Journal of Plant Physiology 190 (2016) 1–14

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

Drought tolerance strategies highlighted by two *Sorghum bicolor* races in a dry-down experiment

Alessandra Fracasso[a,*], Luisa Trindade[b], Stefano Amaducci[a]

[a] Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29122 Piacenza, Italy
[b] Wageningen UR Plant Breeding, Wageningen University and Research Centre, 6708 PD Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 24 October 2015
Accepted 26 October 2015
Available online 14 November 2015

Keywords:
Sorghum bicolor (Moench)
Drought stress
Tolerance indices
Gas exchange
Chlorophyll fluorescence
qRT-PCR

ABSTRACT

Drought stress is the major environmental stress that affects more and more frequently plant growth and productivity due to the current climate change scenario. Unravelling the physiological mechanism underlying the response of plants to water stress and discover traits related to drought tolerance provide new and powerful tools for the selection in breeding programmes. Four genotypes of *Sorghum bicolor* (L.) Moench were screened in a dry-down experiment using different approaches to discover physiological and molecular indicators of drought tolerance.

Different strategies were identified in response to drought among the four genotypes and the two *Sorghum* race allowing to state the tolerance of *durra* race compared to the *caudatum* one and, within the *durra* race, the drought tolerance of the genotype IS22330. It retained high biomass production and high tolerance index, it had a low threshold of fraction of transpirable soil water and high capacity to recover leaf apparatus after drought stress. Furthermore in this study, the expression levels of four genes highlighted that they could be used as proxy for drought tolerance. *Dehdrine* (*DHN*) could be used for screening drought tolerance both in *durra* and in *caudatum* races. *NADP-Malic Enzyme*, *Carbonic Anhydrase* (*CA*) and *Plasma membrane Intrinsic Protein* (*PIP2-5*), being up-regulated by drought stress only in *durra* race, have a more limited, though nonetheless useful application. In the tolerant *durra* genotype IS22330 in particular, the regulation of stomatal openings was strongly related to *NADP-Malic Enzyme* expression.

1. Introduction

The cultivation of biomass crops is a valuable strategy to produce bioenergy and materials for a modern biorefinery provided that they are environmentally sustainable (Guzman et al., 2014) and do not interfere with food production and availability. One way to prevent food vs fuel competition is to cultivate biomass crops on marginal areas (e.g., dry environments) that are not currently being used for the production of food crops. These biomass crops should have the following characteristics: high yielding, low energy inputs, low cost and low nutrient and water requirements (Koçar and Civaş, 2013).

Sorghum bicolor (Moench) is a good candidate crop for bioenergy production (Rooney et al., 2007) because it is adapted to dry environments and provides a large array of raw materials (starch from the grain varieties, sugar from sweet varieties and fibre from biomass and forage varieties). Several breeding programs have been already set up to identify and improve the *sorghum* traits that are linked to the stabilization of crop yield performance under current climate change scenarios. In the last decades, high-yields, drought-tolerance and high water use efficiency are the traits that have been investigated the most (Rooney et al., 2007).

Drought stress is the most important environmental constraint limiting crop productivity in many regions of the world (Cattivelli et al., 2008). It is first detected as a reduction of leaf water potential, changes in cell turgor (Pardossi et al., 1991; Comstock, 2002) and stomatal closure due to root-sourced and/or leaf-sourced abscisic acid production (Davies and Zhang, 1991). This leads to a reduction of intercellular CO_2 concentration, and an increased mesophyll resistance resulting in a modification in photosynthetic metabolism (Pinheiro and Chaves, 2011). Consequently, the

Abbreviations: FTSW, fraction of transpirable soil water; $FTSW_t$, threshold of fraction of transpirable soil water; DAE, days after emergence; TI, tolerance index; DSI, drought susceptibility index; GR, growth rate; LA, leaf area; EL, emitted leaves; RWC, relative water content; Pn, net photosynthetic rate; g_s, stomatal conductance; E, transpiration; VPD, vapour pressure deficit; ΦPSII, photosystem II efficiency; Fv/Fm, maximum quantum yield; qP, photochemical quenching; qNP, non photochemical quenching; qRT-PCR, quantitative real-time PCR.

* Corresponding author. Fax: +39 0523 599 222.
E-mail addresses: alessandra.fracasso@unicatt.it (A. Fracasso), luisa.trindade@wur.nl (L. Trindade), stefano.amaducci@unicatt.it (S. Amaducci).

http://dx.doi.org/10.1016/j.jplph.2015.10.009
0176-1617/

reduction of photosynthetic and CO_2 assimilation rate results in a decrease of cell and plant growth and development (Boyer and Westgate, 2004; Jaleel et al., 2007; Karthikeyan et al., 2007). Drought also inhibits the electron transport rate (Masojídek et al., 1991) increasing the oxidative stress, that can seriously affect the leaf photosynthetic machinery (Ort, 2001). The components of the photosynthetic apparatus, and in particular photosystem II(PSII), located in the thylakoids of the mesophyll cells, is considered to be the component that is most sensitive to drought stress and is the main source of fluorescence signals (Havaux and Strasser, 1992).

Leaf area (Karamanos and Papatheohari, 1999; Tsuj et al., 2003), vegetative growth (Younis et al., 2000), root dry matter (Huang and Gao, 2000; Giuliani et al., 2005), whole plant transpiration rate (Casadebaig et al., 2008; Luquet et al., 2008), photosystem II energy use and non-photochemical quenching (Jagtap et al., 1998; Cousins et al., 2002) are physiological traits that have been measured to assess drought tolerance in plants. Alterations in gene expression in *sorghum* during drought have been studied using different tools: cDNA libraries (Pratt et al., 2005), microarrays (Buchanan et al., 2005; Pasini et al., 2014) and RNA-Seq experiment (Dugas et al., 2011). *Sorghum* genetic maps, developed using anonymous DNA markers (Mace et al., 2009; Ashraf, 2010), have been overcome by the recent sequencing of the *Sorghum bicolor* genome (Paterson et al., 2009) enabling the development of physical maps and a better understanding of positioning of key genes involved in response to various abiotic stress (Dugas et al., 2011; Pasini et al., 2014).

Understanding how gene expression is linked to the physiological response of plants is still a crucial point in the translation of genetic information into a useful set of information for crop production and management (Bruce et al., 2002).

In this study four *sorghum* genotypes were tested in pots with an accurate control of soil water availability and water consumption, carrying out a "dry-down" experiment (Luquet et al., 2008) in standardized experimental conditions (Jones, 2007). During drought stress and after water recovery, various physiological traits (biomass production, phenology, leaf area, transpiration rate, relative water content, chlorophyll fluorescence and gas exchange) were measured. The expression profiles of seven drought- related genes, five of which mapped into a drought-related QTL (Mace and Jordan, 2011), were evaluated under drought conditions using quantitative real-time PCR (Q-PCR).

The aim of the work presented herein was to describe the response of different *sorghum* genotypes to reduced soil water availability combining and linking two different approaches: physiological and transcriptomic characterization, with the purpose of unravelling the complex relationships and responses that drought stress triggers in *sorghum* plants.

2. Materials and methods

2.1. Plant materials and growth conditions

Four *sorghum* genotypes, with contrasting tolerance to drought, were cultivated in July 2012 in a dry down experiment to assess their physiological response to drought during the vegetative stage. The genotypes were selected on the basis of their biomass productivity measured at the pre-flowering stage in field trials carried out in Senegal in 2002 and in 2003 (S. Braconnier (CIRAD), personal communication). The tolerant genotypes were IS10234 and IS22330 while the sensitive ones IS16173 and IS20351. IS10234 and IS16173 belongs to *caudatum* race and IS20351 and IS22330 to the *durra* race. The genotypes are part of germplasm collection of CIRAD and were provided by the CRB-T (Centre de Resources Biologiques Tropicales) CIRAD Montpellier.

Germination of seeds was carried out in Petri dishes at 25 °C and in dark conditions for 3 days. Germinated seeds were planted in plastic pots (16 L capacity), filled with a base layer of sand to guarantee drainage and 8 kg of a soil mixture (24% clay, 64% silt, and 12% sand), that had been previously sieved, dried and homogenized. Each pot was planted with five germinated seeds, that were thinned at the 4th leaf stage so to have one healthy plant per pot. Eighty pots were divided into two groups: drought stress and control (well-watered) treatment and randomly divided in five replications, distributed outdoors in an open space (average of minimum and maximum temperatures 18.3 and 32.5 °C respectively) and daily rearranged to avoid any border effect or light heterogeneity.

In July 2013 the same experimental set up was repeated, in the same location and period of the year (30th– 32nd week of the year), to study the daily course of gas exchange measurements on the genotypes IS20351 and IS22330 that had shown peculiar physiological response to drought in the first experiment. In addition gas-exchange, shoot and root biomass production, tolerance index, drought susceptibility index, growth rate, leaf area, normalized transpiration rate, relative water content and chlorophyll fluorescence were surveyed in order to link this experiment to the previous one carried out in 2012.

2.2. Characterisation of drought dynamics

Total Transpirable Soil Water (TTSW) content was determined as the difference between the Soil Water Content (SWC) at Field Capacity (FC) and that at Wilting Point (WP). Both FC and WP were determined in a preliminary experiment (data not shown). FC was the soil water content left in the soil after complete drainage of excess water while WP was the residual water left in the soil after *sorghum* plants were grown until complete wilting. FC and WP were used to calculate the Fraction of Transpirable Soil Water (FTSW) as follows:

$$\mathrm{FTSW} = \frac{\mathrm{ASWC}}{\mathrm{TTSW}} = \frac{\mathrm{SWC} - \mathrm{WP}}{\mathrm{FC} - \mathrm{WP}}$$

where ASWC is the Available Soil Water Content, calculated subtracting the soil water content at WP from the actual soil water content SWC. During the experiment each pot was weighed three times per day: at 9 am, 1 and 5 pm to estimate its soil water content.

At the 7th leaf developmental stage (33rd DAE, day after emergence), all plants were irrigated until FC, a thin layer of perlite was spread on the soil surface, and the top of the pots was covered with PVC bags. A little slit was cut at the bottom of the plastic bag to allow the *sorghum* plant to grow through it. The slit was sealed with adhesive packing tape around plant collars to minimize evaporation losses from the soil surface. With this method whole-plant transpiration was estimated from the decrease of pot weight between two consecutive weight determinations (Luquet et al., 2008; Xin et al., 2008).

2.3. Physiological measurements

Drought stress was applied from the 7th leaf stage stopping the irrigation until the pots contained 20% of FTSW (0.2 FTSW). From this moment irrigation was applied to maintain 0.2 FTSW in the pots subjected to drought stress: at each weighing event (three times a day) FTSW was calculated and pots were irrigated, if necessary, to bring back FTSW at 0.2 level. After five days at 0.2 FTSW had passed, half of the plants subjected to drought stress were irrigated to reach FC and kept well-watered until the end of the experiment. Two destructive samplings were carried out during the experiment: the first at the end of the stressed period (after five days at 0.2 FTSW,