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South African Journal of Botany 95 (2014) 123–128

Contents lists available at ScienceDirect

South African Journal of Botany

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

Antioxidant enzyme activity, proline accumulation, leaf area and cell membrane stability in water stressed *Amaranthus* leaves

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ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 13 August 2014
Accepted 27 August 2014
Available online 29 September 2014

Edited by J van Staden

Keywords:
A. hybridus
A. hypochondriacus
Antioxidative enzymes
A. tricolor
Cell membrane stability
Leaf area
Leaf water status
Metabolic response
Osmoregulation
Proline
Relative water content
Water stress

ABSTRACT

Traditional crops are extremely important for food production in low income, food-deficit countries (LIFDCs) where they continue to be maintained by socio-cultural preferences and traditional uses. Significant potential exists to improve these crops, one of which is to select for improved productivity during moisture stress conditions. Germplasm of *Amaranthus tricolor*, *Amaranthus hypochondriacus* and *Amaranthus hybridus* were subjected to various screening methods to measure metabolic and physiological changes during water stress. The activities of enzymes involved in the oxygen-scavenging system during abiotic stress conditions (superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR)), free proline production, leaf area (LA), cell membrane stability (CMS), leaf water potential (LWP) and relative water content (RWC) were measured in these three amaranth species during induced water stress. This study showed significant differences in metabolic responses during water deficit of the three species tested. Moisture stress and a decrease in RWC and LWP were first experienced in *A. hybridus* and *A. hypochondriacus*, followed by *A. tricolor*. There was an indirect correlation between leaf water status (RWC and LWP), enzyme activity, proline production and leaf area. The combined effect of GR, APX and SOD could ensure higher levels of regulation of the toxic effect of H_2O_2 which could be associated with drought tolerance in *Amaranthus*. Distinct differences in onset of proline accumulation and the amount of accumulated in leaves upon induced water stress was noticed for the three amaranth species tested. Proline accumulation during water stress conditions in amaranth seems to be indirect and could possibly have a protective role apart from osmoregulation during stress conditions. This contention is supported by the decrease in leaf area and high cell membrane stability for two of the species tested. This study forms part of a project aimed at the development of improved traditional crops to contribute to food production and quality for subsistence farmers in areas with low precipitation or variable rainfall patterns.

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1. Introduction

Water deficit is one of the most common environmental limitations of crop productivity and economic losses, and it is a permanent constrain that farmers face daily (Fuglie, 2007; Hyman et al., 2008). Chronic or sporadic periods of water deficit stress leads to reduced growth and quality in plants, and high losses in yield of 50% and more are experienced (Wang et al., 2003). Exposure of plants to extreme stress conditions such as drought will initiate a diverse set of physiological, morphological and developmental changes in order to survive, as have been widely reported (Gomes et al., 2010; Ozkur et al., 2009). Physiological traits relevant or modified by the responses to water deficits span a wide range of vital processes and there is no single response pattern that is highly correlated with yield under all drought environments (Cattivelli et al., 2008).

Plants have evolved a number of antioxidant enzymes that ameliorate oxidative stress by scavenging toxic oxygen species (Liang et al., 2003; Sun et al., 2007). Superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate reductase (APX) co-operate to reduce the damaging effect of oxygen radicals through the Halliwell–Asada pathway. There are numerous reports on these enzymes protecting plants during oxidative stress initiated by drought (Ahmed et al., 2009; Boogar et al., 2014; Ozkur et al., 2009). Plants able to tolerate drought must therefore be able to increase their defense mechanisms under conditions of severe water deficit. Water stress causes oxidative injury, and the ability to increase the levels of antioxidative capacity or increased levels of antioxidants during water stress can limit membrane damage and enzyme activity can be an important measurement of drought tolerance (Dawood et al., 2014).

Water stress induces numerous metabolic changes in plants, and many plants respond to a decrease in osmotic potential by intracellular

Abbreviations: APX, ascorbate peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; LA, leaf area; LWP, leaf water potential; RWC, relative water content; SOD, superoxide dismutase.

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http://dx.doi.org/10.1016/j.sajb.2014.08.008
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accumulation of organic solutes e.g. ions, alcohols, sucrose or amino acids. One of the most prominent metabolic changes is a tremendous free-proline accumulation (Aziz et al., 1998; Manivannan et al., 2007). The role of proline during stress includes functioning as a compatible osmolite/osmoregulation (Abdalla and El-Khoshiban, 2007; Huang et al., 2000), preventing hyperosmotic stress (Kishor et al., 1995) by balancing concentration differences between the cytoplasm and the central vacuole of the plant cells, is involved in stabilizing proteins/ enzymes and enzyme activities (Gill and Tuteja, 2010), stabilizes subcellular structures (membranes and proteins) (Ashraf and Foolad, 2007; Efeoghu et al., 2009), and is involved in the conservation of nitrogen and energy for a post-stressed period (Hare et al., 1998). Proline is also known to induce expression stress responsive genes, such as drought and salt stress (Chinnusamy et al., 2005; Dawood et al., 2014; Zhu et al., 1998).

The measurement of solute leakage from plant tissue is a proven method for measuring membrane integrity in relation to environmental stresses (Blum, 1998; Blum and Ebercon, 1981; Farooq and Azam, 2006). This technique involves measurements of electrolyte leakage into an aqueous medium, where the degree of cell membrane stability is considered to be one of the best physiological indicators of water stress tolerance (Kocheva et al., 2004; Labuschagne et al., 2008).

Maintenance of turgor pressure during stress is important to preserve metabolic responses in crop species, and is a well recognized mechanism in breeding toward water stress tolerance. The ability of seed propagated plants to adapt to water stress involves either tolerance to cellular dehydration or minimisation of water loss and maintenance of turgor pressure (Morgan, 1992). Relative water content (RWC) allows for the comparison of metabolic changes in the plant, at the same cellular water status (Blum, 1998). RWC further allows the estimation of plant water status in terms of cellular hydration and is under the possible effect of both leaf water potential and osmotic adjustment (Blum, 1998). RWC can be used effectively to evaluate drought tolerance and selection of the most drought tolerant genotypes (Abdalla and El-Khoshiban, 2007).

A renewed interest in the traditional crop *Amaranthus* has developed not only because of its growth abilities, but also because of its good nutritional qualities. As a vegetable crop, *Amaranthus* is a very nutritious summer leafy vegetable. The leaves are rich in protein, iron, calcium and vitamins A, C & D (Brenner et al., 2001). In the present study, the effect of water stress on the activities of the enzymes of the antioxidative pathway (SOD, APX and GR), leaf area (LA), osmoprotection (free proline production), cell membrane stability (CMS), in comparison with changes in leaf turgor maintenance (RWC, LWP), was assessed to obtain an indication of the changes in metabolic traits which could indicate possible mechanisms of drought tolerance in different amaranth genotypes.

2. Materials and methods

2.1. Cultivation, water stress and sampling

Experiments were carried out at the Agricultural Research Council-Roodeplaat, Pretoria, at 25°59″S; 28°35″E and at an altitude of 1200 m above sea level. Three amaranth species, i.e. *Amaranthus hybridus*, *Amaranthus hypochondriacus* and *Amaranthus tricolor* were cultivated in pots in a temperature controlled greenhouse. Cultivation and leaf sampling was done as described by Slabbert and Krüger (2011).

2.2. Analytical procedures

2.2.1. Leaf water status

LWP and RWC measurements were made pre-dawn from control and stressed plants according to the methods described by Slabbert and Krüger (2011).

2.2.2. Antioxidative enzyme activity

Leaf material was sampled midday; freeze dried and stored at −80 °C until subsequent analyses. Samples of 0.04 g of freeze dried material were thoroughly macerated with cold enzyme extraction buffer [0.2% polyvinyl polypyrrolidone (PVP), 0.1 mM EDTA, and 50 mM potassium phosphate, pH 7.8]. The extracted sample was centrifuged (20 000 *g*, 10 min, 2–5 °C) and stored in ice.

Superoxide dismutase (SOD) activity was determined measuring nitrate formation from the oxidation of hydroxyl ammonium chloride at A_{530} as described by Malan et al. (1990) with slight modifications. A 425 µl reaction mixture containing 250 µl of a 65 mM potassium phosphate buffer (KH_2 PO_4) (pH 7.8), 25 µl of 1.5 mM xanthine, 25 µl of 1 mM hydroxyl ammonium chloride ($NH_2OH \cdot HCl$) and 125 µl dH_2O, to which 75 µl of crude plant extract was added. After 20 min at 25 °C, 500 µl of reaction mixture was mixed with 500 µl of each 17 mM sulphanylic acid and 7 mM α-naphtylamine. After a further 20 min at 25 °C, the absorbancy of the reaction mixture was read at A_{530}. The SOD activity is expressed as units per gram dry weight (units g^{-1}, D_W).

The rate of NADPH oxidation over time at A_{340} was used as measure of glutathione reductase (GR) activity using a modified method of Tappel (1978) with slight modifications. The reaction mixture contained 500 µl of 0.1 M Tris–HCl buffer (pH 8.0), 375 µl of 0.5 mM EDTA, 50 µl of 0.25 mM glutathione (GSSG), 50 µl of 0.125 mM NADPH and 25 µl of crude plant extract. GR activity was expressed as units per gram dry weight (units g^{-1}, D_W).

The ascorbate peroxidase (APX) activity was assessed by measuring the oxidation of H_2O_2 by means of a decrease in absorbance at A_{265}, as described by Dalton et al. (1986) with slight modifications. The APX assay was performed in a 1.5-ml quartz cuvette containing 465 µl of 0.25 mM ascorbate, 830 µl of 50 mM KH_2 PO_4 at pH 7.0, 25 µl of 1.0 mM H_2O_2, and 10 µl of crude plant extract. The APX activity was expressed as µgram per µl protein ($\mu g^{-1}/\mu l^{-1}$ protein). Protein was determined according to Bradford (1976), using bovine serum albumin (BSA) as a standard. All enzyme activities were expressed as percentage of controls (Srivastava et al., 1995).

2.2.3. Leaf free proline content

Proline was assayed from freeze dried leave material, using a 3% sulfosalicylic and ninhydrin extraction buffers (Bates et al., 1973). Samples of 0.04 g dry weight of leaves was homogenized with 3% (*w*/*v*) sulfosalicylic acid and centrifuged at 3000 *g* for 10 min. A 200 µl aliquot of the supernatant was mixed with 400 µl of the reagent mixture (30 ml glacial acetic acid, 20 ml phosphoric acid and 1.25 g ninhydrin) and heated in sealed test tubes at 100 °C for 1 h. After cooling down, 4 ml toluene was added to each sample. Proline content was read on a Titertek Multiskan ® Ascent (Titertek Instruments Inc., USA) at A_{520} and expressed as µmoles per gram dry weight ($\mu mol^{-1}/g^{-1}$, D_W).

2.2.4. Leaf area (LA)

LA was measured early morning by a leaf area meter (LI-3100, LI-COR Environmental, USA). Leaves were placed one at a time, with their apical side down, on the conveyer belt passing an interrupted light source and sensor. A digital reading was noted.

2.2.5. Cell membrane stability (CMS)

Leakage of electrolytes from tissue to an external solution was measured by the electron conductivity of the solution according to the method of Sullivan (1972). Each sample (five plants) consisted of 5 leaf disks ($n = 25$), cut with a number 6 cork bore, rinsed well with distilled water. Ten milliliters of deionized water was added to each tube, and the tubes were left at room temperature of approximately 20 °C for 24 h. Conductivity was measured for the water stressed plants (T1), samples were autoclaved for 15 min, and measured again (T2). Similar readings were obtained for the control (C1) and after

autoclaving (C2). Electron conductivity was measured and expressed as:

$$\text{CMS}(\%) = [1\text{-}(\text{T1}/\text{T2})/1\text{-}(\text{C1}/\text{C2})] \times 100.$$

2.2.6. Data analysis

Results were analysed by analysis of variance (ANOVA) at a significance level of ($p \leq 0.05$) and standard deviation of the mean (SDEV) was also calculated for presentation in figures.

3. Results

3.1. Activity of the antioxidant enzymes

An indirect correlation existed between enzyme activity and RWC (Fig. 1) and RWC significantly decreased ($p \leq 0.05$) with increased water stress. Leaf RWC of control plants of all species ranged from 87 to 97% at predawn (data not shown). At maximum stress after 17 days of withholding water, the RWC levels were 33% for *A. hypochondriacus*, 48% for *A. hybridus* and 77% for *A. tricolor*, respectively. After rewatering the RWC recovery was fastest in *A. tricolor* (89%), followed by and *A. hybridus* (85%) and *A. hypochondriacus* (67%) within 2 days.

The tendency toward the increase in SOD activity over time of the genotypes tested (control) may be indicative of a possible ageing effect on SOD activity (Fig. 1). SOD activity markedly increased from days 12 to 17 of water stress and was statistically significant ($p \leq 0.05$) for *A. hypochondriacus* (RWC 33–49%), and *A tricolor* (RWC 76–87%) and *A. hybridus* (RWC 66–89%). The SOD activity (as activity of the controls) increased at an average from 98% (day 3) to 239% (day 17) in *A. hypochondriacus*, from 122% (day 3) to 167% (day 17) in *A. tricolor* and from 74% (day 3) to 128% (day 17) in *A. hybridus*. After rewatering SOD levels dropped in *A. hypochondriacus* (66%), *A. tricolor* (83%) and *A. hybridus* (73%) at a level below that of the control even after four days after re-watering.

The GR activity was generally low for all three species tested (Fig. 1). A significant increase in GR activity ($p \leq 0.05$) was noticed from day 12 to 17 for *A. tricolor*, and only on day 17 for *A. hybridus*. At maximum water stress after 17 days, GR (as activity of the control) increased noticeable for *A. hypochondriacus* (125%), *A. tricolor* (130%) and

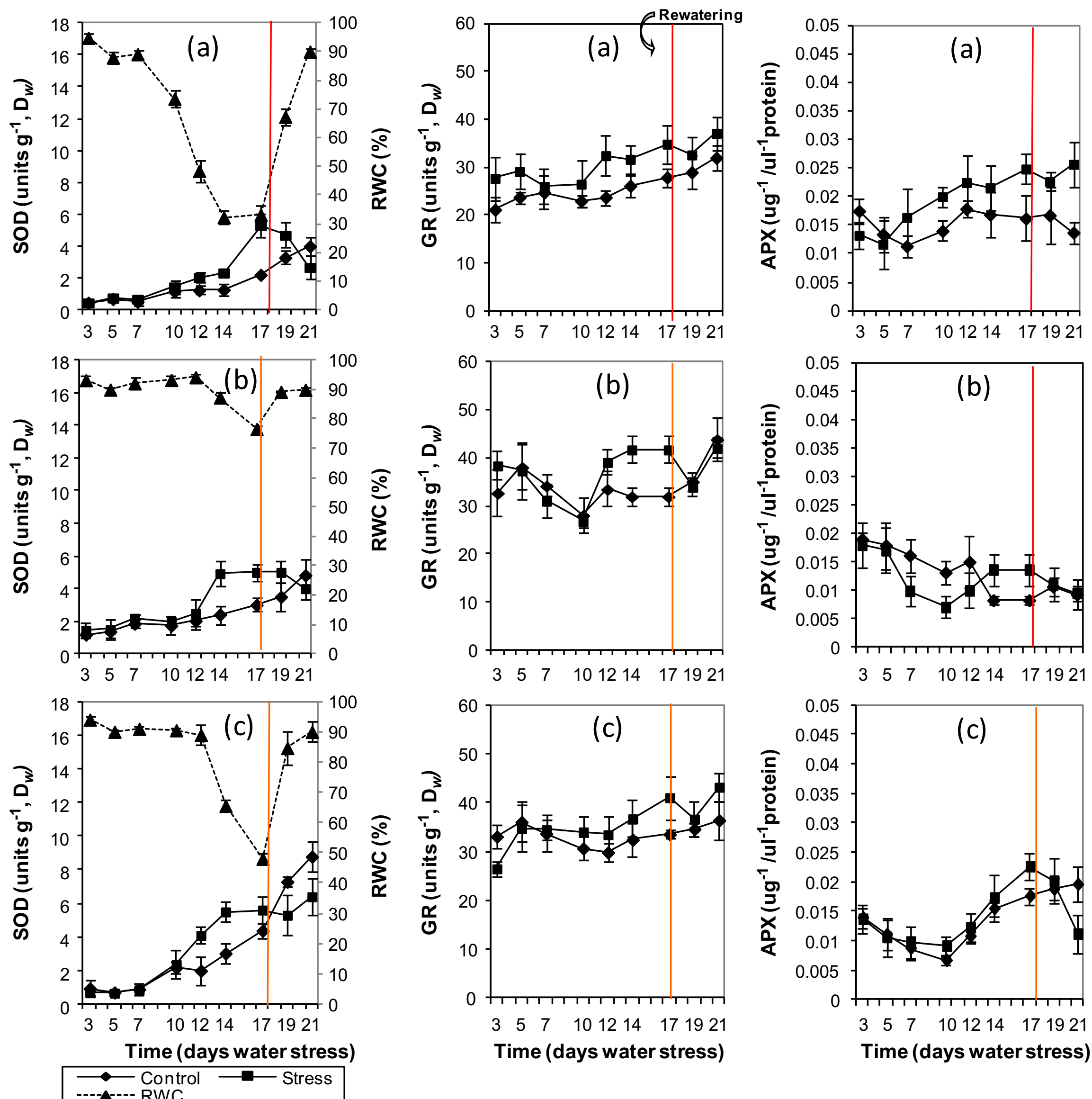

Fig. 1. Effect of increasing water stress (withholding of water) on the CuZn superoxide dismutase (SOD); glutathione reductase (GR) and ascorbic peroxidase (APX) ($\mu g^{-1}/\mu l^{-1}$ protein) levels in (a) *A. hypochondriacus*, *(b) A. tricolor* and *(c) A. hybridus* leaves in relation to RWC. Bars represent the standard deviation of the mean ($n = 5$).

A. hybridus (122%). This increase in GR activity coincided with the decrease in RWC in all three species tested. Recovery of the GR antioxidative activity was stable within 2 days after rewatering in *A. tricolor* (83%). In both *A. hypochondriacus* and *A. hybridus*, the GR activity was still high four days after rewatering (116 and 119%).

APX activity (Fig. 1), in both *A. hypochondriacus* and *A. hybridus* increased significantly ($p \leq 0.05$) over time (ageing effect) which correlated indirectly to RWC. The opposite was true for *A. tricolor* where a decrease in APX was noticed over time, with an increase in APX activity occurring only during the time of low RWC values from day 14 to 17. The average endogenous APX activity was highest for *A. hypochondriacus* (0.015 $\mu g^{-1}/\mu l^{-1}$ protein), followed by *A. hybridus* (0.014 $\mu g^{-1}/\mu l^{-1}$ protein) and *A. tricolor* (0.013 $\mu g^{-1}/\mu l^{-1}$ protein). At maximum water stress of 17 days, the APX (as activity of the control) was the highest in *A tricolor (*165%), followed by *A hypochondriacus* (154%) and *A hybridus* (128%). After rewatering APX in *A. tricolor* was equal to the control within two to four days, while in both *A. hypochondriacus* and *A. hybridus* the APX system seemed to have collapsed after rewatering for *A. hybridus* (57%) and *A. hypochondriacus* (187%) within four days.

3.2. Free proline production

The first signs of proline accumulation ($\mu mol^{-1}/g^{-1}$, D_W) occured after 12 days of withholding water (Fig. 2) for *A. hybridus* (152) and *A. hypochondriacus* (104). Higher levels of proline accumulation occured on day 14 in *A. hypochondriacus* (380), *A. hybridus* (443) and *A. tricolor* (71). Water stress significantly increased proline production ($p \leq 0.05$) and was indirectly correlated to a decrease in RWC on day 14 for *A. hypochondriacus* (32%), *A. hybridus* (65%) and *A. tricolor* (87%). Peak concentrations of proline accumulation ($\mu mol^{-1}/g^{-1}$, D_W) were reached after 14–17 days of severe water deficit, and were highest for A. *hybridus* (455), followed by *A. hypochondriacus* (380) and *A. tricolor* (104). Recovery of proline accumulation ($\mu mol^{-1}/g^{-1}$, D_W) was fastest in *A. tricolor* (24), followed by A. *hypochondriacus* (33) and *A. hybridus* (239), compared to an average of (17) for the controls, within two days after rewatering.

3.3. Leaf area and cell membrane stability

Leaf area (LA) was significantly influenced by water stress, and was directly correlated to leaf water potential (LWP) (Table 1). In all three species the LA decreased significantly ($p \leq 0.05$) by increasing the severity and duration of the water stress from 6 to 18 days. For both *A. hypochodriacus* and *A. hybridus* the LWP decreased from −0.95 and −0.63 MPa after 6 days water stress, to −2.38 and −2.87 MPa within 12 days of water stress respectively. LWP decreased from −0.58 MPa after 6 days water stress, to −2.4 MPa after 18 days water stress in *A. tricolor.*

Cell membrane stability (CMS) in *A. tricolor* and *A. hybridus* decreased only slightly coinciding with a reduction in LWP after 18 days water stress, but was still relatively high at 77 and 66% after 18 days water stress respectively (Table 1). The CMS of *A. hypochodriacus* did however decrease significantly to 32% after 18 days water stress.

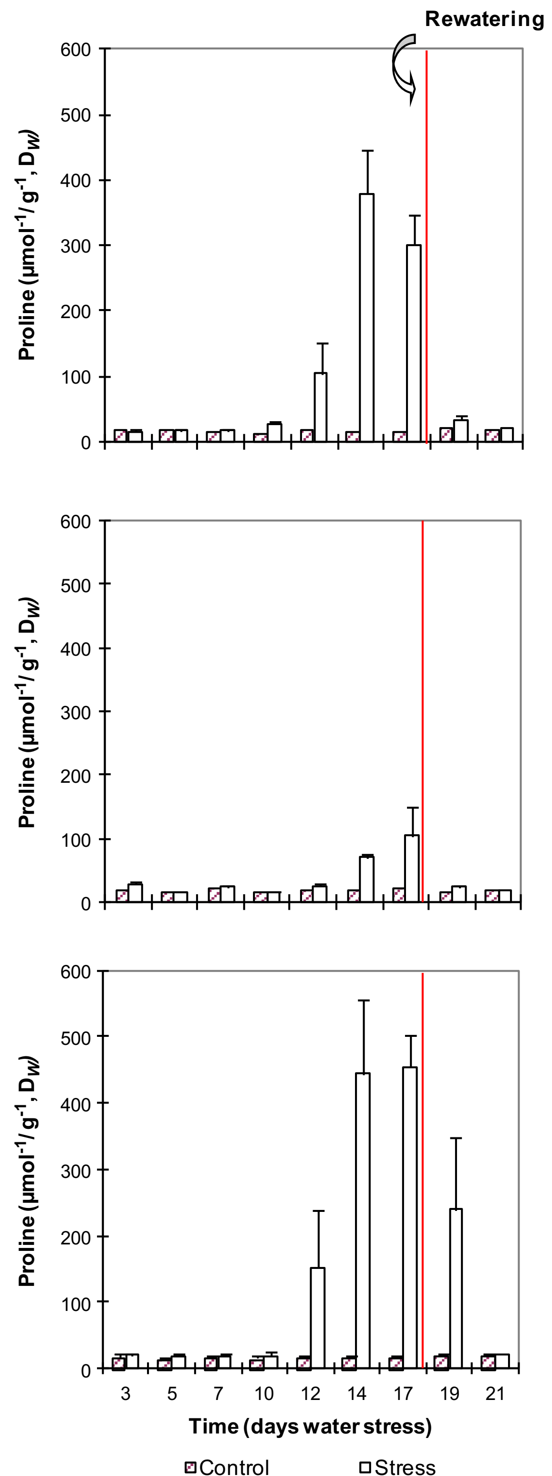

Fig. 2. Effect of increasing water stress and rewatering on proline accumulation in (a) *A. hypochondriacus*, (b) *A. tricolor* and (c) *A. hybridus* greenhouse plants. Bars represent the standard deviation of the mean ($n = 5$).

4. Discussion

4.1. Leaf water status

Drought response indices (period of withholding water) have strong associations with water loss during water deficit conditions, demonstrating that cultivars showing low water loss, are more drought tolerance (Abdalla and El-Khoshiban, 2007; Hassanzadeh et al., 2009). RWC measurement is a general method used to determine leaf water balance in plants during water deficit periods (Uzildaya et al., 2012) and estimates the percentage of water present in the leaf as a fraction of the total volumetric water that the leaf can hold at full turgor (Blum, 1998). When RWC can be maintained in cells and tissues, it allows continuation of the metabolic activity by osmotic adjustment and other traits of adaptation to drought (Blum and Ebercon, 1981). Recovery of RWC after rewatering is a very important and often neglected factor in drought tolerant studies. Speedy recovery of RWC in plants is important factor to consider for food production in marginal areas since periods of drought is typically followed by unpredictable

Table 1
Leaf water potential, leaf area and cell membrane stability in leaves of *A. hypochondriacus*, *A. tricolor* and *A. hybridus* during 18 days water stress and recovery after rewatering.

Drought stress (days)	Leaf water potential (-MPa)	Leaf area[a] (mm^2)	Cell membrane stability (%)
	A. hypochondriacus		
Control	0.58 ± 0.15	0.00	88.48 ± 8.94
5	0.95 ± 0.15	3.96 ± 15.42	89.08 ± 5.79
10	2.38 ± 0.75	−33.57 ± 28.67	90.59 ± 18.41
17 (rewatered)	≤2.38	−72.27 ± 13.88	31.58 ± 14.32
21	0.50 ± 0.09	−4.00 ± 29.09	90.44 ± 14.30
	A. tricolor		
Control	0.53 ± 0.05	0.00	98.80 ± 1.15
5	0.58 ± 0.10	28.73 ± 33.28	86.22 ± 11.75
10	1.18 ± 0.57	−12.74 ± 28.50	81.68 ± 1.74
17 (rewatered)	2.40 ± 1.85	−55.20 ± 48.74	77.19 ± 3.42
21	0.55 ± 0.07	−12.21 ± 21.73	81.06 ± 2.35
	A. hybridus		
Control	0.50 ± 0.08	0.00	89.99 ± 6.38
5	0.63 ± 0.25	0.56 ± 13.07	86.09 ± 9.18
10	2.87 ± 0.25	−37.23 ± 9.11	83.42 ± 10.24
17 (rewatered)	≤2.87	−57.58 ± 15.92	66.31 ± 8.09
21	0.54 ± 0.06	−8.11 ± 4.69	83.72 ± 12.85

Data presented as the average ± Stdev.
[a] Leaf area presented as average difference between stress and control (stress-control).

rainfall patterns. For all species studied, recovery of RWC was achieved within two to four days after rewatering, indicating their ability to recover after severe periods of water stress.

4.2. Antioxidative enzyme activity

The RWC correlated indirectly with the activity of SOD, GR and APX. Our data showed that RWC in *A. hypochondriacus* decreased to a very low 33% after 17 days severe drought stress. Similarly a sharp decline in RWC below 30% was experienced with several Bermuda grass spp. (Hu et al. (2010)), and RWC below 40% with spruce sp. (Blödner et al., 2005) and 45% in maize (Efeoghu et al., 2009) during severe water stress. The water stress-induced increases in SOD, APX and GR activity seemed to work in a concerted fashion to convey drought tolerance by scavenging oxygen radicals. Increased SOD and GR activity occurred after 10–12 days water deficit and for APX already after 7 days moisture stress in two of the species tested. APX activity for *A. tricolor* was higher in the stress than the control from days 14 to 17, similarly APX activity in control plants was higher in *C. spinosa* and *C. gynandra* after 5 and 10 days water stress, indicating vital role in scavenging H_2O_2, in the case of short term stress (Uzildaya et al., 2012). Increased SOD production has been correlated to tolerance to cold stress (Gao et al., 2009), ozone stress (Calatayud et al., 2010) and water stress (Ahmed et al., 2009; Farrant, 2000). SOD is thus rated a critical component of active oxygen scavenging system of plant chloroplasts, and it is known that SOD can respond to an environmental change within a few hours (Scandalios, 1990) as was the case for water stressed amaranth plants. SOD converts superoxide radicals (O^{-2}) into hydrogen peroxide (H_2O_2), and APX uses ascorbate as an electron donor to reduce H_2O_2 to water. The main function of APX is the removal of toxic H_2O_2 and thereby protecting plants during oxidative stress (Kuk et al., 2003).

GR activity increased during severe water stress. GR catalyses the NADP-dependent reduction of GSSG to generate reduced glutathione which plays an important role during the removal of dioxygen under stress conditions (Hakam and Simeon, 1996). The regeneration of GSH from oxidized glutathione (GSSG) by GR is very important since only the reduced form of GSH can take part in the removal of active oxygen species (Foyer and Halliwell, 1976). The enhanced activity of GR in amaranth during water stress conditions recorded in the present study could have been responsible for the maintenance of the reduced glutathione ratio required to protect the regulatory enzymes of the C_4 and Calvin cycles against the inactivation by toxic thiol derivatives. The loss of function observed in plants exposed to biotic and abiotic stress results from oxidative damage to membranes, DNA and proteins (Foyer et al., 1997). Furthermore, there is considerable evidence that glutathione (GSH) plays an important role in the defense systems of plants as an antioxidant as well as in gene activation that lead to stress tolerance and other defense responses stresses.

Increased APX, SOD and GR activities in tolerant plants could reduce the amount of damage caused by various stress conditions (Dawood et al., 2014; Sun et al., 2007; Yin et al., 2005). For the amaranth species tested, the activities of GR, APX and SOD are affected differently during water deficit, with respect to the timing and extend of response. Although the single effect of each enzyme tested could be too low in some cases to play an important role in protecting cells against oxidative damage during drought stress, the combined effect of the increased H_2O_2 scavenging enzyme action in amaranth could ensure higher levels of regulation of the toxic effect of H_2O_2, which could be associated with maintaining the redox balance during oxidative stress. Such control may contribute to increased drought tolerance in *Amaranthus*.

4.3. Leaf free proline content

We found distinct genotypical differences in the effect of severe water deficit on the onset and the amount of proline accumulated. The earliest and highest proline accumulation occurred in *A. hybridus* and *A. hypochondriacus*, while only a slight increase in proline production occurred in *A. tricolor* over the same period of time. The RWC of *A. tricolor* was however high which shows that water stress was not experienced at this time. This means that the increased production of proline in amaranth during water deficit could be an indication of plant water status, rather than drought tolerance, as also documented by Lazcano-Ferrat and Lovatt (1999). Recovery after rewatering was fast in both *A. tricolor* and *A. hypochondriacus* and proline levels decreased when the RWC increased. According to Abdalla and El-Khoshiban (2007) stomata apertures are closed during water stress, photosynthetic rate declines while respiration rate is increased so as to provide some hydrolysate which is prerequisite for raising the osmotic potential, thus increasing cell turgor and eventually growth presumes once more after rewatering. Free proline may be acting as a storage compound for both carbon and nitrogen during water stress when both starch and protein synthesis are inhibited. Such a storage compound might be utilized for growth upon rewatering, and after rewatering the enhanced level of proline decreases rapidly (Hare et al., 1998).

It is possible that both proline accumulation and antioxidative enzyme activities could be used as an index of drought tolerance (Ahmed et al., 2009). The higher proline accumulation accompanied by higher enzyme activities of SOD, APX and CAT could suggest that the antioxidative defense mechanism is activated by increased proline production (Ahmed et al., 2009; Yan et al., 2000). We did not find a positive correlation between proline production and turgor manintenance or leaf area, indicating that proline does not play a role in osmoregulation or osmotic adaptation, as was also found by Sanchez et al. (1998) and Gomes et al. (2010). According to Sanchez et al. (1998) the pea cultivars which accumulated more proline had a lower water contents upon turgor loss. This seems to indicate that proline may play a protective role in minimizing the damage caused by dehydration by stabilizing cellular structures (Sanchez et al., 1998) or modification of cell wall proteome (Maatallah et al., 2010), a fact corroborated by the relative small decrease in CMS during severe water stress in two of the species tested in this study. According to Gomes et al. (2010) high membrane stability in cells of drought-stressed coconut, as indicated by electrolyte leakage measurements and low drought-induced photoinhibition support the hypothesis of the protective role played by proline under severe water stress ($\Psi_{PD} = -1.2$ MPa). According to our results, greenhouse screening for leaf antioxidative enzymes and proline production in amaranth provides a good indication of genotype variation concerning the onset and severity of drought experienced by the plant, the water relations

of the plant and the recovery capacity, both possibly minimizing the damage caused by dehydration. Proline production and activity of the antioxidative enzymes could thus be utilized as selection criteria in breeding programs for drought tolerance in amaranth. Ultimately selection of drought tolerant amaranth genotypes is aimed at improving the productivity of crops on which the poor depend in marginal environments.

Acknowledgments

Execution of this project was possible through the Agricultural Research Council-Roodeplaat and additional funding was provided by The Water Research Commission of South Africa (WRC Report no. 944/1/04). We thank Tania Caetano & Helen Phasha for technical assistance.

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