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Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments via soluble sugar and free proline

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ABSTRACT

Sweet potato (*Ipomoea batatas* (L.) Lam.) is one of the most important carbohydrate rich crops and provides nutritional value-added products. However, its growth and yield is limited by water availability, especially in the rain-dependent zones. The present study investigated the role of arbuscular mycorhizal fungi (AMF) in improving the growth and yield in two cultivars of sweet potato (Tainung 57 and PROC 65-3) under water deficit (WD) and elucidates the morphological and physiological changes upon AMF-inoculation. Root colonization in AMF inoculated sweet potato under well watering was demonstrated in both cv. Tainung 57 (57.72%) and cv. PROC 65-3 (68.75%) and it declined when subjected to water deficit condition, indicating susceptibility to drought conditions. Phosphorus content in AMF inoculated cvs. Tainung 57 and PROC 65-3 were enriched by 8.3 and 5.7 mg g^{-1} DW, respectively, more than those in the AMF un-inoculated plants. Free proline and soluble sugars play a key role in WD-stressed plants with AMF-association by adjusting osmotic potential. In PROC 65-3, free proline and soluble sugars in the leaf tissues of AMF-inoculated plants under 17.5% soil water content (SWC) were maximized (6.27 μmol g^{-1} FW and 53.94 μg g^{-1} DW, respectively). A positive correlation was revealed between osmotic potential and osmolyte accumulation. Leaf osmotic potential (Ψ_s) in plants without AMF-inoculation grown under WD condition declined, leading to total chlorophyll degradation. In contrast, this was enhanced in AMF-inoculated plants under water deficit conditions, leading to increased content of photosynthetic pigments, enhanced maximum quantum yield of PSII (F_v/F_m) and photon yield of PSII (ϕ_{PSII}), increased net photosynthetic rate and growth characteristics. Moreover, the numbers of tubers per plant and tuber fresh weight in AMF-inoculated sweet potato plants under WD stress were significantly increased, especially in cv. Tainung 57 (water deficit sensitive). The study concludes that inoculation of AMF in sweet potato plants improves plant growth characteristics and enhances water deficit tolerance via soluble sugars and free proline accumulation.

1. Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam.], a carbohydrate storage root crop, serves as an important source of food, bioethanol, carotene and micronutrients (Laurie et al., 2012). It is predominantly cultivated in the rain-fed or low precipitation areas (Gomes and Carr, 2001, 2003; Gomes et al., 2005). It has been well established that sweet potato plants grown under water deficit stress exhibit a reduction in growth, yield and impairment in various physiological responses such as low water potential (Ψ_w), pigment degradation, chlorophyll fluorescence, limit on CO_2 assimilation, low stomatal conductance (g_s), and decrease on net photosynthetic rate (P_n) (Haimeirong and Kubota, 2003; van Heerden and Laurie, 2008; Yooyongwech et al., 2014). In a previous study, we screened the native and imported cultivars of sweet potato and categorized PROC 65-3 as a drought-tolerant cultivar, and Tainung 57 as drought-susceptible (Yooyongwech et al., 2013a). The study revealed that these two genotypes serve as good models for the study of drought-tolerant mechanisms, particularly the osmo-regulation system, in sweet potato. In addition, Japanese Yellow has been categorized as a water deficit tolerant cultivar to be grown in rain fed areas. Previously, studies

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have reported that arbuscular mycorrhizal fungi (AMF) inoculation improves morphology, sugar and carotene contents and tuber yield in sweet potato (Farmer et al., 2007; Saraswati et al., 2012; Tong et al., 2013). However, the studies pertaining to physiological attributes of an underlying protective mechanism are limited. Under drought conditions, AMF-inoculated plants contain multiple defense mechanisms such as regulation of aquaporin proteins, phosphorus enrichment and sugar transporter proteins in the roots, accumulation of osmolytes (proline and sugars) in the leaves and roots, and increases in antioxidants (Rodriguez et al., 2004; Wu et al., 2013; Rapparini and Peñuelas, 2014). The accumulation of free proline and soluble sugars in AMF-inoculated plants under drought stress have been well established in several plant species, such as *macadamia* (Yooyongwech et al., 2013b), pistachio (Abbaspour et al., 2012), soybean (Grümberg et al., 2015), *Cyclobalanopsis glauca* (Zhang et al., 2014) and citrus (Wu and Xia, 2006; Wu et al., 2007). In a recent study, it has been shown that in AMF-associated citrus plants grown under water deficit Δ^1-pyrroline-5-carboxylate synthetase (P5CS), a key enzyme of proline biosynthesis, is up-regulated, whereas proline dehydrogenase (PDH), involved in proline degradation, is down-regulated (Zou et al., 2013). Qiao et al. (2012) found that glutathione-*S*-transferase (GST) genes are up-regulated in pigeon pea inoculated with AMF is regulated under drought condition. In addition, non-enzymatic antioxidants such as anthocyanins, carotenoids and ascorbic acid are accumulated in AMF-associated lettuce plants under water deficit stress (Baslam and Goicoechea, 2012). We hypothesized that osmolytes regulated by AMF-association in a drought sensitive cultivar of sweet potato grown under water deficit stress may increase water use efficiency at a cellular level and subsequently improve the growth and tuber yield. The present study involves experimentation on AMF-inoculated plants and w/o AMF in two genotypes of sweet potato, Tainung 57 and PROC 65-3. Physiological, biochemical and morphological traits were assessed when plants were subjected to water deficit stress. Therefore, the objective of this investigation was to elucidate the physiological changes i.e., free proline and total soluble sugars during osmotic adjustment in AMF-inoculated sweet potato plants under water deficit stress.

2. Materials and methods

2.1. Plant materials and water deficit treatments

Two sweet potato genotypes, PROC 65-3 (water deficit tolerant) and Tainung 57 (water deficit sensitive) obtained from Agricultural Extension Group, Phichit province, Thailand, were used as master stock materials (Yooyongwech et al., 2013a). A vine cutting (15 cm in length) without leaf blades was propagated and planted into plastic pots (ø = 20 cm) containing 2 kg mixed soil (*EC* = 2.687 dS m^{-1}; pH 5.5; organic matter = 10.36%; total nitrogen = 0.17%; total phosphorus = 0.07%; total potassium = 1.19%). The soil was sterilized before mixing with arbuscular mycorrhiza fungi (+AMF; 4 g per pot) or without AMF (−AMF). In +AMF treatment, AM fungus powder "Micoriza® provided by Department of Agriculture was inoculated in the soil (25 living spores g^{-1} of *Glomus* sp. and *Acaulospora* sp.). The cuttings planted in the pot culture were incubated in a greenhouse under 500–1,000 μmol m^{-2} s^{-1} photosynthetic photon flux density with a 10 h d^{-1} photoperiod, 28 ± 2 °C ambient temperature and 80 ± 5% relative humidity, for 4 weeks. In all, the experimental design included four treatments: well watering (WW; 47.45% soil water content), water deficit (WD; 17.5% soil water content, by water withholding for 15 days), WW + AMF and WD + AMF.

2.2. AMF colonization

Fresh root samples (3.0 ± 0.5 cm in length) of sweet potato in each treatment were collected, washed by distilled water and then cut into 1.0 cm lengths and kept in 60% ethanol (storage solution). Roots were washed thrice by distilled water, transferred to 10% KOH and incubated in 95 °C for 30 min as cleaning procedure. Cleaned roots were washed by distilled water and then strained using 0.05% (w/v) Trypan blue for 15 min. AMF-colonization in the roots was observed under light microscope (Zeiss, Germany) to count the arbuscules, vesicle and mycorrhizal hyphae according to the method outlined by Brundrett et al. (1996).

2.3. Biochemical changes

Available phosphorus was extracted and determined spectrophotometrically as blue molybdate–phosphate complexes under partial reduction with ascorbic acid (Jackson, 1958). Briefly, five-hundred milligrams of dried leaf samples in each treatment were ground, transferred to 1 mL digestion mixture (0.42 g Se, 14 g $LiSO_4 \cdot 2H_2O$ to 350 mL H_2O_2, and 420 mL H_2SO_4) and then placed on the hot plate (gradually increased from 50 to 150 °C) until the mixture turned back. Five-hundred microliters of 72% $HClO_4$ were added to each sample and heated until the material became colorless. After cooling, the samples were diluted with equal volume of $HClO_4$, filtered (Whatman #42) and then mixed with 0.5 mL of Barton's reagent [25 g ammonium molybdate (400 mL), 1.25 g ammonium meta-vendate (350 mL) and HNO_3 (250 mL)] for 10 min. Total phosphorus (%) were measured at

Table 1
Vine fresh weight, root fresh weight, root dry weight and number of tuber per plant of sweet potato cv. Tainung 57 and PROC 65-3 pretreated with (+) or without AMF (−) subsequently exposed to well-watering (WW; 47.45% SWC) or water deficit (WD; 17.5% SWC) then recovery until harvesting (150 d after sowing).

Cultivar	AMF	Irrigation	Vine fresh weight (g)	Root fresh weight (g)	Root dry weight (g)	Number of tuber per plant
Tainung 57	–	WW	25.70a	5.71bcd	2.93cd	2.67a
	–	WD	14.06b	3.20d	0.88d	2.00b
			(45.29%)	(43.96%)	(69.97%)	(25.09%)
	+	WW	24.15ab	4.26cd	2.32d	2.67a
	+	WD	21.29ab	3.58d	1.16d	2.33ab
			(11.84%)	(15.96%)	(50.00%)	(12.73%)
PROC 65-3	–	WW	23.10ab	8.21ab	5.22b	2.33ab
	–	WD	15.83b	4.81cd	2.52cd	2.00b
			(31.47%)	(41.41%)	(51.72%)	(14.16%)
	+	WW	24.64a	11.46a	9.35a	2.67a
	+	WD	21.15ab	7.00ab	6.08ab	2.33ab
			(14.16%)	(38.92%)	(34.97%)	(12.73%)

Different letters in each column show significant difference at $p \leq 0.01$ by Tukey's HSD. Figs. in parentheses represent growth reduction percentage of water-deficit stressed plants over control in each cultivar.