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PHENOTYPING ROOT SYSTEM ARCHITECTURE OF COTTON (*GOSSYPIUM BARBADENSE* L.) GROWN UNDER SALINITY

SHADY A. MOTTALEB[1*], ESSAM DARWISH[1], MENNA MOSTAFA[2], AND GEHAN SAFWAT[2]

[1]Cairo University, Egypt
[2]October University for Modern Sciences and Arts, Egypt

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Soil salinity causes an annual deep negative impact to the global agricultural economy. In this study, the effects of salinity on early seedling physiology of two Egyptian cotton (*Gossypium barbadense* L.) cultivars differing in their salinity tolerance were examined. Also the potential use of a low cost mini-rhizotron system to measure variation in root system architecture (RSA) traits existing in both cultivars was assessed. Salt tolerant cotton cultivar 'Giza 90' produced significantly higher root and shoot biomass, accumulated lower Na^+/K^+ ratio through a higher Na^+ exclusion from both roots and leaves as well as synthesized higher proline contents compared to salt sensitive 'Giza 45' cultivar. Measuring RSA in mini-rhizotrons containing solid MS nutrient medium as substrate proved to be more precise and efficient than peat moss/sand mixture. We report superior values of main root growth rate, total root system size, main root length, higher number of lateral roots and average lateral root length in 'Giza 90' under salinity. Higher lateral root density and length together with higher root tissue tolerance of Na^+ ions in 'Giza 90' give it an advantage to be used as donor genotype for desirable root traits to other elite cultivars.

Key words: *Gossypium barbadense* L., salinity stress, phenotyping, root system architecture

Soil salinity is estimated to cause losses in crop production of about 27.3 billion US dollars annually (Qadir *et al.* 2014). The effects that excess Na^+ cations present in saline soils have on plant physiology are devastating, ranging from ion toxicity and physiological drought to reactive oxygen species (ROS) formation and cell death (Munns & Tester 2008). Although plants have developed a set of strategies to tolerate salinity stress (Roy *et al.* 2014), the majority of economically important crop plants are considered glycophytes and are severely affected by high Na^+ concentration with an evident trade-off between yield and salinity tolerance.

Salinity tolerance among cotton germplasm varies widely, both intra- and interspecifically where, for example, *Gossypium barbadense* varieties were reported to be more tolerant to salinity than *Gossypium hirsutum* or *Gossypium arboreum* cottons (Abul-Naas & Omran 1974). Phenotypic variability of cotton root traits was reported to be present for root weight, length, volume, total dry matter, and shoot-to-root ratio in *G. hirsutum* germplasm (Basal *et al.* 2003; Aboukheir *et al.* 2008). However, not only very little is known about phenotypic variability of other important RSA traits such as lateral root length and density but also assessing its available variability in Egyptian cotton (*G. barbadense*), an economically valuable species, is lacking.

Plant root system architecture (RSA), the spatial distribution of the root system within the rooting volume, controls the fate of the plant through its efficiency of anchorage to the soil as well as water,

Shady A. Mottaleb (*Corresponding author), Essam Darwish, Agricultural Botany Department – Plant Physiology Division, Faculty of Agriculture, Cairo University, Egypt. E-mail: shady.osama@agr.cu.edu.eg
Menna Mostafa, Gehan Safwat, Faculty of Biotechnology, October University for Modern Sciences and Arts, Egypt

nutrients uptake and abiotic stress tolerance. Modifying RSA traits was reported to contribute in improving grain yield and drought tolerance (Steele *et al.* 2013; Uga *et al.* 2013). Nevertheless, studying RSA is difficult regarding root sampling measurement, and plasticity in response to various environmental stimuli (Julkowska & Testerink 2015). Several methods have been proposed to study RSA, including hydroponics (Tuberosa *et al.* 2002), rhizotrons (Devienne-Barret *et al.* 2006), mini-lysimeters (Udayakumar *et al.* 1998), and PVC tubes (Taylor *et al.* 1991).

The present study aims to investigate the existence of variability in RSA under salinity stress in Egyptian cotton as well as identify potential new traits beneficial for salinity tolerance. Using a morphological and physiological approach to study two Egyptian cotton cultivars differing in salinity tolerance, cv. 'Giza 45' (salt sensitive) and cv. 'Giza 90' (salt tolerant), evidence will be provided that phenotypic variability in RSA is present in Egyptian cotton cultivars. Also, the possibility of phenotyping this variability will be described using a simple mini-rhizotron system. Finally some recommendations on the optimum type of substrates and conditions to be used for an accurate phenotyping of Egyptian cotton roots at seedling stage are given.

MATERIAL AND METHODS

The present study was carried out during January–July 2016 in the Plant Physiology division, Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Egypt.

Plant material

Egyptian cotton (*Gossypium barbadense* L.) cultivars 'Giza 90' (salt tolerant) and 'Giza 45'(salt sensitive) were used in our experiments, both obtained from the Cotton Research Institute, Agricultural Research Centre, Giza, Egypt.

Mini-rhizotron description

Mini-rhizotron system allows a non-destructive study of root development during early stages of seedling growth. It usually contains a thin layer of substrate that directs the roots to grow in 2D conditions, facilitating the monitoring and measurement of root system morphology and architecture. The mini-rhizotron used in our experiments consisted of two glass sheets of 30 × 30 cm separated by a 3 mm wide glass separator. The inner space available for culture substrate was approximately 250 cm^3.

Experimental design and plant growth conditions

Experiments were arranged as a randomized design with 3–5 replicates, and were repeated two times to confirm results. Two different experiments were performed using this mini-rhizotron in the present research work as follows:

Experiment 1

The substrate of this experiment included peat moss and sieved fine sand mixture (2:1). After the mini-rhizotron was filled with the substrate, the two glass sheets were placed over each other and sealed from the corners and below with paper clips and sellotape to keep substrate from being lost and was perforated at the bottom to allow drainage. Mini-rhizotrons were kept in a growth chamber under the following conditions: 25°C, 60% humidity, photon irradiance of 100 $\mu E/m^2/s$ and 16/8h light/dark cycle). Mini-rhizotrons were placed vertically under the angle of 70° in the growth chamber and covered with black plastic bags to provide dark conditions for roots.

Experiment 2

The substrate placed in mini-rhizotrons of this experiment consisted of sucrose-free ¼ strength MS medium (Murashige & Skoog 1962) supplemented with agarose gel 1.2% and pH was adjusted to 5.8. The same growing conditions were maintained as experiment 1.

Seed sterilization and cultivation

Seeds were sterilized in commercial sodium hypochlorite solution 1% for 10 min and then rinsed three times with tap water, and then left to germinate for two days in Petri dishes lined with water-soaked filter paper. In both experiments, one germinated seed from cultivar Giza 90 was planted at approximately equal spacing from the other cultivar Giza 45 per mini-rhizotron.

Salinity stress treatment

In Experiment 1, two days old seedlings were transferred to mini-rhizotrons and randomly divided into two groups. The first group was the control and

was watered with 100 ml of ¼ MS medium every two days during the two weeks. The second group was treated with ¼ MS medium containing 150 mM NaCl every two days also for two weeks.

In Experiment 2, using mini-rhizotron containing solid MS nutrient medium, salinity stress was imposed from the beginning using solid ¼ strength MS medium containing 150 mM NaCl, where 2 days old seedlings of both cotton cultivars were transferred to four mini-rhizotrons. In the case of control conditions, another four seedlings of both cultivars were transferred to NaCl free solid ¼ strength MS medium.

Salinity tolerance physiological traits

Leaf relative water content (RWC) was calculated according to Weatherly (1950). Fresh and dry weights were determined with the accuracy of 0.001 g on the regular lab scale. Dry weight of root and shoot tissues were measured after drying the material for 48 h at 70°C. Free proline concentration in leaves was determined according to Bates *et al.* (1973) using 0.5 g dry weight samples. Na^+ and K^+ cation contents of shoot or root dry weight samples were extracted by 0.1 M HCl solution (Garciadeblas *et al.* 2003). Determination of Na^+ and K^+ cation contents was realized using a flame photometer (Jenway PFP-7, Bibby Scientific Limited, UK).

Image capturing and analysis of RSA

Mini-rhizotrons were scanned with a Canon MG2400 series Scanner at 200 dpi at 14 days plant age. Scanned images RSA parameters were processed and quantified using EZ-Rhizo software (Armengaud *et al.* 2009). Data were collected from 3 individual seedlings per treatment per experiment. RSA parameters of control conditions were not quantified, since the roots were too entangled for the EZ-Rhizo software to detect. All data were cleared from outliers.

Statistical analysis of data

Differences among means were tested by a two-way ANOVA followed by Duncan *post hoc* test. In the case of RSA parameters, differences between means were tested by *t*-test. Differences were considered statistically significant at $P < 0.05$ in all analyses. Both statistical analyses were carried out using IBM SPSS Statistics V. 20 (IBM, USA).

RESULTS

Cv. 'Giza 90' accumulates lower Na^+ and higher proline contents than 'Giza 45'

RWC was significantly decreased by almost 10% under 150 mM NaCl treatment in both cultivars but with no differences between them (Table 1). Nevertheless, salinity treatment significantly increased the content of Na^+ but not K^+ in both shoots and roots as compared to plants under control conditions. These increases in Na^+ concentrations were significantly higher in cv. 'Giza 45' making it less efficient than cv. 'Giza 90' in eliminating Na^+ to the outside of root cells. This was reflected by a significantly less decrease in K^+ / Na^+ ratio in cv. 'Giza 90' roots under salinity. However, cv. 'Giza 90' showed similar values to "Giza 45" under control conditions. In addition, the ability to synthesize proline under salinity stress in cv. 'Giza 45' was found to be significantly lower than cv. 'Giza 90'. Leaf proline contents of cv. 'Giza 90' were one-fold higher than cv. 'Giza 45' under salinity stress, being *ca.* 1.93 and 4.17 mg/g leaf dry weight basis in cvs. 'Giza 45' and 'Giza 90', respectively. Surprisingly, the synthesis of proline in cv. 'Giza 90' leaves under control conditions was similar to the value of cv. 'Giza 45' leaf proline under salinity conditions.

Salinity stress severely hinders root growth rate of cv. 'Giza 45'

Hidden lateral roots in the thin layer of substrate (Figure 1) made possible to only examine and measure growth of the main root of both cotton cultivars. Root growth rate of both cultivars was significantly hindered under 150 mM NaCl treatment compared with control (Table 1). Nevertheless, root system of cv. 'Giza 45' was apparently more sensitive to salinity as the decrease in its main root growth rate was more significant than cv. 'Giza 90' under 150 mM NaCl, giving values of approximately 11.1 and 16.6 mm/day, respectively.

Cv. 'Giza 90' gives higher root and shoots biomass under salinity

Harvesting intact root system from peat moss/sand substrate was difficult to achieve in both treatments of experiment 1, because significant root biomass was lost in harvesting. However, in mini-rhizotron containing solid MS medium, the whole in-

tact root system was easily harvested and washed. The effect of 150 mM NaCl was detrimental to shoots of both cultivars (Table 1) being more significantly pronounced in the case of cv. 'Giza 45'.

Root system architecture (RSA) traits under salinity of cv. 'Giza 90' is highly superior than 'Giza 45'

Solid MS media in mini-rhizotrons permitted a clear and accurate measurement of the whole root system of both cotton cultivars (Figure 2). Nonetheless, the vigorous growth under control treatment led to overlapping of lateral roots of both cultivars which prevented its analysis using EZ-Rhizo root image analysis software (Figure 2 and Supplementary Figure 1). This, however, was not the case under 150 mM NaCl, where root growth was slower and less dense. Results in Table 2 show the effect of salinity on RSA of both cultivars. Overall, the morphology of cv. 'Giza 90' was considerably superior under both control and 150 mM NaCl conditions (Figure 2). The total root system size of cv. 'Giza 90' was 2.5 folds over cv. 'Giza 45' under 150 mM NaCl. This was attributed to the significantly longer main root length of cv. 'Giza 90' as compared to cv. 'Giza 45' as well as almost a 2.5 folds bigger cumulative lateral roots length. This might imply that the effect of 150 mM NaCl on lateral roots of cv. 'Giza 45' was more profound and more detrimental. This was also reflected in cv. 'Giza 45' as a longer main root as a ratio of total root size of *ca.* 45%, which clearly demonstrates an extreme effect on lateral root growth than on main root comparing with 'Giza 90' (*ca.* 19%). Finally, this was also evident in 'Giza 90' giving higher number of lateral roots and average lateral root length values, which was almost the double in size than that of cv. 'Giza 45' in both cases.

DISCUSSION

Assessing salinity tolerance of 'Giza 90' and 'Giza 45' cotton cultivars

Egyptian cotton varieties are classified according to their salinity tolerance into three groups; salt sensitive, moderate salt tolerant and salt tolerant (Ashour & Abd-El'Hamid 1970). Curiously, early reports considered cv. 'Giza 45' salt tolerant, (El-Zahab 1971) while more recent reports classify it as salt sensitive (El-Kadi *et al.* 2006). Thus, it was important in our work to assess the degree of seedling stage salinity tolerance of 'Giza 45' and 'Giza 90'

T a b l e 1

Salinity tolerance traits studied in cvs. 'Giza 90' and 'Giza 45' cotton plants (14 days age) under control and 150 mM NaCl treatment (Experiments 1 and 2)

Experiment	Trait	Control		150 mM NaCl	
		'Giza 90'	'Giza 45'	'Giza 90'	'Giza 45'
1	Leaf RWC [%]	82.1 ± 1.8^{a}	83.2 ± 1.5^{a}	73.5 ± 1.3^{b}	77.5 ± 0.5^{b}
	Root growth rate [mm/d]	28.0 ± 2.0^{a}	19.0 ± 1.9^{b}	16.6 ± 2.5^{b}	11.1 ± 1.0^{c}
	Leaf proline [mg/g]	2.22 ± 0.12^{b}	1.53 ± 0.20^{c}	4.17 ± 0.32^{a}	1.93 ± 0.03^{bc}
	Leaf Na^+ [nmol/mg]	89.8 ± 9.4	60.6 ± 7.1^{a}	430.8 ± 37.2^{b}	648.4 ± 44.4^{c}
	Root Na^+ [nmo/mg]	310.5 ± 21.6^{a}	438.1 ± 61.5^{a}	716.6 ± 35.9^{b}	891.3 ± 57.3^{c}
	Leaf K^+ [nmol/mg]	282.2 ± 44.5^{a}	247.5 ± 53.8^{a}	302.2 ± 28.5^{a}	301.4 ± 29.2^{a}
	Root K^+ [nmol/mg]	251.1 ± 40.0^{a}	300.5 ±16.2^{a}	394.4 ± 61.0^{a}	343.4 ± 5.8^{a}
	Leaf K^+/Na^+	3.3 ± 0.9^{a}	4.2 ± 1.0^{a}	0.7 ± 0.0^{b}	0.5 ± 0.0^{b}
	Root K^+/Na^+	0.8 ± 0.1^{a}	0.7 ± 0.1^{ab}	0.5 ± 0.1^{bc}	0.4 ± 0.0^{c}
2	Shoot DWT [mg]	186.5 ± 2.6^{a}	92.7 ± 4.2^{b}	68.2 ± 10.3^{b}	25.3 ± 6.9^{c}
	Root DWT [mg]	126.2 ± 1.0^{a}	20.7 ± 1.4^{b}	104.7 ± 11.4^{a}	14.1 ± 2.7^{b}
	Shoot/Root	1.5 ± 0.7^{b}	4.5 ± 0.5^{a}	1.3 ± 0.6^{b}	2.4 ± 0.6^{b}

Each value represents the mean ± standard error of 3 replicates. Means with identical letters in the same row are not significantly different ($P > 0.05$) according to Duncan test. (Abbreviations: RWC – relative water content; DWT – dry weight)

cultivars using simple physiological measurements before starting root phenotyping.

High salinity reduces vegetative and reproductive growth of cotton (Gorham *et al.* 2010). Both plant height and leaf expansion are negatively affected in saline soils where the differentiation of nodes is suppressed (Ahmed 1994). These effects are however less accentuated in tolerant as in the case 'Giza 90' where both its shoot and root biomass are significantly higher than cv. 'Giza 45'. Salinity level of 150 mM NaCl was reported to reduce the elongation of the taproot of cotton plants by 60% over control plants (Zhong & Lauchli 1993). The severity of this level of salinity on the water relations of both cultivars was assessed by measuring the relative water content (RWC) in leaves (Table 1), a trait that measures of water deficit in the leaf that reflecting the dynamic water balance between water flow into and out of the tissue (Sinclair & Ludlow 1985). It is clear that under this moderate stress, the stomata are compelled to adjust their conductance to maintain more or less stable water balance in the leaves and prevent further water losses to maintain cell and tissue turgor, and this effect was similar on both cultivars.

The apparent higher efficiency of cv. 'Giza 90' in Na^+ exclusion or sequestration inside the cell vacuole might depend on the level of transcription of transporters and activity of responsible transporters such as *SOS1* and *NHX*, respectively. Also, significantly lower foliar Na^+ content accumulated in cv. 'Giza 90' was detected. This is an important trait to protect the leaves photosynthetic machinery from any damage induced by excessive Na^+ involving several mechanisms such as Na^+ xylem loading, Na^+ retrieval from the xylem and Na^+ retrieval from the shoots (Karley *et al.* 2000; Davenport *et al.* 2007). A lower Na^+ concentration in the leaves is usually expressed in more salinity tolerance. Furthermore, cell depolarization occurs under salinity makes K^+ uptake more problematic, causing a massive K^+ efflux resulting in a depletion of the cytosolic K^+ pool (Shabala & Munns 2012). Nevertheless, our results do not show any significant perturbation in K^+ levels under 150 mM NaCl treatment neither in roots nor in shoots, indicating that stress level imposed was not very severe nor extended in time (Table 1). Cytosolic K^+/Na^+ ratio, and not the absolute quantity of Na^+ *per se*, seems to determine cell metabolic competence and, ultimately, the ability of a plant to survive in saline environments (Shabala & Cuin 2008) and, thus, higher K^+/Na^+ ratio could reflect more salinity tolerance, which might seem to be the case in cv. 'Giza 90'. Nevertheless, its higher K^+/Na^+ ratio is attributed to a higher Na^+ efflux and not from higher K^+ retention (Table 1).

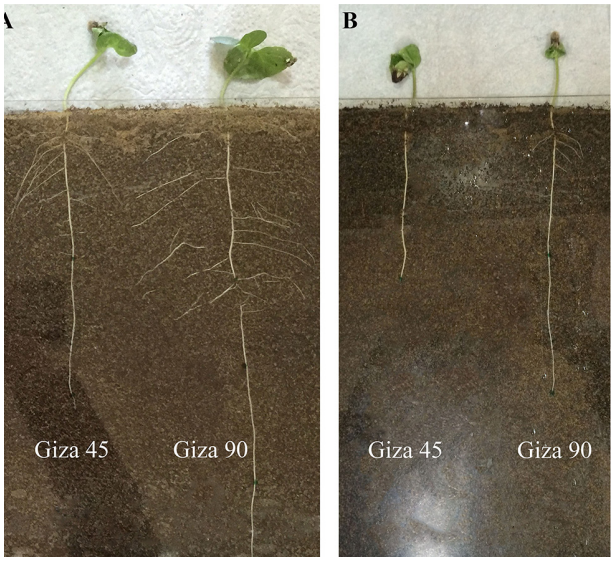


Figure 1. Root system morphology of cvs. 'Giza 90' and 'Giza 45' cotton plants (14 days age) under A) control and B) 150 mM NaCl conditions (Experiment 1)

Under salinity, cells adjust their osmotic potential by accumulating many compatible solutes which also perform many other important functions. Oosterhuis and Wullschleger (1988) reported that cotton has more osmotic adjustment capabilities than other major crops. Moreover, significant differences among cotton cultivars for osmotic potential exist, suggesting that genotypic variation for osmoregulation in cotton is wide (Quisenberry *et al.* 1982). All in all, it is extensively reported that proline concentration increases in cotton with increasing soil salinity (He *et al.* 2007). It is apparent that under our experimental conditions, proline content of cv. 'Giza 90' is significantly higher than cv. 'Giza 45', giving it a superior ability to maintain its turgor under salinity, results similarly reported by El-Kadi *et al.* (2006).

Challenges in studying RSA of cotton

Root phenotype of plant seedling can be a sound predictor of later stages of plant development (Tuberosa *et al.* 2002). However, a problem we faced in studying roots of early stage cotton plants in

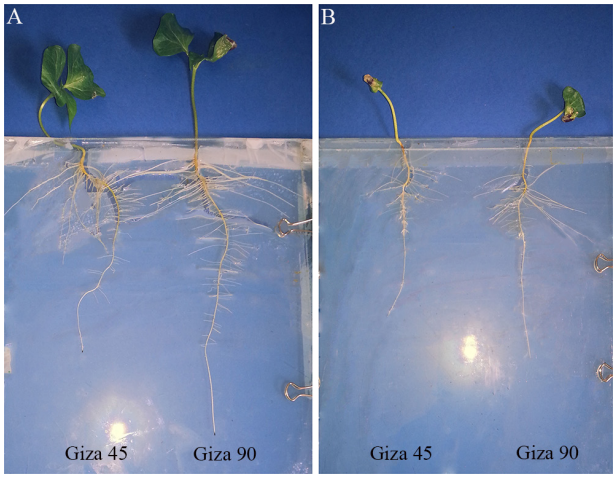
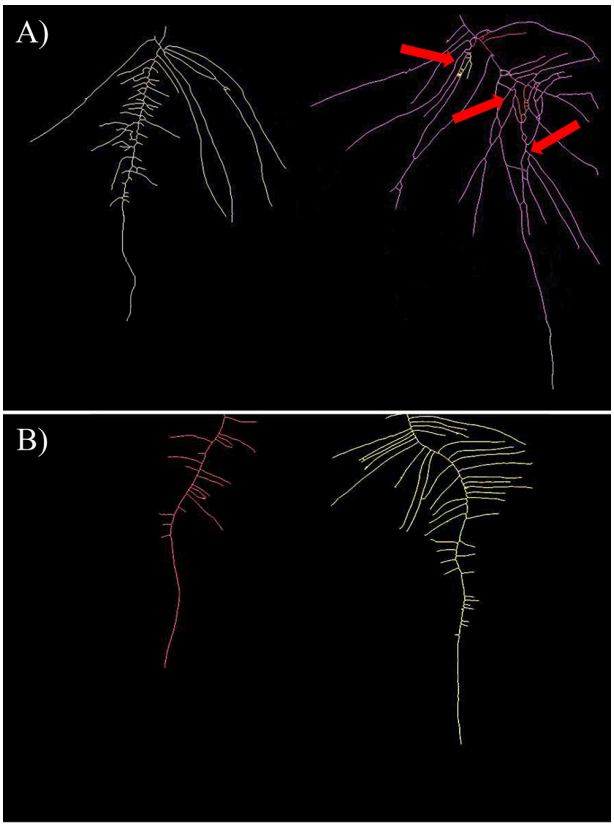


Figure 2. Root system morphology of cvs. 'Giza 45' and 'Giza 90' cotton plants (14 days age) under A) 150 mM NaCl and B) control conditions (Experiment 2)



Suppl. Figure 1. Processed image by EZ-Rhizo software of root system morphology of cvs. 'Giza 90' and 'Giza 45' cotton plants (14 days age) under A) control and B) 150 mM NaCl conditions (Experiment 2). Red arrows indicate entangled regions erroneously detected by the software

mini-rhizotron, in contrast to other crops such as tomato (Darwish *et al.* 2016), was that some lateral roots were either hidden into the soil layer and not showing over neither the front nor back glass plates of the mini-rhizotron to be scanned for subsequent image analysis. Also, some lateral roots showed at back side only. The main cause of this problem is that the lateral roots of cotton emerge on the main root in a 3D manner, which decreases the efficiency of studying RSA in 2D mini-rhizotrons, as in the case of our experiment. A possible solution to avoid this problem is to force the growth of lateral roots in an even more 2D growth by decreasing the spacing between the two mini-rhizotron glass plates even lower than 3 mm. This, however, will probably put a mechanical strain which might affect the main root growth making its growth pattern not be reliable to study. Thus, solid MS medium was used to provide a translucent environment needed to detect all lateral roots and study their RSA. Another problem we faced was that, under control conditions, the root growth was very vigorous and led to the overlapping and entanglement of lateral roots in many zones (Figure 2). This problem leads to a faulty detection of roots in Ez-Rhizo software and gave erroneous results (Supplementary Figure 1). This could be overcome by studying RSA under control conditions at earlier stages of growth (e.g. 10 days age). For this reason, it was only possible for us to study the RSA of cvs. 'Giza 45' and 'Giza 90' only under salinity stress.

Identifying potential root traits in Egyptian cotton desirable for salinity tolerance

A significant phenotypic variability in *G. hirsutum* cotton, i.e. root length, root fresh weight, root dry weight, lateral root number, lateral root dry weight, total root dry weight, root volume, and root-to-shoot ratio was reported in previous studies (Basal *et al.* 2003; AbouKheir *et al.* 2008). This variability, however, seems to be much lower in genotypes adapted to humid and high-rainfall conditions (Quisenberry *et al.* 1981). In the case of our experiments, substantial variation in root traits was detected between cvs. 'Giza 90' and 'Giza 45'. The analyzed data of mini-rhizotron root system images (Experiment 2) with EZ-Rhizo software (Table 2) shows that cv. 'Giza 90' root system architecture

under salinity conditions was significantly higher in various parameters including total root size, main root length, cumulative lateral root length, average lateral root length, number of lateral roots, length of basal and branched zones, and depth. This was also the case with main root growth rate. On the other hand, several other parameters did not show any significant difference such as length of apical zone and number of lateral roots per cm of main root. These impressive root system characteristics of the salinity tolerant cultivar cv. 'Giza 90' suggest that the allocation of photosynthate from the source to the roots is more effective than in cv. 'Giza 45', which is finally translated as a higher root biomass as shown earlier (Table 1). The body of literature published on the effect of salinity on root traits of cotton in general, and RSA in particular, is very limited (Gorham *et al.* 2010). However, a number of different root morpho-physiological traits have been proposed to be implicated as important mechanisms that impart drought tolerance in cotton, which might be beneficial in salinity tolerance as well. These include distance from transition zone to the first main lateral root, taproot weight, number of lateral roots, seedling vigour, rapidity of root system development, and root to shoot ratio and longer taproot length (Pace *et al.* 1999). Our results show that cv. 'Giza 90' possesses several of the aforementioned traits that are beneficial under drought and probably under salinity stress as well. For example, the production of significantly denser and longer lateral roots in the top soil is desired traits and especially in saline soils because salinity is lower at these areas and becomes more concentrated in deeper layers. This high density of lateral roots permits a more efficient extraction of less salinised water from topsoil and consequently the plants become less susceptible to dehydration. This trait present in salt tolerant cv. 'Giza 90' cotton suggests its advantage as a donor genotype for this particular desirable root trait to other elite cotton cultivars in any of the ongoing breeding programs for salinity and /or drought tolerance.

CONCLUSIONS

Salt tolerant 'Giza 90' cotton cultivar showed superior shoot/root biomass, higher K^+/Na^+ ratio and proline content. This superiority also holds true regarding the majority of root system architecture (RSA) parameters. The possibility of phenotyping of cotton RSA at early stage could be predictor for later developmental stages, using a mini-rhizotron system which was demonstrated being more accurate using solid MS media than peat moss/sand as substrate. Phenotypic variation in potential beneficial root traits for salinity tolerance, such as a longer and denser lateral roots in branched zone, in the

T a b l e 2

Root system architecture (RSA) parameters of cvs. 'Giza 45' and 'Giza 90' cotton plants (14 days age) measured using EZ-Rhizo software under 150 mM NaCl salinity stress (Experiment 2)

Root system architecture (RSA) parameter	'Giza 45'	'Giza 90'
Main root "MR" length [cm]	14.47 ± 0.12	19.01 ± 0.72*
Lateral roots "LR" cumulative length [cm]	19.64 ± 4.66	97.56 ± 3.98*
Total root size "cumulative length of LR and MR" [cm]	35.38 ± 6.05	120.43 ± 8.56*
Number of lateral roots per main root (#)	19 ± 0	43 ± 4*
Average length of lateral roots [cm]	0.93 ± 0.18	1.94 ± 0.17*
Average lateral root length as ratio of main root length [%]	6 ± 0	9 ± 0*
Main root length as ratio of total root size [%]	45 ± 4	19 ± 2*
Length of basal zone [cm]	1.04 ± 0.92	0.74 ± 0.54
Length of branched zone [cm]	8.42 ± 0.22	18.63 ± 1.87*
Length of apical zone [cm]	6.29 ± 2.10	7.30 ± 0.55

Each value represents the mean ± standard error of 3 replicates. Means with asterisk (*) in the same row are significantly different ($P \leq 0.05$) according to t-test.

case of 'Giza 90' cultivar, was identified. This low-cost approach using inexpensive material and open source software will allow a rapid and cost effective phenotyping of root systems present in cotton germplasm available in developing countries. The obtained results in this work will hopefully open the door for future studies including additional accessions and salinity levels allowing performing accurate correlation studies between each of the RSA and salinity tolerance parameters.

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