South African Journal of Botany 103 (2016) 61–69

Contents lists available at ScienceDirect

South African Journal of Botany

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

Physiological responses of selected African sorghum landraces to progressive water stress and re-watering☆

N. Devnarain [a,b], B.G. Crampton [b,c], R. Chikwamba [a], J.V.W. Becker [d], M.M. O'Kennedy [a,*]

[a] Council for Scientific and Industrial Research (CSIR) Biosciences, Pretoria, South Africa
[b] Department of Plant Science, University of Pretoria, Pretoria, South Africa
[c] Forestry and Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa
[d] African Centre for Gene Technologies (ACGT), Pretoria, South Africa

ARTICLE INFO

Article history:
Received 8 April 2015
Received in revised form 18 August 2015
Accepted 7 September 2015
Available online 28 November 2015

Edited by L Sebastiani

Keywords:
Carotenoid
Chlorophyll
Drought tolerance
Physiology
Proline
Sorghum bicolor

ABSTRACT

Sorghum is particularly drought tolerant compared with other cereal crops and is favoured for subsistence farming in water scarce regions of the world. This study was conducted to identify South African sorghum landraces with superior drought tolerance compared with a drought-tolerant breeding line (P898012). Seedlings of 14 South African sorghum landrace accessions were initially screened for drought tolerance by assessing percentage leaf water content (LWC) during progressive water deficit. Four landraces (designated LR5, LR6, LR35, and LR36) recorded higher LWC than P898012. These were subsequently evaluated with P898012 during the reproductive growth stage, for their physiological responses to mild (4 days) and severe (6 days) water stress treatments and a moderate re-watered treatment on day 7. Plant height, soil moisture, and LWC were measured during harvests. Chlorophyll, carotenoid, and proline contents were quantified. All five genotypes maintained LWC above 80% during mild and severe stress treatments. For LR35 and LR36, LWC were recorded within 8% less in comparison to their well-watered controls following the moderate re-watered treatment. Significantly higher chlorophyll and carotenoid contents were recorded for both LR6 and LR35 in comparison to P898012 during severe stress. When LWC was reduced in LR36 (to 73.68%) and LR35 (to 73.51%), their proline content significantly increased by 14- and 16-fold, respectively. In this study, we have identified four previously uncharacterised sorghum genotypes exhibiting drought tolerance and described their physiological responses during water deficit and moderate re-watering. Aside from their application to breeding, these landraces are valuable resources to elucidate genetic mechanisms that enable drought tolerance in South African sorghum.

1. Introduction

Drought is a complex environmental stress and major constraint to crop productivity (reviewed by Mishra and Singh, 2010; Farooq et al., 2012). It is a global problem that may have profound effects on agriculture and food security, especially upon agricultural systems which depend on rain as their primary source of water (Bray et al., 2000; Rosegrant et al., 2002). Subsistence and small-scale farmers, particularly those living in the semi-arid areas of Africa and Asia, are vulnerable to the impacts of drought as they often lack essential resources for additional agricultural inputs and irrigation systems (Glantz, 1987; Leichenko and O'Brien, 2002). While most primary cereal crop species are sensitive to hot and dry climates, sorghum [*Sorghum bicolor* (L.) Moench] is recognised as a remarkably drought-tolerant species and is favoured for subsistence farming in water scarce, impoverished regions of the world (House, 1985; McKersie and Leshem, 1994; Wani et al., 2012).

Sorghum, which is indigenous to Africa, is a close relative of sugarcane and cereals such as maize and pearl millet. It is a versatile crop and the utilisation of the whole plant is far-reaching; consequently, sorghum is grown for food, animal feed, fibre, fuel and used for some industrial purposes (Wall and Ross, 1970; House, 1985; Paterson et al., 2009). Sorghum is the third most important grain crop cultivated in South Africa after maize and wheat (Sorghum Section 7 Committee, 2007). Worldwide, sorghum is the fifth most important grain crop with 62 million tonnes produced during 2013 (Wani et al., 2012; FAO, 2015). Although grain sorghum exhibits resilience to the effects of water stress, particular growth stages in its lifecycle are susceptible to drought stress. The early vegetative stage and reproductive stages

Abbreviations: LWC, leaf water content; c_a, chlorophyll *a*; c_b, chlorophyll *b*; $c_{(x+c)}$, carotenoids; GS II, growth stage II; Mod-RW, moderate re-watered; MS, mild stress; SMC, soil moisture content; SS, severe stress; *Tchl*, total chlorophyll.

☆ This research was conducted at Biosciences, Council for Scientific and Industrial Research (CSIR), Meiring Naudé Road, Pretoria, South Africa.

* Corresponding author at: CSIR Biosciences, PO Box 395, Pretoria 0001, South Africa. Tel.: +27 12 841 3773; fax: +27 12 841 2185.
E-mail addresses: NDevnarain@csir.co.za (N. Devnarain), Bridget.Crampton@fabi.up.ac.za (B.G. Crampton), RChikwamba@csir.co.za (R. Chikwamba), JBecker@csir.co.za (J.V.W. Becker), mokennedy@csir.co.za (M.M. O'Kennedy).

http://dx.doi.org/10.1016/j.sajb.2015.09.008
0254-6299/

(pre- and post-flowering) of sorghum are vulnerable to the effects of water deficit (Tuinstra et al., 1997; Kebede et al., 2001; Wani et al., 2012). A drought period during the early seedling stage of sorghum may inhibit establishment of the crop (McKersie and Leshem, 1994). The water demand of sorghum is greatest during the pre-flowering reproductive growth stage (Anon, 2008). Water stress during pre- and post-flowering stages impacts grain development and yield of the crop (McKersie and Leshem, 1994). Therefore, the ability to withstand water deficit at these stages is critical to productivity.

Plants may exhibit various biochemical and physiological mechanisms to ameliorate the effects of drought (Tuinstra et al., 1997; Bray et al., 2000). The process in which plants are able to grow and complete their lifecycle before soil moisture becomes limiting represents the drought escape mechanism. Drought avoidance involves features which aid in decreasing the amount of water loss by the plant while drought tolerance encompasses stabilising mechanisms that protect cellular and metabolic integrity and function at the tissue or cellular level (Tuinstra et al., 1997; Blum, 2011). These mechanisms may work synergistically to bring about successful tolerance during periods of drought.

Water is essential for the myriad of biological processes which contribute to sustaining life. Consequently, periods of water deficit have profound effects on the physiology of all organisms, especially sedentary plants. Aside from a plants' response to continuous water stress, it is important to consider the effect of re-hydration on plant physiology. In field environments, water availability is subject to cyclical changes and unpredictable climatic conditions therefore, intermittent rains may follow a drought period (Izanloo et al., 2008; reviewed by Mishra and Singh, 2010). A plants' prompt biochemical response to a re-hydration event is a good indicator of recovery which is dependent on the severity of the preceding water stress. Intensive research has been conducted to understand plant responses to water deficit only; however, work describing the effects of water stress and re-watering on plants are limited (Takele, 2010; reviewed by Xu et al., 2010; Filippou et al., 2011).

Water stress in plants may manifest as decreased leaf water content and chlorophyll contents. The leaf water content is a measure of plant stress and severe decreases may contribute to structural interruptions of important biological functions in plants leading to injury or tissue death (McKersie and Leshem, 1994). Total chlorophyll content as well as chlorophyll *a* and *b* contents are indicators of overall plant health and directly influence a plants' ability to absorb light for photosynthesis (Malkin and Niyogi, 2000). This is crucial to maintaining vital processes of the plant system.

Some plant protective mechanisms may be activated during abiotic stress, such as increased production of pigments and organic osmolytes. Carotenoids, which include carotenes and xanthophylls, are pigments closely associated with chlorophylls and play a role in light absorption and photosynthesis (reviewed by Britton, 1995; Malkin and Niyogi, 2000). They also provide photoprotection during abiotic stress. The amino acid, proline is an important compatible osmolyte which has been found to accumulate in plants during stress (Bray et al., 2000; Ashraf and Foolad, 2007). Proline is suggested to serve an important protective role against abiotic stress in plants due to its distinct biochemical properties which enable this amino acid to have a neutral charge at physiological pH, not affect cellular metabolism and scavenge harmful reactive oxygen species (Van Rensburg et al., 1993; Bray et al., 2000; reviewed by Kavi Kishor et al., 2005).

The aim of this study was to identify drought-tolerant African sorghum genotypes and subsequently evaluate their physiological responses to progressive water stress and subsequent moderate re-watering. The first objective was to screen 14 sorghum landrace accessions for drought tolerance at the seedling stage together with a known drought susceptible (ICSV112) and tolerant breeding line (P898012) during progressive water stress. The second objective was to evaluate the physiological responses of those landraces which compared favourably with P898012 in the seedling stage screen, together with P898012, during progressive water deficit (mild and severe stress) and a moderate re-watered treatment at the drought-sensitive growth stage (GS) II of development. The early seedling and pre-flowering reproductive stages of sorghum are sensitive to water stress. Drought tolerance at these stages are important for plant survival and grain yield.

2. Materials and methods

2.1. Plant material

The seeds of sorghum [*Sorghum bicolor* (L.) Moench] lines and landrace accessions were obtained from the Agricultural Research Council Grain Crops Institute (ARC-GCI) and the National Plant Genetic Resources Centre of the Department of Agriculture, Forestry and Fisheries (DAFF), South Africa, respectively. P898012 is a public genotype that was bred at Purdue University, USA, and exhibits pre-flowering and post-flowering drought resistance (Casas et al., 1993; Kumar et al., 2011; Yu et al., 2013). ICSV112 was bred at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center, India, and its cultivation is recommended for rainy seasons or as an irrigated crop during post-rainy seasons (Anon, 1988).

2.1.1. Seedling water stress screen

Three seeds of P898012 (drought tolerant), ICSV112 (drought susceptible) and each landrace accession, were planted in 12 cm diameter plastic plant pots, lined with filter paper in the bottom and filled with 624 g of an autoclaved soil mix consisting of ½ red soil: ½ river sand: 1 vermiculite: 2 compost. The soil was thoroughly wet with water before seeds were sown at a depth of 2 cm and each pot was supplemented with 50 mL Nutrifeed [Starke Ayres (Pty) Ltd., RSA] nutrient solution (1 g/L). There were three replicates per genotype and stress time point. Pots were randomly placed in a controlled growth room facility with a 16 h light/8 h dark photoperiod at a total energy in the visible region measured by an AccuPAR LP-80 ceptometer (Decagon Devices, Inc., USA) to be 312 μmol.m^{-2} s^{-1}. A HOBO® U10 Temp/RH series data logger (Onset®, USA) was used to monitor the temperature at 15 min intervals for the duration of the experiment. The temperature was maintained at a range of 26–29 °C. Seedlings were grown to the five leaf stage by daily watering with 50 mL water. Before the onset of stress, each pot was watered with 150 mL water and supplemented with 50 mL Nutrifeed (2 g/L). Water was withheld from pots for 6, 7, 8, and 9 days.

2.1.2. Drought simulation at reproductive GS II

One hundred seeds per sorghum genotype (P898012, LR5, LR6, LR35, and LR36) were surface decontaminated by exposure to sodium hypochlorite (1% v/v NaOCl, JIK® Reckitt Benckiser Group plc., RSA) for 10 min followed by a 3× rinse with sterile deionised water. For seed germination, 4 kg of autoclaved soil mix (Section 2.1.1) was wet with 500 mL water whereafter it was placed in plastic trays (35 × 25 cm). Surface decontaminated seeds were sown into the soil at a depth of 2 cm and thereafter, the soil was watered with 200 mL water. Trays were watered daily with 400 mL water and maintained at a 16 h light/8 h dark photoperiod at a total energy in the visible region at 150 μmol.m^{-2} s^{-1}. The temperature was maintained at a range of 24–29 °C.

One week old sorghum seedlings were transferred to individual 1 L, 15 cm plastic, filter paper-lined plant pots filled with 1 kg autoclaved soil mix (Section 2.1.1). The soil was saturated with 250 mL water before planting seedlings and each pot was supplemented with 50 mL Nutrifeed (1 g/L). Appropriately labelled pots were randomly arranged in the growth room. Seedlings were grown for 8 weeks after seeds were sown and pots were watered daily with 80 mL water during weeks 1–4, 100 mL water during weeks 5–6, and 120 mL water from