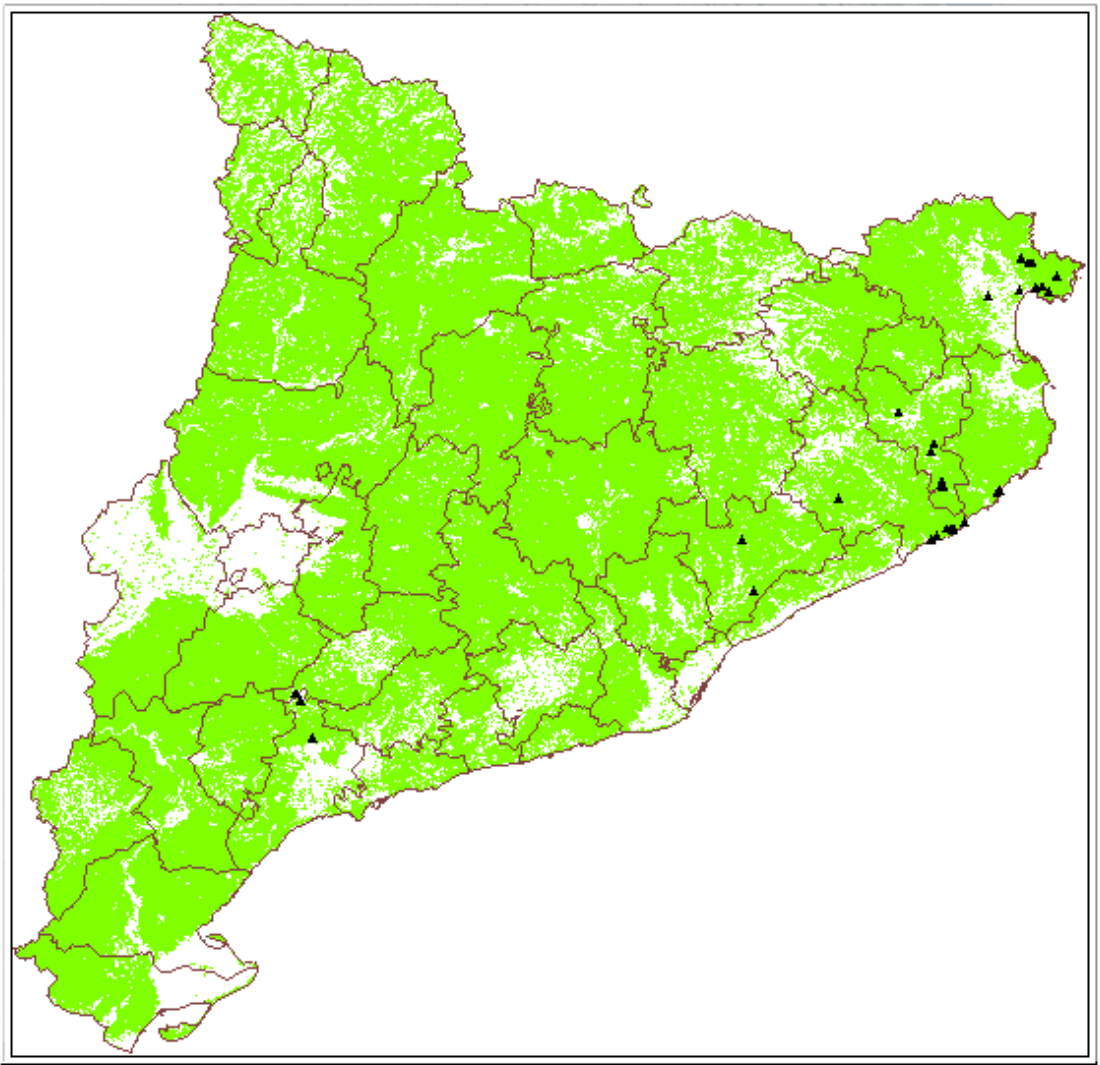
I. Annex Maps



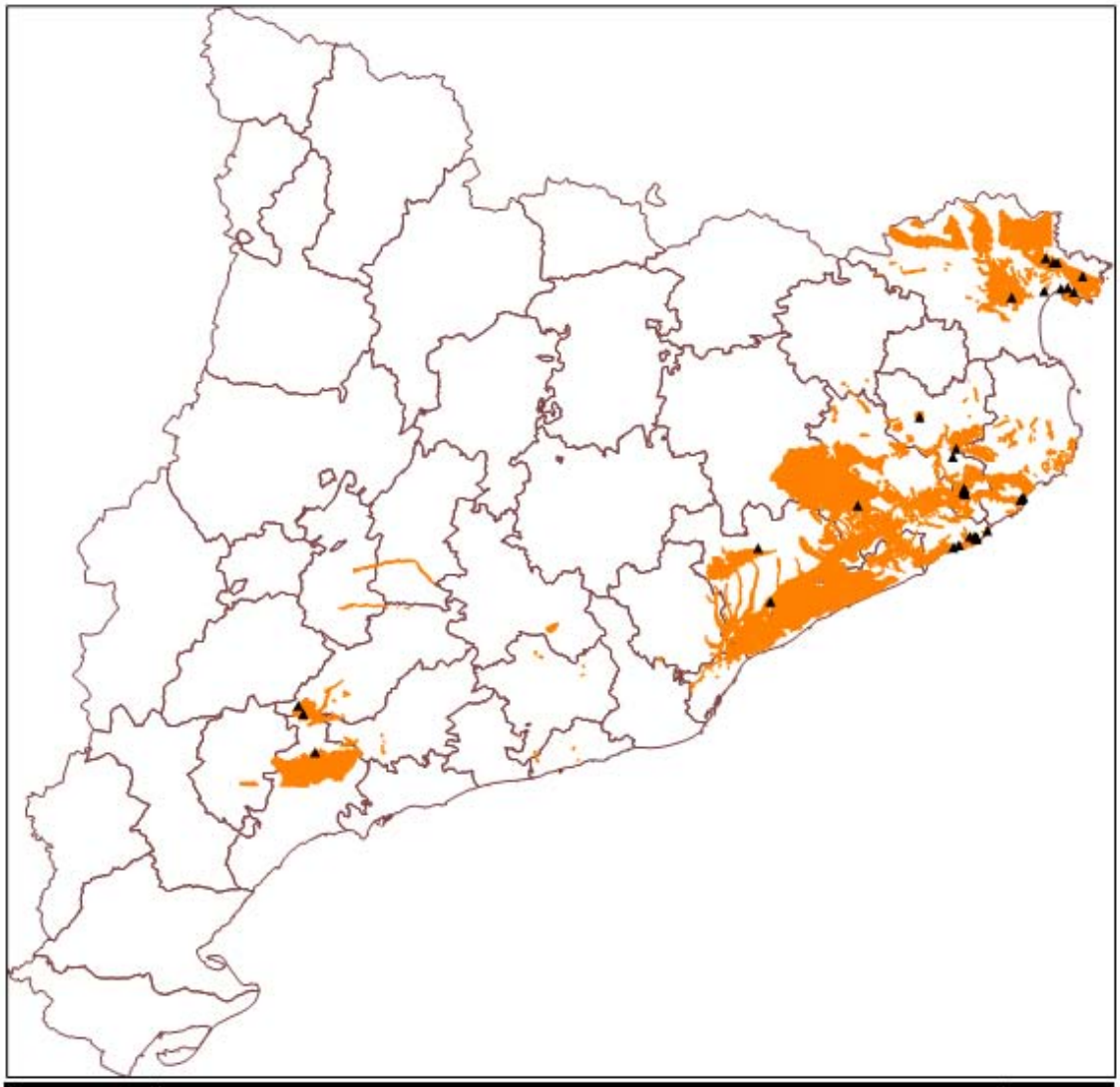
MAP 1	Geographically referenced sites where *Arabidopsis thaliana* had been previously located in Catalonia	
Map base: Catalonia map divided by regional boundaries		**Scale :** 0 50 km



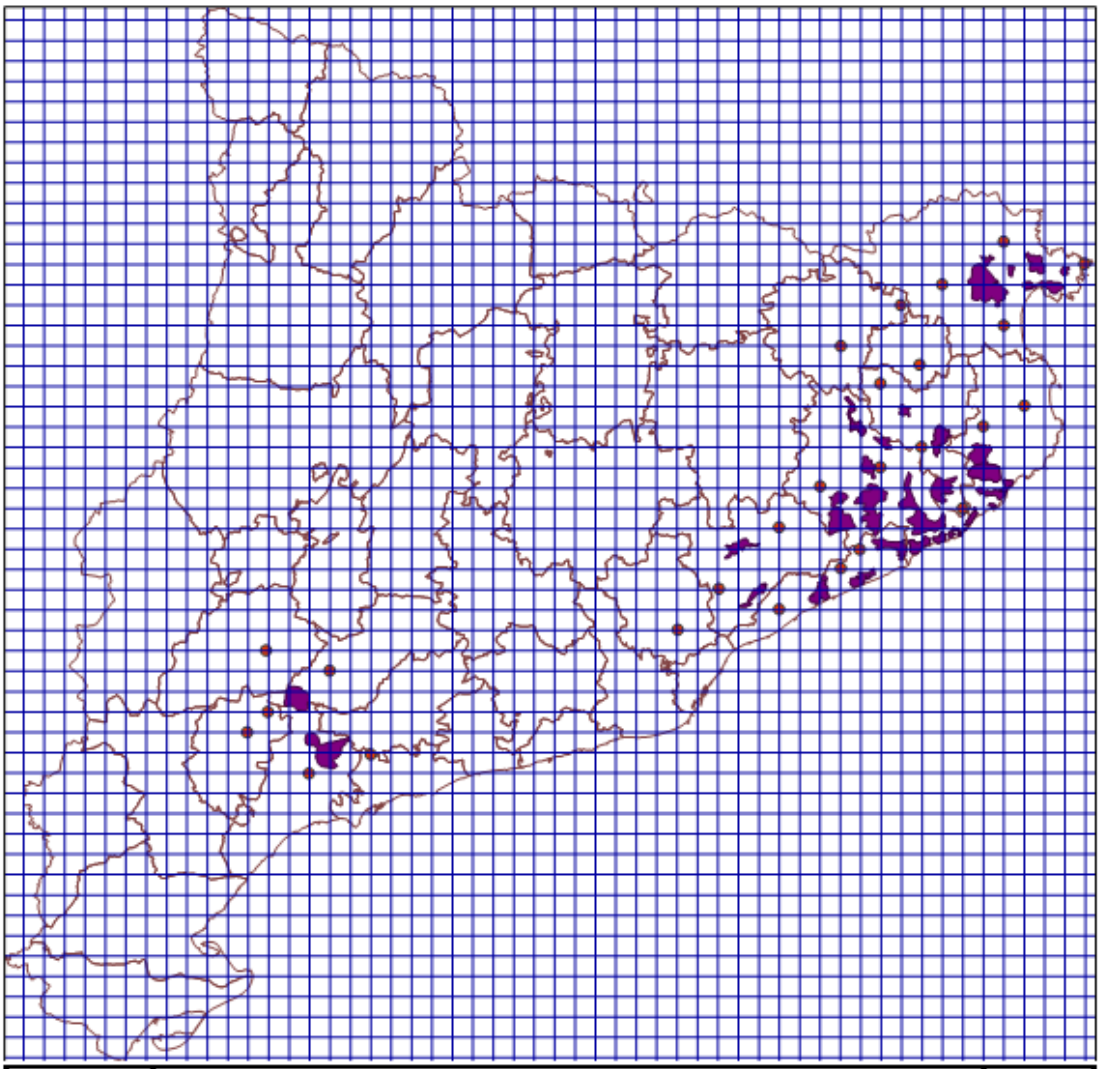
MAP 2	Benchmarks (black triangles) upon binary map of Alimetry (0 = white ; 1= blue = [0 – 850m])	
Map base: Catalonia map divided by regional boundaries (maroon lines)		**Scale :** 0 50 km



MAP 3	Benchmarks (black triangles) and binary map of land uses (0=white; 1= green = sandy, soil with sparse vegetation, rainfed fruit trees, thickets and meadows, sclerophyllous forest and residential areas)	
Map base: Catalonia map divided by regional boundaries (maroon lines)		**Scale :** 0 50 km



MAP 4	Benchmarks (black triangles) and binary map of Geology (0= white; 1= orange = granite, granitoid, granodiorite and hornfels)	
Map base: Catalonia map divided by regional boundaries (maroon lines)		**Scale :** 0 50 km



MAP 5	Predicted areas obtained from the interception of the three maps (purple polygons) with net of 5 x 5 km used to select the random outside points (red points)	
Map base: Catalonia map divided by regional boundaries (maroon lines)		**Scale :** 0 50 km

II. Annex Tables

Annex Table 1. Deme name, source, U.T.M. coordinates, location, year of first identification and status of the population during the evaluated years.

Deme ID	Source	UTM (x)	UTM (y)	Location	2007	2011 (population size)	2012 (population size)	2013 (population size)	2014 (population size)	2015 (population size)
SC 1	K.Bomblies	455110	4618929	Inland	Found	Not found	-	-	-	
SC 2	K.Bomblies	469504	4613719	Inland	Found	Not found	-	-	Refound (10 - 20 plants)	Persists (20 - 30 plants)
SC 3	K.Bomblies	464382	4617067	Inland	Found	Not found	-	-	-	Refound (10 - 20 plants)
SC 4	K.Bomblies	467159	4612212	Inland	Found	Not found	-	-	-	
Bom 1	K.Bomblies	489804	4617871	Coastal	Found	Not found	-	-	-	
Bom 3	K.Bomblies	505372	4629341	Coastal	Found	Not found	-	-	-	
Bom 5	K.Bomblies	504753	4628880	Coastal	Found	Not found	-	-	-	
Bom 6	K.Bomblies	494024	4619415	Coastal	Found	Not found	-	-	-	
Bom 7	K.Bomblies	494056	4619500	Coastal	Found	Not found	-	-	-	
Bom 11	K.Bomblies	493498	4619835	Coastal	Found	Not found	-	-	-	
Bom 13	K.Bomblies	493280	4618325	Coastal	Found	Not found	-	-	-	
Bom 20	K.Bomblies	480288	4648847	Inland	Found	Not found	-	-	-	
Bom 21	K.Bomblies	492359	4619923	Coastal	Found	Not found	-	-	-	
Ant 1	Anthos	502481	4677885	Coastal	Found	Not found	-	-	-	
Ant 2	Anthos	510282	4679317	Coastal	Found	Not found	-	-	-	
Ant 3	Anthos	517532	4679117	Coastal	Found	Not found	-	-	-	
PO1	Anthos	513471	4686163	Coastal	Found	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)
PR1	Anthos	330512	4578975	Inland	Found	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)
PR4	Anthos	331677	4577004	Inland	Found	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)
RO1	Anthos	514342	4679952	Coastal	Found	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Disappears (HC)

Annex Table 1. Continuation...

Deme ID	Source	UTM (x)	UTM (y)	Location	2007	2011 (population size)	2012 (population size)	2013 (population size)	2014 (population size)	2015 (population size)
RO2	Anthos	515998	4680193	Coastal	Found	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)
RO3	Anthos	519614	4682924	Coastal	Found	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)
V1	Anthos	512474	4686157	Inland	Found	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (> 50 plants)
V4	Anthos	510705	4687328	Inland	Found	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)
C3	K.Bomblies	488311	4639153	Inland	Found	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Disappears (NC)	-	-
LG4	K.Bomblies	491003	4631631	Inland	Found	Persists (1 - 10 plants)	Disappears (HC)	-	-	-
LG5	K.Bomblies	491118	4631016	Inland	Found	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)
LG7	K.Bomblies	490788	4630667	Inland	Found	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)
LG8	K.Bomblies	491256	4630235	Inland	Found	Persists (1 - 10 plants)	Disappears (NC)	-	-	-
LM2	K.Bomblies	489123	4641204	Inland	Found	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)
LLO1	K.Bomblies	488445	4617295	Coastal	Found	Persists (1 - 10 plants)	Disappears (HC)	-	-	-
PA10	K.Bomblies	504788	4628914	Coastal	Found	Persists (10 - 20 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)
T5	K.Bomblies	493932	4619788	Coastal	Found	Persists (10 - 20 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Disappears (HC)	-
T6	K.Bomblies	496641	4621417	Coastal	Found	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)
T7	K.Bomblies	493986	4619635	Coastal	Found	Persists (1 - 10 plants)	Disappears (HC)	-	-	-
JBB	K.Bomblies	483914	4613996	Coastal	Found	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)

Annex Table 1. Continuation...

Deme ID	Source	UTM (x)	UTM (y)	Location	2007	2011 (population size)	2012 (population size)	2013 (population size)	2014 (population size)	2015 (population size)
A1	S.Busoms	469130	4645107	Inland	-	New (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)
A2	S.Busoms	469126	4645129	Inland	-	New (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)
A5	S.Busoms	470427	4643878	Inland	-	New (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (40 - 50 plants)	Persists (40 - 50 plants)
AM	S.Busoms	466250	4652252	Inland	-	New (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)
LG6	S.Busoms	490867	4630573	Inland	-	New (1 - 10 plants)	Disappears (NC)	-	-	-
LLM1	S.Busoms	488354	4641151	Inland	-	New (1 - 10 plants)	Disappears (HC)	-	-	-
LM11	S.Busoms	489073	4641177	Inland	-	New (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)
LLO2	S.Busoms	486340	4616926	Coastal	-	New (30 - 40 plants)	Persists (30 - 40 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (10 - 20 plants)
O3	S.Busoms	467719	4644561	Inland	-	New (10 - 20 plants)	Persists (20 - 30 plants)	Persist (10 - 20 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)
O4	S.Busoms	467714	4644580	Inland	-	New (1 - 10 plants)	Disappears (NC)	-	-	-
PR2	S.Busoms	330462	4578956	Inland	-	New (10 - 20 plants)	Persist (10 - 20 plants)	Persist (10 - 20 plants)	Persist (10 - 20 plants)	Persists (1 - 10 plants)
PR3	S.Busoms	331933	4578321	Inland	-	New (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)
PR5	S.Busoms	331729	4577086	Inland	-	New (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (> 50 plants)
SFG9	S.Busoms	502068	4625549	Coastal	-	New (30 - 40 plants)	Persists (20 - 30 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (> 50 plants)
T1	S.Busoms	494117	4618398	Coastal	-	New (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)
T10	S.Busoms	493847	4618331	Coastal	-	New (10 - 20 plants)	Persists (10 - 20 plants)	Persists (1 - 10 plants)	Disappears (NC)	-

Annex Table 1. Continuation...

Deme ID	Source	UTM (x)	UTM (y)	Location	2007	2011 (population size)	2012 (population size)	2013 (population size)	2014 (population size)	2015 (population size)
T11	S.Busoms	492166	4618894	Coastal	-	New (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (30 - 40 plants)
T12	S.Busoms	494382	4618443	Coastal	-	New (1 - 10 plants)	Disappears (HC)	-	-	-
T13	S.Busoms	494084	4618942	Coastal	-	New (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (> 50 plants)
T2	S.Busoms	494113	4618445	Coastal	-	New (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)
T3	S.Busoms	494121	4618441	Coastal	-	New (1 - 10 plants)	Disappears (NC)	-	-	-
T4	S.Busoms	494537	4618856	Coastal	-	New (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Disappears (HC)
T8	S.Busoms	494438	4618511	Coastal	-	New (30 - 40 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Disappears (HC)	Refound (1 - 10 plants)
T9	S.Busoms	493780	4618456	Coastal	-	New (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (30 - 40 plants)
V2	S.Busoms	512429	4686145	Inland	-	New (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Persists (1 - 10 plants)	Disappears (NC)
V3	S.Busoms	511824	4686169	Inland	-	New (40 - 50 plants)	Persists (20 - 30 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)
CALA	S.Busoms	495575	4619864	Coastal	-	-	New (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)
CE	S.Busoms	468887	4646387	Inland	-	-	New (1 - 10 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)
HIP	S.Busoms	467528	4649434	Inland	-	-	New (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)	Persists (10 - 20 plants)
LLO3	S.Busoms	485082	4615657	Coastal	-	-	New (10 - 20 plants)	Persists (20 - 30 plants)	Persists (30 - 40 plants)	Persists (30 - 40 plants)
LP1	S.Busoms	461620	4656602	Inland	-	-	New (> 50 plants)	Disappears (HC)	-	Refound (10 - 20 plants)
LP2	S.Busoms	462415	4655728	Inland	-	-	New (20 - 30 plants)	Persists (20 - 30 plants)	Persists (20 - 30 plants)	Persists (10 - 20 plants)

Annex Table 1. Continuation...

Deme ID	Source	UTM (x)	UTM (y)	Location	2007	2011 (population size)	2012 (population size)	2013 (population size)	2014 (population size)	2015 (population size)
SCF	S.Busoms	472172	4634689	Inland	-	-	New (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)
VIN	S.Busoms	289449	4489170	Coastal	-	-	-	New (> 50 plants)	Persists (> 50 plants)	Persists (> 50 plants)
PO2	S.Busoms	515312	4686449	Coastal	-	-	-	-	-	New (10 - 20 plants)

Annex Table 2. Deme name; location (Coastal/Inland); *AtHKT1;1* allele type (C, T or MIX); *AtMOT1* allele type (C, V or MIX); demes selected and number of plants per deme used in some of the experiments performed in this study. Figure's number in parentheses.

Deme ID	Location	*AtHKT1;1* allele type	*AtMOT1* allele type	425-SNPs genotyping (F15, F16)	Reciprocal transplant BLA-SCF 2013 /2014 (F17, F18, F23)	Common garden assay with soil from BLA-SCF (F19)	Soil samples from March 2013/2014/ 2015 (F21)	Plants from natural habitat collected on March of 2013/2014/2015 (F22)	Irrigation / hydroponics with NaCl (F24, F25, F26)
A1	Inland	C	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
A2	Inland	C	C	-					
A5	Inland	C	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
AM	Inland	C	C	-	0 / 10		3 / 3 / 3	2 / 8 / 5	12 / 9
C3	Inland	C	C	2	10 / 0	10	3 / 3 / 3	2 / 8 / 5	
CALA	Coastal	C	V	-					
CE	Inland	C	C	-					
HIP	Inland	C	C	-					
JBB	Coastal	MIX	C	2					
LG4	Inland	-	-	1					
LG5	Inland	C	C	7	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
LG6	Inland	-	C	-					
LG7	Inland	C	-	12	10 / 10	10	3 / 3 / 3	2 / 8 / 5	

Annex Table 2. Continuation...

Deme ID	Location	*AtHKT1;1* allele type	*AtMOT1* allele type	425-SNPs genotyping (F15, F16)	Reciprocal transplant BLA-SCF 2013 /2014 (F17, F18, F23)	Common garden assay with soil from BLA-SCF (F19)	Soil samples from March 2013/2014/ 2015 (F21)	Plants from natural habitat collected on March of 2013/2014/2015 (F22)	Irrigation / hydroponics with NaCl (F24, F25, F26)
LG8	Inland	-	-	1					
LLM1	Inland	-	-	-					
LLO1	Coastal	C	C	6					
LLO2	Coastal	C	MIX	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
LLO3	Coastal	C	C	-			3 / 3 / 3	2 / 8 / 5	
LM11	Inland	-	-	-	10 / 0	10			
LM2	Inland	C	C	5	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
LP1	Inland	C	C	-					
LP2	Inland	C	C	-					
O3	Inland	C	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
O4	Inland	-	-	-					
PA10	Coastal	C	C	3	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
PO1	Coastal	C	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
PR1	Inland	C	C	-			3 / 3 / 3	2 / 8 / 5	
PR2	Inland	C	C	-					

Annex Table 2. Continuation...

Deme ID	Location	*AtHKT1;1* allele type	*AtMOT1* allele type	425-SNPs genotyping (F15, F16)	Reciprocal transplant BLA-SCF 2013 /2014 (F17, F18, F23)	Common garden assay with soil from BLA-SCF (F19)	Soil samples from March 2013/2014/ 2015 (F21)	Plants from natural habitat collected on March of 2013/2014/2015 (F22)	Irrigation / hydroponics with NaCl (F24, F25, F26)
PR3	Inland	C	C	-			3 / 3 / 3	2 / 8 / 5	
PR4	Inland	C	C	-					
PR5	Inland	C	C	-					
RO1	Coastal	MIX	MIX	-					
RO2	Coastal	MIX	MIX	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
RO3	Coastal	C	C	-					
SCF	Inland	C	C	-	0 / 10		3 / 3 / 3	2 / 8 / 5	
SFG9	Coastal	MIX	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
T1	Coastal	C	V	-			3 / 3 / 3	2 / 8 / 5	12 / 9
T10	Coastal	-	-	-					
T11	Coastal	C	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
T12	Coastal	-	-	-					
T13	Coastal	MIX	C	-	0 / 10		3 / 3 / 3	2 / 8 / 5	12 / 9
T2	Coastal	C	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	
T3	Coastal	-	-	-					

Annex Table 2. Continuation...

Deme ID	Location	*AtHKT1;1* allele type	*AtMOT1* allele type	425-SNPs genotyping (F15, F16)	Reciprocal transplant BLA-SCF 2013 /2014 (F17, F18, F23)	Common garden assay with soil from BLA-SCF (F19)	Soil samples from March 2013/2014/ 2015 (F21)	Plants from natural habitat collected on March of 2013/2014/2015 (F22)	Irrigation / hydroponics with NaCl (F24, F25, F26)
T4	Coastal	C	C	-					
T5	Coastal	MIX	C	1	10 / 0	10	3 / 3 / 3	2 / 8 / 5	
T6	Coastal	C	V	4	10 / 10	10	3 / 3 / 3	2 / 8 / 5	
T7	Coastal	-	-	1					
T8	Coastal	C	C	-	10 / 10	10			
T9	Coastal	C	MIX	-			3 / 3 / 3	2 / 8 / 5	
V1	Inland	C	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
V2	Inland	C	C	-					
V3	Inland	C	C	-	10 / 10	10	3 / 3 / 3	2 / 8 / 5	12 / 9
V4	Inland	C	C	-					
VIN	Coastal	C	C	-					
Pla-1	Coastal	-	-	1					
Se-0	Coastal	-	-	1					
Ts-1	Coastal	-	-	1					
Hh-0	Inland	-	-	1					

Annex Table 2. Continuation...

Deme ID	Location	*AtHKT1;1* allele type	*AtMOT1* allele type	425-SNPs genotyping (F15, F16)	Reciprocal transplant BLA-SCF 2013 /2014 (F17, F18, F23)	Common garden assay with soil from BLA-SCF (F19)	Soil samples from March 2013/2014/ 2015 (F21)	Plants from natural habitat collected on March of 2013/2014/2015 (F22)	Irrigation / hydroponics with NaCl (F24, F25, F26)
LG	Inland	-	-	2					
SC 1	Inland	-	-	3					
SC 2	Inland	-	-	6					
SC 3	Inland	-	-	9					
SC 4	Inland	-	-	7					
Bom 1	Coastal	-	-	5					
Bom 3	Coastal	-	-	2					
Bom 5	Coastal	-	-	4					
Bom 6	Coastal	-	-	1					
Bom 7	Coastal	-	-	2					
Bom 11	Coastal	-	-	1					
Bom 13	Coastal	-	-	2					
Bom 20	Inland	-	-	2					
Bom 21	Coastal	-	-	3					

Annex Table 3: List of candidate genes obtained from the detected locus with F_{st} higher than 0,25 and main functions described in TAIR webpage (http://www.arabidopsis.org).

ID	Gene	Name	Involved in
G1	At1G73660	SIS8	MAPK cascade, flowering, response to salt stress, sugar mediated signalling pathway
G2	At1G73650	-	Flavonoid biosynthetic process, lipid metabolic process, regulation of defence response, response to UV-B
G3	At1G73610	-	Lipid catabolic process
G4	At1G73580	CAR3	Calcium dependent lipid binding, abscisic acid activated signalling pathway
G5	At1G73540	NUDT21	Metal ion binding, hydrolase activity
G6	At1G73500	MKK9	MAPK cascade, stress-activated protein, response to fungus, salt stress and wounding
G7	At1G73490	-	RNA binding
G8	At1G73480	-	Hyperosmotic salinity response, lipid metabolic process, response to abscisic acid, cold and water deprivation
G9	At1G73440	-	Calcium ion binding
G10	At1G73360	HDG11	Plant-type cell wall loosening, regulation of transcription
G11	At1G73340	-	Electron carrier activity, oxidation-reduction process
G12	At2G40580	-	Protein amino acid phosphorylation
G13	At2G40560	-	Protein kinase activity
G14	At2G40540	KT2	Potassium ion transmembrane trasport, regulation of meristem growth
G15	At2G40490	HEME2	Chlorophyll and porphyrin biosynthetic process, response to cytokinin
G16	At2G40470	LBD15	Lateral organ boundaries
G17	At2G40460	-	Oligopeptide transport, response to nematode
G18	At2G40420	-	Amino acid transport, response to fructose and sucrose
G19	At2G40370	LAC5	Lignin catabolic process, oxidation-reduction process, response to copper ion

Annex Table 4: ANOVA between coastal and inland regions of 4 climatic variables obtained from Climatic Atlas of Catalonia (http://www.opengis.uab.cat/acdc/)

	Location	Mean	Std Dev	DF	F Value	P > F
Annual precipitation (mm)	Coastal	661,73	71,63	1	2,87	0,0948
	Inland	716,35	113,45			
Annual mean temperature (ºC)	Coastal	15,50	1,02	1	0,26	0,1731
	Inland	14,19	0,98			
Annual evapotranspiration (L)	Coastal	707,81	123,6	1	1,34	0,2059
	Inland	730,54	144,72			
Annual water deficit (L)	Coastal	200,08	25,78	1	0,85	0,3622
	Inland	179,35	18,94			

Annex Table 5: Mean ± Standard Deviation of pH, Carbonate content (%), Water Holding Capacity (mL/g), Organic Matter (%) and mineral nutrients in soil from the habitat of 13 coastal and 13 inland *A. thaliana* demes in 2013, 2014 and 2015. ANOVA between locations (coastal/inland) of 3 samples from each site and year. Regression line between the variable analysed and the logarithm of meters to sea.

	Year	Location	N	Mean	Std Dev	F Ratio	Prob > F	R^2 (m to sea)
pH	2013	Coastal	39	6,80	0,44	21,45	<,0001	0,275
		Inland	39	7,21	0,32			
	2014	Coastal	39	6,94	0,35	7,19	0,009	0,201
		Inland	39	7,18	0,45			
	2015	Coastal	39	6,92	0,36	11,05	0,0014	0,258
		Inland	39	7,21	0,39			
$CaCO_3$ (%)	2013	Coastal	39	2,71	1,58	204,31	<,0001	0,712
		Inland	39	13,97	4,82			
	2014	Coastal	39	2,51	0,99	146,16	<,0001	0,617
		Inland	39	13,74	5,93			
	2015	Coastal	39	3,21	1,48	181,00	<,0001	0,700
		Inland	39	16,85	6,38			
WHC (mL/g)	2013	Coastal	39	9,69	3,56	122,79	<,0001	0,563
		Inland	39	17,91	2,88			
	2014	Coastal	39	8,17	2,56	188,93	<,0001	0,652
		Inland	39	17,03	3,13			
	2015	Coastal	39	9,07	2,13	302,50	<,0001	0,676
		Inland	39	19,66	3,21			
O. M. (%)	2013	Coastal	39	3,21	1,11	3,46	0,0669	0,058
		Inland	39	3,66	1,00			
	2014	Coastal	39	3,10	1,09	6,91	0,0104	0,110
		Inland	39	3,70	0,90			
	2015	Coastal	39	3,22	1,12	7,29	0,0085	0,122
		Inland	39	3,83	0,84			
Chloride (mg/g)	2013	Coastal	39	1,89	0,37	114,07	<,0001	0,609
		Inland	39	1,15	0,20			
	2014	Coastal	39	1,84	0,28	149,99	<,0001	0,615
		Inland	39	1,20	0,15			
	2015	Coastal	39	2,05	0,31	191,34	<,0001	0,643
		Inland	39	1,21	0,21			
Sulphate (mg/g)	2013	Coastal	39	53,39	16,44	130,57	<,0001	0,696
		Inland	39	19,11	7,88			
	2014	Coastal	39	48,42	24,01	58,95	<,0001	0,457
		Inland	39	17,10	5,00			
	2015	Coastal	39	63,13	22,62	102,21	<,0001	0,660
		Inland	39	23,22	7,52			

Annex Table 5: Continuation...

	Year	Location	N	Mean	Std Dev	F Ratio	Prob > F	R^2 (m to sea)
Na (mg/g)	2013	Coastal	39	88,57	35,60	64,47	<,0001	0,452
		Inland	39	38,60	12,07			
	2014	Coastal	39	87,72	27,46	59,12	<,0001	0,485
		Inland	39	49,25	13,06			
	2015	Coastal	39	111,52	29,21	81,32	<,0001	0,527
		Inland	39	61,88	16,61			
K (mg/g)	2013	Coastal	39	102,50	51,98	64,90	<,0001	0,231
		Inland	39	221,87	77,95			
	2014	Coastal	39	108,62	66,11	42,40	<,0001	0,419
		Inland	39	223,07	88,80			
	2015	Coastal	39	115,48	79,77	46,90	<,0001	0,366
		Inland	39	248,40	91,64			
Na/K Ratio	2013	Coastal	39	1,08	0,61	69,45	<,0001	0,612
		Inland	39	0,21	0,13			
	2014	Coastal	39	1,11	0,68	51,10	<,0001	0,540
		Inland	39	0,27	0,18			
	2015	Coastal	39	1,47	1,15	35,76	<,0001	0,456
		Inland	39	0,31	0,23			
Ca (mg/g)	2013	Coastal	39	672,07	96,26	0,61	0,4386	0,015
		Inland	39	690,04	107,66			
	2014	Coastal	39	701,87	88,31	0,12	0,7344	0,006
		Inland	39	708,77	90,26			
	2015	Coastal	39	757,26	119,92	0,01	0,9181	0,001
		Inland	39	760,00	113,96			
Mg (mg/g)	2013	Coastal	39	174,53	61,04	61,17	<,0001	0,579
		Inland	39	87,18	29,75			
	2014	Coastal	39	174,45	71,67	33,29	<,0001	0,391
		Inland	39	94,41	45,64			
	2015	Coastal	39	173,32	56,85	44,40	<,0001	0,414
		Inland	39	97,94	40,01			
Ca/Mg Ratio	2013	Coastal	39	4,42	1,93	35,16	<,0001	0,401
		Inland	39	9,28	4,89			
	2014	Coastal	39	4,76	2,15	25,04	<,0001	0,309
		Inland	39	10,02	6,40			
	2015	Coastal	39	4,89	1,89	34,92	<,0001	0,355
		Inland	39	9,21	4,28			
Mo (mg/g)	2013	Coastal	39	0,03	0,02	23,24	<,0001	0,114
		Inland	39	0,02	0,01			
	2014	Coastal	39	0,03	0,01	15,15	0,0002	0,092
		Inland	39	0,02	0,01			
	2015	Coastal	39	0,03	0,02	12,85	0,0006	0,081
		Inland	39	0,02	0,01			

Annex Table 5: Continuation...

	Year	Location	N	Mean	Std Dev	F Ratio	Prob > F	R^2 (m to sea)
Co (mg/g)	2013	Coastal	39	0,18	0,14	5,91	0,0174	0,086
		Inland	39	0,26	0,14			
	2014	Coastal	39	0,13	0,09	16,60	0,0001	0,239
		Inland	39	0,24	0,15			
	2015	Coastal	39	0,17	0,13	12,10	0,0008	0,193
		Inland	39	0,29	0,16			
As (mg/g)	2013	Coastal	39	0,12	0,06	1,10	0,2986	0,031
		Inland	39	0,14	0,11			
	2014	Coastal	39	0,09	0,04	4,85	0,0307	0,073
		Inland	39	0,13	0,11			
	2015	Coastal	39	0,12	0,06	5,77	0,0187	0,144
		Inland	39	0,18	0,13			
Cd (mg/g)	2013	Coastal	39	0,06	0,06	14,93	0,0002	0,185
		Inland	39	0,18	0,19			
	2014	Coastal	39	0,07	0,07	14,18	0,0003	0,158
		Inland	39	0,19	0,18			
	2015	Coastal	39	0,09	0,10	8,33	0,0051	0,098
		Inland	39	0,19	0,20			
Cu (mg/g)	2013	Coastal	39	14,43	26,59	3,55	0,0632	0,054
		Inland	39	5,77	7,71			
	2014	Coastal	39	13,99	27,76	3,12	0,0812	0,065
		Inland	39	5,55	7,61			
	2015	Coastal	39	15,49	28,75	3,17	0,0789	0,059
		Inland	39	6,63	8,60			
Fe (mg/g)	2013	Coastal	39	23,49	11,76	4,03	0,0482	0,083
		Inland	39	29,59	15,06			
	2014	Coastal	39	19,63	10,02	8,94	0,0038	0,192
		Inland	39	29,55	18,59			
	2015	Coastal	39	16,81	9,66	4,55	0,0362	0,098
		Inland	39	21,88	11,34			
Li (mg/g)	2013	Coastal	39	0,03	0,01	0,52	0,474	0,015
		Inland	39	0,02	0,02			
	2014	Coastal	39	0,02	0,01	0,13	0,7196	0,005
		Inland	39	0,02	0,01			
	2015	Coastal	39	0,02	0,01	0,37	0,5438	0,011
		Inland	39	0,02	0,01			
Mn (mg/g)	2013	Coastal	39	35,20	18,30	0,14	0,7094	0,016
		Inland	39	36,71	17,17			
	2014	Coastal	39	31,20	18,60	0,66	0,4187	0,002
		Inland	39	34,58	17,95			
	2015	Coastal	39	38,97	20,49	0,47	0,4942	0,003
		Inland	39	41,87	16,17			

Annex Table 5: Continuation...

	Year	Location	N	Mean	Std Dev	F Ratio	Prob > F	R^2 (m to sea)
Ni (mg/g)	2013	Coastal	39	0,17	0,11	3,85	0,0534	0,145
		Inland	39	0,23	0,16			
	2014	Coastal	39	0,14	0,09	6,07	0,016	0,156
		Inland	39	0,21	0,15			
	2015	Coastal	39	0,18	0,11	5,31	0,0239	0,147
		Inland	39	0,26	0,19			
P (mg/g)	2013	Coastal	39	14,29	11,82	2,81	0,0977	0,055
		Inland	39	23,11	31,66			
	2014	Coastal	39	13,31	15,31	1,06	0,306	0,024
		Inland	39	18,69	29,52			
	2015	Coastal	39	9,12	10,46	5,72	0,0192	0,097
		Inland	39	18,83	23,77			
Rb (mg/g)	2013	Coastal	39	0,14	0,09	8,04	0,0059	0,189
		Inland	39	0,24	0,22			
	2014	Coastal	39	0,16	0,11	11,49	0,0011	0,206
		Inland	39	0,30	0,25			
	2015	Coastal	39	0,19	0,15	3,53	0,064	0,105
		Inland	39	0,28	0,24			
S (mg/g)	2013	Coastal	39	31,46	27,93	1,03	0,3131	0,003
		Inland	39	38,20	30,71			
	2014	Coastal	39	31,53	30,92	5,12	0,0265	0,030
		Inland	39	17,64	21,57			
	2015	Coastal	39	20,47	26,36	0,01	0,9309	0,004
		Inland	39	21,02	29,19			
Se (mg/g)	2013	Coastal	39	1,53	0,83	13,85	0,0004	0,191
		Inland	39	0,97	0,41			
	2014	Coastal	39	1,68	0,79	19,26	<,0001	0,285
		Inland	39	1,01	0,48			
	2015	Coastal	39	1,95	0,85	18,41	<,0001	0,226
		Inland	39	1,20	0,65			
Sr (mg/g)	2013	Coastal	39	2,74	0,91	15,12	0,0002	0,144
		Inland	39	1,94	0,90			
	2014	Coastal	39	2,72	1,19	11,83	0,001	0,105
		Inland	39	1,93	0,75			
	2015	Coastal	39	3,22	1,11	11,60	0,0011	0,117
		Inland	39	2,46	0,80			
Zn (mg/g)	2013	Coastal	39	23,62	35,00	0,06	0,8136	0,000
		Inland	39	25,39	30,17			
	2014	Coastal	39	22,28	36,37	0,30	0,5878	0,000
		Inland	39	26,50	31,21			
	2015	Coastal	39	23,27	32,83	1,38	0,2445	0,013
		Inland	39	33,13	41,40			

Annex Table 6: Mean ± Standard Deviation of mineral nutrients (µg/g dry weight) and Na/K and Ca/Mg ratios in leaves from *A. thaliana* plants growing naturally in the field in 13 coastal and 13 inland habitats. ANOVA between locations (coastal/inland) of 2 samples from each deme in 2013, 8 samples from each deme in 2014 and 5 samples from each deme in 2015.

Element	Location	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Na	Coastal	195	931,52	978,00	1	100,85	<,0001
	Inland	195	232,13	240,34			
K	Coastal	195	26180,50	8720,60	1	13,42	0,0003
	Inland	195	29653,69	10624,16			
Na/K	Coastal	195	0,04	0,05	1	80,38	<,0001
	Inland	195	0,01	0,01			
Ca	Coastal	195	30927,21	9130,73	1	53,32	<,0001
	Inland	195	38365,88	11610,72			
Mg	Coastal	195	4482,05	1555,83	1	46,34	<,0001
	Inland	195	3603,39	1035,19			
Ca/Mg	Coastal	195	7,54	3,01	1	98,33	<,0001
	Inland	195	11,55	5,04			
As	Coastal	195	0,76	0,84	1	0,69	0,4073
	Inland	195	0,70	0,46			
Cd	Coastal	195	0,48	0,44	1	2,28	0,1314
	Inland	195	0,55	0,46			
Co	Coastal	195	1,11	1,26	1	16,79	<,0001
	Inland	195	1,72	1,75			
Cu	Coastal	195	7,15	3,50	1	1,66	0,1982
	Inland	195	6,76	2,52			
Fe	Coastal	195	466,42	569,52	1	0,85	0,3578
	Inland	195	519,50	611,52			
Li	Coastal	195	1,63	1,41	1	2,64	0,1049
	Inland	195	1,42	1,23			
Mn	Coastal	195	117,60	86,76	1	10,92	0,001
	Inland	195	94,15	55,10			
Mo	Coastal	195	3,48	3,25	1	0,01	0,9244
	Inland	195	3,45	3,45			
Ni	Coastal	195	1,01	0,61	1	2,46	0,1177
	Inland	195	1,10	0,59			
P	Coastal	195	4674,26	2497,60	1	1,18	0,2787
	Inland	195	4916,97	2065,54			
Rb	Coastal	195	8,27	6,79	1	22,93	<,0001
	Inland	195	5,64	4,20			
S	Coastal	195	8996,94	3859,40	1	1,58	0,2095
	Inland	195	9502,62	4372,92			
Se	Coastal	195	8,75	10,61	1	2,05	0,1525
	Inland	195	7,43	8,01			
Sr	Coastal	195	123,80	137,90	1	2,52	0,1128
	Inland	195	107,65	51,07			
Zn	Coastal	195	59,65	38,60	1	3,61	0,0582
	Inland	195	71,03	77,97			

Annex Table 7: Mean ± Standard Deviation of mineral nutrients (mg/g) in soil from the coastal common garden (BLA) and the inland common garden (SCF) collected in April of 2013, 2014 and 2015. ANOVA between common garden sites (BLA/SCF) of 8 samples from each site and year.

	Year	Site	N	Mean (mg/g)	Std Dev (mg/g)	DF	F Ratio	Prob > F
Na	April 2013	BLA	8	59,93	10,06	1	20,61	0,0005
		SCF	8	39,84	7,46			
	April 2014	BLA	8	49,27	9,60	1	34,33	<,0001
		SCF	8	26,40	5,45			
	April 2015	BLA	8	74,04	11,34	1	49,31	<,0001
		SCF	8	34,34	11,28			
K	April 2013	BLA	8	124,63	21,02	1	15,80	0,0014
		SCF	8	88,75	14,50			
	April 2014	BLA	8	113,96	13,10	1	59,6632	<,0001
		SCF	8	60,72	14,44			
	April 2015	BLA	8	114,75	7,38	1	408,6975	<,0001
		SCF	8	46,29	6,10			
Na/K	April 2013	BLA	8	0,49	0,12	1	0,49	0,4947
		SCF	8	0,46	0,09			
	April 2014	BLA	8	0,44	0,12	1	0,1011	0,7552
		SCF	8	0,46	0,16			
	April 2015	BLA	8	0,65	0,11	1	4,0874	0,0627
		SCF	8	0,77	0,14			
Ca	April 2013	BLA	8	723,14	65,12	1	4,05	0,0638
		SCF	8	612,47	141,26			
	April 2014	BLA	8	817,90	76,05	1	0,09	0,764
		SCF	8	807,65	56,44			
	April 2015	BLA	8	615,03	147,48	1	9,17	0,009
		SCF	8	784,95	58,74			
Mg	April 2013	BLA	8	186,59	18,04	1	185,32	<,0001
		SCF	8	65,76	17,46			
	April 2014	BLA	8	178,69	42,27	1	23,65	0,0003
		SCF	8	104,26	9,35			
	April 2015	BLA	8	170,53	25,13	1	33,25	<,0001
		SCF	8	116,61	8,23			
Ca/Mg	April 2013	BLA	8	3,94	0,82	1	198,28	<,0001
		SCF	8	9,42	0,74			
	April 2014	BLA	8	4,80	1,17	1	47,27	<,0001
		SCF	8	7,76	0,37			
	April 2015	BLA	8	3,59	0,60	1	148,42	<,0001
		SCF	8	6,74	0,41			

Annex Table 7: Continuation...

	Year	Site	N	Mean (mg/g)	Std Dev (mg/g)	DF	F Ratio	Prob > F
As	April 2013	BLA	8	0,17	0,04	1	35,61	<,0001
		SCF	8	0,07	0,03			
	April 2014	BLA	8	0,17	0,04	1	22,24	0,0003
		SCF	8	0,10	0,01			
	April 2015	BLA	8	0,25	0,04	1	15,75	0,0014
		SCF	8	0,19	0,02			
Cd	April 2013	BLA	8	0,03	0,02	1	8,96	0,0097
		SCF	8	0,01	0,00			
	April 2014	BLA	8	0,02	0,01	1	0,36	0,5586
		SCF	8	0,03	0,01			
	April 2015	BLA	8	0,03	0,01	1	1,70	0,2134
		SCF	8	0,03	0,00			
Co	April 2013	BLA	8	0,10	0,02	1	31,50	<,0001
		SCF	8	0,06	0,01			
	April 2014	BLA	8	0,09	0,03	1	19,64	0,0006
		SCF	8	0,04	0,01			
	April 2015	BLA	8	0,09	0,02	1	19,42	0,0006
		SCF	8	0,06	0,01			
Cu	April 2013	BLA	8	4,20	1,52	1	33,96	<,0001
		SCF	8	1,00	0,34			
	April 2014	BLA	8	8,30	1,21	1	330,68	<,0001
		SCF	8	0,51	0,09			
	April 2015	BLA	8	10,51	1,38	1	395,10	<,0001
		SCF	8	0,79	0,12			
Fe	April 2013	BLA	8	19,80	2,08	1	0,00	0,9979
		SCF	8	19,80	6,17			
	April 2014	BLA	8	21,02	5,09	1	10,07	0,0068
		SCF	8	30,51	6,76			
	April 2015	BLA	8	14,04	3,09	1	4,59	0,0502
		SCF	8	17,06	2,52			
Li	April 2013	BLA	8	0,02	0,01	1	0,89	0,3622
		SCF	8	0,03	0,01			
	April 2014	BLA	8	0,01	0,00	1	36,78	<,0001
		SCF	8	0,03	0,01			
	April 2015	BLA	8	0,01	0,00	1	58,33	<,0001
		SCF	8	0,02	0,00			
Mg	April 2013	BLA	8	105,34	20,42	1	17,36	0,001
		SCF	8	65,76	17,46			
	April 2014	BLA	8	141,19	52,72	1	3,81	0,0714
		SCF	8	104,26	9,35			
	April 2015	BLA	8	170,53	25,13	1	33,25	<,0001
		SCF	8	116,61	8,23			

Annex Table 7: Continuation...

	Year	Site	N	Mean (mg/g)	Std Dev (mg/g)	DF	F Ratio	Prob > F
Mn	April 2013	BLA	8	29,94	10,68	1	0,12	0,7378
		SCF	8	27,94	12,62			
	April 2014	BLA	8	22,21	5,96	1	9,42	0,0083
		SCF	8	14,67	3,57			
	April 2015	BLA	8	22,27	6,36	1	0,07	0,791
		SCF	8	21,59	3,26			
Mo	April 2013	BLA	8	0,02	0,01	1	0,06	0,8054
		SCF	8	0,03	0,01			
	April 2014	BLA	8	0,05	0,02	1	18,03	0,0008
		SCF	8	0,01	0,00			
	April 2015	BLA	8	0,04	0,02	1	6,30	0,025
		SCF	8	0,02	0,00			
Ni	April 2013	BLA	8	0,18	0,06	1	24,52	0,0002
		SCF	8	0,07	0,02			
	April 2014	BLA	8	0,08	0,02	1	0,57	0,4644
		SCF	8	0,08	0,01			
	April 2015	BLA	8	0,10	0,02	1	1,96	0,1838
		SCF	8	0,11	0,01			
Rb	April 2013	BLA	8	0,13	0,03	1	30,73	<,0001
		SCF	8	0,07	0,01			
	April 2014	BLA	8	0,24	0,13	1	4,67	0,0484
		SCF	8	0,14	0,03			
	April 2015	BLA	8	0,25	0,12	1	16,58	0,0011
		SCF	8	0,08	0,01			
Se	April 2013	BLA	8	0,60	0,10	1	134,11	<,0001
		SCF	8	1,63	0,20			
	April 2014	BLA	8	0,44	0,11	1	157,24	<,0001
		SCF	8	1,77	0,28			
	April 2015	BLA	8	0,78	0,09	1	247,55	<,0001
		SCF	8	3,13	0,41			
Sr	April 2013	BLA	8	2,02	0,18	1	1,12	0,3087
		SCF	8	2,27	0,63			
	April 2014	BLA	8	1,73	0,37	1	100,01	<,0001
		SCF	8	3,12	0,14			
	April 2015	BLA	8	1,52	0,43	1	38,76	<,0001
		SCF	8	2,90	0,46			
Zn	April 2013	BLA	8	9,98	2,12	1	42,53	<,0001
		SCF	8	3,69	1,71			
	April 2014	BLA	8	10,59	3,04	1	63,12	<,0001
		SCF	8	1,94	0,47			
	April 2015	BLA	8	14,92	2,68	1	195,77	<,0001
		SCF	8	1,50	0,39			

Annex Table 8: Mean ± Standard Deviation of mineral nutrients (µg/g) in leaves from *A. thaliana* coastal and inland plants cultivated in a coastal common garden (BLA) and inland common garden (SCF) fields. ANOVA between locations (coastal/inland) of plants harvested in April of 2013 and 2014 in both sites.

	site	Year	Location	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Na	BLA	2013	Coastal	79	759,45	209,11	1	19,31	<,0001
			Inland	100	950,13	300,46			
		2014	Coastal	84	706,51	289,13	1	8,89	0,0032
			Inland	100	842,31	352,06			
	SCF	2013	Coastal	81	618,88	228,40	1	1,34	0,2487
			Inland	100	667,34	275,46			
		2014	Coastal	83	681,96	305,52	1	5,90	0,016
			Inland	100	583,97	263,34			
K	BLA	2013	Coastal	79	42599,81	16050,57	1	0,07	0,7969
			Inland	100	43408,31	20784,94			
		2014	Coastal	84	37367,45	17831,23	1	0,58	0,4465
			Inland	100	35281,52	20734,54			
	SCF	2013	Coastal	81	80231,87	46738,30	1	6,83	0,0099
			Inland	100	63017,66	27919,37			
		2014	Coastal	86	34194,93	38152,41	1	0,11	0,7406
			Inland	100	32896,95	8815,88			
Na/K	BLA	2013	Coastal	79	0,02	0,01	1	6,58	0,0114
			Inland	100	0,03	0,01			
		2014	Coastal	84	0,02	0,01	1	6,05	0,0148
			Inland	100	0,03	0,02			
	SCF	2013	Coastal	81	0,01	0,01	1	1,84	0,1776
			Inland	100	0,01	0,01			
		2014	Coastal	86	0,02	0,01	1	15,80	<,0001
			Inland	100	0,02	0,01			
Ca	BLA	2013	Coastal	79	51604,51	17255,70	1	7,65	0,0065
			Inland	100	70108,24	56072,54			
		2014	Coastal	84	112427,05	62030,33	1	0,62	0,4328
			Inland	100	105376,26	64796,73			
	SCF	2013	Coastal	81	61106,97	17269,42	1	3,96	0,0484
			Inland	100	55174,70	18535,49			
		2014	Coastal	86	65555,87	97869,70	1	0,05	0,8264
			Inland	100	68688,20	103805,14			
Mg	BLA	2013	Coastal	79	5080,23	1791,55	1	22,13	<,0001
			Inland	100	7822,47	4753,19			
		2014	Coastal	84	19461,59	15548,61	1	0,06	0,8056
			Inland	100	18931,44	14864,00			
	SCF	2013	Coastal	81	7246,04	2098,96	1	6,87	0,0097
			Inland	100	8664,47	4246,89			
		2014	Coastal	86	8971,00	13194,36	1	0,12	0,732
			Inland	100	9631,88	14042,37			

Annex Table 8: Continuation...

	site	Year	Location	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Ca/Mg	BLA	2013	Coastal	79	10,99	3,95	1	0,17	0,6819
			Inland	100	10,54	8,57			
		2014	Coastal	84	7,14	2,75	1	0,99	0,3203
			Inland	100	6,78	2,44			
	SCF	2013	Coastal	81	8,57	1,54	1	7,45	0,0072
			Inland	100	7,43	3,31			
		2014	Coastal	86	7,32	2,00	1	2,17	0,1467
			Inland	100	7,13	1,00			
As	BLA	2013	Coastal	79	1,11	0,65	1	0,04	0,8373
			Inland	100	1,09	0,54			
		2014	Coastal	84	5,13	6,34	1	0,06	0,8045
			Inland	100	5,34	5,73			
	SCF	2013	Coastal	81	0,53	0,17	1	16,29	<,0001
			Inland	100	0,42	0,17			
		2014	Coastal	86	2,31	6,71	1	0,01	0,921
			Inland	100	2,22	6,40			
Cd	BLA	2013	Coastal	79	0,50	0,16	1	0,79	0,3762
			Inland	100	0,53	0,19			
		2014	Coastal	84	0,72	1,02	1	0,66	0,4171
			Inland	100	0,85	1,17			
	SCF	2013	Coastal	81	0,60	0,28	1	2,10	0,1498
			Inland	100	0,54	0,19			
		2014	Coastal	86	1,73	2,53	1	0,02	0,8776
			Inland	100	1,68	2,72			
Co	BLA	2013	Coastal	79	0,90	0,32	1	0,82	0,3657
			Inland	100	0,95	0,39			
		2014	Coastal	84	2,06	1,78	1	0,00	0,9817
			Inland	100	2,06	1,82			
	SCF	2013	Coastal	81	1,19	0,44	1	19,05	<,0001
			Inland	100	0,90	0,34			
		2014	Coastal	86	2,02	3,07	1	0,30	0,5874
			Inland	100	1,80	2,73			
Cu	BLA	2013	Coastal	79	12,17	4,51	1	20,27	<,0001
			Inland	100	16,62	7,08			
		2014	Coastal	84	31,42	28,75	1	1,17	0,2812
			Inland	100	36,04	31,65			
	SCF	2013	Coastal	81	12,37	3,37	1	0,04	0,8481
			Inland	100	12,47	3,03			
		2014	Coastal	86	14,45	21,64	1	0,03	0,8609
			Inland	100	15,00	22,02			

Annex Table 8: Continuation...

	site	Year	Location	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Fe	BLA	2013	Coastal	79	292,23	177,61	1	0,53	0,469
			Inland	100	315,08	189,71			
		2014	Coastal	84	924,56	1091,40	1	0,02	0,889
			Inland	100	946,22	1101,02			
	SCF	2013	Coastal	81	1379,53	724,75	1	0,86	0,3543
			Inland	100	1264,85	756,09			
		2014	Coastal	86	3056,87	3542,85	1	0,50	0,4786
			Inland	100	2734,49	2840,80			
Li	BLA	2013	Coastal	79	0,41	0,22	1	1,36	0,2449
			Inland	100	0,37	0,18			
		2014	Coastal	84	1,85	2,28	1	0,16	0,6891
			Inland	100	1,98	2,36			
	SCF	2013	Coastal	81	4,10	1,97	1	21,80	<,0001
			Inland	100	2,79	1,22			
		2014	Coastal	86	7,98	12,68	1	0,27	0,6021
			Inland	100	7,10	11,00			
Mn	BLA	2013	Coastal	79	120,51	67,45	1	16,49	<,0001
			Inland	100	227,34	220,62			
		2014	Coastal	84	174,60	157,65	1	0,01	0,9426
			Inland	100	176,22	160,44			
	SCF	2013	Coastal	81	213,91	67,20	1	0,78	0,3773
			Inland	100	242,62	280,33			
		2014	Coastal	86	231,60	336,91	1	0,00	0,9524
			Inland	100	228,80	325,01			
Mo	BLA	2013	Coastal	79	7,43	4,08	1	0,02	0,884
			Inland	100	7,32	4,87			
		2014	Coastal	84	13,99	14,49	1	0,13	0,7216
			Inland	100	13,29	12,87			
	SCF	2013	Coastal	81	2,20	1,14	1	3,10	0,0805
			Inland	100	1,88	0,99			
		2014	Coastal	86	1,43	1,94	1	1,31	0,2539
			Inland	100	1,16	1,37			
Ni	BLA	2013	Coastal	79	1,40	0,51	1	0,63	0,4279
			Inland	100	1,51	0,99			
		2014	Coastal	84	2,57	2,61	1	0,02	0,8983
			Inland	100	2,53	2,40			
	SCF	2013	Coastal	81	2,14	0,61	1	1,41	0,2363
			Inland	100	1,98	0,95			
		2014	Coastal	86	3,37	5,04	1	0,20	0,656
			Inland	100	3,07	4,52			

Annex Table 8: Continuation...

	site	Year	Location	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
P	BLA	2013	Coastal	79	7226,82	2718,43	1	45,15	<,0001
			Inland	100	15221,80	10108,18			
		2014	Coastal	84	17123,87	16132,87	1	0,26	0,61
			Inland	100	18324,64	17094,39			
	SCF	2013	Coastal	81	6932,94	2949,00	1	7,56	0,0068
			Inland	100	9402,83	7345,24			
		2014	Coastal	86	5181,91	5276,09	1	0,51	0,4773
			Inland	100	5786,56	6654,15			
Rb	BLA	2013	Coastal	79	8,98	7,73	1	0,40	0,5279
			Inland	100	9,82	7,72			
		2014	Coastal	84	23,29	21,79	1	0,09	0,7613
			Inland	100	22,41	19,10			
	SCF	2013	Coastal	81	8,19	3,53	1	6,61	0,0112
			Inland	100	6,82	2,74			
		2014	Coastal	86	17,95	31,65	1	0,24	0,6254
			Inland	100	15,89	27,69			
Se	BLA	2013	Coastal	79	5,14	3,09	1	11,04	0,0011
			Inland	100	8,16	7,24			
		2014	Coastal	84	8,18	9,90	1	1,22	0,2701
			Inland	100	9,92	12,23			
	SCF	2013	Coastal	81	16,07	11,72	1	5,66	0,0187
			Inland	100	21,41	15,32			
		2014	Coastal	86	25,85	45,12	1	1,00	0,3181
			Inland	100	19,72	41,47			
Sr	BLA	2013	Coastal	79	179,68	65,05	1	1,22	0,2721
			Inland	100	165,34	87,74			
		2014	Coastal	84	140,54	100,95	1	0,18	0,6741
			Inland	100	146,87	111,40			
	SCF	2013	Coastal	81	208,47	60,20	1	14,89	0,0002
			Inland	100	174,99	39,52			
		2014	Coastal	86	190,11	233,39	1	0,12	0,7314
			Inland	100	202,11	259,81			
Zn	BLA	2013	Coastal	79	88,43	38,13	1	9,92	0,002
			Inland	100	133,85	120,48			
		2014	Coastal	84	204,82	230,49	1	1,25	0,2655
			Inland	100	269,37	530,20			
	SCF	2013	Coastal	81	105,09	93,39	1	1,84	0,1769
			Inland	100	126,92	99,90			
		2014	Coastal	86	369,36	719,20	1	0,75	0,3867
			Inland	100	287,02	619,29			

Annex Table 9: Statistical analysis of fitness results from progeny of crossings *(male x female)* (27 plants from *coastal x inland* (C x I) ; 27 plants from *inland x coastal* (I x C) for each treatment) and parental plants (9 parents inland (Par I); 9 parents coastal (Par C) for each treatment) cultivated on hydroponics treated with 0, 50 and 100 mM NaCl. Comparisons for all pairs of means using Tukey-Kramer HSD test.

Treatment (NaCl)	Type comparison	Dif (nº siliques)	Std Err Dif	HSD	p-Value
0 mM	C x I - Par I	11,000	19,262	-39,731	0,9404
	I x C - Par I	37,481	19,262	-13,249	0,2189
	I x C - C x I	26,481	13,620	-9,391	0,2196
	I x C - Par C	24,815	19,262	-25,916	0,5735
	Par C - Par I	12,667	23,591	-49,466	0,9497
	Par C - C x I	1,667	19,262	-49,064	0,9998
50 mM	C x I - Par I	19,296	9,474	-5,654	0,1848
	I x C - Par I	110,963	9,474	86,012	<,0001
	I x C - C x I	91,667	6,699	74,024	<,0001
	I x C - Par C	20,852	9,474	-4,099	0,1333
	Par C - Par I	90,111	11,603	59,553	<,0001
	Par C - C x I	70,815	9,474	45,864	<,0001
100 mM	C x I - Par I	6,556	5,090	-6,849	0,5736
	I x C - Par I	39,630	5,090	26,225	<,0001
	I x C - C x I	33,074	3,599	23,596	<,0001
	I x C - Par C	4,963	5,090	-8,441	0,764
	Par C - Par I	34,667	6,233	18,25	<,0001
	Par C - C x I	28,111	5,090	14,707	<,0001

Annex Table 10: Mean ± Standard Deviation of mineral nutrients (mg/g) and Na/K and Ca/Mg ratios in soil from the habitat of 12 coastal sites with plants homozygous for the strong allele of *AtHKT1;1* (C) and 6 coastal sites containing also the weak allele of *AtHKT1;1* (T). ANOVA between the presence of *AtHKT1;1* weak allele of 2 soil samples collected in March and 3 soil samples collected in May of 2013, 2014 and 2015.

	AtHKT1;1	N	Mean (mg/g)	Std Dev (mg/g)	DF	F Ratio	Prob > F
Na	C	180	99,019	32,007	1	38,2628	<,0001
	T	90	77,258	18,029			
K	C	180	104,669	83,500	1	14,4598	0,0002
	T	90	141,476	63,515			
Na/K	C	180	1,822	1,829	1	12,6672	0,0004
	T	90	1,006	1,840			
Ca	C	180	720,957	105,631	1	0,9976	0,3187
	T	90	707,842	103,861			
Mg	C	180	166,730	65,303	1	15,4146	0,0001
	T	90	136,330	54,566			
Ca/ Mg	C	180	5,142	2,590	1	7,2288	0,0076
	T	90	5,972	2,215			
As	C	180	0,122	0,082	1	9,4072	0,0024
	T	90	0,155	0,090			
Co	C	180	0,182	0,203	1	3,9951	0,0466
	T	90	0,236	0,223			
Cu	C	180	2,990	2,380	1	101,7238	<,0001
	T	90	13,039	13,317			
Mo	C	180	0,032	0,027	1	17,4333	<,0001
	T	90	0,047	0,030			
Ni	C	180	0,145	0,082	1	31,1163	<,0001
	T	90	0,215	0,124			
Rb	C	180	0,151	0,106	1	20,941	<,0001
	T	90	0,218	0,129			
Se	C	180	1,792	1,174	1	16,7863	<,0001
	T	90	1,245	0,690			
Zn	C	180	7,891	7,525	1	28,1734	<,0001
	T	90	18,000	23,908			
Cd	C	180	0,065	0,065	1	0,1744	0,6766
	T	90	0,061	0,071			
Fe	C	180	19,627	13,699	1	0,2909	0,5901
	T	90	20,493	9,652			
Li	C	180	0,021	0,013	1	0,0481	0,8265
	T	90	0,021	0,012			
Mn	C	180	32,786	21,403	1	0,0003	0,9855
	T	90	32,838	24,695			
P	C	117	12,309	12,911	1	0,9351	0,3348
	T	71	14,030	9,777			
S	C	120	47,183	43,507	1	2,3916	0,1238
	T	61	37,668	28,517			
Sr	C	180	2,673	0,830	1	2,2327	0,1363
	T	90	2,501	1,041			

Annex Table 11: Mean ± Standard Deviation of plant mineral nutrients (µg/g dry weight) and Na/K and Ca/Mg ratios in leaves from *A. thaliana* plants growing naturally in 18 coastal habitats. ANOVA between *AtHKT1;1* allele type (strong (C)/ weak (T)) of 21 "C" and 8 "T" plants collected in 2013, 99 "C" and 21 "T" plants collected in 2014 and 53 "C" and 16 "T" plants collected in 2015.

	AtHKT1;1	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Na	C	173	748,432	780,291	1	126,405	<,0001
	T	45	2350,613	1086,298			
K	C	173	25961,128	8327,931	1	2,583	0,1095
	T	45	23742,082	7942,707			
Na/K	C	173	0,031	0,030	1	135,699	<,0001
	T	45	0,111	0,068			
Ca	C	173	30195,037	9123,078	1	7,497	0,0067
	T	45	34437,054	9769,959			
Mg	C	173	4527,788	1552,999	1	1,543	0,2155
	T	45	4208,957	1455,413			
Ca/Mg	C	173	7,230	2,782	1	12,866	0,0004
	T	45	9,026	3,700			
Co	C	173	0,875	0,717	1	20,695	<,0001
	T	45	1,589	1,522			
Cu	C	173	6,606	2,945	1	9,226	0,0027
	T	45	8,301	4,546			
Li	C	173	1,488	1,338	1	7,238	0,0077
	T	45	2,105	1,484			
Ni	C	173	0,982	0,554	1	9,298	0,0026
	T	45	1,300	0,841			
Sr	C	173	109,363	109,105	1	7,349	0,0072
	T	45	173,430	226,678			
As	C	173	0,837	0,649	1	3,518	0,0723
	T	45	1,106	1,099			
Cd	C	173	0,410	0,314	1	2,989	0,0853
	T	45	0,515	0,511			
Fe	C	173	501,213	591,982	1	0,677	0,4114
	T	45	584,064	637,712			
Mn	C	173	118,243	85,289	1	2,753	0,0985
	T	45	96,383	44,671			
Mo	C	173	3,174	3,016	1	0,705	0,4019
	T	45	3,606	3,287			
Rb	C	173	7,731	6,110	1	0,027	0,8695
	T	45	7,563	6,014			
S	C	173	9168,493	3627,805	1	3,447	0,0647
	T	45	10366,141	4638,228			
Se	C	173	8,077	10,671	1	2,071	0,1516
	T	45	10,769	12,974			
Zn	C	173	62,065	40,946	1	0,965	0,3271
	T	45	55,620	31,534			

Annex Table 12: Mean, Variance, minimum and maximum value and total difference between them of Na content (mg/g) and Na/K ratio of soil samples from T1 (site with plants homozygous for the strong allele of *AtHKT1;1*) and T13 (site containing a mixture of plants with the strong and weak allele of *AtHKT1;1*) collected twice a month from February to May of 2014 and 2015.

Year	Site	Soil element	Mean	Var (σ^2)	Min Value	Max Value	Diff (Max-Min)
2014	T1 (C)	Na (mg/g)	93,34	117,76	73,32	117,81	44,49
		Na/K	1,15	0,17	0,49	1,99	1,5
	T13 (C / T)	Na (mg/g)	61,16	360,15	28,55	94,16	65,61
		Na/K	1,52	1,40	0,31	4	3,69
2015	T1 (C)	Na (mg/g)	114,58	143,57	92,21	131,62	39,40
		Na/K	1,54	0,07	1,24	2,03	0,79
	T13 (C / T)	Na (mg/g)	106,62	1982,10	53,81	200,05	146,24
		Na/K	1,18	0,31	0,34	2,11	1,77

Annex Table 13: Repeated Measures ANOVA between means of growth (rosette diameter, mm) of 20 plants with the weak allele and 20 plants with the strong allele of *ATHKT1;1* cultivated in each common garden (BLA/SCF) in 2014 and 2015 over time (independent measures of growth taken once a week during 6 weeks).

	Site	Year	F Test Value	Exact F	DF	Den DF	Prob>F
AtHKT1	BLA	2014	0,13	4,77	1	38	0,0352
Time	BLA	2014	47,57	261,62	6	33	<,0001
Time * AtHKT1	BLA	2014	0,28	1,55	6	33	0,1917
AtHKT1	BLA	2015	3,67	212,81	1	38	<,0001
Time	BLA	2015	125,50	1108,54	6	33	<,0001
Time * AtHKT1	BLA	2015	3,23	28,52	6	33	<,0001
AtHKT1	SCF	2014	0,00	0,00	1	38	0,9719
Time	SCF	2014	29,14	160,29	6	33	<,0001
Time * AtHKT1	SCF	2014	0,19	1,05	6	33	0,4092
AtHKT1	SCF	2015	0,04	2,35	1	38	0,1308
Time	SCF	2015	52,13	460,48	6	33	<,0001
Time * AtHKT1	SCF	2015	0,06	0,54	6	33	0,7734

Annex Table 14: Mean ± Standard Deviation of fitness (silique number produced) and flowering time (measured as days from germination when the first flower appeared) and ANOVA between 20 plants with the weak allele (T) and 20 plants with the strong allele (T) of *ATHKT1;1* cultivated in each common garden (BLA/SCF) in the spring of 2014 and 2015.

	Site	Year	*AtHKT1;1*	N	Mean	Std Dev	DF	F Ratio	Prob > F
Silique number	BLA	2014	C	20	396,30	112,54	1	7,1544	0,011
			T	20	310,80	88,15			
		2015	C	20	221,00	32,41	1	70,5115	<,0001
			T	20	147,10	35,68			
	SCF	2014	C	20	152,40	29,98	1	0,3384	0,5642
			T	20	146,45	34,55			
		2015	C	20	105,67	27,77	1	0,3426	0,5606
			T	20	101,47	27,81			
Flowering time	BLA	2014	C	20	69,4	6,09	1	19,79	<,0001
			T	20	61,65	4,86			
		2015	C	20	46,65	6,17	1	10,15	0,0029
			T	20	40,8	5,43			
	SCF	2014	C	20	71,95	6,28	1	0,94	0,3395
			T	20	70,05	6,14			
		2015	C	20	53,65	6,67	1	0,50	0,4822
			T	20	52,15	6,70			

Annex Table 15: Mean ± Standard Deviation of plant mineral nutrients content (µg/g dry weight) and Na/K and Ca/Mg ratios in leaves from *A. thaliana* plants growing in the coastal (BLA) or inland (SCF) common gardens. ANOVA between *AtHKT1;1* allele type (strong (C)/ weak (T)) of 10 plants T13c, T13t, JBBc, JBBt in each site collected in April of 2014 and 2015.

	Site	Year	*AtHKT1;1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Na	BLA	2014	C	20	431,07	236,84	1	39,318	<,0001
			T	20	2775,46	1655,19			
		2015	C	20	202,34	205,98	1	38,9539	<,0001
			T	20	1822,67	1142,61			
	SCF	2014	C	20	239,08	172,41	1	56,1092	<,0001
			T	20	1052,81	454,20			
		2015	C	20	260,02	100,37	1	74,5515	<,0001
			T	20	861,32	294,83			
K	BLA	2014	C	20	34246,83	12701,08	1	0,5573	0,4599
			T	20	31407,26	11314,72			
		2015	C	20	42603,18	8684,92	1	6,1101	0,018
			T	20	36251,20	7526,00			
	SCF	2014	C	20	26528,41	5318,59	1	0,7391	0,3953
			T	20	27991,65	5445,04			
		2015	C	20	32692,90	4074,27	1	1,8631	0,1803
			T	20	30277,95	6782,74			
Na/K	BLA	2014	C	20	0,015	0,01	1	18,9115	<,0001
			T	20	0,112	0,10			
		2015	C	20	0,005	0,00	1	38,3858	<,0001
			T	20	0,053	0,03			
	SCF	2014	C	20	0,01	0,01	1	44,7745	<,0001
			T	20	0,04	0,02			
		2015	C	20	0,01	0,00	1	52,0293	<,0001
			T	20	0,03	0,01			
Ca	BLA	2014	C	20	40023,76	21996,70	1	2,6789	0,1099
			T	20	54144,18	31697,61			
		2015	C	20	37311,37	6363,56	1	1,5025	0,2278
			T	20	34817,34	6504,21			
	SCF	2014	C	20	30923,57	7997,70	1	1,9978	0,1657
			T	20	35084,45	10457,34			
		2015	C	20	37358,73	7306,38	1	1,7625	0,1922
			T	20	41725,34	12766,37			
Mg	BLA	2014	C	20	5489,68	2619,87	1	1,816	0,1858
			T	20	6639,45	2773,99			
		2015	C	20	7216,99	2042,86	1	2,0225	0,1631
			T	20	6418,46	1460,23			
	SCF	2014	C	20	4531,67	1069,71	1	3,7011	0,0619
			T	20	5189,98	1094,34			
		2015	C	20	4933,44	534,11	1	4,6126	0,0382
			T	20	5399,27	809,70			

Annex Table 15: Continuation...

	Site	Year	*AtHKT1;1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Ca/Mg	BLA	2014	C	20	7,241	2,25	1	0,4611	0,5012
			T	20	7,708	2,11			
		2015	C	20	5,363	0,94	1	0,3625	0,5507
			T	20	5,553	1,05			
	SCF	2014	C	20	6,87	1,10	1	0,1822	0,6719
			T	20	6,72	1,07			
		2015	C	20	7,67	1,75	1	0,0114	0,9154
			T	20	7,72	1,74			
As	BLA	2014	C	20	8,088	8,08	1	0,0174	0,8958
			T	20	7,723	9,38			
		2015	C	20	1,246	0,51	1	1,9868	0,1668
			T	20	1,647	1,17			
	SCF	2014	C	20	0,57	0,39	1	1,0924	0,3025
			T	20	0,46	0,23			
		2015	C	20	0,16	0,06	1	0,6458	0,4266
			T	20	0,18	0,10			
Cd	BLA	2014	C	20	0,903	0,88	1	0,1133	0,7382
			T	20	0,805	0,95			
		2015	C	20	0,131	0,03	1	0,01	0,9211
			T	20	0,130	0,03			
	SCF	2014	C	20	0,78	0,30	1	0,0719	0,79
			T	20	0,76	0,23			
		2015	C	20	0,36	0,17	1	0,0649	0,8002
			T	20	0,35	0,10			
Co	BLA	2014	C	20	2,399	1,97	1	0,0838	0,7737
			T	20	2,614	2,68			
		2015	C	20	0,861	0,22	1	0,3193	0,5753
			T	20	0,925	0,46			
	SCF	2014	C	20	1,34	0,49	1	4,6689	0,0371
			T	20	1,06	0,29			
		2015	C	20	0,65	0,24	1	1,5809	0,2163
			T	20	0,84	0,63			
Cu	BLA	2014	C	20	50,096	45,82	1	0,1961	0,6604
			T	20	43,856	43,25			
		2015	C	20	10,189	2,26	1	5,4023	0,0256
			T	20	12,337	3,46			
	SCF	2014	C	20	7,09	1,24	1	1,3387	0,2545
			T	20	7,75	2,22			
		2015	C	20	7,87	2,16	1	0,9736	0,3300
			T	20	7,35	0,85			

Annex Table 15: Continuation...

	Site	Year	*AtHKT1;1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Fe	BLA	2014	C	20	224,062	65,59	1	3,7078	0,0617
			T	20	291,574	142,42			
		2015	C	20	272,331	108,55	1	0,0004	0,9840
			T	20	271,772	60,29			
	SCF	2014	C	20	298,15	110,40	1	8,3404	0,0064
			T	20	217,25	59,23			
		2015	C	20	492,50	117,49	1	1,2808	0,2648
			T	20	543,52	163,82			
Li	BLA	2014	C	20	2,426	2,66	1	0,9932	0,3253
			T	20	1,669	2,11			
		2015	C	20	0,185	0,06	1	4,0159	0,0522
			T	20	0,268	0,18			
	SCF	2014	C	20	5,03	1,68	1	5,3785	0,0259
			T	20	3,98	1,13			
		2015	C	20	1,20	0,57	1	3,3312	0,0742
			T	20	1,54	1,50			
Mn	BLA	2014	C	20	60,289	27,91	1	0,5297	0,4712
			T	20	67,983	38,15			
		2015	C	20	90,672	11,40	1	0,3272	0,5707
			T	20	88,047	17,07			
	SCF	2014	C	20	149,67	32,56	1	5,7645	0,0214
			T	20	128,10	23,52			
		2015	C	20	77,49	21,21	1	0,8549	0,361
			T	20	85,96	35,04			
Mo	BLA	2014	C	20	14,703	14,22	1	0,0325	0,858
			T	20	15,636	18,28			
		2015	C	20	13,255	4,69	1	0,08	0,7789
			T	20	13,768	6,61			
	SCF	2014	C	20	0,93	0,58	1	0,3612	0,5514
			T	20	0,83	0,40			
		2015	C	20	2,32	1,66	1	0,2962	0,5894
			T	20	2,07	1,31			
Ni	BLA	2014	C	20	3,011	2,61	1	0,0006	0,9811
			T	20	3,032	3,06			
		2015	C	20	0,341	0,10	1	4,4273	0,0420
			T	20	0,404	0,09			
	SCF	2014	C	20	1,73	0,50	1	1,3014	0,2611
			T	20	1,56	0,40			
		2015	C	20	0,45	0,11	1	0,3395	0,5636
			T	20	0,47	0,14			

Annex Table 15: Continuation...

	Site	Year	AtHKT1;1	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
P	BLA	2014	C	20	5658,805	1703,69	1	1,5362	0,2228
			T	20	5043,129	1425,63			
		2015	C	20	7193,762	1212,15	1	0,6548	0,4234
			T	20	7476,448	985,67			
	SCF	2014	C	20	3539,05	951,96	1	2,3746	0,1316
			T	20	4218,89	1728,14			
		2015	C	20	4994,00	939,95	1	22,5129	<,0001
			T	20	3752,37	697,17			
Rb	BLA	2014	C	20	21,465	16,54	1	0,0209	0,8857
			T	20	22,356	22,02			
		2015	C	20	7,122	1,96	1	0,1419	0,7085
			T	20	6,855	2,50			
	SCF	2014	C	20	10,58	5,49	1	1,6676	0,2044
			T	20	8,68	3,60			
		2015	C	20	4,92	5,08	1	0,2722	0,6049
			T	20	5,80	5,56			
Se	BLA	2014	C	20	11,119	12,23	1	0,0004	0,9840
			T	20	11,040	12,68			
		2015	C	20	13,204	4,40	1	5,9755	0,0193
			T	20	17,435	6,37			
	SCF	2014	C	20	10,68	9,11	1	2,7184	0,1074
			T	20	7,13	3,12			
		2015	C	20	23,47	8,55	1	2,2182	0,1446
			T	20	19,74	7,19			
Sr	BLA	2014	C	20	184,713	126,20	1	0,026	0,8728
			T	20	178,439	119,81			
		2015	C	20	51,883	10,31	1	0,0329	0,8571
			T	20	51,253	11,64			
	SCF	2014	C	20	110,78	34,25	1	0,8062	0,3749
			T	20	120,30	32,77			
		2015	C	20	137,45	42,47	1	0,0019	0,9656
			T	20	138,01	38,28			
Zn	BLA	2014	C	20	223,745	191,10	1	0,0239	0,8779
			T	20	236,566	317,63			
		2015	C	20	55,512	17,82	1	3,9203	0,0550
			T	20	102,76	105,21			
	SCF	2014	C	20	353,49	569,01	1	1,5625	0,2189
			T	20	185,11	197,82			
		2015	C	20	69,88	19,82	1	0,0367	0,8492
			T	20	71,63	35,81			

Annex Table 16: Repeated Measures ANOVA between means of growth (rosette diameter, mm) of 10 plants with the strong allele and 10 plants with the weak allele of *ATHKT1;1* cultivated in potting mix soil irrigated with nutrient solution plus 0, 50 and 100 mM NaCl over time (independent measures of growth taken every 3-4 days during 3 weeks).

	NaCl Treatment	F Test Value	Exact F	DF	Den DF	Prob>F
AtHKT1	0 mM	0,00	0,02	1	18	0,8844
Time	0 mM	81,04	175,58	6	13	<,0001
Time * *AtHKT1*	0 mM	0,40	0,87	6	13	0,5446
AtHKT1	50 mM	1,26	22,60	1	18	**0,0002**
Time	50 mM	51,41	111,39	6	13	<,0001
Time * *AtHKT1*	50 mM	6,53	14,16	6	13	**<,0001**
AtHKT1	100 mM	0,22	4,01	1	18	0,0605
Time	100 mM	32,74	70,94	6	13	<,0001
Time * *AtHKT1*	100 mM	0,10	0,21	6	13	0,9683

Annex Table 17: Mean ± Standard Deviation of fitness (silique number produced) ad ANOVA between 10 plants with the strong allele (C) and 10 plants with the weak allele (T) of *ATHKT1;1* cultivated in potting mix soil irrigated with nutrient solution plus 0, 50 and 100 mM NaCl.

NaCl Treatment	*AtHKT1;1*	N	Mean (Silique number)	Std Dev (Silique number)	DF	F Ratio	Prob > F
0 mM	C	10	156,7	28,92	1	0,1929	0,6657
	T	10	163,3	37,70			
50 mM	C	10	103,2	30,20	1	21,32	0,0002
	T	10	169,4	33,81			
100 mM	C	10	20,3	9,74	1	0,1243	0,7285
	T	10	22,3	15,06			

Annex Table 18: Repeated Measures ANOVA between means of growth (rosette diameter, mm) of 20 plants with the strong allele and 20 plants with the weak allele of *ATHKT1;1* during two weeks of exposure to 0, 50 and 100 mM NaCl in the hydroponic solution over time (independent measures of growth taken every 3-4 days during 2 weeks).

	NaCl Treatment	F Test Value	Exact F	DF	Den DF	Prob>F
AtHKT1	0 mM	0,01	0,25	1	41	0,6194
Time	0 mM	57,32	544,53	4	38	<,0001
Time * *AtHKT1*	0 mM	0,04	0,39	4	38	0,8121
AtHKT1	50 mM	0,01	0,60	1	42	0,4445
Time	50 mM	40,40	393,87	4	39	<,0001
Time * *AtHKT1*	50 mM	0,70	6,82	4	39	0,0003
AtHKT1	100 mM	0,02	0,90	1	37	0,3481
Time	100 mM	23,84	202,65	4	34	<,0001
Time * *AtHKT1*	100 mM	0,32	2,75	4	34	0,044

Annex Table 19: Mean ± Standard Deviation of rosette fresh weight (g). ANOVA between 20 plants with the strong allele of *AtHKT1;1* (C) and 20 plants with the weak allele of *AtHKT1;1* (T) after exposed to 0, 50 or 100 mM NaCl in the hydroponic solution during 2 weeks.

NaCl Treatment	*AtHKT1;1*	N	Mean (g)	Std Dev (g)	DF	F Ratio	Prob > F
0 mM	C	20	1,074	0,438	1	0,2531	0,6228
	T	20	1,176	0,375			
50 mM	C	20	0,912	0,195	1	7,9306	0,0137
	T	20	1,167	0,167			
100 mM	C	18	0,605	0,247	1	0,3689	0,5533
	T	20	0,547	0,110			

Annex Table 20: Mean ± Standard Deviation of plant mineral nutrients (µg/g dry weight) and Na/K and Ca/Mg ratios in leaves from *A. thaliana* plants growing hydroponically under 0, 50 and 100 mM NaCl. ANOVA between *AtHKT1;1* allele type (strong (C)/ weak (T)) of 10 plants T13c, T13t, JBBc, JBBt per each treatment.

	NaCl Treatment	*AtHKT1;1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Na	0 mM	C	20	1266,18	3892,76	1	54,03	<,0001
		T	20	7617,97	732,57			
	50 mM	C	20	29829,86	13243,59	1	36,03	<,0001
		T	20	80622,57	37415,25			
	100 mM	C	20	56638,33	29515,46	1	21,28	<,0001
		T	20	106826,69	37171,49			
K	0 mM	C	20	58822,42	16122,38	1	7,16	0,0107
		T	20	47388,59	11632,03			
	50 mM	C	20	45263,17	12005,53	1	0,02	0,8849
		T	20	45776,99	11395,26			
	100 mM	C	20	61542,18	7794,44	1	21,03	<,0001
		T	20	43395,00	12598,16			
Na/K	0 mM	C	20	0,02	0,01	1	44,22	<,0001
		T	20	0,18	0,11			
	50 mM	C	20	0,69	0,33	1	28,75	<,0001
		T	20	1,90	1,01			
	100 mM	C	20	0,98	0,70	1	29,11	<,0001
		T	20	2,70	1,25			
Ca	0 mM	C	20	46589,67	12464,17	1	0,13	0,7226
		T	20	48064,29	14457,33			
	50 mM	C	20	39066,19	10427,38	1	0,25	0,6194
		T	20	40864,01	13237,32			
	100 mM	C	20	37966,74	9790,93	1	2,03	0,1629
		T	20	42785,79	11133,08			
Mg	0 mM	C	20	4510,01	1851,00	1	0,00	0,9913
		T	20	4515,76	1578,09			
	50 mM	C	20	4322,18	1293,36	1	0,02	0,8875
		T	20	4386,89	1696,08			
	100 mM	C	20	4107,12	1500,09	1	1,83	0,1845
		T	20	4777,59	1579,84			
Ca/Mg	0 mM	C	20	11,70	4,81	1	0,16	0,6915
		T	20	11,22	2,70			
	50 mM	C	20	9,37	2,50	1	0,44	0,5126
		T	20	9,90	2,81			
	100 mM	C	20	10,01	2,89	1	0,45	0,5063
		T	20	9,45	2,32			

Annex Table 20: Continuation...

	NaCl Treatment	*AtHKT1;1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
As	0 mM	C	20	0,30	0,22	1	1,46	0,2336
		T	20	0,38	0,23			
	50 mM	C	20	0,43	0,29	1	0,33	0,5669
		T	20	0,48	0,33			
	100 mM	C	20	0,41	0,33	1	1,37	0,2488
		T	20	0,56	0,44			
Cd	0 mM	C	20	0,45	0,32	1	0,01	0,9429
		T	20	0,45	0,34			
	50 mM	C	20	0,51	0,24	1	0,60	0,4435
		T	20	0,45	0,27			
	100 mM	C	20	0,66	0,43	1	1,11	0,2981
		T	20	0,54	0,28			
Co	0 mM	C	20	0,29	0,20	1	0,05	0,8252
		T	20	0,28	0,17			
	50 mM	C	20	0,22	0,08	1	0,15	0,7004
		T	20	0,23	0,10			
	100 mM	C	20	0,24	0,13	1	0,00	0,9471
		T	20	0,24	0,11			
Cu	0 mM	C	20	14,47	8,11	1	1,31	0,2585
		T	20	18,11	12,20			
	50 mM	C	20	16,87	7,07	1	0,10	0,7549
		T	20	17,60	8,23			
	100 mM	C	20	16,17	8,59	1	0,02	0,9025
		T	20	15,86	7,15			
Li	0 mM	C	20	0,21	0,26	1	0,93	0,3415
		T	20	0,15	0,11			
	50 mM	C	20	0,18	0,14	1	0,23	0,6363
		T	20	0,16	0,13			
	100 mM	C	20	0,18	0,15	1	0,02	0,8761
		T	20	0,18	0,15			
Mn	0 mM	C	20	190,37	149,30	1	0,29	0,5908
		T	20	171,71	60,32			
	50 mM	C	20	146,17	36,89	1	0,06	0,8109
		T	20	149,12	44,21			
	100 mM	C	20	140,13	46,45	1	0,03	0,8616
		T	20	142,22	26,63			
Mo	0 mM	C	20	31,39	13,18	1	3,90	0,0625
		T	20	35,03	17,19			
	50 mM	C	20	30,83	14,58	1	0,27	0,6088
		T	20	33,54	19,93			
	100 mM	C	20	27,01	14,09	1	3,37	0,0744
		T	20	36,53	17,68			

Annex Table 20: Continuation...

	NaCl Treatment	*AtHKT1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Ni	0 mM	C	20	3,41	3,33	1	0,08	0,7794
		T	20	3,72	3,80			
	50 mM	C	20	3,24	2,70	1	0,40	0,5283
		T	20	2,78	2,01			
	100 mM	C	20	3,68	3,84	1	1,14	0,2921
		T	20	2,65	2,03			
P	0 mM	C	20	14507,83	11384,39	1	0,80	0,3766
		T	20	12137,06	4911,39			
	50 mM	C	20	15300,25	5279,72	1	0,17	0,6866
		T	20	14582,39	6388,45			
	100 mM	C	20	13301,90	3963,70	1	2,69	0,1092
		T	20	15363,58	3863,71			
Rb	0 mM	C	20	3,06	2,33	1	0,12	0,7281
		T	20	3,32	2,64			
	50 mM	C	20	1,79	1,07	1	1,61	0,2121
		T	20	2,33	1,66			
	100 mM	C	20	2,06	1,71	1	0,55	0,4628
		T	20	2,57	2,45			
Se	0 mM	C	20	20,59	20,73	1	0,55	0,4607
		T	20	25,98	26,24			
	50 mM	C	20	24,28	26,30	1	0,11	0,7415
		T	20	26,84	24,96			
	100 mM	C	20	23,28	25,63	1	0,77	0,3863
		T	20	31,63	32,71			
Sr	0 mM	C	20	18,35	13,29	1	0,38	0,5407
		T	20	15,96	12,15			
	50 mM	C	20	10,33	5,00	1	0,27	0,6063
		T	20	9,50	5,53			
	100 mM	C	20	9,55	4,34	1	0,94	0,3387
		T	20	11,67	8,37			
Zn	0 mM	C	20	173,14	111,06	1	0,01	0,9389
		T	20	170,56	108,23			
	50 mM	C	20	198,16	70,17	1	0,45	0,5081
		T	20	182,06	88,76			
	100 mM	C	20	215,25	102,70	1	1,41	0,2434
		T	20	184,89	52,89			

Annex Table 21: Mean ± Standard Deviation of mineral nutrients (mg/g) and Na/K and Ca/Mg ratios in soil from the habitat of 11 coastal sites with plants homozygous for the strong allele of *AtMOT1* (C) and 7 coastal sites containing plants with the weak allele of *AtMOT1* (V). ANOVA between the presence of *AtMOT1* weak allele of 2 soil samples collected in March and 3 soil samples collected in May of 2013, 2014 and 2015.

	AtMOT1	N	Mean (mg/g)	Std Dev (mg/g)	DF	F Ratio	Prob > F
Na	C	176	91,313	32,814	1	0,1032	0,7482
	V	112	92,476	24,810			
K	C	176	129,386	77,173	1	11,5832	0,0008
	V	112	97,377	78,801			
Na/K	C	176	1,284	1,808	1	9,4007	0,0024
	V	112	1,967	1,896			
Ca	C	176	715,869	108,394	1	0,021	0,885
	V	112	717,711	100,028			
Mg	C	176	152,679	52,463	1	1,728	0,1897
	V	112	162,753	77,571			
Ca/ Mg	C	176	5,383	2,339	1	0,0937	0,7598
	V	112	5,475	2,740			
As	C	168	0,123	0,075	1	4,8895	0,0278
	V	112	0,146	0,099			
Cd	C	168	0,064	0,062	1	0,0328	0,8565
	V	112	0,063	0,074			
Co	C	168	0,179	0,142	1	4,1352	0,043
	V	112	0,231	0,281			
Cu	C	168	7,283	10,524	1	5,8487	0,0162
	V	112	4,626	6,039			
Fe	C	168	20,377	11,522	1	0,5948	0,4412
	V	112	19,198	13,932			
Li	C	164	0,022	0,014	1	3,4919	0,0627
	V	112	0,019	0,010			
Mn	C	168	35,411	24,696	1	5,7549	0,0171
	V	112	28,891	18,046			
Mo	C	168	0,038	0,028	1	0,7146	0,3987
	V	112	0,035	0,031			
Ni	C	168	0,162	0,079	1	1,2916	0,2567
	V	112	0,176	0,130			
P	C	136	14,366	12,875	1	7,1916	0,008
	V	52	9,278	7,422			
Rb	C	168	0,191	0,129	1	10,9044	0,0011
	V	112	0,145	0,092			
Se	C	168	1,466	0,885	1	8,4736	0,0039
	V	112	1,842	1,277			
Sr	C	168	2,440	0,853	1	17,2601	<,0001
	V	112	2,886	0,919			
Zn	C	168	14,793	18,823	1	25,0457	<,0001
	V	112	5,661	5,237			

Annex Table 22: Mean ± Standard Deviation of plant mineral nutrients (µg/g dry weight) and Na/K and Ca/Mg ratios in leaves from *A. thaliana* plants growing naturally in 18 coastal habitats. ANOVA between *AtMOT1* allele type (strong (C) / weak (V)) of 18 "C" and 11 "V" plants collected in 2013, 85 "C" and 35 "V" plants collected in 2014 and 49 "C" and 20 "V" plants collected in 2015.

	AtMOT1	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Na	C	152	1170,253	1164,924	1	6,28	0,013
	V	66	793,603	551,516			
K	C	152	25213,084	8056,146	1	0,61	0,4339
	V	66	26170,910	8804,087			
Na/K	C	152	0,051	0,055	1	2,88	0,0909
	V	66	0,038	0,044			
Ca	C	152	31436,679	8874,180	1	0,76	0,384
	V	66	30227,780	10524,327			
Mg	C	152	4306,065	1337,182	1	4,68	0,0315
	V	66	4775,583	1744,552			
Ca/Mg	C	152	7,820	2,842	1	2,43	0,1207
	V	66	7,118	3,503			
As	C	152	0,615	0,424	1	10,60	0,0013
	V	66	0,856	0,649			
Cd	C	152	0,365	0,289	1	17,57	<,0001
	V	66	0,583	0,466			
Co	C	152	0,898	0,587	1	8,39	0,0042
	V	66	1,310	1,513			
Cu	C	152	6,805	2,803	1	0,98	0,3225
	V	66	7,302	4,482			
Fe	C	152	410,838	262,027	1	0,00	0,9967
	V	66	411,001	270,341			
Li	C	152	1,591	1,358	1	0,15	0,6951
	V	66	1,672	1,466			
Mn	C	152	108,375	73,446	1	2,32	0,1293
	V	66	126,064	90,048			
Mo	C	152	4,610	2,885	1	92,54	<,0001
	V	66	1,088	1,082			
Ni	C	152	0,973	0,602	1	6,11	0,0142
	V	66	1,188	0,560			
P	C	152	5451,367	2087,330	1	5,16	0,0241
	V	66	4723,858	2357,807			
Rb	C	152	8,137	6,213	1	2,65	0,105
	V	66	6,684	5,667			
Se	C	152	6,809	7,376	1	14,11	0,0002
	V	66	12,835	16,345			
Sr	C	152	95,180	50,519	1	19,98	<,0001
	V	66	185,709	238,340			
Zn	C	152	58,842	31,208	1	1,17	0,2805
	V	66	65,093	53,312			

Annex Table 23: Repeated Measures ANOVA between means of growth (rosette diameter, mm) of 20 plants with the weak allele and 20 plants with the strong allele of *AtMOT1* cultivated in each common garden site (BLA/SCF) in 2014 and 2015 over time (independent measures of growth taken once a week during 6 weeks).

	Site	Year	F Test value	Exact F	N DF	D DF	Prob > F
AtMOT1	BLA	2014	0,264	10,045	1	38	0,003
Time	BLA	2014	26,373	145,049	6	33	<,0001
Time * *AtMOT1*	BLA	2014	0,792	4,354	6	33	0,0024
AtMOT1	BLA	2015	0,156	9,036	1	58	0,0039
Time	BLA	2015	89,599	791,458	6	53	<,0001
Time * *AtMOT1*	BLA	2015	1,690	14,927	6	53	<,0001
AtMOT1	SCF	2014	0,002	0,058	1	38	0,8112
Time	SCF	2014	32,546	179,001	6	33	<,0001
Time * *AtMOT1*	SCF	2014	0,066	0,365	6	33	0,8955
AtMOT1	SCF	2015	0,049	2,860	1	58	0,0962
Time	SCF	2015	57,823	510,771	6	53	<,0001
Time * *AtMOT1*	SCF	2015	0,312	2,753	6	53	0,021

Annex Table 24: Mean ± Standard Deviation of fitness (silique number produced) and flowering time (measured as days from germination when the first flower appeared). ANOVA between 20 plants with the weak allele (V) and 20 plants with the strong allele (C) of *AtMOT1* cultivated in each common garden site (BLA/SCF) in the spring of 2014 and 2015.

	Site	Year	*AtMOT1*	N	Mean	Std Dev	DF	F Ratio	Prob > F
Silique number	BLA	2014	C	20	332,75	116,59	1	6,13	0,0178
			V	20	463,95	206,22			
		2015	C	20	124,90	29,99	1	9,37	0,0033
			V	20	149,70	32,71			
	SCF	2014	C	20	151,75	34,22	1	0,26	0,6129
			V	20	157,85	41,09			
		2015	C	20	110,80	28,88	1	2,86	0,096
			V	20	98,73	26,31			
Flowering time	BLA	2014	C	20	63,55	6,33	1	20,59	<,0001
			V	20	72,3	5,86			
		2015	C	20	42,5	5,16	1	73,72	<,0001
			V	20	55,4	4,31			
	SCF	2014	C	20	71,2	6,11	1	0,15	0,7045
			V	20	70,4	7,10			
		2015	C	20	48,25	5,18	1	5,01	0,0312
			V	20	52,15	5,82			

Annex Table 25: Mean ± Standard Deviation of plant mineral nutrients (µg/g dry weight) and Na/K and Ca/Mg ratios in leaves from *A. thaliana* plants growing in BLA and SCF common gardens. ANOVA between *AtMOT1* allele type (strong (C)/ weak (V)) of 10 plants LLO2c, LLO2v, T9c, T9v from 2014 and 2015 field-based cultivation.

	Site	*AtMOT1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Mo	BLA	C	40	5,842	3,779	1	23,031	<,0001
		V	40	2,595	2,008			
	SCF	C	40	4,697	2,398	1	53,8216	<,0001
		V	40	1,672	1,024			
Na	BLA	C	40	414,968	163,324	1	69,4711	<,0001
		V	40	187,137	56,676			
	SCF	C	40	333,523	77,885	1	80,8827	<,0001
		V	40	188,764	65,553			
K	BLA	C	40	40601,687	11453,098	1	0,5703	0,4524
		V	40	38623,571	11970,200			
	SCF	C	40	34924,333	8771,417	1	0,8998	0,3458
		V	40	33145,233	7985,117			
Na/K	BLA	C	40	0,012	0,006	1	32,1747	<,0001
		V	40	0,005	0,002			
	SCF	C	40	0,010	0,004	1	29,9206	<,0001
		V	40	0,006	0,003			
Ca	BLA	C	40	34561,805	8917,104	1	3,589	0,0619
		V	40	38648,033	10323,834			
	SCF	C	40	38869,268	18309,267	1	0,5581	0,4573
		V	40	35926,961	16888,581			
Mg	BLA	C	40	6172,852	2292,927	1	0,8422	0,3616
		V	40	6760,326	3336,915			
	SCF	C	40	5253,310	1587,747	1	0,8327	0,3643
		V	40	4940,133	1480,009			
Ca/Mg	BLA	C	40	6,088	2,072	1	0,2784	0,5992
		V	40	6,314	1,746			
	SCF	C	40	7,259	2,206	1	0,0293	0,8646
		V	40	7,178	2,052			
As	BLA	C	40	0,802	1,235	1	0,075	0,7849
		V	40	0,868	0,896			
	SCF	C	40	1,085	0,766	1	2,2777	0,1353
		V	40	1,458	1,363			
Cd	BLA	C	40	0,266	0,167	1	0,1561	0,6938
		V	40	0,252	0,167			
	SCF	C	40	0,421	0,279	1	0,9745	0,3266
		V	40	0,481	0,259			
Co	BLA	C	40	0,780	0,294	1	3,9992	0,049
		V	40	0,939	0,408			
	SCF	C	40	0,959	0,288	1	2,0313	0,1581
		V	40	1,078	0,441			

Annex Table 25: Continuation...

	Site	*AtMOT1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Cu	BLA	C	40	10,543	6,629	1	3,3442	0,0713
		V	40	8,480	2,644			
	SCF	C	40	10,047	4,081	1	0,4812	0,4899
		V	40	10,745	4,884			
Fe	BLA	C	40	409,477	267,905	1	4,5863	0,0354
		V	40	568,085	384,232			
	SCF	C	40	1259,409	1116,585	1	1,387	0,2425
		V	40	1575,980	1281,945			
Li	BLA	C	40	0,769	0,692	1	2,6106	0,1102
		V	40	1,060	0,903			
	SCF	C	40	2,331	2,036	1	2,3617	0,1284
		V	40	3,128	2,572			
Mn	BLA	C	40	82,216	24,351	1	3,2413	0,0757
		V	40	91,275	20,485			
	SCF	C	40	110,141	35,846	1	1,5963	0,2102
		V	40	123,545	56,718			
Ni	BLA	C	40	0,401	0,269	1	1,6896	0,1975
		V	40	0,484	0,296			
	SCF	C	40	1,073	0,508	1	1,3363	0,2512
		V	40	1,206	0,519			
P	BLA	C	40	5494,421	1788,647	1	0,0204	0,8867
		V	40	5550,823	1741,285			
	SCF	C	40	4939,335	1420,813	1	1,7812	0,1859
		V	40	4532,350	1304,237			
Rb	BLA	C	40	10,141	7,682	1	0,653	0,4215
		V	40	8,848	6,588			
	SCF	C	40	7,789	2,493	1	0,4204	0,5186
		V	40	8,284	4,133			
Se	BLA	C	40	15,699	7,380	1	0,0022	0,963
		V	40	15,608	9,835			
	SCF	C	40	7,169	6,460	1	0,5659	0,4542
		V	40	8,269	6,616			
Sr	BLA	C	40	78,701	44,929	1	1,1159	0,2941
		V	40	89,414	45,777			
	SCF	C	40	89,258	40,546	1	0,4139	0,5219
		V	40	94,946	38,498			
Zn	BLA	C	40	67,716	30,788	1	0,5984	0,4415
		V	40	62,643	27,784			
	SCF	C	40	89,678	92,705	1	0,5765	0,45
		V	40	76,783	54,265			

Annex Table 26: Repeated Measures ANOVA between means of growth (rosette diameter, mm) of 16 plants with the strong allele and 16 plants with the weak allele of *ATMOT1* during three weeks of exposure to 0, 50 and 100 mM NaCl in the hydroponic solution over time (independent measures of growth taken every 3-4 days during 3 weeks).

	NaCl treatment	F Test value	Exact F	N DF	D DF	Prob>F
AtMOT1	0 mM	0,002	0,060	1	35	0,8086
Time	0 mM	24,368	151,084	5	31	<,0001
Time * *AtMOT1*	0 mM	0,246	1,523	5	31	0,2115
AtMOT1	50 mM	0,045	1,568	1	35	0,2188
Time	50 mM	20,734	128,549	5	31	<,0001
Time * *AtMOT1*	50 mM	0,321	1,993	5	31	0,1075
AtMOT1	100 mM	0,214	7,499	1	35	0,0096
Time	100 mM	26,544	164,571	5	31	<,0001
Time * *AtMOT1*	100 mM	2,231	13,833	5	31	<,0001

Annex Table 27: Mean ± Standard Deviation of leaf and root fresh weight (g). ANOVA between 16 plants with the strong allele of *AtMOT1* (C) and 16 plants with the weak allele of *AtMOT1* (V) after being exposed to 0, 50 or 100 mM NaCl in the hydroponic solution during 3 weeks.

	NaCl treatment	*AtMOT1*	N	Mean (g)	Std Dev (g)	DF	F Ratio	Prob > F
Leaf Fresh Wt	0 mM	C	16	1,20	0,22	1	0,96	0,3439
		V	16	1,10	0,19			
Root Fresh Wt	0 mM	C	16	0,31	0,08	1	3,07	0,1018
		V	16	0,26	0,03			
Leaf Fresh Wt	50 mM	C	16	0,60	0,17	1	10,05	0,0068
		V	16	0,84	0,24			
Root Fresh Wt	50 mM	C	16	0,20	0,08	1	0,30	0,5908
		V	16	0,21	0,05			
Leaf Fresh Wt	100 mM	C	15	0,21	0,12	1	7,86	0,0141
		V	16	0,35	0,05			
Root Fresh Wt	100 mM	C	15	0,10	0,03	1	5,06	0,0411
		V	16	0,14	0,02			

Annex Table 28: Mean ± Standard Deviation of plant mineral nutrients content (µg/g dry weight) and Na/K and Ca/Mg ratios in leaves from *A. thaliana* plants growing hydroponically under 0, 50 and 100 mM NaCl. ANOVA between *AtMOT1;1* allele type (strong (C)/ weak (V)) of 8 plants LLO2c, LLO2v, T9c, T9v per each treatment.

	NaCl Treatment	*AtMOT1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Mo	0 mM	C	16	15,21	16,09	1	5,3516	0,0267
		V	16	5,88	6,91			
	50 mM	C	16	21,51	13,57	1	21,1832	<,0001
		V	16	6,54	2,51			
	100 mM	C	15	23,54	10,09	1	55,4933	<,0001
		V	16	6,06	1,67			
Na	0 mM	C	16	1110,95	870,65	1	0,0519	0,8211
		V	16	1026,17	1331,20			
	50 mM	C	16	53877,30	27374,80	1	5,4531	0,0258
		V	16	35487,53	18633,57			
	100 mM	C	15	96687,94	52980,99	1	7,1806	0,0115
		V	16	58739,70	28378,80			
K	0 mM	C	16	54030,96	12236,40	1	0,5158	0,4774
		V	16	51550,12	8547,10			
	50 mM	C	16	40379,50	8719,33	1	4,1905	0,0487
		V	16	48590,15	14194,06			
	100 mM	C	15	41044,53	11049,96	1	0,154	0,6973
		V	16	42456,59	9896,21			
Na/K	0 mM	C	16	0,02	0,02	1	0,0239	0,878
		V	16	0,02	0,03			
	50 mM	C	16	1,41	0,86	1	6,581	0,015
		V	16	0,80	0,52			
	100 mM	C	15	2,72	2,00	1	5,2215	0,0291
		V	16	1,54	0,91			
Ca	0 mM	C	16	29385,06	5482,59	1	0,0839	0,7738
		V	16	28869,62	5341,26			
	50 mM	C	16	31091,69	8252,42	1	1,0878	0,3045
		V	16	28420,01	6875,05			
	100 mM	C	15	34987,36	11218,60	1	6,2202	0,018
		V	16	27812,89	5042,43			
Mg	0 mM	C	16	3518,43	818,80	1	1,7731	0,1916
		V	16	3933,11	1053,52			
	50 mM	C	16	3502,56	834,68	1	1,0502	0,3129
		V	16	3779,86	766,12			
	100 mM	C	15	3727,31	905,36	1	0,6461	0,4274
		V	16	4012,86	1114,91			

Annex Table 28: Continuation...

	NaCl Treatment	*AtMOT1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Ca/Mg	0 mM	C	16	8,72	2,31	1	0,7805	0,383
		V	16	7,96	2,90			
	50 mM	C	16	9,31	3,24	1	2,1857	0,1488
		V	16	7,83	2,64			
	100 mM	C	15	9,71	3,57	1	5,5515	0,0248
		V	16	7,35	2,26			
As	0 mM	C	16	0,25	0,25	1	1,1698	0,2868
		V	16	0,18	0,11			
	50 mM	C	16	0,20	0,11	1	0,2798	0,6004
		V	16	0,18	0,11			
	100 mM	C	15	0,26	0,09	1	0,1788	0,6752
		V	16	0,28	0,16			
Cd	0 mM	C	16	0,39	0,38	1	0,0311	0,861
		V	16	0,41	0,36			
	50 mM	C	16	0,44	0,36	1	0,0001	0,9919
		V	16	0,44	0,48			
	100 mM	C	15	0,35	0,17	1	0,8256	0,3703
		V	16	0,43	0,33			
Co	0 mM	C	16	0,61	1,26	1	1,3842	0,2473
		V	16	0,27	0,22			
	50 mM	C	16	0,14	0,05	1	0,4358	0,5137
		V	16	0,13	0,04			
	100 mM	C	15	0,18	0,08	1	0,0607	0,8069
		V	16	0,19	0,06			
Cu	0 mM	C	16	11,30	2,78	1	10,4921	0,0026
		V	16	8,81	1,82			
	50 mM	C	16	14,06	3,76	1	7,201	0,0113
		V	16	11,24	2,35			
	100 mM	C	15	13,57	5,32	1	0,6614	0,4221
		V	16	12,42	2,85			
Fe	0 mM	C	8	125,89	38,62	1	1,021	0,3294
		V	8	109,66	23,95			
	50 mM	C	8	123,29	25,08	1	0,9876	0,3372
		V	8	111,93	20,42			
	100 mM	C	8	187,74	66,23	1	3,3889	0,0869
		V	8	141,80	24,40			
Li	0 mM	C	16	0,14	0,15	1	0	0,9997
		V	16	0,14	0,08			
	50 mM	C	16	0,15	0,24	1	0,0529	0,8195
		V	16	0,13	0,19			
	100 mM	C	15	0,20	0,10	1	0,0508	0,8231
		V	16	0,21	0,14			

Annex Table 28: Continuation...

	NaCl Treatment	*AtMOT1*	N	Mean (µg/g)	Std Dev (µg/g)	DF	F Ratio	Prob > F
Mn	0 mM	C	16	119,56	24,84	1	7,1283	0,0114
		V	16	100,70	17,71			
	50 mM	C	16	123,73	25,33	1	11,3579	0,0019
		V	16	98,32	18,99			
	100 mM	C	15	137,97	48,23	1	2,5834	0,1674
		V	16	125,50	17,03			
Ni	0 mM	C	16	1,87	1,31	1	0,3333	0,5674
		V	16	2,16	1,69			
	50 mM	C	16	1,79	1,23	1	0,0363	0,85
		V	16	1,87	1,28			
	100 mM	C	15	1,66	1,27	1	1,5115	0,2279
		V	16	2,22	1,36			
P	0 mM	C	16	8920,81	3571,02	1	0	0,9984
		V	16	8918,63	3068,47			
	50 mM	C	16	13123,92	2957,55	1	0,0998	0,754
		V	16	12802,66	3051,59			
	100 mM	C	15	14799,85	4099,96	1	0,429	0,5171
		V	16	14001,86	3007,33			
Rb	0 mM	C	16	2,50	0,91	1	1,0589	0,3105
		V	16	2,20	0,88			
	50 mM	C	16	1,28	0,41	1	2,2646	0,1419
		V	16	1,70	1,07			
	100 mM	C	15	1,53	0,81	1	0,542	0,467
		V	16	1,77	1,01			
Se	0 mM	C	16	12,87	9,80	1	0,2599	0,6134
		V	16	11,37	8,16			
	50 mM	C	16	11,98	9,27	1	1,0829	0,3056
		V	16	9,22	6,19			
	100 mM	C	15	13,28	7,17	1	0,0691	0,7943
		V	16	14,01	8,68			
Sr	0 mM	C	16	17,10	20,29	1	0,8898	0,352
		V	16	12,48	6,57			
	50 mM	C	16	10,55	11,47	1	0,9599	0,3343
		V	16	7,68	4,62			
	100 mM	C	15	13,46	5,70	1	0,3551	0,5554
		V	16	11,89	8,86			
Zn	0 mM	C	16	90,32	21,70	1	1,2347	0,2741
		V	16	98,75	24,30			
	50 mM	C	16	126,75	48,69	1	1,2311	0,2752
		V	16	146,76	57,35			
	100 mM	C	15	162,68	96,83	1	0,0389	0,8449
		V	16	157,15	66,76			

Annex Table 29: Repeated Measures ANOVA between means of growth (rosette diameter, mm) of 4 Col-0 plants and 4 *mot1* knockout mutant plants during three weeks of exposure to 0, 50 and 100 mM NaCl in the hydroponic solution over time (independent measures of growth taken every 3-4 days during 3 weeks).

	NaCl Treatment	F Test value	Exact F	N DF	D DF	Prob>F
Accession	0 mM	0,04	0,21	1	6	0,6623
Time	0 mM	152,04	60,81	5	2	0,0163
Time * Accession	0 mM	3,20	1,28	5	2	0,4937
Accession	50 mM	0,49	2,94	1	6	0,1372
Time	50 mM	595,01	238,00	5	2	0,0042
Time * Accession	50 mM	4,27	1,71	5	2	0,4089
Accession	100 mM	3,11	18,67	1	6	0,005
Time	100 mM	127,85	51,14	5	2	0,0193
Time * Accession	100 mM	22,69	9,08	5	2	0,1022

Annex Table 30: Mean ± Standard Deviation of leaf and root fresh weight (g) and root ferric reducing capacity (nmol $Fe^{2+} \cdot g^{-1}$ root fresh Wt $\cdot h^{-1}$). ANOVA between 4 Col-0 plants and 4 *mot1* knockout mutant plants after being exposed to 0, 50 or 100 mM NaCl in the hydroponic solution during 3 weeks.

	NaCl Treatment	Accession	N	Mean	Std Dev	DF	F Ratio	Prob > F
Leaf Fresh Wt (g)	0 mM	Col-0	4	2,33	0,04	1	3,82	0,0984
		mot1	4	1,52	0,83			
	50 mM	Col-0	4	2,30	0,11	1	4,65	0,0746
		mot1	4	1,58	0,66			
	100 mM	Col-0	4	0,51	0,30	1	4,89	0,0407
		mot1	4	0,95	0,20			
Root Fresh Wt (g)	0 mM	Col-0	4	0,42	0,05	1	0,05	0,8313
		mot1	4	0,41	0,13			
	50 mM	Col-0	4	0,35	0,03	1	0,95	0,3674
		mot1	4	0,40	0,10			
	100 mM	Col-0	4	0,23	0,05	1	0,17	0,6914
		mot1	4	0,24	0,02			
Fe Reducing Capacity (nmol $\cdot g^{-1} \cdot h^{-1}$)	0 mM	Col-0	4	113,55	6,92	1	98,45	<,0001
		mot1	4	52,43	10,20			
	50 mM	Col-0	4	104,25	14,75	1	24,81	0,0025
		mot1	4	48,38	16,91			
	100 mM	Col-0	4	46,76	16,50	1	1,10	0,3342
		mot1	4	57,83	13,12			