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Using the model perennial grass Brachypodium sylvaticum to engineer resistance to multiple abiotic stresses

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Abstract

We are using the perennial model grass Brachypodium sylvaticum to identify combinations of transgenes that enhance tolerance to multiple, simultaneous abiotic stresses. The most successful transgene combinations will ultimately be used to create improved switchgrass (Panicum virgatum L.) cultivars. To further develop B. sylvaticum as a perennial model grass, and facilitate our planned transcriptional profiling, we are sequencing and annotating the genome. We have generated ~40x genome coverage using PacBio sequencing of the largest possible size selected libraries (18, 22, 25 kb). Our initial assembly using only long-read sequence contained 320 Mb of sequence with an N50 contig length of 316 kb and an N95 contig length of 40 kb. This assembly consists of 2,430 contigs, the largest of which was 1.6 Mb. The estimated genome size based on c-values is 340 Mb indicating that about 20 Mb of presumably repetitive DNA remains yet unannotated. Significantly, this assembly is far superior to an assembly created from paired-end short-read sequence, ~100x genome coverage. The short-read-only assembly contained only 226 Mb of sequence in 19k contigs. To aid the assembly of the scaffolds into chromosome-scale assemblies we produced an F1 mapping population and have generated 480 individuals using a genotype by sequence approach.

One of the reasons for using B. sylvaticum as a model system is to determine if the transgenes adversely affect perenniality and winter hardness. Toward this goal, we examined the freezing tolerance of wild type B. sylvaticum lines to determine the optimal conditions for testing the freezing tolerance of the transgenics. A survey of seven accessions noted significant natural variation in freezing tolerance. Seedling or adult Ain-1 plants, the line used for transformation, survived an 8 hour challenge down to -6°C and 50% survived a challenge down to -9°C. Thus, we will be able to easily determine if the transgenics compromise freezing tolerance.

In the effort to develop biotechnological tools for perennial grass improvement, we have completed the transformation of B. sylvaticum with constructs containing 20 genes shown to be associated with enhanced abiotic stress tolerance in monocots. In addition, we have transformed plants with constructs containing a combination of genes (i.e. SARK::IPT- Ubi::HSR1::Ubi::NHX1) in order to simultaneously overexpress genes associated with drought + heat tolerance + salt tolerance. We generated single copy insert T1 lines for all constructs and the generation and bulking of homoygous T1 lines is well underway. In addition to our B. sylvaticum transgenics, we transformed B. distachyon with many of the same genes. Some of the transgenic B. distachyon plants subjected to a combined stress of both drought and salinity were able to produce higher yields than wild type plants. Our results indicate a great potential for the development of grasses with improved performance and yield in water-limited areas.

B. Sylviaticum as a model system

B. sylvaticum plants are compact, self-fertile, diploid, possess a relatively small genome (~340Mb), can go from seed to seed in 3 months and are perennial. (A) Three accessions at flowering stage. The pots are 10 cm tall. (B) While controlled crosses. The outcrossing rate is ~5% under greenhouse conditions. (C) A high-density genetic map to order contigs and polish the final assembly with 100X Illumina PE generated 40x genome coverage from libraries created from DNA sheared to 20 kb before size

Generation of B. sylvaticum transgenic plants

We are using B. sylvaticum to optimize transgenic approaches to engineer tolerance to multiple abiotic stresses. Previously we identified 20 genes that enhance tolerance to salt, drought or heat in other plants. We have transformed all of these genes into B. sylvaticum, some under the control of multiple promoters. We will test the stress tolerance of these lines and conduct transcriptional profiling to identify transgenes that work through different mechanisms. We will also test the freezing tolerance and perenniality of the lines to identify unwanted side effects. Using all of this information, we will select combinations of transgenes that should enhance tolerance to multiple abiotic stresses. These combinations will be transformed into B. sylvaticum and characterized. The best combinations will ultimately be transformed into switchgrass. The table shows the current status of our transformation efforts. We have created transgenics and identified homoygous, single-insert lines for all constructs. Bulking seed for further characterization is underway.

Freezing tolerance of B. sylvaticum

To ensure that our transgenic approaches to enhance abiotic stress tolerance do not compromise perenniality or hardiness, we will test the freezing tolerance and perenniality of the transgenic lines. Toward this goal, we have optimized test conditions. As expected, B. sylvaticum gains freezing tolerance after vernalization as shown in (A) where the plant vernalized for 4 days at 4°C survived a challenge of -4°C for 8 hours and the non-vernallized plant died. We compared the freezing tolerance of seven natural accessions and noted significant natural variation. The freezing tolerance of 8 hours at various temperatures is shown in (B) for seedlings and in (C) for adult plants after flowering and seed set. Using these conditions we will be able to test the freezing tolerance of the transgenic lines.

Phenotypic analysis of transgenic Brachypodium plants

T0 and T1 transgenic B. sylvaticum transgenic plants growing in the greenhouse (A). Typical experiment for the assessment of stress tolerance of transgenic plants (B). Stress treatments of transgenic B. distachyon expressing OsMADS57 (transcription factor), or OsHYD1 or OsDWF5 (associated with Brassinosteroid synthesis and signaling) started at the 4th leaf stage. Plants were continuously irrigated with the saline solution (25 mM NaCl) to mimic conditions that are typical of poor quality soils and water. The transgenic conductivities of the watering solutions flowing through the pots at the end of the experiment was about 4.5-5.0 dS/m (equivalent to 45 mM NaCl). At the tillering stage, watering was halted for 10 days and resumed using a solution containing 25 mM NaCl until harvest (C). The expression of OsMADS57 (D), OsHYD1 (E) and OsDWF5 (F) resulted in enhanced tolerance of the transgenic plants after a stress episode with yields similar to those displayed under normal watering conditions.