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Research Article

Different drought conditions could modulate growth responses of *Arabidopsis thaliana* through regulation of mRNA expression of genes encoding plasma membrane PIN proteins

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Abstract

Plant responses to drought stress are accompanied with changes in growth pattern via phytohormones such as auxin. It is, however, debatable a relationship between growth and different aspects of polar auxin transport such as mRNA level of some key genes in polar auxin transport under water deficit. In this study growth parameters and mRNA expression of genes encoding plasma membrane PIN proteins were evaluated in roots and shoots of *Arabidopsis thaliana* grown on nutrient media with different water potentials of -0.2, -0.5 and -0.9 MPaat 0, 24, 48, 120 and 192 hours after drought induction. The results showed different patterns of growth and *PINs*mRNA expression. These patterns indicated regulation of growth and *PINs* mRNA expression by organ type,developmental mechanisms, duration and intensity of drought stress.They showed a relationship between growth and *PINs* mRNA under drought stress. Although cooperation of all *PIN* genesis essential for growth, *PIN3* in roots and *PIN1* in shoots might have determinant role in regulation of growth responses to drought stress. Therefore, it is suggested that different drought conditions could modulate the *Arabidopsis*growth responses via changing *PINs* mRNA levels to establish a balance between vegetative growth and survival under water deficit.

Keywords: *Arabidopsis thaliana*,Drought stress,Growth pattern,mRNA expression, *PIN* genes,Real time PCR

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Introduction

Optimum plant growth and development are limited by undesirable changes in environmental conditions which are called abiotic stresses. Drought stress as one of the most important environmental stresses not only threatens plant survival but also affects human life negatively by decreasing plant productivity (Seki et al., 2002; Mahajan and Tuteja, 2005). During evolution, plants have developed various strategies in order to avoid drought stress, tolerate or adapt to water deficit by providing changes in their life cycle, morphology, physiology, and gene expression (Griffiths and Parry, 2002; Yordanovet al., 2003; Bray, 2004; Verslues and Bray, 2004). Some studies have shown that a part of these changes is related to the function and amount of plant growth regulators in response to drought stress (Dreher and Callis, 2007; Wang et al., 2008; Engelberth and Engelberth, 2009; Zhang et al.,2009a; Peleg and Blumwald, 2011). With the exception of abscisic acid as a well-known stress hormone (Bray, 1997; Schachtmanet al.,2008), auxin can play a crucial role in response to abiotic stresses as well. Since some of the most known growth and development processes such as cell division and elongation, development of the embryo, root initiation, tropistic responses (phototropism and gravitropism), apical dominance, vascular differentiation and fruit

ripening are controlled by auxin (Eckardt, 2001; Jenik and Barton, 2005; Ljunget al.,2005; Prusinkiewiczet al.,2009; Mano et al.,2010; Zhao, 2010), thus the action and importance of auxin in the maintenance of plant survival and homeostasis under abiotic stresses such as drought stress is undeniable (Seo and Park, 2009; Zhang et al., 2009a). Auxin function in response to environmental stimuli and stresses is related to auxin biosynthesis/catabolism, auxin signaling and its transport and distribution throughout plant especially polar auxin transport (Kerr et al.,2007; Petrásek and Friml, 2009). The polar auxin transport provides auxin gradient and auxin maxima to promote normal plant growth and determines plant architecture (Blilouet al., 2005; Vanneste and Friml, 2009). PIN proteins (PIN-FORMED) as the auxin efflux carriers are the key components in the polar auxin transport and growth phenomena (Tealeet al., 2006; Peer et al., 2011). Eight subgroups of PIN proteins have been introduced in *Arabidopsis* which on the basis of their amino acid sequences and functions are divided into PIN1-PIN8. All PIN proteins are found in plasma membrane except PIN5, PIN6 and PIN8 which are located in the endoplasmic reticulum membrane (Tealeet al., 2006; Petrásek and Friml, 2009; Peer et al.,2011). Some studies have shown that different stresses can affect PIN proteins and polar auxin transport. For example, increase in basipetal auxin transport and reversible effect on polar auxin transport in *Avena*mesocotyl section under osmotic condition (Sheldrake, 1979) and also changes of expression profile of *PIN*s in *Sorghum bicolor* under drought stresses (Shenet al., 2010) and up-regulation of *OsPIN3t* in rice seedlings treated with 20% polyethylene glycol (Zhang et al., 2012) have been reported.

Changes in growth patterns are the most common responses to drought stress and so far, few studies have been conducted to understand how plants regulate their growth responses to drought stress. One of the unknown mechanisms is related to relationship between plant growth, and polar auxin transport under drought stress. This mechanism possesses many aspects, which study of all aspects under drought needs extensive and continuous research. Therefore, at the first step to achieve this objective, this study attempted to answer the main questions below.

1- Does drought stress regulate growth responses of roots and shoots through change in mRNA expression of genes encoding plasma membrane PIN proteins? 2- Does the regulation depend on intensity and duration of drought stress?

Finally we attempted to find relationships between growth patterns and *PINs* mRNA expression of roots and shoots in *Arabidopsis thaliana* seedlings grown under different drought conditions. To answer the questions, in this study, growth patterns of *Arabidopsis* seedlings as a model plant were studied under water control condition (Ψw =-0.2 MPa), mild (Ψw =-0.5 MPa) and severe (Ψw =-0.9 MPa) drought stresses *in vitro* during 192 hours of drought induction. Simultaneously, the expression patterns of *PIN* genes(*PIN1*, *PIN2*, *PIN3*, *PIN4*, *PIN7.1* and *PIN7.2*) at mRNA level of roots and shoots of *Arabidopsis thaliana* seedlings were evaluated in roots and shoots at 0, 24, 48, 120 and 192 hours after drought induction.

Materials and Methods

Plant Material and Growth Condition

The sterilized wild type seeds of *Arabidopsis thaliana* ecotype Columbia (Col-0) were placed on the solid MS basal medium (Murashige and Skoog, 1962) containing 0.8% (w/v) agar, pH = 5.85 at 4°C for 24 h for stratification. The seeds were grown vertically in a growth chamber at 22±2°Cunder long day conditions (16 h light/8 h dark).

Drought Stress Induction

According to a primary experiment (data not shown),MS media with similar nutrient concentration and different amount of agar including 0.8, 2 and 4% (w/v) were prepared in order to induce different water potentials (Ψw) of -0.2, -0.5 and -0.9 MPa as control, mild and severe drought inducer respectively. Therefore, in this experiment, no osmoticum compounds such as mannitol or polyethylene glycol (PEG) were added to the nutrient media as drought inducers. The water potentials of the media were measured using a pressure plate (15 Bar Pressure Plate Extractor). Then 4-day old seedlings were transferred to the media and were grown vertically under the above-mentioned growth condition. Plants exposed to different drought conditions were splitted into two groups. In the first group, the changes of growth parameters were determined daily within 192 hours (8 days) after drought induction. Simultaneously, from

the second group, plant samples were obtained after 0, 24, 48, 120 and 192 hours in order to measure *PIN* genes mRNA expression.

mRNA Expression Analysis

RNAs were extracted separately from roots (1 cm of the root apex) and shoots of treated seedlings using RNeasy mini plant kit (Qiagen). The amounts and purity of RNAs were measured by uv-spectrophotometr and detection of ratios 260/280 and 260/230 (in all treatments the ratios were close to 2). Then, 1 µg of RNAs were treated with DNaseI (Fermentase).The treated RNAs were used for cDNA synthesis using RevertAid First Strand cDNA Synthesis kit (Fermentase). Real time PCR was performed according to the recommended instruction of SYBR Premix EX Taq, TliRNaseH Plus (TAKARA) kit for StepOnePlus applied biosystem thermal cycler with three step PCR program (5s at 95°C, 30s at 54°C and 30s at 72°C) in three replicates. Efficiencies of all pair primers and an optimum concentration of input cDNA were determined via standard curves obtained from amplification plots between C_T and amounts of 1:10 of cDNA which were prepared in a serial dilution from the control sample.Efficiencies of primers for root and shoot samples were separately calculated. The relative expression of each gene as the fold expression was calculated through Pfaffle's method (2001). For normalization of gene expression, *ACTIN* gene was used as the endogenous reference gene. The cDNA sequences of all genes of interest were aligned to find dissimilar regions in order to design the specific primers for each gene. Primers were designed using Beacon designer software (Beacon Designer 7.5) and blasted with NCBI blast tools. Accession numbers for the genes including *ACTIN* (AT3G18780); *PIN1* (AT1G73590); *PIN2* (AT5G57090); *PIN3* (AT1G70940); *PIN4* (AT2G01420); *PIN7.1* (AT1G23080.1); *PIN7.2* (AT1G23080.2) were obtained from TAIR database (http://www.arabidopsis.org/). The sequences of the used primers for each gene were:

ACTIN, Forward: 5'GTATCGCTGACCGTATGAG3',
Reverse: 5'CTGCTGGAATGTGCTGAG 3';

PIN1, Forward:

5'CTCAAGGCTTATCTGCGACAC3',
Reverse: 5'AGTTAGAGTTCCGACCACCAC3;'

PIN2, Forward:

5'CTCGTCACGGTTACACTAATAG3',
Reverse: 5'TCATACTTCTGCCTCCTCTTC3';

PIN3,Forward:

5'AGTGGAGATTTCGGAGGAGAAC3',
Reverse:5'GGAGCAAGTTTGTTTAGACCATTC3';

PIN4, Forward:

5'CGAAAGAGTGGTGGTGATG3',
Reverse: 5'ATGTGTTCCGTTGTTGCC3';

PIN7.1, Forward:

5'AACAAAGCTGGTCCGATGAAC3',
Reverse: 5'TGTAGTCCGTTAGGCACTTCC3';

PIN7.2, Forward: 5'GCATGGACCATCCGACAG3',
Reverse: 5'GGACCACGACAACAATCAAG3'.

Results

Growth Patterns under Drought Stress

The results of growth parameters (for 192 hours) including number of lateral roots, primary root length and number of rosette leaves under control condition (Ψw = -0.2 MPa), mild (Ψw = -0.5 MPa) and severe drought stresses (Ψw = -0.9 MPa) were presented in Figs. 1 to 3.

Lateral Root Pattern

Under severe drought stress (Fig. 1), the first lateral root was emerged at 72 h after drought induction, while under mild drought stress the emergence of the first lateral root was happened 24 hours later. However, no lateral root was observed in seedlings grown under control condition ($P < 0.05$). At 192 hours, total number of lateral roots in seedlings under severe drought stress was almost two-fold than seedlings under mild drought stress ($P < 0.05$) and the total number of lateral roots increased as the elapsed time of drought induction increased

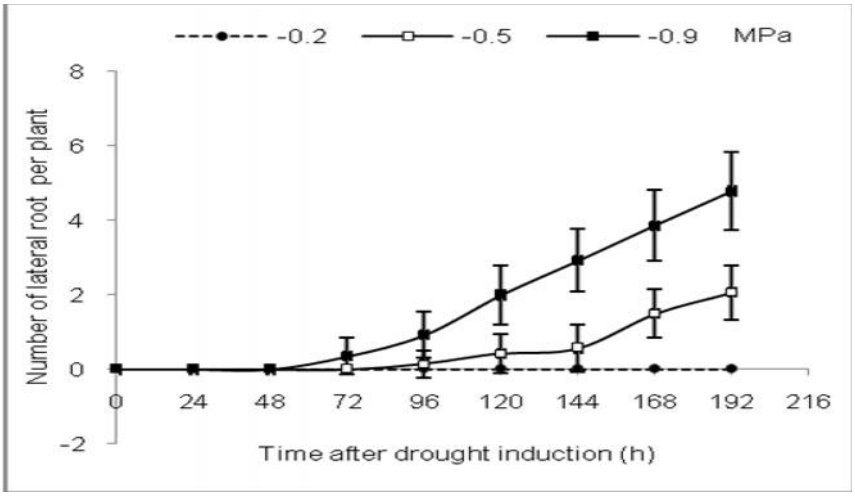

Fig. 1 Lateral root number in seedlings of *Arabidopsis thaliana* grown under control condition (Ψw = -0.2 MPa), mild (Ψw = -0.5 MPa) and severe (Ψw = -0.9 MPa) drought stresses during 192 h of drought induction. Ψw indicates water potential. MPa indicates megapascal. Data are mean (n = 14) bar lines are ±SD

Primary Root Pattern

The results of primary root length (Fig. 2) showed that during drought induction for 192 hours, primary root lengths increased differently in control seedlings and seedlings grown under mild and severe drought stresses. Although the maximum average primary root length was observed at 192 h in seedlings grown under mild drought stress, at this time, the seedlings grown under severe drought stress had the shortest primary root length ($P < 0.05$). In addition, the primary root elongation under control condition surprisingly was always lower than mild drought stress.

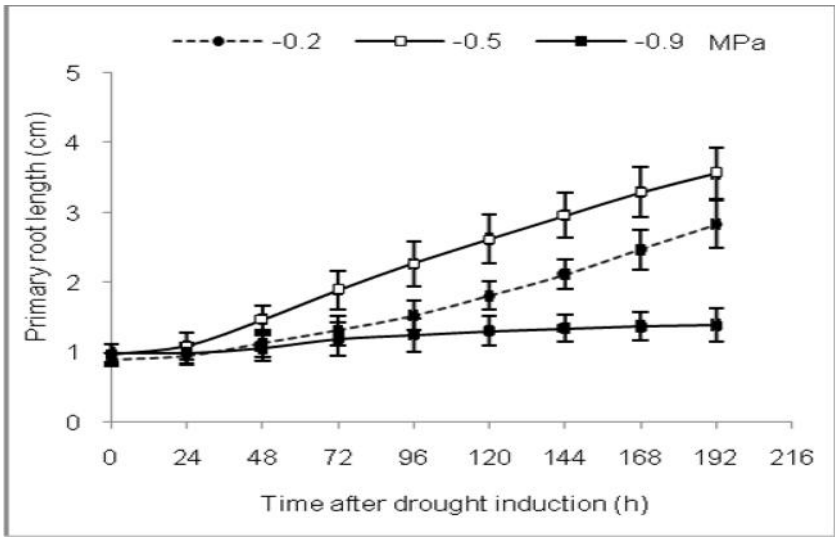

Fig. 2 Primary root length of *Arabidopsis thaliana* under control condition (Ψw = -0.2 MPa), mild (Ψw = - 0.5MPa) and severe (Ψw = -0.9 MPa) drought stresses during 192 h of drought induction. Ψw indicates water potential. MPa indicates megapascal Data are mean (n = 14) bar lines are ±SD

Shoot Pattern

The number of rosette leaves (Fig. 3) showed that the primitive leaf appearance (except of two primary cotyledonary leaves) was observed at 72 h after transferring seedlings to the medium with different water potentials of – 0.2 MPa or- 0.5 MPa, while the leaves appeared at 96 h under severe drought condition. At the end of the experiment (192 h after drought induction), 93% and 100% of seedlings grown under control and mild drought conditions had six leaves respectively. In contrast under severe drought stress, no seedlings had six leaves and mostly (85.7%) possessed five leaves. This was indicative of a delay in leaf formation under severe drought condition.

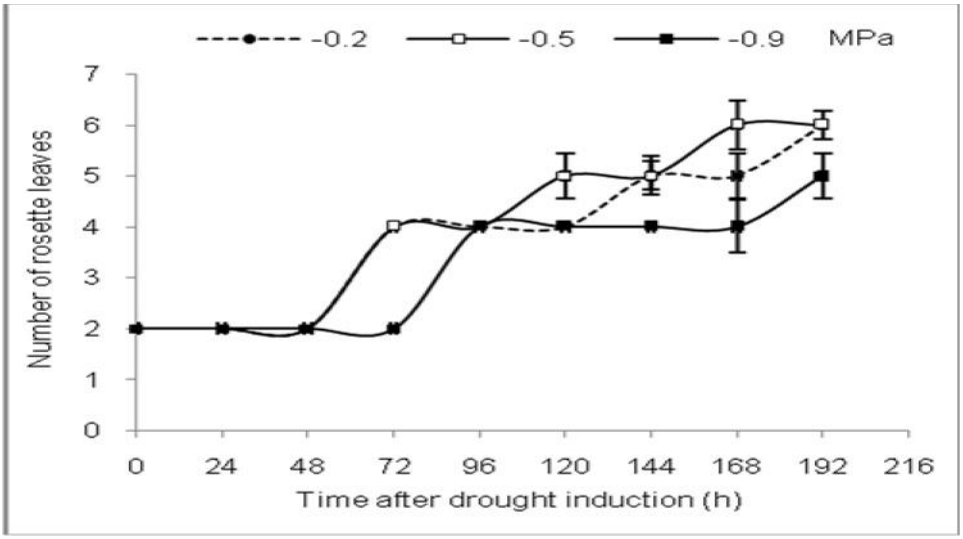

Fig. 3 Number of rosette leaves in seedlings of *Arabidopsis thaliana* grown under control condition (Ψw = -0.2 MPa), mild (Ψw = -0.5 MPa) and severe (Ψw = -0.9 MPa) drought stresses during 192 h of drought induction. Ψw indicates water potential. MPa indicates megapascal. Data are mean (n = 14) bar lines are ±SD

PIN Genes mRNA Expression under Drought Stress

Transcriptional (mRNA) levels of *PIN* genes (*PIN1, PIN2, PIN3, PIN4, PIN7.1* and *PIN7.2*) at 0, 24, 48, 120 and 192 hours of drought induction for roots (1 cm of root apex) and shoots of *Arabidopsis thaliana* seedlings grown on MS media with water potentials of - 0.2, – 0.5 and - 0.9 MPa were presented in figures 4 and 5. The *PIN*s mRNA expression profiles represented different levels of up- and down-regulation of *PIN* genes under three water potentials in roots and shoots.

PIN Genes mRNA Expression in Roots

The qRT-PCR results of roots (1 cm of root apex) showed that the expression of all *PIN* genes mRNA under control and drought conditions during 192 h after drought induction (Fig. 4) had approximate similarities and fluctuations. Different levels of down-regulation of all *PIN* genes were observed under three drought conditions (water potentials of -0.2, -0.5 and -0.9 MPa) at the first 24 h. Then, under control and severe drought conditions, the relative mRNA expression of all *PINs* (except *PIN3* and *PIN7.2* under severe drought stress) reached to maximum level significantly at 120 h and after that suddenly decreased up to 192 h. The most abundant mRNA (about 80-fold) was related to *PIN1*and *PIN4* under severe drought and control conditions at 120 h, respectively. Under mild drought stress, the highest up-regulation of *PIN1, PIN3* and *PIN4* mRNA was seen at 192 h ($P < 0.05$). However, the expression of *PIN2* and *PIN7.1* mRNAs was remained approximately constant after 120 h. Meanwhile, the relative abundance of all *PIN* genes mRNAs under mild drought stress was significantly higher than

their control condition ($P < 0.05$) at 192 h. Under mild and severe drought conditions, expression of *PIN7.1* and *PIN 7.2* mRNAs was remarkably higher than their values in control condition at 192 h. However, the highest expression ($P < 0.05$) of *PIN7.2* splice variant (about 60-fold) was observed under mild drought stress at 120 h.

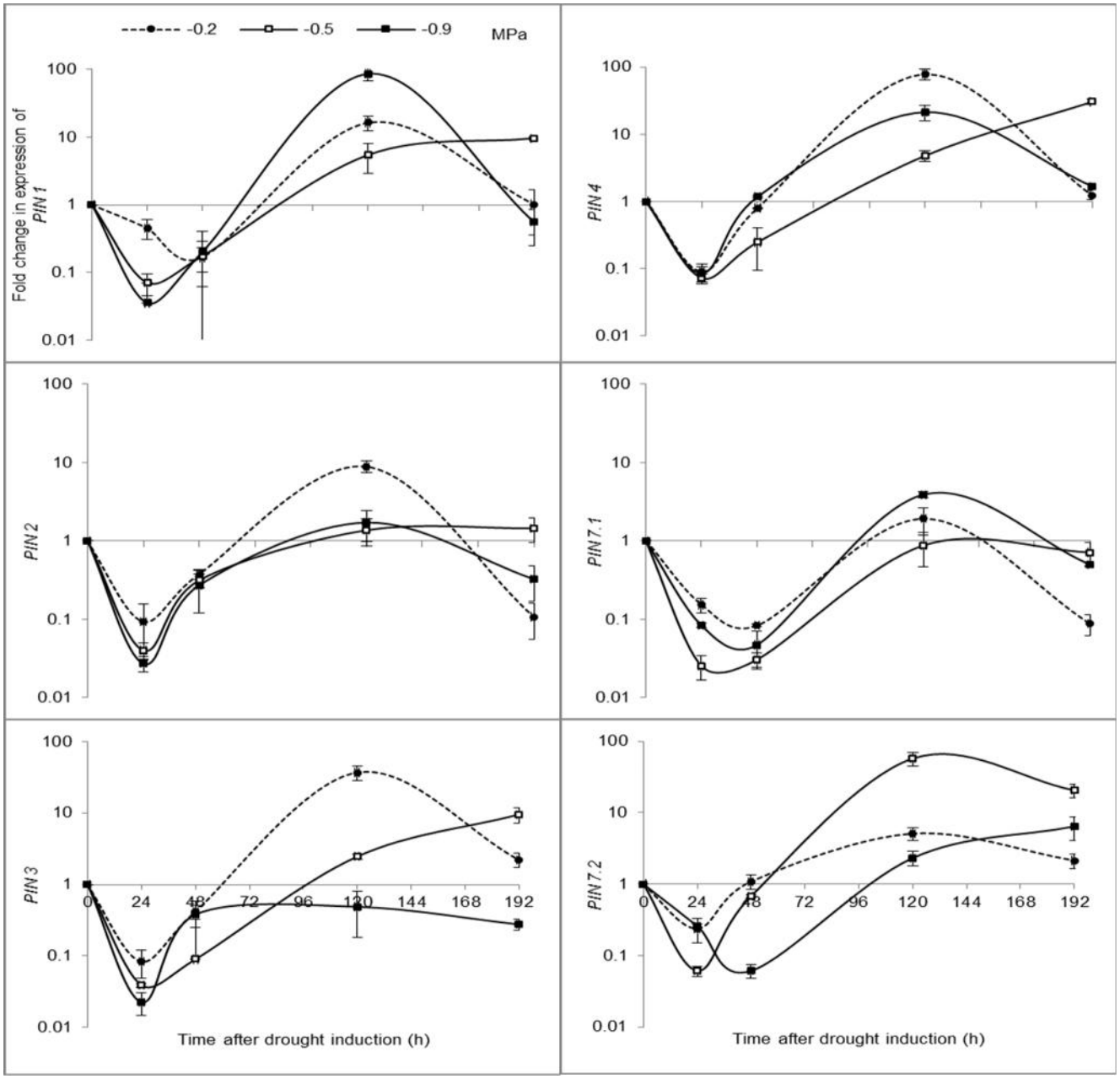

Fig. 4 Expression of *PIN1*, *PIN2*, *PIN3*, *PIN4*, *PIN7.1* and *PIN7.2* mRNA in roots (1 cm of root apex) of *Arabidopsis thaliana* seedlings grown under control condition (Ψw = -0.2 MPa), mild (Ψw = -0.5 MPa) and severe (Ψw = -0.9 MPa) drought stresses at 0, 24, 48, 120 and 192 hours after drought induction. Ψw indicates water potential. MPa indicates megapascal. Data are mean (n = 3) and bar lines are ±SD

PIN Genes mRNA Expression in Shoots

PIN genes mRNA expression in shoots grown under each water potential of -0.2, -0.5 and -0.9 MPa had almost similar profiles (Fig. 5). Under control condition, there was a wave-like succession of up-and down- regulation of PIN genes mRNA expression for all PIN genes mRNA (except of *PIN7.1* mRNA), The highest level of expression (about 7000-fold) was observed for *PIN2* mRNA under control condition at 120 h ($P < 0.05$) in comparison with others. The fluctuations of *PIN* genes mRNA expression under mild and severe drought stresses were less than the control condition. However, under drought stresses (mild and severs), a remarkable resemblance was evidenced in expression patterns of *PIN3*, *PIN4*, *PIN7.1* and *PIN7.2* mRNAs. The highest level of up-regulation of the mentioned genes took place significantly at 48 h, while their expression was down-regulated in seedlings grown under control condition. Moreover, at 48 h, relative expression of *PIN3*, *PIN4*, *PIN7.1* and *PIN7.2* mRNAs under mild and severe drought conditions were significantly higher than control condition. Under drought conditions, the maximum (about 3700-fold) and the minimum (about 0.3-fold) *PIN* transcripts were attributed to *PIN1* mRNA of seedlings grown under severe drought condition at 24 h and 192 h, respectively ($P < 0.05$).

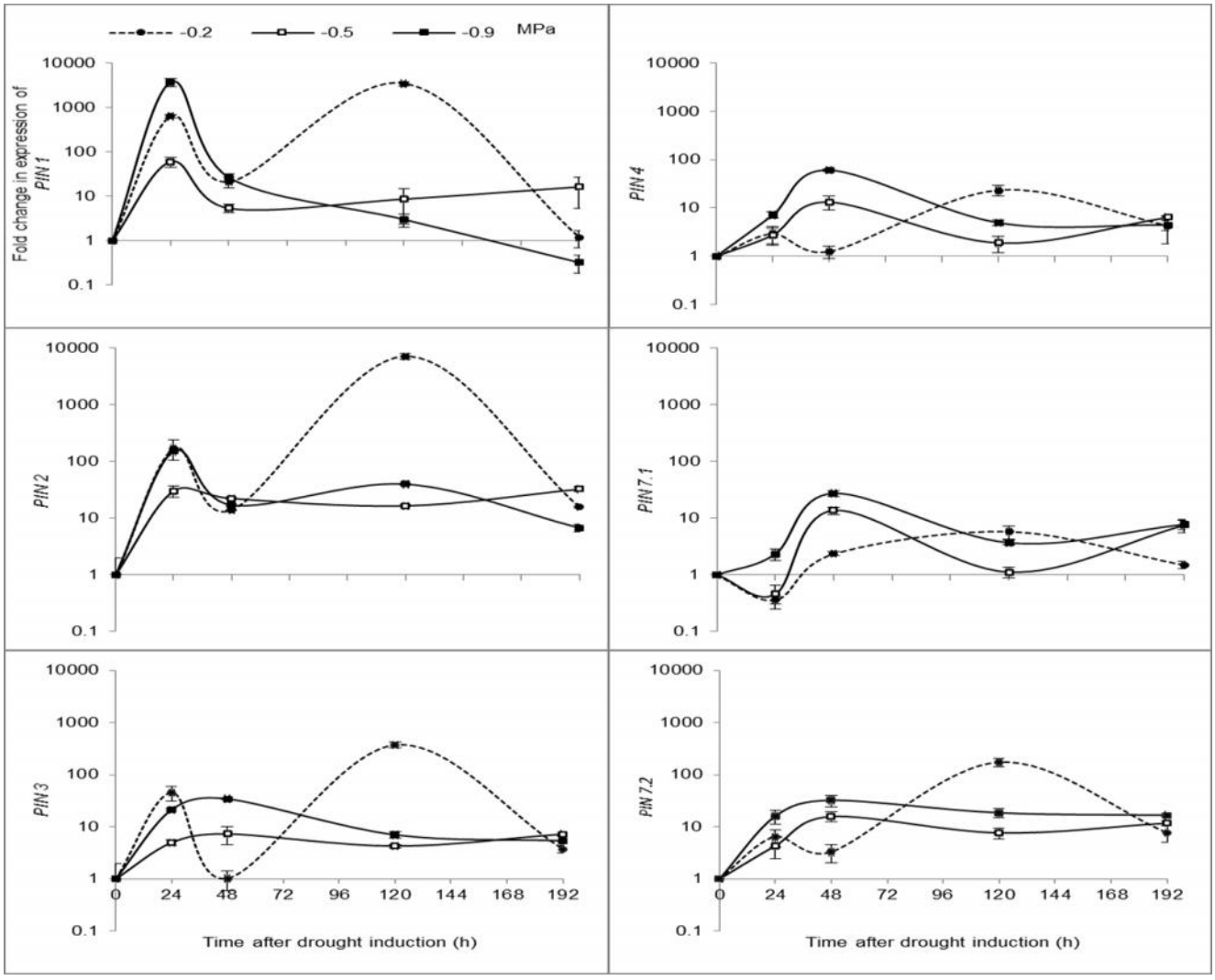

Fig. 5 Expression of *PIN1*, *PIN2*, *PIN3*, *PIN4*, *PIN7.1* and *PIN7.2* mRNA in shoots of *Arabidopsis thaliana* seedlings grown under control condition (Ψw = -0.2 MPa), mild (Ψw = -0.5 MPa) and severe (Ψw = -0.9 MPa) drought stresses at 0, 24, 48, 120 and 192h after drought induction. Ψw indicates water potential. MPa indicates megapascal Data are mean (n = 3) and the bar lines are ±SD

Discussion

Plant growth and development are the result of genetic, physiological and biochemical processes in response to environmental factors such as drought stress (Chapin III et al., 1987; Wu et al., 2007; Zlatev and Lidon, 2012). Depending on the intensity and duration of drought stress (Kalefetoglu and Ekmekci,2005; Neumann, 2008), water deficit can target one or more of these processes and cause induction of proper responses to drought stress (Kalefetoglu and Ekmekci, 2005; Farooq et al., 2009; Claeys and Inzé, 2013). The results of this study showed that different level of water potentials (– 0.2, -0.5 and – 0.9 MPa) for 192 hours can cause different patterns of growth and *PINs* mRNA expression in *Arabidopsis thaliana*.

Growth Responses under Drought Stress

Root Growth under Drought Conditions

After 192 h of drought induction, total number of lateral root under severe (Ψw = - 0.9 MPa) drought stress was approximately two-fold than the mild (Ψw = - 0.5 MPa) one, while no lateral rootswere formed under control condition (Ψw = - 0.2 MPa). Similar results for increasing lateral root number under severe water deficit were reported in desert cactus (Dubrovsky and Gómez-Lomelí, 2003) and *Triticale* (Zhang et al.,2009b). In contrast, it was shown that water potential of less than -0.5 MPa provided by PEG 8000 (van der Weeleet al., 2000) decreased lateral root number in *Arabidopsis thaliana*. The contrast could be related to the difference between van der Weele and our methods to induce drought stress in MS medium. Previous studies reported that osmoticum compounds such as PEG or mannitolwhich were used for drought induction could be taken up by plant roots and also had adverse effects on plant growth (Lawlor, 1970; Janes, 1974; Yaniv and Werker, 1983, Jacominiet al.,1988; Lipavska and Vreugdenhil, 1996; Fritz and Ehwald, 2010). More lateral root formation under severe drought stress in our experiment might be due to using not any osmoticum compounds in the MS medium.

During 192 h of drought induction, the longest and the shortest primary root was observed under mild and severe drought stresses, respectively. During 192 h, elongation of primary root length of seedlings grown under control condition was slower than seedlings under mild drought stress and faster compared to severe drought condition. Almost similar results were reported by van der Weeleet al.,(2000) for *Arabidopsis thaliana.* They indicated that the order of elongation rate of primary roots was seen under moderate stress (-0.23 and -0.51 MPa), control condition (-0.1 MPa) and high stress levels (-0.8 and -1.2 MPa) during 3-4 days after application of treatments, respectively. The higher primary root length under mild drought stress in comparisonwith control condition might be due to quick adaptation of seedlings in order to complete their life cycle faster. This mechanism seemed to be a proper way to escape from drought stress which has been considered as drought avoidance strategies (Blum, 2005; Kalefetoglu and Ekmekci,2005; Riveroet al., 2007; Harbet al., 2010; Claeys and Inzé, 2013). However the fast primary root elongation could be the result of more allocation of biomass toward roots to uptake more water (Zlatev and Lidon, 2012) or due to the main role of root tip in response to water deficit (Shimazakiet al., 2005).

The lowest primary root growth coincided with the maximum number of lateral root formation under severe drought stress probably reflects the shifting root growth pattern toward creation of more lateral roots to provide maximum surface for water absorption. The reduction of primary root growth and increase in lateral root in *Arabidopsis thaliana* grown under 150 mMNaCl was also reported (Wanget al.,2009).

Shoot Growth under Drought Conditions

After 192 h of drought induction, six leaves were observed in seedlings grown under mild drought stress and control condition, while noticeable delay in leaf formation (4-5 leaves in seedlings) was observed under severe drought stress. The results showed that mild drought stress did not have any effect on leaf formation but severe drought stress could adversely influence leaf initiation. The reduction of leaf number in *Arabidopsis thaliana* grown under water potentials -0.6 and -1.1 MPa was reported by Hummel et al.,(2010). It is suggested that the number of rosette leaves and time of their formation could be a proper indicator of shoot growth pattern of young *Arabidopsis* seedlings under drought conditions.

Drought Stress and *PIN*Genes mRNA Expression

Should we assume that *PIN* genes mRNA expression in both roots and shoots of seedlings grown under control condition (Ψw = - 0.2 MPa) followed regular patterns (Figs. 4 and 5) then, the pattern of *PIN* genes mRNA expression could be under natural mechanisms of plant growth and development. In contrast, any changes induced by drought stresses indicated that expression of *PIN* genes mRNA was probably regulated by duration and intensity of drought stress.

The effects of developmental stages and environmental stimuli such as gravity and light on *PIN* gene expression have been demonstrated (Petrásek and Friml, 2009; Friml, 2010). Moreover, in this experiment, different patterns of *PIN* mRNA expression in roots and shoots of *Arabidopsis* seedlings indicated that not only *PIN* genes mRNAs were expressed differently in each organ but also roots and shoots could respond to different levels of drought stress via change in patterns of *PINs* mRNA expression. Shenet al.,(2010) showed different expression profiles of *PIN* genes in root and leave of *Sorghum bicolar*grown under drought stress. In addition, considering the regulatory effects of auxin concentration on *PIN* genes transcription (Vietenet al., 2005; Paponoveet al., 2008), it can be suggested that these fluctuations in *PIN* genes mRNA expression might be related to the changes of auxin concentration and vice versa under drought conditions. It was reported that drought stress could decrease auxin concentration and cause drought tolerance in *Arabidopsis* (Seoet al., 2009) and rice (Zhang et al.,2009a).

PIN7 Splice Variant mRNAs under Drought Stress in Roots and Shoots

In this study, although the existence of both *PIN7* mRNAs (*PIN7.1* and *PIN7.2* splice variants) was observed under three water potentials (-0.2, -0.5 and -0.9 MPa) during 192 h, the relative expression of *PIN7.2* mRNAs were significantly more than *PIN7.1* mRNAs. This result was in agreement with previous studies (English et al., 2010). They indicated that there is difference in amount of some splicing variants of specific genes and splice variants were divided into minor and major forms based on their occurrence frequency. Although a precise role has not been defined for alternative splicing in plants, it has been suggested that alternative splicing is an important process in the regulation of gene expression. It could be proposed that *PIN7.2* mRNAs may mediate the regulation of *PIN7.1* mRNAs translation and/or the maintenance of *PIN7.1* mRNAs stability by RNA-RNA interaction. Other scientists have also emphasized the role of alternative splicing variants in RNA stability, RNA localization and the effective translation of mRNA (Eckardt, 2002; Faustino and Cooper, 2003; Reddy, 2007). In addition, in previous studies the importance of RNA-RNA interaction in the regulation of cellular processes like pre-mRNA splicing, gene silencing and RNA localization have been explained (Vermaet al., 1997; Schmitz and et al.,2010; Hartswoodet al., 2012; Menzelet al., 2012). High ratio of *PIN7.2* transcripts to *PIN7.1* in the roots and shoots under mild and severe drought stresses probably indicated an adaptive mechanism in response to drought stress at the gene expression level. In rice (*Oryza sativa*), two splicing forms were identified for a MAP-kinase gene. *OsMAPK5* spliced form could be involved in drought tolerance and disease resistance (Xiong and Yang, 2003). However, it has been proposed that alternative splicing and splice variants of genes in plants can play a specific role in response to stress and other environmental signals (Shi et al., 2002; Kazan, 2003; Kong et al., 2003; Barbazuket al., 2011).

Relationshipbetween Growth Responses and *PIN*Genes mRNA Expression under Drought Conditions

Relationship between Root Growth and PIN Genes mRNA Expression in Root

A well-known model which illustrates role of PIN proteins for auxin distribution in *Arabidopsis* root (Kleine-Vehn and Friml, 2008; Krecekeet al., 2009; Petrásek and Friml, 2009; Peer et al., 2011) could elucidate the relationship between root growth and expression of *PIN* genes in roots under drought stress. According to this model and also patterns ofroot growth and *PIN* genes expression in this study, three possible events for *PINs* regulation under different levels of drought stresses (Ψw = -0.2, -0.5 and -0.9 MPa) at the mRNA level could be proposed:

First event: under severe drought stress (Ψw = -0.2 MPa), up-regulation of *PIN1* and *PIN4* especially after 48 h (Fig. 4) could cause an increase in auxin flow toward root tips, while simultaneously the expression of *PIN3* and *PIN2* was not increased to redistribute

auxin and adjust its concentration in the root tips. This led to reduction of auxin amount in the upper region of the root tips and root elongation zone.Therefore, it could inhibit elongation of primary roots. The formation of local auxin concentration in the upper region of the roots which might be due to the feedback mechanisms of the auxin accumulation in the root tips (Vietenet al., 2007) could induce initiation of lateral root primordia and consequently the lateral root formation. It was reported that primary root elongation was inhibited by the high auxin concentration, while lateral root could be formed in such condition (Tealeet al., 2005). In addition, auxin accumulation in the root tips and inhibition of its redistribution to the upper region probably caused auxin depletion in root tips through down-regulation of *PIN1* and *PIN4* after 120 h. Changes in metabolism of auxin in response to drought stress could be other reason for reducing auxin (Seoet al., 2009) and consequently depletion of auxin in the root tips.Loss of auxin maxima in the root tips also might inhibit primary root elongation. Koprivovaet al.,(2010) reported that inhibition of glutation synthesis could destroy auxin accumulation in the root tips and inhibit primary root elongation. Thus, having maximum number of lateral root and the shortest primary root in seedlings grown under the severe drought stress might be the consequences of such event. (Figs. 1 and 2).

Second event: Under mild drought stress (Ψw = -0.5 MPa), the up-regulation of *PIN1*, *PIN2*, *PIN3* and *PIN4* mRNA after 48 h occurred with slower rate than the control seedlings (Fig. 4). Thus, no peak of *PINs* mRNA expression was formed during 192 h and this probably established a mild gradient of auxin concentration between root apex and upper region of the roots. It seemed that under mild drought stress, the extreme status of auxin concentration (auxin excess or auxin depletion) was not formed in the root tips and it led to accelerate primary root elongation and low rate of lateral root formation (Figs. 1 and 2).

Third event: Under the control condition (Ψw = -0.2 MPa), higher expression of *PIN3* and *PIN2* mRNA (in comparisonwith severe drought stress) after 48 h of drought induction (Fig. 4) could compensate up-regulation of *PIN1* and *PIN4* mRNA and likely caused adjustment of auxin concentration in the root tips and upper region of the roots.Therefore, primary roots were significantly longer than roots grown under the severe drought stress and no lateral roots were formed during 192 h of drought induction (Figs. 1 and 2).

Relationship between Shoot Growth and PIN Genes Expression in Shoot

Emergence of a leaf is a result of complex processes at the shoot apical meristem (SAM) to form a leaf primordium. Cell division and expansion into the leaf primordium lead to the leaf out growth (Veit, 1998; Micol and Hake, 2003; Fleming, 2006). Benkováet al.,(2003) reported that existence of an auxin gradient and accumulation of auxin at the tip of leaf primordial causes leaf formation. The local auxin concentration can be attributed to the role of PIN proteins at shoot apical meristem. According to previous studies at the shoot apical meristem PIN1 exports auxin from SAM to its flank and induces cell division and leaf primordium initiation. Then PIN1, PIN2 and PIN3 which are localized at the upper side of epidermal cells of the leaf primordial, can conduct the stream of auxin toward the future leaf tips. However, weak expression of PIN4 in the leaf primordial and expression of PIN3 and PIN7 in cotyledons was reported (Scarpellaet al., 2010; Peer et al., 2011; Guenotet al., 2012). Expression of PIN7 in the leaf primordium has been also attributed to its role in transferring auxin into provascular tissue (Tsugekiet al.,2009). Thus, dynamic expression of *PIN* genes mRNA in this study may indicate that probably PIN proteins by regulating auxin distribution could provide a specific local concentration of auxin at the shoot apical meristem and the leaf primordial. In this regard, it could be suggested that drought stress through the alteration of *PINs* mRNA expression and consequently auxin concentration could schedule leaf initiation and leaf emergence.

As Vietenet al.,(2007) described, auxin level can regulate transcription of *PIN* genes in a feedback mechanism.Therefore, by observing up-regulation of all *PIN* gens mRNAs (except *PIN7.1*) under control condition at 24 and 120 hours, and their down regulation at 48 and 192 hours it could be assumed that auxin concentration might directly control *PIN* genes expression at mRNA level.

However a relative stability in *PINs* mRNA expression under mild and severe drought stresses might represent the effect of unknown factors induced by drought stress on expression of *PIN* genes at mRNA level.

In addition, according to the profile of *PIN1* mRNA expression in shoots, low level of *PIN1* mRNA under mild drought stress before 72 h probably established a specific concentration of auxin which could induce leaf initiation earlier than the severe drought stress. Under severe drought stress higher expression of *PIN1, PIN3, PIN4* and *PIN7* mRNAs during first 48 h probably led to a delay in leaf formation before 72 h.

Conclusion

The results of this study showed that different levels of water potential (-0.2, -0.5 and -0.9 MPa as control, mild and severe drought conditions, respectively) could induce different growth patterns in *Arabidopsis thaliana* seedlings during 192 hours of drought induction. In addition mRNAs levels of *PIN* genes (*PIN1, PIN2, PIN3, PIN4, PIN7.1* and *PIN7.2*) in roots and shoots under three drought conditions and at 0, 24, 48, 120 and 192 hours indicated that expression of *PINs* mRNA could be regulated by developmental mechanisms, duration and intensity of drought stressand organ type.With regard to the patterns of growth and *PINs* mRNA expressionunder different drought conditions it seemed that young *Arabidopsis thaliana* seedlings could modify their growth response to different drought conditions by changing *PINs* mRNA levels for survival.

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Conflict of interest

The authors declare that they have no conflictof interest.

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