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SlARF4 Under Expression Improves Tolerance to Salinity in Tomato (*Solanum lycopersicum*)

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Abstract
Understanding physiological and molecular basis of plant response and tolerance to environmental constraints and abiotic stresses is important to improve plant survival, crop yield and quality. Auxin controls many aspects of plant growth and development. This phytohormone acts on the transcriptional regulation of target genes, mainly through Auxin Response Factors (ARF). However, its role in stress responses remains so far poorly studied. The current study provide clues supporting the involvement of an auxin response factor; ARF4 in tomato tolerance to salt stress. Using a reverse genetic approach, we found that the down-regulation of an auxin response factor; *SlARF4* improves tomato tolerance to salt by promoting root development and density and maintaining good development and high chlorophyll content under salt stress conditions. Our results bring new elements on auxin involvement in plant stress responses and underline the role of *ARF4* in salt tolerance. These provide new insights into tomato selection and breeding for environmental stress-tolerant.

Keywords: Auxin, tomato, ARF4, tolerance, salinity.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the major crops with significant economic and scientific interests and as in the case of other crop plants, environmental stresses negatively affect tomato growth, productivity and quality [1]. Developing new tomato cultivars with enhanced tolerance to abiotic stress can give a significant impact on global food production in many regions. Plant responses to environmental stresses are extremely complex and require the involvement of many actors; sensors, phytohormones, transcriptional factors, genes and proteins in order to prevent damage and ensure survival [2]. Several phytohormones such as abscisic acid, ethylene, salicylic acid and jasmonate were identified as key regulators in various stress responses. Auxin controls many plant growth and development processes throughout the plant life cycle, strikingly, its putative role in modulating plant growth under stress responses remains poorly understood. Auxins act via three types of transcription regulators, auxin response factors (ARFs), Aux/IAA proteins and TOPLESS (TPS) [3–5]. 22 ARFs were identified, isolated and well characterized in tomato [6]. We have recently shown that the expression several genes belonging to ARF gene family was significantly regulated by salt and drought stresses. Among the regulated genes, ARF4 expression was significantly induced in tomato plants in response to salt stress [7]. Additionally, SlARF4 regulatory region was enriched in cis-acting elements specific to salt stress response [7]. ARF4 down-regulated and co-suppressed plants showed a stress tolerance phenotype namely an increase in chlorophyll content and curled leaves [8]. In this work, we show new findings on Sl-ARF4 down-regulated tomato plants supporting the role of this gene in tomato tolerance to salinity.

MATERIAL AND METHODS

Tomato plants (*cv MicroTom*) MT wildtype and ARF4 overexpressing (ARF4-OX) and ARF4 downregulated (ARF4-AS) seeds were grown in MS/2 medium for 7 days. One week plants were then grown hydroponically for two weeks in BD liquid medium (BROUGHTON & DILLWORTH) [9]. Salt stress was performed by adding 100 mM or 150 mM of NaCl to the culture medium for 2 weeks. Shoot and root dry weights, primary root length and lateral root number were determined. Chlorophyll a and b content was calculated as described by Bassa et al., 2012 [10].

RESULTS AND DISCUSSION

Shoot and root dry weight was investigated in wildtype and ARF4 transgenic plant lines in salt stress conditions. In the absence of stress, dry weight was significantly higher in ARF4-Ox and ARF4-AS as compared to wildtype. The average of shoot fresh weight was 2,5 and 1,75 times higher than that of wildtype plants (Fig 1A). NaCl application induced a decrease in this parameter in the three tomato lines. In response to 150 mM NaCl, shoot dry weight decreased by 70% in ARF4-Ox plants and only by 35% in the ARF4-AS plants (figure 11A). In roots, the reduction of dry weight was around 70%, 78% and 33% for the wildtype and ARF4-Ox plants lines respectively (Fig 1B). The reduction in plant weight was reported in many plant species in response to salinity [11–13]. Less reduction in plant weights was although reported in response to salinity in some tolerant varieties or species such as tomato, wheat and maize [14-16]. As described above, a decrease in shoot and root fresh weights was observed in the wild-type and the ARF4-transgenic lines in response to salt and drought stresses. However, the reduction in plant weight was less pronounced in the ARF4-AS plants which suggest that ARF4-AS plants might tolerate better salt stress than the other tomato lines. Root development was investigated in ARF4 transgenic lines in response to different concentrations of NaCl. Our results showed a strong reduction in primary root length in ARF4-OX plants (by 65%) while the decrease was nearby 40% and 15% in wildtype and ARF4-AS plants in response to salt stress respectively (Fig 1C). Lateral roots number was also determined in the three lines under stress condition. Our results showed that lateral roots number significantly increased with the increase of NaCl concentrations in wild type and in ARF4-AS plants (Fig 1D). The increase was around increases by 25% and 40% in wildtype and ARF4-AS respectively while the ARF4-OX showed a significant reduction of in root density (nearby 50%) when exposed to 150 Mm of NaCl. Root system is the first organ to encounter salinity [17]. Root development can severely be affected by environmental stresses [18]. In Arabidopsis, root length and development was significantly reduced in response to salinity [19]. In this study, we noticed a decrease in primary root length in the wild type and in the ARF4 transgenic lines in response to salt or drought stress conditions as compared with normal conditions. However, less reduction was reported in the ARF4-AS. Several studies have linked plant stress tolerance with root length and density (Furdi, Velicevici, Petolescu & Popescu 2013). In barley, Munns, James & Läuchli (2006) had noticed the salt tolerance was correlated with the better root growth rates [22]. It has been demonstrated that tolerant cultivars tend to have deeper root systems compared to susceptible ones for several plant species such as sunflower (*Helianthus Annuus L.),* wheat (*Triticum aestivum*) [23][24], Rice (*Oryza sativa*) [25] and cotton (*Gossypium hirsutum*) [26]. ARF4-AS plants showed less reduction in primary root length coupled with an increase in lateral roots number. ARF4 was found to be implicated in defining root architecture in Arabidopsis under optimal growth conditions through the control of lateral root emergence [27] which suggest that this gene might play an important role in root architecture in stress conditions and thus contribute to tomato tolerance to salinity.

Investigating chlorophyll a and b content in ARF4 transgenic lines and wildtype plants subjected to salt stress revealed a significant decrease in chlorophyll a and b content in wildtype and ARF4-OX with the increase of NaCl concentration. ARF4-AS plants showed less reduction in chlorophyll b content with the increase of NaCl concentration while a significant increase in chlorophyll a content was observed in response to 150 mM of NaCl (Fig 1E-F). Photosynthesis is among the primary processes to be affected by salinity [22].

Stress-induced decrease in chlorophyll content have been reported in several plant species including tomato [28], bread wheat [29]. Zarafshar et al., (2014) explained this decrease as the result of pigment photo-oxidation and chlorophyll degradation [30]. Maintaining chlorophyll content at high levels is accepted as an important indicator of salt tolerance in plants. It is stated that plants with high chlorophyll content under salinity stress are more tolerant to salt [30]. ARF4-AS displayed a high chlorophyll a and b contents under salt stress conditions suggesting that ARF4-AS plants might tolerate better salt stress.

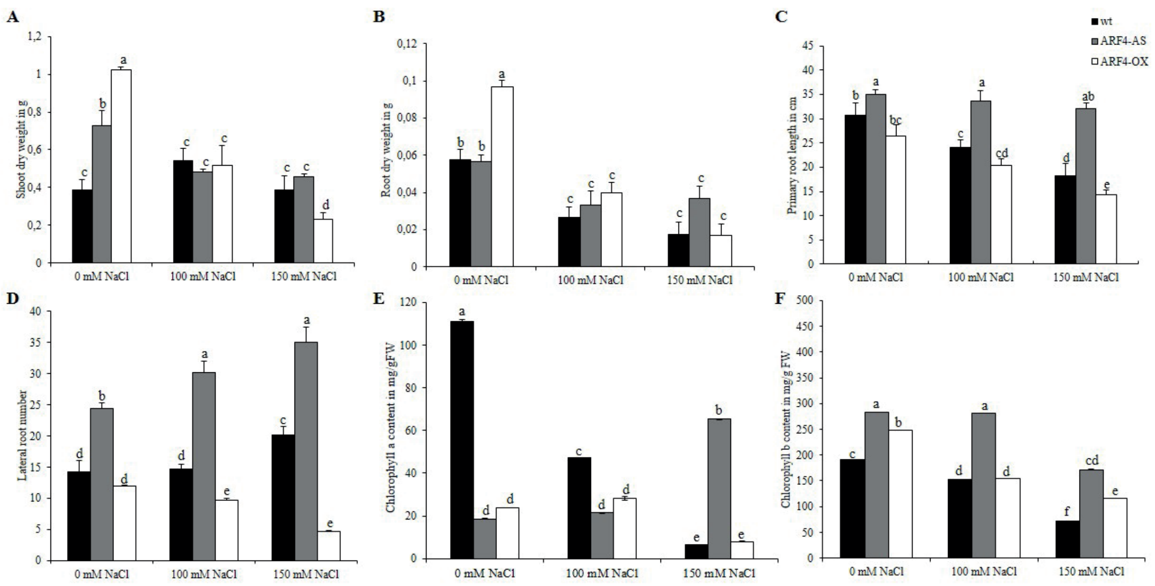

Fig 1. Growth parameters and chlorophyll content in wildtype (Wt), ARF4-AS and ARF4-OX plant lines exposed to different concentrations of NaCl. (A) shoot and (B) root dry weight, (C) primary root length, (D) lateral root number, (E) chlorophyll a and (F) chlorophyll b content. Salt stress was performed on three weeks tomato plants for two weeks by adding 100mM of NaCl or 150 mM of NaCl. Bars with different letters indicate the statistical significance (p<0,05) according to Student Newman-Keuls test.

CONCLUSION

In this work, we found that the down-regulation of *SlARF4* increases root length and lateral root number resulting in a better development of root system which might increase water acquisition and thus stimulate plant growth under adverse conditions. At the physiological level, a higher chlorophyll a and b content under salt stress was observed in ARF4 downregulated plants compared to the wildtype suggesting that the photosynthetic activity of ARF4-AS plants was less affected by salt stress. Altogether, *SlARF4* under expression might improves significantly tomato tolerance to salinity.

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