In silico selection of *Arabidopsis thaliana* ecotypes with enhanced stress tolerance

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Climate models predict increased occurrences of combined abiotic and biotic stress. Unfortunately, most studies on plant stress responses include single or double stress scenarios only. Recently, we established a multi-factorial system in *Arabidopsis thaliana* (*Arabidopsis*) to study the influence of simultaneously applied heat, drought, and virus. Our transcriptome analysis revealed that gene expression under multi-factorial stress is not predictable from single stress treatments. Combined heat and drought stress reduced expression of defense genes and genes involved in R-mediated disease responses, which correlated with increased susceptibility of *Arabidopsis* to virus infection. Eleven genes were found to be differentially regulated under all stress conditions. Assuming that regulated expression of these genes is important for plant fitness, *Arabidopsis* ecotypes were clustered according to their expression. Interestingly, ecotypes showing a close correlation to stressed Col-0 prior stress treatment showed improved growth under stress conditions. This result suggests a functional relevance of these genes in stress tolerance.

Due to their sessile lifestyle, plants are exposed to a wide range of environmental stimuli and stresses. Gradual geographic variations in environmental conditions worldwide lead to the selection of distinct geographic varieties called ecotypes that are adapted to the local conditions.1-3 Adaptations are associated with mutant selection that is manifested in altered genome sequences resulting in changes in circadian clock, growth rate, mineral nutrition, biotic, and abiotic resistance factors. Within the last years, numerous studies on plant responses to biotic and abiotic stresses have been published. However, only a few studies have considered multi-factorial stress situations, more likely to mimic the natural situation.4-6 Recently, we have developed a multi-factorial test system, allowing simultaneous application of heat, drought, and virus stress. Transcriptome and metabolome analysis was applied for comparison of molecular and biochemical responses of *Arabidopsis* plants exposed to these multi-factorial stress scenarios.7 We demonstrated that combined stress treatment alters gene networks involved in stress and signaling in an unpredictable manner. Signaling networks induced by virus infection are completely destroyed, which might explain increased virus susceptibility of triple-stressed plants.7 Moreover, comparative analysis of differentially expressed genes revealed stress-regulated candidate genes specifically expressed in one or more situations. Only 11 genes were found to be differentially expressed under all experimental stress conditions tested, and some of them had already been associated with stress (Fig. 1A).7 Regulated expression of these genes was considered to be important for plant performance under stress conditions.

Under the assumption that *Arabidopsis* ecotypes showing expression patterns of these genes similar to stressed Col-0 plants prior to stress treatment would be more tolerant to subsequent stress conditions, we attempted to identify such ecotypes and to test their stress response. For the selection of natural accessions relevant for responses to environmental changes, the expression levels of the 11 genes for each ecotype have been determined using data from the Weigel lab provided by a natural variation eFP-Browser.8,9 Unfortunately, only data from 8 of the 11 genes were available. Nevertheless, a heat map comparing the expression levels within the accession numbers and the 8 genes revealed ecotypes closely clustering to our stress conditions: NFE1, Li-2:1, and En-T, Er-0, and Can-0 (Fig. 1B).

To study if ecotypes clustering near to our stress conditions might show enhanced stress tolerance, seeds of the *Arabidopsis* ecotypes Can-0 and En-T, as well as control ecotypes Col-0 and Van-0 were obtained from the European *Arabidopsis* Stock Centre (Nottingham, UK) for further analysis. As the 11 genes have been found to be regulated under single stress as well as under different abiotic and biotic stress combinations, we decided to mimic natural growth conditions regardless of controlled temperatures and humidity. Therefore, as indicated in Figure 2A, plant cultivation of different *Arabidopsis* ecotypes was first started in a climate chamber, and after 10 d plants were transferred into the greenhouse providing increased average temperatures ranging from 24–30 °C (day) to 21–24 °C (night) and humidity varying from 28% to 80%. Plants at the age of 21 d have been exposed to controlled drought stress for 4 d as described by Prasch and

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Sonnewald, 2013. Afterwards, leaves from 20 plants either treated with drought stress or well-watered plants were sampled and biomass was determined.

Figure 2B illustrates a significant reduction in biomass for all drought-stressed plants compared with their well-watered controls at the time of harvest. However, biomass as the percentage of the corresponding control during drought stress revealed 61% and 63% for Col-0 and Van-0, respectively, whereas the ecotypes Can-0 and En-T retained more than 72% of their biomass compared with their respective controls. These biomass comparisons allowed us to classify the ecotypes into 2 groups, Col-0 and Van-0 as well as Can-0 and En-T. Interestingly, Can-0 and En-T did not only perform better under combined stress conditions, but they have been identified as ecotypes closely clustering next to the 11 commonly regulated genes under single and multi-parallel stress (see above). These findings suggest that there is a positive relationship between the expression level of the selected genes and tolerance against diverse stress conditions.

In our first study investigating molecular responses of plants to triple stress, namely heat, drought, and virus, we were able to identify 11 genes differentially regulated in single and multi-parallel stress combinations. In this study, we selected ecotypes showing differential regulation of those genes compared with Col-0. Analyzing these ecotypes under controlled drought stress in “field near” conditions, we demonstrated that the identified genes can be used as indicators of stress tolerance. Together these results show that genetic variants found in nature provide an important tool to verify a number of candidate genes at the same time and moreover, highlight the importance to study genes not only found to be regulated under single stress, but also during multi-factorial stress.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Figure 2. Design of the ecotype stress experiment under “field near” conditions and biomass determination. (A) Scheme illustrating the experimental design for exposing different Arabidopsis ecotypes to controlled drought stress under elevated temperatures. Ten days after germination in a climate chamber providing 22 °C days and 18 °C nights plants were transferred in the greenhouse with increased temperatures ranging from 24–30 °C (day) to 21–24 °C (night). After exposing plants to 4 d of drought stress whole rosettes were harvested and biomass was determined. (B) Fresh weight of whole rosettes from drought stressed plants and control plants for each ecotype. Data points represent an average of 17–19 plants ± SD.