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Four Decades of Progress in Monitoring and Modeling of Processes in the Soil-Plant-Atmosphere System: Applications and Challenges

Thermographic imaging: assessment of drought and heat tolerance in Spanish germplasm of *Brachypodium distachyon*

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Abstract

The annual grass Brachypodium distachyon has been recently recognized as the model plant for functional genomics of temperate grasses, including cereals of economic relevance like wheat and barley. Sixty-two lines of B. distachyon were assessed for response to drought stress and heat tolerance. All these lines, except the reference genotype BD21, derive from specimens collected in 32 distinct locations of the Iberian Peninsula, covering a wide range of geo-climatic conditions. Sixteen lines of Brachypodium hybridum, an allotetraploid closely related to B. distachyon were used as reference of abiotic-stress well-adapted genotypes. Drought tolerance was assessed in a green-house trial. At the rosette-stage, no irrigation was applied to treated plants whereas their replicates at the control were maintained well watered during all the experiment. Thermographic images of treated and control plants were taken after 2 and 3 weeks of drought treatment, when stressed plants showed medium and extreme wilting symptoms. The mean leaf temperature of stressed (LTs) and control (LTc) plants was estimated based upon thermographic records from selected pixels (183 per image) that strictly correspond to leaf tissue. The response to drought was based on the analysis of two parameters: LTs and the thermal difference (TD) between stressed and control plants (LTs – LTc). The response to heat stress was based on LTc. Comparison of the mean values of these parameters showed that: 1) Genotypes better adapted to drought (B. hybridum lines) presented a higher LTs and TD than B. distachyon lines. 2) Under high temperature conditions, watered plants of B. hybridum lines maintained lower LTc than those of B. distachyon. Those results suggest that in these species adaptation to drought is linked to a more efficient stomata regulation: under water stress stomata are closed, increasing foliar temperature but also water use efficiency by reducing transpiration. With high temperature and water availability the results are less definite, but still seems that opening stomata allow plants to increase transpiration and therefore to diminish foliar temperature.

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Keywords: abiotic plant stress; phenotyping; stomatal regulation; thermal imaging

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1. Introduction

One of the current challenges in plant breeding is the development of new crop varieties that maintain good yields under the environmental constraints in a climate change scenario. Either if classical breeding or biotechnological approaches are attempted, this goal is hampered by the complex genetic basis of plant adaptation to abiotic stresses, in particular, to drought and high temperatures. Identification of genes involved in adaptive response to those stresses, and development of molecular markers for assisted selection in segregant progenies, are a primary need.

The easy handling, small genome and increasing box of analytical tools available for model plants make them the best choice for the molecular dissection of agronomically important traits in crops with large complex genomes like wheat (16000 Mb). However, the transferability of such knowledge from the model to the crop species requires, on one hand, a high degree of synteny and colinearity between their genomes. On the other hand, the growth conditions and demands of the model must be similar to those of the crop when traits under study are related to plant response to external agents like water availability and temperature. These are the main reasons why the monocot annual grass *Brachypodium distachyon* (272 Mb) has been recognized, over *Arabidopsis* and rice, as the most suitable model plant for functional genomics of temperate grasses, including cereals of economic relevance like wheat and barley [1].

Leaf temperature (LT), or indexes based on LT changes under controlled conditions, has potential for identifying plants with tolerance to water and heat stress [2]. In most cases, the plant's first response to low water availability is to maintain water content close to the unstressed level by limiting water loss. This is achieved in the short term mainly by stomatal closure. The consequent transpiration decreases, causing an increase in LT. Therefore LT monitoring can be use to estimate stomatal response and water stress tolerance [3]. Nevertheless, stomatal conductance is only one of many factors that affect LT, so care should be taken to maximize the potential of this technique [4]. One solution is to make use of reference surfaces, where the observed LT is compared with the temperature that the same plant attains without water availability limitations or with the temperature attained by a dry surface. Thermographic cameras are accurate and precise devices to monitor LT, so they might be a useful tool to identify differences in stomatal response.

In the present study, our objective has been to set up a thermographic image-based methodology to assess drought and heat response in *Brachypodium distachyon*. Our next aim is to identify tolerant and resistant *Brachypodium* genotypes to be crossed. This will allow the development of suitable materials for the functional genomic analysis of genes involved in an enhanced performance of cereal crops to rainfed and high temperature conditions. With that in mind we have conducted the phenotypic analysis on a collection of lines derived from wild accessions of varied geographical origins. This kind of germplasm resources covers most of the genetic variation related to adaptive response to abiotic stresses within a species [5].

2. Materials and Methods

2.1. Plant material collection

Sixty-two lines of *B. distachyon* were assessed for response to drought stress. All these lines, except the reference genotype BD21, derive from specimens collected in 32 distinct locations of the Iberian Peninsula (Figure 1), covering a wide range of geo-climatic conditions: altitude above the sea from 350 to 1500 m; annual rainfall from 300 to 1000 mm; annual mean temperature from 2.5 to 17.5 ºC. Sixteen lines of *Brachypodium hybridum*, an allotetraploid closely related to *B. distachyon* [6] were used as reference of abiotic-stress well-adapted genotypes [7].

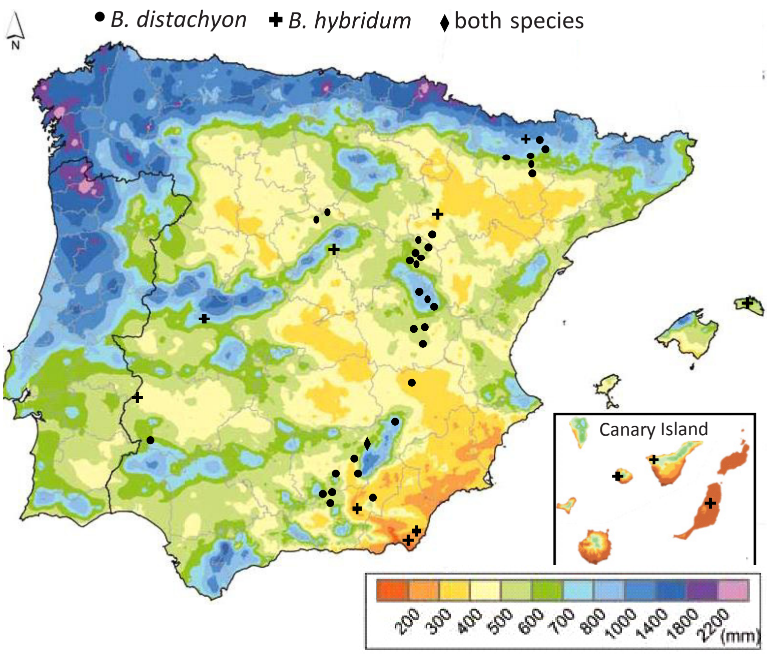

Figure 1. Geographical origin of *B. distachyon* and *B. hybridum* lines used on this study represented on a map showing the mean annual rainfall of the Iberian Peninsula and the Balearic and Canary Island.

2.2. Experiment set up and direct measurements

Lines of *B. distachyon* and *B. hybridum* were assessed for response to drought stress and heat tolerance in a green-house trial. The experiment consisted of four blocks. In each block all lines were planted in individual 10x10 cm plastic pots and randomly distributed. At the rosette-stage, no irrigation was applied to treated plants (three blocks) whereas their replicates at the control (1 block) were maintained well watered during all the experiment. A cooling system maintained green-house temperature bellow 27ºC during the whole experiment.

Thermographic images of treated and control plants were taken after 2 and 3 weeks of drought treatment with a FLIR SC305 camera (Inframetrics, FLIR Systems Inc, OR, US). The camera has a resolution of 320x240 pixels and thermal sensibility of 0.01ºC from -20°C to +120°C. Images were taken from a zenithal position at 1.2 m height over the pots. Images were visualized with the software ThermaCAM Researcher Professional® 2.10 and saved as Excel files in which a temperature was recorded for each single pixel. The mean leaf temperature of stressed (LTs) and control (LTc) plants was estimated based upon the thermographic records from selected pixels (183 per image) that strictly correspond to leaf tissue. Simultaneously, visual color images were taken with a digital camera CANON IXUS 100IS to evaluate the wilting symptoms (Figure 2).

The response to drought was based on the analysis of two parameters: LTs and the thermal difference (TD) between stressed and control plants (LTs – LTc). The response to heat stress was based on LTc.

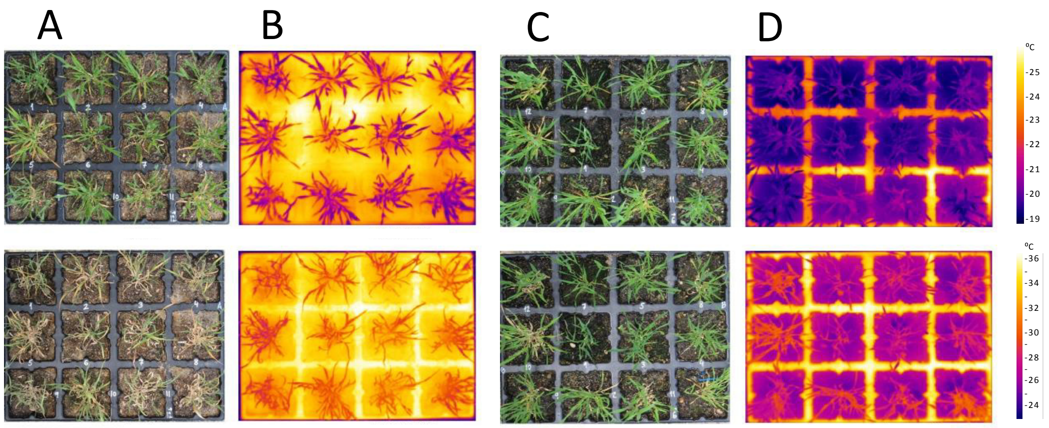

Figure 2. Visual and thermographic images of stressed (A, B) and control (C, D) plants of twelve of the *B. distachyon* lines after 2 weeks (upper images) and 3 weeks (lower images) of drought treatment. Color codes of measured temperatures are included.

3. Results

Thermographic images were able to distinguish between soil and leaf temperature in the stressed and control plants (Figure 2). Lower temperatures were registered in the wet soil of control treatments, whereas higher temperatures were recorded in soils of treated blocks after 3 weeks of drought treatment. After 2 weeks of drought treatment, stressed plants showed medium wilting symptoms in the visual color image and were still able to maintain LTs clearly bellow soil temperature, as it is shown by the contrasts between purple plants and yellow soil (Fig. 2B, upper image). After 3 weeks of drought treatment, stressed plants showed extreme wilting symptoms in the visual color image and even if LTs increase the thermographic images allow distinguishing between leafs and the background soil temperature (Fig. 2B, lower image). Experimental conditions were warmer after 3 weeks of drought treatment (control soil temperature 28 ºC) than after 2 weeks (control soil temperature 20 ºC).

Comparison of the mean values of the selected parameters (Table 1) showed that genotypes better adapted to drought (*B. hybridum* lines) presented a higher LTs and, as a consequence, a larger TD between stressed and control plants than *B. distachyon* lines. Under high temperature conditions (3 weeks after drought), watered plants of *B. hybridum* lines maintained LTc lower than those of *B. distachyon*, but the statistical difference was less significant than with warm conditions (2 weeks after drought).

Table 1. Comparison of drought and heat response in *B. distachyon* and *B. hybridum* lines based upon thermographic images taken after 2 and 3 weeks of drought treatment. LTs: leaf temperature on drought stressed plants; LTc: leaf temperature of control plants; TD = LTs-LTc.

	2 weeks after drought[a]			3 weeks after drought[b]		
	LTs	TD	LTc	LTs	TD	LTc
B. distachyon	22.53	2.10	20.46	30.13	1.77	28.95
B. hybridum	22.54	2.96	19.58	31.44	2.99	28.45
Student-t	ns	*	**	***	***	*

ns: $p > 0.05$; *: $0.05 > p > 0.01$; **: $0.01 > p > 0.001$; ***: $0.001 > p$

a: Green-house outer temperature, 14 ºC; 2weeks-stressed soil temperature, 25 ºC

b: Green-house outer temperature, 27 ºC; 3weeks-stressed soil temperature, 35 ºC.

4. Conclusions

Those results suggest that in these species adaptation to drought is linked to a more efficient stomata regulation [8]. So, under water stress stomata of more tolerant genotypes are closed, increasing foliar temperature but also water use efficiency by reducing transpiration. With high temperature and water availability the results are less definite, but still seems that opening stomata allow plants to increase transpiration and therefore to diminish foliar temperature.

Acknowledgements

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