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Response of water deficit-stressed *Vigna unguiculata* performances to silicon, proline or methionine foliar application

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ABSTRACT

Environmental stresses are increasing over time adversely affecting crop productivity. To face these stresses, plants adopt endogenous mechanisms, but they are not enough in most cases. Therefore, the effect of exogenous application of proline, silicon (Si) or methionine on growth and yield criteria, leaf physio-biochemical properties and leaf anatomical structure of cowpea plants grown under three levels of water deficit (W_0; 60, W_1; 40 and W_2; 20% of soil water holding capacity) was investigated. The water level W_0 was adequate to optimum cowpea plant growth and specified as a control. Results obtained indicated that growth criteria (i.e., shoot dry weight, plant height, leaf area and number of branches per plant), yield characteristics (i.e., dry seed weight and biological yield per plant, and 100-seed weight), contents of leaf chlorophylls *a* and *b*, total carotenoids, shoot and seed nutrients (i.e., N, P and K), and leaf relative water content and membrane stability index were significantly decreased, while activity of leaf antioxidant enzymes such as peroxidase (POX), superoxide dismutase (SOD) and catalase (CAT), content of leaf proline and electrolyte leakage, and shoot Si were significantly increased under water deficit stress (W_1 and W_2) conditions compared to the control (W_0). However under water deficit stress, foliar application of proline, Si or methionine seemed to overcome the harmful effects of water deficit stress, at varying degrees, on the abovementioned cowpea plants characters, which were improved, compared to the corresponding controls. The Si was the most helpful one, where it increased growth criteria (i.e., shoot dry weight, plant height, leaf area and number of branches per plant), yield characteristics (i.e., dry seed weight and biological yield per plant, and 100-seed weight), contents of leaf chlorophylls *a* and *b*, total carotenoids, shoot and seed nutrients (i.e., N, P and K), and leaf relative water content and membrane stability index, and further increased shoot Si content and leaf antioxidant enzyme activities of cowpea plants compared to those of either proline or methionine application. For leaf anatomical features, the width of midvein and xylem, and the thickness of midvein, phloem and xylem tissues, and palisade and spongy tissues of leaf blade were decreased with increasing the water deficit stress; however, foliar spray of Si improved all histological features compared to those of untreated plants.

1. Introduction

Cowpea (*Vigna unguiculata* L.) is an important legume crop for human and livestock feed because it is a cheap source of protein. Cowpea vines and leaves, or its silage is often used as forage for feeding the livestock and pigs. The residues of seed production are an important by-product in sub-Saharan Africa (Singh et al., 2010), and pod husks obtained after threshing are also used for feeding the livestock (Oluokun, 2005).

Water deficit (commonly known as drought) is an abiotic stress, which negatively affects performances of crop plants. Under prolonged water deficit, many crop plants are dehydrated and died. Water deficit stress reduces the plant-cell's water potential and turgor elevating solute concentrations in the cytosol and extracellular matrices. The negative effects of water deficit on mineral nutrition (nutrient uptake and transport) and metabolism lead to a decrease in leaf area and alteration in assimilate partitioning among the organs. Responses to water deficit are complex and various mechanisms are adopted by plants when they encounter water deficit. They include water deficit escape by rapid development allowing plants to finish their cycle before death, water deficit avoidance by, for example, increasing water uptake and reducing transpiration rate by the reduction of stomatal conductance and

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Table 1
Some physical and chemical properties of the investigated soil.

Property	Value
Soil particles distribution:	
Sand (%)	24.7
Silt (%)	27.3
Clay (%)	48.0
Textural class	Clay
Silicon content (SiO_3^{2-}; mg kg^{-1} soil)	9.68
Organic matter (%)	1.09
Calcium carbonate ($CaCO_3$; %)	1.25
Field capacity (%)	34.52
EC_e[a] (dS m^{-1} in soil paste extract)	1.14
pH (Soil paste)	8.07
Ions (mmolc L^{-1} in soil paste extract):	
Ca^{2+}	3.52
Mg^{2+}	2.33
Na^{+}	3.22
K^{+}	2.40
HCO_3^{-}	1.88
Cl^{-}	5.57
SO_4^{2-}	4.02
Available nutrient (mg kg^{-1} soil):	
N	107
P	8.85
K	165

[a] EC_e means electrical conductivity.

leaf area, water deficit tolerance by maintaining tissue turgor via osmotic adjustment allowing plants to maintain growth, and resisting the severe stress through other survival mechanisms (Jones, 2004).

Nowadays, a few numbers of materials are used to alleviate water deficit stress effects in plants. Some of these products that potentially improve water deficit stress tolerance are inorganic or organic substances. Silicon (Si) is the second most abundant element on the earth surface; however its role in plant biology has been poorly understood. So, the attempts to associate the Si with metabolic or physiological activities have been inconclusive (Epstein, 1994). Recently, many works have demonstrated to improve plant growth and productivity of crop plants under water deficit stress (Kaya et al., 2006; Shen et al., 2010; Shi et al., 2014, 2016). Proline plays a major role in the process of osmotic adjustment in many different organisms including higher plants (Hasegawa et al., 2000) to increase their water deficit stress tolerance. It increases the concentration of the culture osmotic components in order to equalize the osmotic potential of the cytoplasm (Wated et al., 1983). It also acts as a free radical scavenger to alleviate water deficit stress effects (Okuma et al., 2000). Another substance to test its role in alleviating the water deficit stress effects, methionine is one of the essential amino acids that participate in a variety of physiological functions (e.g., it is involved in single carbon metabolism, building block of proteins and as a component of the universal activated methyl donor *S*-adenosylmethionine) (Giovanelli et al., 1985; Golan et al., 2005).

All previous studies have handled the ameliorative effects of one substance/antioxidant alone on water deficit stressed-crops, while the current study evaluated the influences of three substances (i.e., proline, silicon or methionine) on mitigation of water deficit stress effects on cowpea plants. In the present study, we have evaluated the roles of Si, proline and methionine in increasing water deficit stress tolerance in cowpea and maintaining plant growth and its relative water content, and protecting cowpea plant cells from oxidative damage under water deficit stress. The results presented here provide new insight into evaluating the role of three substances (i.e., proline, silicon or methionine) in improving water deficit stress tolerance and alleviating water deficit stress-induced damage in cowpea plants by the action of these substances on physio-biochemical attributes, antioxidant defense system, and water use efficiency.

2. Materials and methods

2.1. Growth conditions, experimental design and treatments

During two successive growing summer seasons (2014 and 2015), two pot experiments were carried out in a greenhouse of Soil Science Department, Faculty of Agriculture, Zagazig University, Sharkyya Governorate, Egypt.

Healthy cowpea (*Vigna unguiculata* L., cv. Doki 331) seeds were obtained from Vegetable Research Section, Horticulture Research Institute, Agriculture Research Centre, Giza, Egypt. After washing with distilled water, sterilizing using 1% (v/v) sodium hypochlorite for approximately 2 min and washing thoroughly again with distilled water,

Table 2
Effects of water deficit stress and foliar spray with proline, silicon (Si) or methionine on growth and yields characteristics of cowpea plants.

Source of variation	Parameters							
	Plant height (cm)	No. of leaves $plant^{-1}$	Leaf area (cm^2)	Shoot dry weight $plant^{-1}$ (g)	Number of pods $plant^{-1}$	Dry seed weight $plant^{-1}$ (g)	Biological yield $plant^{-1}$ (g)	100-seed weight (g)
Water deficit (W)	*	*	*	*	*	*	*	*
W_0 (60% of WHC[a])	50.1 ± 4.0a	22.7 ± 1.8a	41.8 ± 3.3a	13.78 ± 1.1a	16.3 ± 1.3a	18.6 ± 1.5a	41.8 ± 3.3a	26.1 ± 2.1a
W_1 (40% of WHC)	42.1 ± 3.4b	17.4 ± 1.4b	35.2 ± 2.8b	9.67 ± 0.77b	13.4 ± 1.1b	14.6 ± 1.2b	33.1 ± 2.6b	22.6 ± 1.8b
W_2 (20% of WHC)	24.8 ± 2.0c	11.2 ± 0.9c	18.5 ± 1.5c	4.29 ± 0.34c	3.7 ± 0.4c	4.8 ± 0.4c	11.9 ± 0.9c	14.1 ± 1.0c
Antioxidant (A)[c]	*	*	*	*	*	*	*	*
DW[b]	34.7 ± 2.8c	13.6 ± 1.1c	25.1 ± 2.0d	7.06 ± 0.56c	9.4 ± 0.7c	11.0 ± 0.8c	25.1 ± 2.0c	19.1 ± 1.5d
Proline (6.0 mM)	39.4 ± 3.2a	17.2 ± 1.4b	31.2 ± 2.5b	9.16 ± 0.73b	11.5 ± 0.8b	13.0 ± 1.0b	29.8 ± 2.4b	20.6 ± 1.5b
Si (2.0 mM)	40.6 ± 3.1**a**	18.6 ± 1.5**a**	33.4 ± 2.7**a**	10.51 ± 0.84**a**	12.5 ± 0.9a	14.4 ± 1.1**a**	33.0 ± 2.6**a**	22.5 ± 1.8**a**
Methionine (4.0 mM)	37.3 ± 3.0b	16.9 ± 1.4b	28.3 ± 2.3c	8.77 ± 0.72b	11.0 ± 0.8b	12.3 ± 1.0b	27.8 ± 2.2b	18.6 ± 1.6c
W × A interactions	*	*	*	*	*	*	*	*

Values are means ± SE (n = 9). Mean values in the same column for each trait followed by the same letter are not significantly different according to Duncan's multiple range test at $P \le 0.05$.
[a] WHC means soil water holding capacity.
[b] DW means distilled water, W means water deficit, and * means significant at $P \le 0.05$.
[c] Antioxidant; all parameters are means of measurements under the three levels of water deficit (60, 40 and 20% of WHC).