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What does *Arabidopsis* natural variation teach us (and does not teach us) about adaptation in plants?

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Sessile organisms such as plants have to develop adaptive responses to face environmental change. In *Arabidopsis 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. The strength of *Arabidopsis* resides in the huge amount of genomic data and molecular tools available leading to the identification of many polymorphisms responsible for phenotypic variation. Remaining limitations to clearly understand how *Arabidopsis* adapts to its environment, that is the complexity of the genetic architecture and the lack of ecological data, should be partially solved thanks to the development of new methods and the acquisition of new data.

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Introduction

Adaptation in plants commonly refers to their ability to successfully grow and reproduce in contrasted and sub-optimal environments. Plant adaptation interests a large community of scientists, from ecologists and molecular geneticists working on fundamental mechanisms of adaptation to crop breeders looking for natural variants which could optimize environmental resources and provide targets for breeding programs. This long-time interest engaged an array of different approaches on many cultivated or wild plant species. Among them, *Arabidopsis thaliana* was initially used because its characteristics made it an efficient model for molecular genetics, leading to the complete sequencing of its genome. Despite their high efficiency in understanding fundamental mechanisms of plant biology, induced mutation-based studies were less well able to address the question of plasticity and adaptation, mainly because of the highly polygenic bases of the underlying traits and lack of finely quantitative mutant

screens. Many *Arabidopsis* lines derived from natural populations (accessions) are used in several disciplines [1–3] to investigate the genetic features that allowed *Arabidopsis* to colonize rapidly a large spectrum of environments displaying many biotic and abiotic challenges [4]. In this review, recent advances in understanding plant adaptation are presented, focusing on natural variation in *Arabidopsis* for mineral and water use efficiency as well as ion toxicity/deficiency tolerance, as major discriminating physiological functions in suboptimal environments.

What are the strategies to investigate natural variation?

Two main strategies are followed to decompose *Arabidopsis* natural variation. First, *Arabidopsis* natural accessions can be used directly to identify polymorphisms associated with adaptation using linkage disequilibrium (LD) mapping [5^{*}]. LD mapping statistically links sequence polymorphisms to phenotype, and potentially identifies candidate genes relevant at a wide scale of genetic variation. This method should be more and more useful as genomic data on 1001 accessions accumulates [6]. However, many limitations, in particular the high rate of false-positive and -negative due to a strong population structure at the worldwide scale [5^{*}], have to be solved to efficiently study adaptation by this means. Currently, the second strategy, mapping in segregating populations such as F2 or RIL sets derived from crosses between two or more parental accessions, is still the most popular way to identify genomic locations exerting a control on a complex phenotypic trait in a given environment, that is quantitative trait loci (QTL). RIL sets are particularly interesting to study plant responses to the environment as they can be cloned indefinitely in the autogamous *Arabidopsis*, allowing researchers to replicate them in many different conditions. In addition to the classical RIL population segregating solely for two parental alleles, new types of RIL sets are derived from intercrosses of several parents to enlarge the range of present alleles [7] but potentially giving rise to very complicated patterns of epistasis.

Environmental plasticity at different levels of integration

In order to deal with changing and challenging environmental conditions, plants exhibit a wide range of integrated responses which usually display complex quantitative variation. First, transcriptomic profiles are largely affected in response to stress [8]. Environmental stresses then impose strong constraints on specific plant physiological parameters such as stomatal conductance, water use efficiency (i.e. the ratio of dry matter gained to water loss) [9^{*}]

or ion homeostasis [10[•],11]. Finally, depending on soil characteristics, nutrient or water availability, plants can have extreme divergent morphologies and development. For example, temporal and spatial variations in the availability of nutrients such as phosphate or nitrogen have a major influence on root system architecture and the root/shoot ratio [12].

Among *Arabidopsis* accessions, a wide variation in those responses has been observed [9[•],13–15]. Using several methods of investigation such as global principal component analysis, one can define groups of individuals according to their responses to a specific stress. Those studies allow the identification of extreme accessions that could be the most suitable to set up segregating populations and investigate the genetic causes of response variation.

One of the first approach toward studying integrated stress responses is to analyse the correlations between different traits. Within RIL populations, these correlations may reflect common genetic bases which should then be supported by QTL colocalizations between traits. For example, several correlations and/or QTL colocalizations have been identified for growth-related traits and mineral concentrations [10[•],11,16] or carbohydrate contents [17] and natural variation can help validate models explaining integrative traits' variation from metabolic or ionic variables [17,18]. The most straightforward hypothesis for QTLs colocalization is the presence of a pleiotropic locus, reflecting true independent effects of one locus on distinct traits or causality relationship between traits after one is affected by the locus. Nevertheless, at this level of resolution, this could also be due to genetic linkage between two different loci with independent phenotypic effects [11].

Another approach to identify stress-related loci is to compare QTLs or correlations among traits in environmental conditions where plants are challenged at different intensities. Often, most of the isolated QTLs are specific to a given condition, revealing the complexity of the underlying traits' plasticity [10[•],11,16,19]. Otherwise, under growth-limiting nitrogen conditions, for example the *SO3.1* locus increased its impact on sulphate content, indicating that the strength of the QTL control on a trait – or pathway – can be environment-dependent [20]. Overall, in many studies, most QTLs controlling mineral concentrations as well as growth-related traits have relatively small contributions, indicating the relative difficulty to clone the genes underlying QTLs that are directly related to whole plant physiology [10[•],11,16,19].

Molecular diversity supporting natural variation

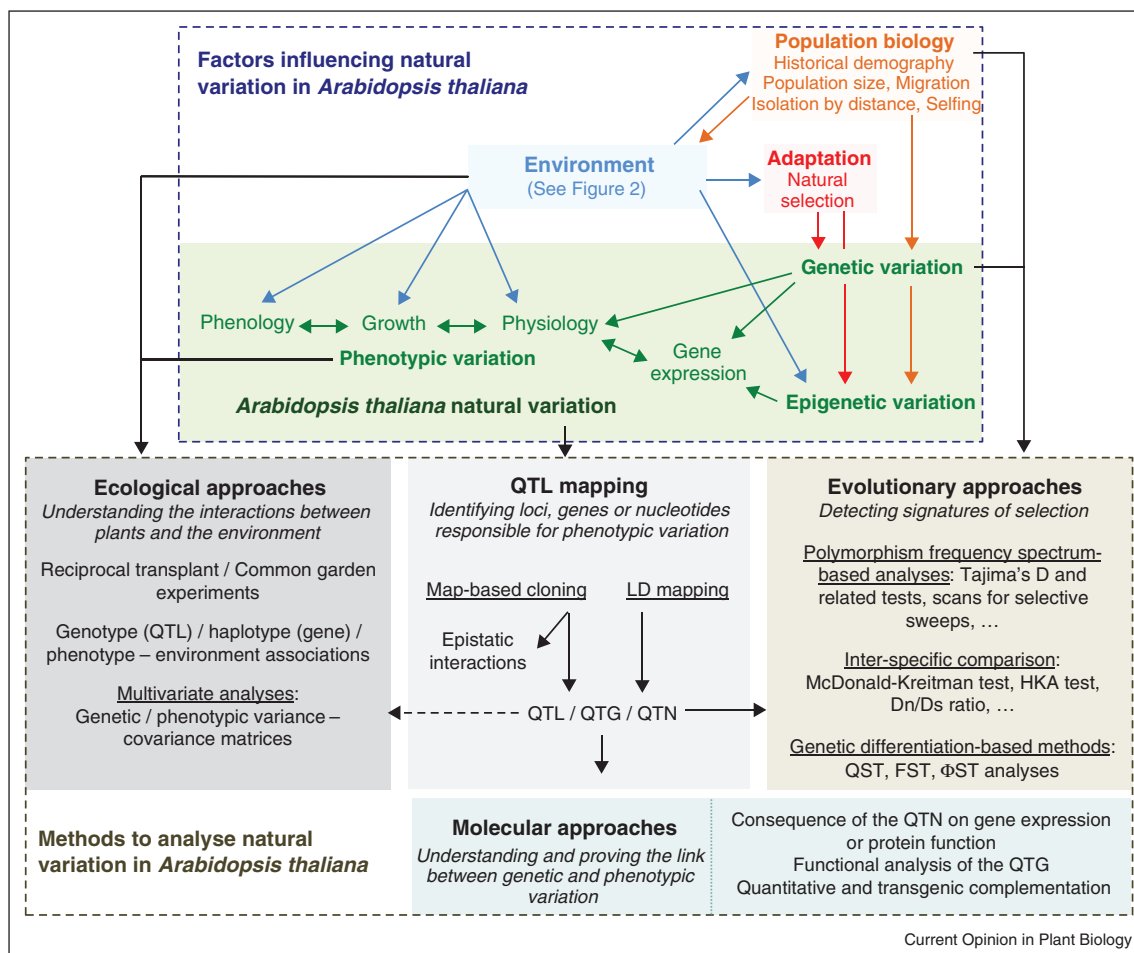
Depending on the mapping method, identifying genes (QTG) or nucleotides (QTN) underlying a QTL can be a

long, laborious and risky task. It not only requires high-resolution mapping strategies but also candidate gene/nucleotide confirmation approaches, one of the ultimate proofs being transgenic complementation of parental or KO lines with the two contrasted alleles, or gene copies mutated at specific sites.

Nevertheless, identifying the causal QTG or QTN is of great support to understand how adaptation shapes natural phenotypic variation through genetic or epigenetic modifications (Figure 1). The first interest of the functional and physiological approaches is to understand the molecular mechanism and biological pathway by which they affect the trait of interest. The nature of the causal polymorphism can be a sequence modification resulting in a non-synonymous change in functionally important amino-acids [20], premature stop codons [21] or variation in transcript accumulation [22,23]. For example, variation in plant molybdenum (Mo) content is mostly explained by a deletion in the promoter of the *MOT1* gene, which is responsible for lower gene expression and lower Mo accumulation in multiple accessions [24[•],25]. Functional analyses then provided evidence that *MOT1* encodes a Mo transporter controlling plant Mo content.

Once the QTG or QTN are identified, one can assess the species-level relevance of gene haplotypes regarding adaptation. In *Arabidopsis* populations, a genome-wide excess of rare alleles has been described [26] as would result from multiple mutations fixed independently in essentially selfing populations and, unsurprisingly, several QTNs isolated so far correspond to rare alleles, such as *RAS1*^[Sha] [21] and *APR2*^[Sha] [20]. The adaptive significance of rare alleles is difficult to test: individually, they are certainly not evolutionarily important at the species scale, but can play a major role in plant adaptation to local and specific environments, in response to combinations of constraints or trade-offs. It is also possible that the low frequency of individual variants is combined with the occurrence of several independent variants of similar phenotypic consequences in different backgrounds. For example, such allelic heterogeneity – two independent mutations in *HMA5*, a Cu transporter – was identified as responsible for variation in basal Cu tolerance [27[•]]. Even if the two identified alleles appeared deleterious on high-Cu soil, one can wonder if the occurrence of distinct mutations in the same transporter can have a significant evolutionary role in other environments, or if they simply result from neutral genetic drift. When a causal QTN is shared by several accessions, one can investigate the association between haplotypes, phenotypes and natural environments. For example, a deficient allele at *HKT1* results in an increase in shoot Na⁺ content (as confirmed by both linkage and association mapping studies) and consequently salt tolerance (probably via enhanced Na⁺ compartmentalization), and is more frequent in

Figure 1



Schematic recapitulation of different approaches to study the factors influencing Arabidopsis natural variation. The top part describes the interplay between different factors influencing natural variation (with the different arrows representing effects of one factor on another); the bottom part describes the strategies and tools used to understand this variation (with black arrows between boxes representing established information required in the next step – solid arrow – or useful in the next step – dotted arrow). QTL = quantitative trait locus; LD = linkage disequilibrium; QTG = quantitative trait gene; QTN = quantitative trait nucleotide(s).

populations in coastal locations or saline areas [22,28**], suggesting adaptation to saline soil. The best experimental support to demonstrate local adaptation would be reciprocal transplant experiments between accessions (Figure 1; [29]) but those approaches are still rarely undertaken in Arabidopsis.

Finally, a search for molecular traces of selection at the QTG can be performed to infer the adaptive significance of QTL. Several approaches are available (Figure 1) and consist in the statistical comparison of the observed pattern of polymorphisms at the locus of interest to the one expected under neutral evolution (reviewed in Refs. [29,30]). Because the neutral model is very sensitive to demographic factors, which is a great concern in Arabidopsis [31], genome-wide patterns of polymorphisms

and/or nucleotidic patterns from close relatives, have to be used as reference. Those approaches successfully helped inferring the adaptive significance of known QTLs such as genes of the flowering time control network [32] but also identifying gene under positive or balancing selection that could be important for plant adaptation [33]. A nice example of the use of those molecular and evolutionary approaches to infer the functional and adaptive significance of a QTG in Arabidopsis is the recent paper of Todesco *et al.* [34*], which shows how an hyperactive allele of *ACD6*, resulting in pathogen tolerance, can be maintained in the species despite its negative effect on plant biomass accumulation. More such complete approaches remains to be implemented on the very quantitative plant physiological responses to abiotic stress.

From causal molecular variants to adaptation and predictive biology

Integrating genotype/phenotype/environment information in the light of evolution is hampered by complex relationships between these determinants at the species scale.

First, phenotype prediction from genotypes is not trivial particularly because of the complicated pattern of epistatic interactions usually controlling a response to the environment [35••]. Conditional interactions, in which an increase in the QTL's phenotypic effect is observed in a given heterogeneous genetic background, are commonly detected and possibly lead to phenotypes not observed in the parental accessions [36]. Co-adaptive interactions are identified when the direction of the effect at one locus is changed by the allele present at another locus; this usually involves an interacting locus with no additive effect on its own. A better survey of the importance of epistasis in adaptation can be obtained by increasing – to a certain extent – the genetic diversity explored, by increasing the number of parents investigated; nested association mapping for example developed in maize on connected RIL sets derived from diverse densely sequenced parents, is promising and probably more efficient than association mapping for this purpose [37]. In addition to complexity at the locus level, complexity at the phenotypic level, that is pleiotropy and possible trade-off effects through adaptive traits [34•], make adaptive predictions even more complex. Tools are efficiently developed at different integration scales to understand the structure of variation: for example regulatory network construction combined with QTL mapping reveals how gene expression controls pathways [38]. However, understanding the complexity at one given integration scale is not necessarily the most informative in the context of adaptation, and it happens that integrative phenotypes are sometimes mainly controlled by a few strong-effect pleiotropic loci in *Arabidopsis* [39••]. Combined with linkage mapping, structural equation modelling or path analysis – on the basis of the analysis of variance/covariance among multiple variables – is an interesting tool to understand the functional relationships between adaptive traits at different integration scales and to disentangle the specific effects of individual polymorphisms [40,41].

Phenotype estimation/prediction for a given genotype in a given environment is another step necessary to conclude on adaptation. This is particularly challenging since *Arabidopsis* is known to be very plastic in response to macro- and micro-environmental fluctuations. Standardization of protocols and development of phenotyping platforms reducing micro-environmental variation within and between experiments will help produce reproducible phenotypes in targeted environments [42]. However, even using shared and precisely detailed protocols, considerable uncontrolled variation remains [43•] and integrating results

from different experiments is limited [16,19]. Apart from these difficulties inherent to controlled-condition phenotyping, a minimal set of information about the environment and ecology of natural *Arabidopsis* accessions is often lacking, thus making it impossible to conclude on adaptation (Figure 2). In this context, results from field experiments are necessary and could be more conclusive; in addition, laboratory results are not always directly relevant to what happens in the more complex natural settings [35••,44]. Generally, life history traits such as germination or flowering time, seem to be very structuring at the *Arabidopsis* species scale and generate functional trade-off, as reported between flowering time and water use efficiency [9]. However, QTL mapping coupled with a better knowledge of local environmental constraints experienced by specific accessions is promising and yet efficient to identify relevant polymorphisms that affect adaptation to particular constraints [28••].

Perspectives

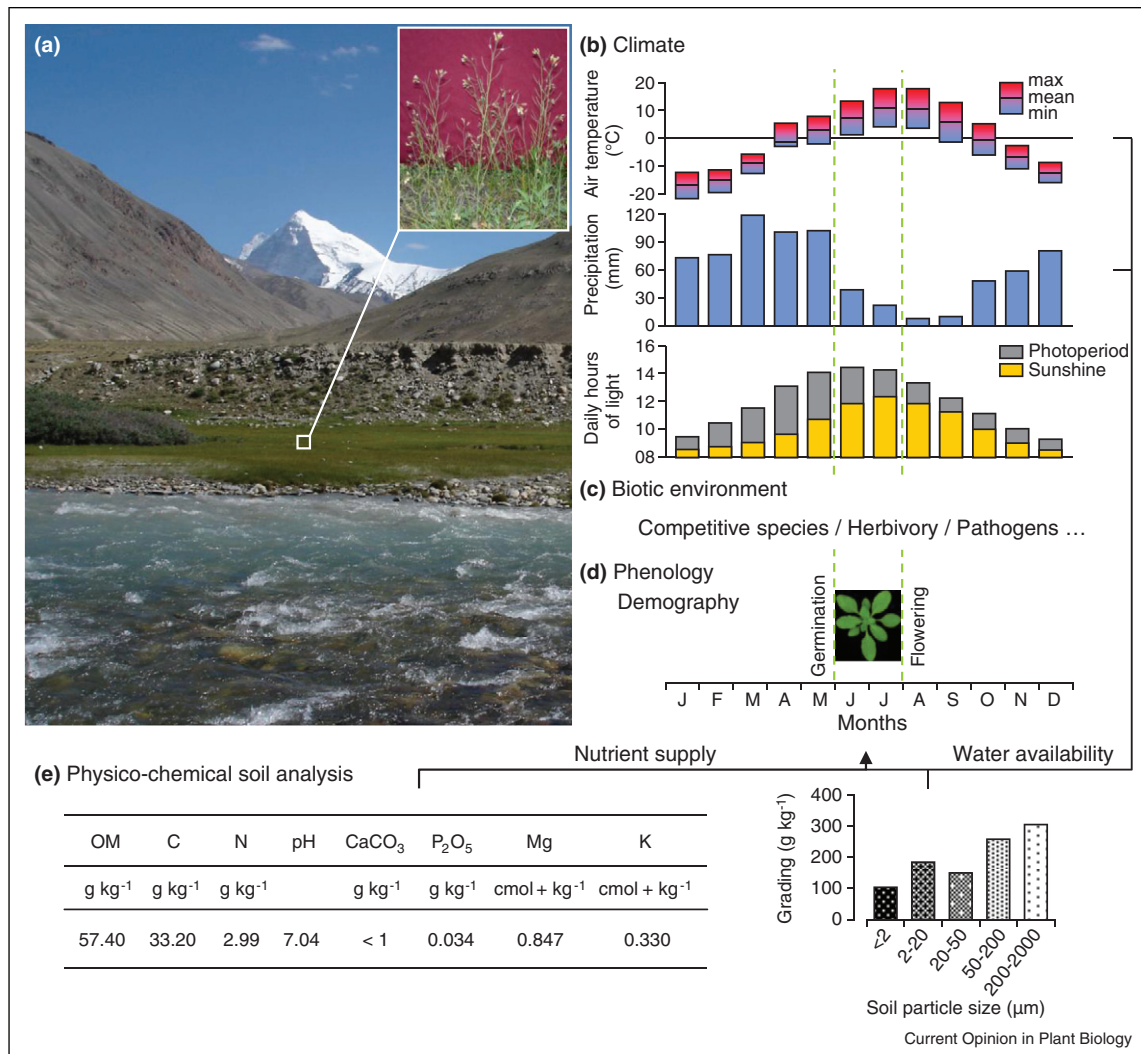
During the last decade, researchers have begun to decode the mechanisms underlying adaptations of *Arabidopsis* to its environment. Nevertheless, many difficulties remain in order to understand how the different levels of variation relate to the respective environments, constituting plant adaptation, especially for very finely quantitative traits like those related to whole plant physiology. A constant improvement of approaches and the development of new resources are already contributing to solving these issues.

Clearly, high-throughput phenotyping platforms are more needed than ever to allow repeated phenotyping of thousands of individuals in extremely stable conditions and compare results across series of successive experiments. In parallel, field experiments should also become common for both mapping and validation purposes [3], and researchers need to move beyond obviously adaptive traits (development and phenology are the most studied factors in field experiments so far) toward physiology and stress response.

In parallel, it becomes obvious that the *Arabidopsis* community has to improve the description of the ecology and environment of *Arabidopsis* populations to more finely decode plant adaptation [45]. Some of the most interesting environmental factors to understand plant physiology and response to abiotic stress can be expected to be heterogeneously distributed across the environment, requiring local characterization.

In addition, we have to be prepared to consider all possible sources of genetic variation: even if no example for metabolic or physiological traits is available in *A. thaliana* yet, natural phenotypic variation in *Arabidopsis* is also explained by structural modifications of the genome such as gene translocation [46], duplication [47] and

Figure 2



Insights into a wild *Arabidopsis* population ecology ('*Shahdara*').

(a) Typical environment of the high Shokhdara valley (Tajikistan, Central Asia), as can be seen at the end of July around 3500 m altitude (N 37° 21.440' / E 72° 28.072'). Mountains are mostly dry, except for a limited strip at the valley floor next to the river: this is where *Arabidopsis*' population '*Shahdara*' is growing (inset). Interpretation of the only available environmental variables at this scale **(b)**; approximate climatic data interpolated from the nearest meteorological stations, obtained from the WorldClim database www.worldclim.org is impossible without a better knowledge of the precise environment encountered by the plants (e.g. **(c)**, **(d)** and **(e)**; OM = organic matter). Here, very limited precipitation during the flowering period (July/August) could be interpreted as a potential drought constraint, if one does not realize that the Shokhdara river, carrying water from melting snow in the neighbouring high-mountains, is irrigating the valley summer-long. Description of the 'original site of collection of *Shahdara*' (including physico-chemical soil analyses): *Neo-Shahdara* is from www.inra.fr/vast/collections.htm (Loudet O.).

epigenetic variation [48]. Such variations are not yet adequately covered by current deep sequencing data on accessions [46]. In *Arabidopsis halleri*, Zn hyperaccumulation tolerance was shown to be explained partly by copy number expansion of the metal pump HMA4 [49], hereby illustrating that important aspects of plant adaptation can be revealed by comparison to the close relatives of *Arabidopsis*.

Association mapping will help identifying causal variants relevant at the species scale, but these approaches still

need to overcome major limitations like an extensive population structure (at least at the species scale), an excess of rare alleles (including rare functional variants), loci with small individual effect or subject to epistatic interactions (especially for very integrative traits) and genetic/allelic heterogeneity. Complementary to linkage mapping approaches, it starts to become possible to combine some advantages of the two strategies (dual linkage/association mapping [44]; nested association mapping) but some limitations remain (reviewed recently by Bergelson and Roux [3]). One of the answers may be a

change of scale [31], for example toward regional-scale samples, and much is probably to be learnt also from segregating populations derived from crosses between related genotypes (including between distinct individuals from a single local population [50]). This would reveal the extent of phenotypic variation and transgression on which natural selection is acting. A particular approach to this was recently described in *Arabidopsis lyrata*, involving deep-sequencing of neighbouring populations to search for candidate loci involved in adaptation to serpentine soils [51].

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