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Key genes involved in desiccation tolerance and dormancy across life forms

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ABSTRACT

Desiccation tolerance (DT, the ability of certain organisms to survive severe dehydration) was a key trait in the evolution of life in terrestrial environments. Likely, the development of desiccation-tolerant life forms was accompanied by the acquisition of dormancy or a dormancy-like stage as a second powerful adaptation to cope with variations in the terrestrial environment. These naturally stress tolerant life forms may be a good source of genetic information to generate stress tolerant crops to face a future with predicted higher occurrence of drought. By mining for key genes and mechanisms related to DT and dormancy conserved across different species and life forms, unique candidate key genes may be identified. Here we identify several of these putative key genes, shared among multiple organisms, encoding for proteins involved in protection, growth and energy metabolism. Mutating a selection of these genes in the model plant *Arabidopsis thaliana* resulted in clear DT-, dormancy- and other seed-associated phenotypes, showing the efficiency and power of our approach and paves the way for the development of drought-stress tolerant crops. Our analysis supports a co-evolution of DT and dormancy by shared mechanisms that favour survival and adaptation to ever-changing environments with strong seasonal fluctuations.

1. Introduction

Climate model simulations predict that by 2100 the climate will be several degrees warmer than it is now [1]. Precipitation will decrease in most tropical and mid-latitude regions, expanding the area of global dryland by ~10% and severely reducing yields for primary crops like corn, wheat and rice [1]. Even if rain increases in these areas, only few of them will get enough rain to compensate the increased evaporation demand of a warmer atmosphere [1]. Moreover, although some high-latitude areas may become more climatically viable for crops, the quality, depth and hydraulic properties of soils in these areas might not be suitable for sustained agricultural production [2]. Considering the devastating impact of drought on global agriculture, feeding a world with an increasing population that may reach 9 billion by 2050 will be a challenge [3]. In this context, it is essential to breed for plant varieties which are optimally adapted to the changing environment and maximally stress tolerant [3]. Genes that confer stress tolerance can be mined from several sources, but particularly from organisms that are naturally able to survive variable and extreme conditions, such as those that are desiccation tolerant and dormant.

Desiccation tolerant organisms have the ability to withstand severe drought by surviving drying to equilibrium below 0.1 g of water per gram dry weight yet resume normal metabolism upon rehydration [4]. Dormant organisms survive extended periods of unfavourable conditions by dramatically reducing or suspending normal progression of life activities or development [5]. When the conditions become supportive to life, they resume normal metabolism.

Desiccation tolerance (DT) is a key trait in ancient non-vascular plants [6]. However, most of the modern vascular plants have retained DT only in a few specialized structures, such as seed and pollen. Seeds (termed orthodox) of more than 90% of the modern angiosperms for which data are available can tolerate desiccation

Abbreviations: ABA, abscisic acid; DSDS50, days of seed dry storage required to reach 50% germination; DT, desiccation tolerance; PEG, polyethylene glycol.

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[7]. Among these, there is a small number of species (about 135), termed "resurrection plants" [8] that are able to acquire DT in their vegetative tissues and to survive repeated cycles of desiccation and rehydration [9,10]. In these seeds and plants, DT combined with dormancy ensures that germination or resumption of full metabolic activity will occur only at the appropriate time to optimize seedling establishment, influences the rate at which species expand their range and determines the chances of survival or extinction upon climate change [11]. Therefore, both desiccation tolerant seeds and resurrection plants are promising genetic resources for improving abiotic stress tolerance in crop species [3].

We hypothesize that the mechanisms of DT and dormancy arouse as solutions to the same problem: survival in fluctuating environments. Several recent studies have focused on cellular mechanisms and gene expression patterns associated with DT and dormancy as two independent phenomena. However, little is known about genetic traits that are conserved in desiccation tolerant and dormant life forms. Therefore, we have adopted a cross-species approach, using published gene expression data sets on re-induction of DT in germinated seeds, induction of DT in a nematode and desiccation responses of resurrection plants and a lichen to search for conserved DT and dormancy genes. By doing this, a core set of genes was identified for which *Arabidopsis thaliana* T-DNA insertion mutants had a high incidence of DT, dormancy and seed-associated phenotypes. Our results support the hypothesis of co-evolution of DT and dormancy as a strategy to survive fluctuating environments.

2. Material and methods

2.1. Data set collection and detection of orthologs

Desiccation tolerance-related transcriptome profiling data were obtained from six available databases. The databases referred to experiments that monitored: (1) the transcriptional response of germinated desiccation sensitive *A. thaliana* seeds at the stage of radicle protrusion, to the re-induction of DT by polyethylene glycol (PEG, GEO Series accession number GSE64227) or by abscisic acid (ABA, GEO Series accession number GSE62876); the overlap between these two independent data sets was used for the comparison with the other sets; (2) the transcriptional responses of germinated *Medicago truncatula* seeds with protruded radicle lengths of 2.7–2.9 mm to re-induction of DT [12]; (3) the responses of *Craterostigma plantagineum* leaves to desiccation (desiccation for 15 days, reaching 5% of relative water content, RWC) compared to fully hydrated leaves [13]; (4) the responses of leaves of the monocot *Sporobolus stapfianus* to desiccation ($0.5\,g\,H_2O\,g^{-1}\,dw^{-1}$) compared to fully hydrated leaves (GEO Series accession number GSE64900); (5) the responses of desiccation sensitive "dauer" larva of *Caenorhabditis elegans* to the induction of DT by preconditioning (4 days 98% relative humidity, RH) [14]; and (6) the responses of thalli of the lichen *Cladonia rangiferina* to desiccation (desiccation for 24 h, reaching 0% RWC) compared to fully hydrated thalli [15].

In the case of both *A. thaliana* data sets, Affymetrix ARAGene 1.1ST Arrays were used and linear modelling (limma [16]) was applied with thresholds for absolute fold change of $\geq$1.0 (on a $\log_2$ scale) and $\leq$0.01 for *P*-value. For *M. truncatula,* Medtr_v1.0 12×135 K arrays (synthesized by Roche NimbleGen) were used [12] with thresholds for absolute fold change of $\geq$1.0 (on a $\log_2$ scale) and $\leq$0.01 for *P*-value. Fold change values from the *C. plantagineum* data set were calculated based on expression values of all assembled transcripts provided by Rodriguez et al. [13] and the absolute value for fold change was set as $\geq$2.0 (on a $\log_2$ scale). The *S. stapfianus* data set was derived from a custom oligoarray, 7×60mer probes per contig constructed from 454 sequencing of pooled cDNAs, synthesized and hybridized with single dye labelling by Roche NimbleGen (Madison, WI). The expression data was also analysed using linear modelling with thresholds for absolute fold change of $\geq$1.0 (on a $\log_2$ scale) and $\leq$0.01 for *P*-value. The different fold-change cut offs were used in an attempt to compensate for the different experimental set-ups that generated the data.

C. plantagineum sequences were downloaded from NCBI (www.ncbi.nlm.nih.gov). CDS sequences of *M. truncatula* genes (version Mt3.5v3) were downloaded from LegumeIP (http://plantgrn.noble.org/LegumeIP/). *S. stapfianus* sequences were obtained from M. Oliver (unpublished). *C. elegans* and *C. rangiferina* sequences were downloaded from Ensembl (http://www.ensembl.org/index.html). The sequences were compared against the whole *A. thaliana* genome using BLASTP in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To test the robustness of our analysis, we also compared the sequences of *C. plantagineum* and *S. stapfianus* against the whole *M. truncatula* genome using BLASTP in LegumeIP (http://plantgrn.noble.org/LegumeIP/blast.do), resulting in no appreciable differences.

Orthologs were defined as hits with lowest Expect value (E-value), with a threshold of $\leq 10^{-20}$. Multiple hits were considered orthologs when the difference between their E-values and the lowest hit's E-value was smaller than 10^{-10}. To search for orthologs, the sequence of each transcript was compared with the whole genome sequence of *A. thaliana* (TAIR10) using online tools available on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The analysis was conducted for transcripts with increased and decreased abundance separately, so only genes with similar transcript expression profiles across different species would be selected.

2.2. Phenotypic characterization

Genes were selected for phenotypic characterization because they exhibited seed preferential mRNA expression patterns in *A. thaliana* (when the ratio between their maximum expression value in seeds and the maximum expression value in leaves or roots was higher than 2). Expression values were obtained from the Botany Array Resource (BAR) Expression Browser [17] and belonged to at least one of the following Gene Ontology (GO) categories: *response to stress*, *response to stimulus* or *seed development*. T-DNA insertion lines were obtained from the Nottingham Arabidopsis Stock Centre (Suppl. Fig. 1 and Table 1). T-DNA insertions were confirmed using PCR. Seeds of plants homozygous for the mutations and WT (Col-0) seeds were grown on Rockwool, in a growth chamber (20 °C day, 18 °C night), with 16 h of light ($35\,W\,m^{-2}$), and watered with Hyponex nutrient solution ($1\,g\,l^{-1}$, http://www.hyponex.co.jp). Seeds obtained from these lines were bulk harvested in three replicates of at least two plants and were phenotyped to evaluate the ability to re-establish DT according to the protocol described by Maia et al. [18] for re-induction of DT in germinated seeds at appearance of first root hairs (stage IV in Ref. [18]). Averages were calculated and a Student's *t*-test was performed.

The degree of seed dormancy was evaluated as the number of Days of Seed Dry Storage required to reach 50% germination (DSDS50) according to Alonso-Blanco et al. [19]. In summary, all the measurements of germination percentages for each line at the various times during seed storage were used for probit regression on a logarithm time scale applying the regression module of the statistical package SPSS, version 10.0.6 [19]. Seed longevity was estimated based on the performance of the seeds in a germination test after undergoing accelerated aging. In order to accelerate aging, seeds were stored for six days in a closed container above a saturated NaCl solution (80% relative humidity) at 40 °C in the dark. Seed vigour was assessed as the ability of the seeds to germinate at high temperature (at 30 °C) or on NaCl (125 mM). The germination experiments and germination scoring were performed as

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