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Molecular and Biochemical Changes of Indole-3-Acetic Acid in the Expanding Leaves of Barley (*Hordeum vulgare* L.) under Salinity Stress

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Abstract

Indole-3-acetic acid (IAA) is a major natural auxin that plays a crucial role in many developmental and physiological processes in plants and in plants' tolerance to abiotic stress such as salinity. Salinity is a major abiotic stress that threatens many important crops such as barley. In this study, IAA biosynthesis genes of the YUC family and the IAA transport genes of the PIN family were identified in barley. Eight YUC genes (*HvYUCs*) and 8 PIN genes (*HvPINs*) were identified in barley. Phylogenetic analysis revealed that the majority of YUC and PIN genes were evolutionarily related to their orthologues in arabidopsis and rice. Conserved domain analysis revealed the presence of domains characteristic of these gene families. The results of IAA quantification showed a rapid decrease in IAA concentration upon exposure to salinity (at day 1 of salinity stress). After this rapid decrease, IAA concentration increased to normal levels compared with the control plants (at day 2, 4, and 8 of salinity stress). The transcription profile analysis of *HvYUCs* and *HvPINs* genes revealed differential changes in gene transcription level at different time points of salinity stress. This suggests a key role of IAA in barley as a physiological response to salinity stress.

Keywords:Abiotic stress, Auxin, Genes, HvYUCs, HvPINs.

1. Introduction

Salinity is a major abiotic stress that threatens the growth and reproduction of different crop plants. In fact, salinity stress results in great losses in the yield of many important crops. Moreover, a high percentage of land worldwide cannot be cultivated due to high salinity while a high percentage of the world population is undernourished. According to the Food and Agriculture Organization (FAO) (2018), about one out of every nine people is undernourished. Therefore, good efforts are needed to increase crop production to meet the increasing demand for food by the world's population.

Salinity stress affects the growth of plants in two phases. During the first phase, it causes osmotic stress that reduces water absorption by plants and eventually leads to growth retardation. Growth retardation is a consequence of the inhibition of photosynthesis. Photosynthesis is inhibited due to low CO_2 concentration, which results from stomatal closure under water deficit in the first phase of salinity stress (Sudhakar *et al*., 2001; Abogadallah 2010; Marti *et al.*, 2011). In the second phase, salt ions accumulate in plant tissues, resulting in salt toxicity (Munns and Tester 2008).

Plants are complex biological systems and they can respond to salinity stress through a wide variety of molecular, biochemical, physiological, and morphological changes. The response of plants to salinity stress depends on the genotype, species, and developmental stage. Many strategies have been developed by plants to minimize the negative effects of salinity stress (Prasch and Sonnewald 2015). Under stress conditions, plants reallocate energy and nutrients toward defense responses to save their reproductive capacity. Changes in the allocation of energy and nutrients under stress conditions are mediated by complex signaling networks. Moreover, this redirection of resources by the stressed plants has many consequences, including early flowering and a reduction in the accumulation of biomass (Munns and Gilliham 2015; Prasch and Sonnewald 2015).

Barley (*Hordeum vulgare*) is the fourth largest crop worldwide and is cultivated in relatively dry areas (Distelfed *et al.,* 2014; Hiei *et al.,* 2014). Unlike wheat (*Triticum aestivum*), barley is used mainly as animal feed. The barley genome is diploid (2n=2x=14), with a size of about 5.1 Gb (Bennett and Leitch 2012). Under environmental stresses, barley exhibits more stress tolerance than wheat; however, barley yield is estimated to decrease under future stress conditions such as increased salinity (Ingvordsen *et al.,* 2015).

Plant hormones were shown to play an important role in the adaptation of plants to different abiotic stresses through several responses (Peleg and Blumwald 2011; Bielach *et al.,* 2017). Indeed, a deep understanding of the role played by plant hormones and their interactions under

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abiotic stress might pave the way for the targeted genetic engineering of stress-tolerant crops (Wani *et al.,* 2016). Many previous studies have shown the important role of plant hormones in response to salinity stress (Javid *et al.,* 2011; Kaya *et al.,* 2009; Ryu and Cho 2015).

As a major plant hormone, auxin is a key regulator of plant growth and development, and many other biological processes (Vanneste and Friml 2009; Balzan *et al.,* 2014). Auxin homeostasis in plants is regulated by the *de novo* biosynthesis, transport, conjugation/deconjugation, and inactivation (Korasick *et al.,* 2013). Indole-3-acetic acid (IAA) is the most common auxin in plants (Korasick *et al.,* 2013). In poplar plants (*Populus euphratica and Populus x canescens*), free IAA was decreased in the xylem of salinity treated plants for 48 h (Junghans *et al.,* 2006). The level of phytohormones, including IAA, was also decreased in creeping bentgrass (*Agrostis stolonifera*) plants under salinity (Krishnan and Merewitz 2015). A decrease in IAA levels under salinity stress was also shown in rice (*Oryza sativa*) and tomato (*Solanum lycopersicum*) (Nilsen and Orcutt 1996; Dunlap and Binzel 1996, respectively). Long-term exposure (10, 15, and 22 days) of tomato plants to 100 mM NaCl resulted in a highly significant decrease of IAA level with treatment time (Ghanem *et al.,* 2008). In maize (*Zea mays*), a salinity-tolerant genotype maintained the level of IAA in its root under salinity, whereas the level of Indole Butyric acid (IBA) was increased in its leaves (Zörb *et al.,* 2013). An increase in IAA level was shown in wild species of common bean (*Phaseolus vulgaris*), whereas a decrease in its level was shown in the cultivated species (Yurekli *et al.,* 2004).

There are two biosynthetic pathways for IAA: tryptophan-dependent and tryptophan-independent (Woodward and Bartel 2005). Based on numerous recent studies, the tryptophan-independent pathway is not a major pathway for auxin biosynthesis in plants (Kasahara 2016). The tryptophan-dependent pathway was shown to occur via different pathways: the indole-3-acetamide (IAM) pathway; the indole-3-pyruvic acid (IPA) pathway; the tryptamine (TAM) pathway; and the indole-3-acetaldoxmide (IAOX) pathway (Mano and Nemoto 2012). From an evolutionary point of view, it was suggested that either IAM and/or IPA is the major auxin biosynthetic pathway in plants (Mano and Nemoto 2012). Moreover, many biochemical and genetic studies have demonstrated the critical role of the IPA biosynthetic pathway in auxin biosynthesis in many plant species such as maize, rice, *Arabidopsis thaliana*, and *Marchantia polymorpha* (Kasahara 2016). Indeed, this pathway is the first completely identified biosynthesis pathway of auxin, and it is shown to be conserved in land plants (Mashiguchi *et al.,* 2011; Kasahara 2016). The two main gene families in the IPA pathway are TAA1/TAR (aminotransferases) and the YUC (flavin monooxygenases) gene families (Zhao 2012). The YUC genes catalyze the rate-limiting step in auxin biosynthesis (Zhao 2012). Orthologs of the *TAA1/TAR* genes and of *YUC* genes are widely distributed in vascular and nonvascular plants (Kasahara 2016).

A major aspect of auxin biology is its differential distribution, which is achieved by polar auxin transport (Balzan *et al.,* 2014). The polar transport of auxin is crucial for normal development and in response to external environmental changes. Four types of auxin transporters were identified in plants: the PIN-FORMED (PIN) exporters, the ATP-binding cassette (ABC)-B/multi-drug resistance/P-glycoprotein (ABCB/MDR/PGP) subfamily of ABC transporters, the AUXIN1/LIKE-AUX1 (AUX/LAX) importers, and the newly described PIN-LIKES (PILS) proteins (Balzan *et al.,* 2014). Auxin transport was mostly studied in Arabidopsis and in monocot models such as rice, maize, *Sorghum bicolor*, and *Brachypodium distachyon* (Balzan *et al.,* 2014). In a latest review, short- as well as long-distance auxin transport was shown to be critical for different physiological responses of plants under different environmental conditions (Korver *et al.,* 2018).

The objective of this study was to investigate the contribution of IAA in the response of barley plants to salinity stress. To fulfill this objective, changes in IAA concentration at the biochemical and molecular levels in barley plants under salinity stress were monitored. Changes in the level of IAA in the expanding leaf of barley plants under salinity stress were recorded in a time course. Moreover, a time course of the transcription profile of selected IAA biosynthesis and transport genes in the expanding barley leaves was tested under salinity stress.

2. Materials and Methods

2.1. Identification of auxin biosynthesis and transport genes in barley

For the identification of barley orthologous genes, gene sequences for auxin biosynthesis (YUC genes) and transport (PIN genes) of *Arabidopsis thaliana* were retrieved from TAIR (https://www.arabidopsis.org/). These sequences were then blasted against the rice genome (Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/index.shtml). Rice YUC and PIN genes were then blasted against the barley genome IPK (http://webblast.ipk-gatersleben.de/barley_ibsc/). High score hits were chosen for the alignment analysis and construction of a phylogenetic tree. The putative protein sequences for barley (HvYUC) and the (HvPIN) gene family were aligned using ClustalW and then a phylogenetic tree was constructed using the Neighbor-Joining method and the bootstrap analysis, with 1,000 replicates in the MEGA software version X (*Kumar et al.,* 2018). The conserved motifs in *HvYUC* and *HvPIN* genes were derived using the NCBI conserved domain search (https://www.ncbi.nlm.gov/Structure/cdd/wrpsb.cgi). The gene structure for all HvYUCs and HvPINs were retrieved from Ensembl Plants (https://plants.ensembl.org/Hordeum_vulgare). The protein transmembrane topology of *HvPIN* genes was predicted by using the TMHHM Server v2.0 (Krogh *et al.,* 2001).

2.2. Primer design for barley YUC and PIN genes

Gene sequences of HvYUCs and HvPINs were used to design primers using Primer3 (http://primer3.ut.ee/) (Table 1).

Table.1 Primer sequences and PCR product size of auxin biosynthesis and transport genes of barley.

Target gene	Forward primer 5' ------ 3'	Reverse primer 5' --------- 3'	Product size (bp)
HvYUC2	AGTGAGGGCA AGAGAGTCCA	CTATGCAGTTGGA GCGTTCA	240
HvYUC3	GGAAGCGACTT CTTCAGTGG	GGGTACCAGCTGT GTTGGTT	210
HvYUC6	GCGCTAGCAAA GATCAGGTC	GTGAAGCCGACG GAGTAGAG	247
HvYUC7	TCGTTGGATCT GGAAACTCC	GGTCATTGGACCC ATTTTTG	244
HvPIN4	GCTTCAACCAG TCCGACTTC	GCTCCTTGTTCGA GTTGGAG	243
HvPIN2	GAGGACCTCCA CATGTTCGT	CTGTTCCCGAAGC TGAAGTC	176
HvPIN7	GACCCGTTCCA GGTCAACTA	CGAGTTGGTGAGT GTGGAGA	168
HvPIN8	GTATCCCGTTG CTGAAAGGA	CTGGCCCTCATGG TATGTCT	215
SALM	GGGAGATTGGC TCTGGAAAT	GCCTCTTGGGTGT GGTTTAG	110

2.3. Plant growth and salinity stress

Barley (*Hordeum vulgare* cv Morex) grains were surface sterilized by 70% ethanol for 1 min, then 1% sodium hypochlorite for 10 min followed by 3 times (each for 5 min) rinse with sterile water. The grains were then germinated on two sheets of moist filter paper in glass dishes in the darkness for 2 days at 22˚C. Afterwards, 120 germinated grains were transferred to perlite rooting medium in 64-well trays. There were 4 trays: 2 for the control and 2 for the salinity stress. Seedlings were kept to grow for 5 days under conditions of 22/20˚C day/night, 70% humidity, 16/8 light/dark at 100 µmol/m^2/s. During growth, barley seedlings were supplemented by half-strength Hoagland's solution (Hoagland No. 2 basal salt mixture, Sigma-Aldrich, USA). At day 7 of germination (5 days after transfer to pots), a group of plants (60 plants in 2 trays) were exposed to salinity stress of 100 mM as the final NaCl concentration. This final NaCl concentration was given in 25 mM increments each day from day 7 of germination until day 11. A volume of 1 L of NaCl solution and half-strength Hoagland's solution was added to the bottom of each tray. Another group (60 plants in 2 trays) is the control group that was given half-strength Hoagland's solution. Afterwards, the expanding leaves were harvested at days 1, 2, 4, and 8 after their exposure to the final salt concentration (100 mM NaCl). Leaf samples were snap frozen in liquid nitrogen and kept at −80˚C for IAA analysis and RNA isolation. Five expanding leaves from five randomly chosen plants were pooled in one biological replicate and three biological replicates were sampled for each treatment (control and salinity stress).

2.4. RNA isolation

Total RNA was isolated using Quick-RNA^{TM}Plant Miniprep-Zymo following the manufacturer's instructions (Zymo Research Corporation, USA). RNA was detected quantitatively using Epoch Microplate Spectrophotometer (BIOTEK, USA). RNA was purified from DNA contamination using RQ1 RNase-Free DNase (Promega, USA). 1.0 µg of total RNA was used to synthesize the complementary DNA strand (cDNA) using GoScript^{TM} Reverse Transcription System (Promega, USA).

2.5. Semi-quantitative PCR

Specific primers of HvYUCs and HvPINs were used to test the expression of these genes. The following PCR mixture of a total volume of 20 µL was prepared:10 µL of 2x PCR master mix (i-MAX II, iNtRON Biotechnology, Korea), 1 µL cDNA (0.2 µg), 1 µL gene-specific forward primer (10 pmol μL^{-1}), 1 µL gene-specific reverse primer (10 pmol μL^{-1}), and free-nuclease water. The genes were amplified in 35 cycles according to the following PCR program: initial denaturation at 95˚C for 2 min, denaturation at 95˚C for 30 s, annealing at 58˚C for 30 s, extension at 72˚C for 30 s, and final extension at 72˚C for 5 min. S-adenosyl-L-methionine-dependent methyl-transferase superfamily (*SALM*) was used as a reference gene (Hua *et al*. 2015). The PCR products were visualized on agarose (2%) gel that was run for 1 hour at 100 V.

2.6. Determination of free IAA concentration

About 500 mg of barley leaf tissue was taken from each biological sample for the quantification of free IAA. Three biological samples from the control and the salinity stress groups were quantified. Free IAA was quantified using the Plant Indole 3 Acetic Acid (IAA) ELISA kit according to the manufacturer's instructions (Sunlong, China). Free IAA concentration was expressed in ng/g fresh weight.

2.7. Statistical analysis

The concentration of free IAA in the different barley samples was analyzed using Student's *t*-test. A difference in means less than 0.05 was considered significant.

3. Results

3.1. Identification of YUC and PIN genes in barley

A BLAST search of the 11 YUC genes and 8 PIN genes of *Arabidopsis* was executed in the rice genome database. In this search, the highest hit score revealed 6 *OsYUC* and 5 *OsPIN* genes. The sequences of *OsYUCs* and *OsPINs* were blasted against the barley genome IPK database. Eight (8) *HvYUC* genes and 8 *HvPIN* genes were identified and named *HvYUC1–HvYUC8* and *HvPIN1–HvPIN8*, respectively (Table 2). Two homologs, which were named *HvYUC3* and *HvYUC4*, were identified for *OsYUC3*. Moreover, *OsYUC4* showed two homologs, which were named *HvYUC5* and *HvYUC6* in the barley genome. One homolog that was named *HvYUC1*, *HvYUC2*, *HvYUC7* and *HvYUC8*, was identified in barley for each *OsYUC1*, *2*, *5*, and *6*, respectively. Three homologs were found for *OsPIN1* and were named *HvPIN1*, *2*, and *3*. Two homologs were found for *OsPIN3* and were named *HvPIN5* and *HvPIN6*. For *HvPIN2*, *4*, and 5, one homolog each was found and named *HvPIN4*, *HvPIN7*, and *HvPIN8*, respectively.

Table 2. Auxin biosynthesis (YUCs) and transport genes (PINs) in barley and in the two model plants, *Arabidopsis* and rice.

Gene abbreviation	Barley gene ID	*Arabidopsis* orthology locus	Rice
HvYUC1	HORVU3Hr1G057950	*AtYUC1*	*OsYUC1*
		AT4G32540	LOC_Os01g45760
HvYUC2	HORVU3Hr1G069580	*AtYUC2*	*OsYUC2*
		AT4G13260	LOC_Os01g53200
HvYUC3	HORVU5Hr1G125560	*AtYUC3*	*OsYUC3*
		AT1G04610	LOC_Os07g25540
HvYUC4	HORVU5Hr1G050630	*AtYUC3*	*OsYUC3*
		AT1G04610	LOC_Os07g25540
HvYUC5	HORVU2Hr1G116980	*AtYUC5*	*OsYUC4*
		AT5G43890	LOC_Os04g03980
HvYUC6	HORVU2Hr1G001820	*AtYUC5*	*OsYUC4*
		AT5G43890	LOC_Os04g03980
HvYUC7	HORVU1Hr1G022530	*AtYUC10*	*OsYUC5*
		AT1G48910	LOC_Os11g10140
HvYUC8	HORVU7Hr1G017620	*AtYUC11*	*OsYUC6*
		AT1G21430	LOC_Os12g08780
HvPIN1	HORVU7Hr1G038700	*AtPIN1*	*OsPIN1*
		AT1G73590	LOC_Os06g12610
HvPIN2	HORVU6Hr1G076110	*AtPIN1*	*OsPIN1*
		AT1G73590	LOC_Os06g12610
HvPIN3	HORVU4Hr1G026690	*AtPIN1*	*OsPIN1*
		AT1G73590	LOC_Os06g12610
PHvPIN4	HORVU7Hr1G110470	*AtPIN2*	*OsPIN2*
		AT5G57090	LOC_Os06g44970
HvPIN5	HORVU1Hr1G091030	*AtPIN3*	*OsPIN3*
		AT1G70940	LOC_Os01g45550
HvPIN6	HORVU3Hr1G057630	*AtPIN3*	*OsPIN3*
		AT1G70940	LOC_Os01g45550
HvPIN7	HORVU3Hr1G094000	*AtPIN5*	*OsPIN4*
		AT5G16530	LOC_Os01g69070
HvPIN8	HORVU3Hr1G067670	*AtPIN8*	*OsPIN5*
		AT5G15100	LOC_Os01g51780

Phylogenetic analysis using the full-length protein sequences of AtYUCs, OsYUCs, and HvYUCs showed that all the YUCs can be divided into three clades [Figure 1A (green lines)]. The first one is the largest clade, which includes 7 HvYUCs, 5 OsYUCs, and 9 AtYUCs. This clade includes HvYUC1, 3, 4, 5, 6, 7, and 8, OsYUC1, 3, 4, 5, and 6, and AtYUC1, 3, 4, 5, 7, 8, 9, 10, and 11. Clade II includes AtYUC2, OsYUC2, and HvYUC2, and Clade III has only AtYUC6. The phylogenetic tree of HvYUCs showed that these genes can be divided into three clades [Figure 1B (green line)]. HvYUC1, 3, 4, 5, and 6 are included in Clade I. Clade II includes HvYUC2 and Clade III includes HvYU7 and HvYUC8. Analysis of conserved domains revealed that all HvYUC genes have CzcO (predicted flavoprotein CzcO associated with the cation diffusion facilitator CzcD) (accession number domain COG2072) (Supplementary data file S1).

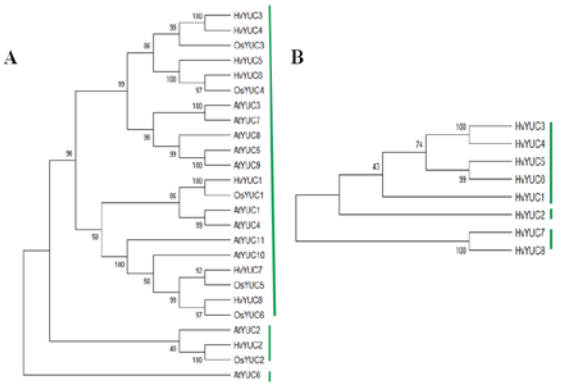

Figure 1. Phylogenetic analysis of YUC family genes. A. Phylogenetic tree of YUC genes in Arabidopsis (11 AtYUC genes), rice (6 OsYUC), and barley (8 HvYUC). B. Phylogenetic tree of YUC genes in barley. The unrooted trees were constructed by MEGAX software using the Neighbor-joining (NJ) method.

Phylogenetic analysis using the full-length protein sequences of AtPINs, OsPINs, and HvPINs showed that all the PIN genes can be divided into three clades [Figure 2A (green lines)]. The largest clade is clade I, which includes HvPIN4, 5, 7, and 8, OsPIN2, 4, and 5, and AtPIN2, 3, 4, 5, 6, 7, and 8. Clade II includes HvPIN1, 2, and 3, OsPIN1, and AtPIN1. HvPIN6 and OsPIN3 are included in clade III. The phylogenetic tree of HvPINs showed that these genes could be divided into two clades [Figure 2B (green lines)]. Clade I includes HvPIN1, 2, 3, 4, and 6 and clade II includes HvPIN5, 7, and 8.

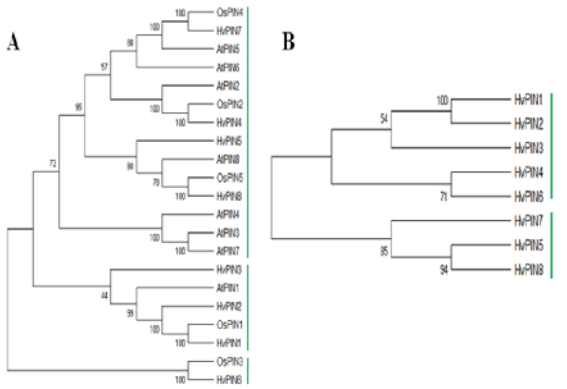

Figure 2. Phylogenetic analysis of PIN family genes. A. Phylogenetic tree of PIN genes in Arabidopsis (8 AtPINs), rice (5 OsPINs), and barley (8 HvPINs). B. Phylogenetic tree of PIN genes in barley. The unrooted trees were constructed by MEGAX software using the Neighbor-joining (NJ) method.

Gene structures of HvYUC and HvPIN genes showed differences in the number of exons and introns and the direction of transcription for each gene (Figures 3 and 4, respectively). Analysis of conserved domains revealed that all HvPIN genes have the membrane transport domain (accession number pfam03547), which is characteristic of auxin efflux carrier proteins (Supplementary data Table S1). Similar to other plant PINs, all HvPIN proteins except HvPIN6 and HvPIN7 have two hydrophobic segments located at the N- and C- termini and linked by a central hydrophilic loop (Figure 5 and Supplementary file S1). All HvPIN proteins possess 8 or 9 transmembrane helices except for HvPIN6 and HvPIN7, which have 0 and 5 transmembrane helices, respectively.

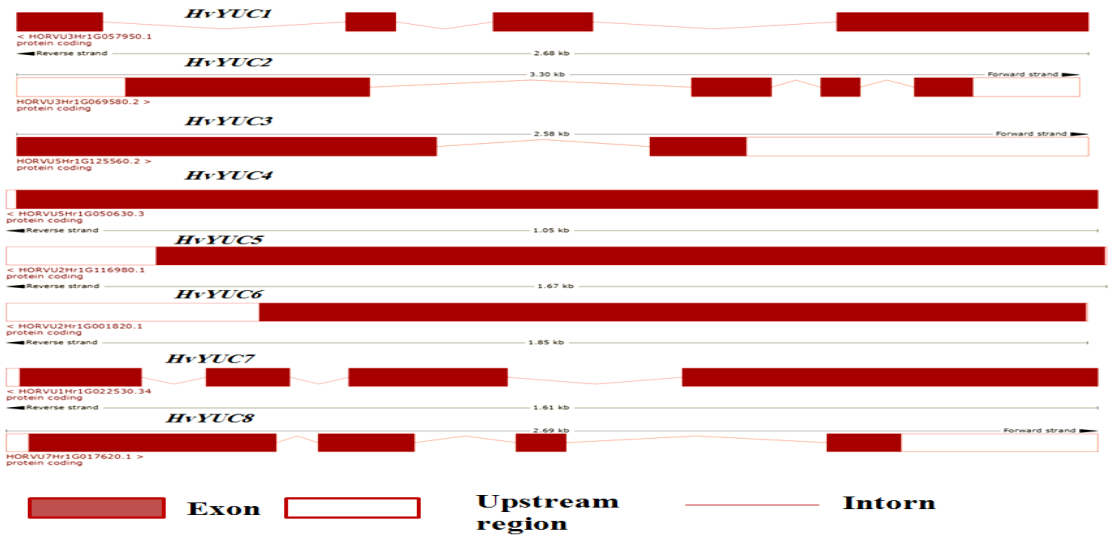

Figure 3. Gene structure of HvYUC genes

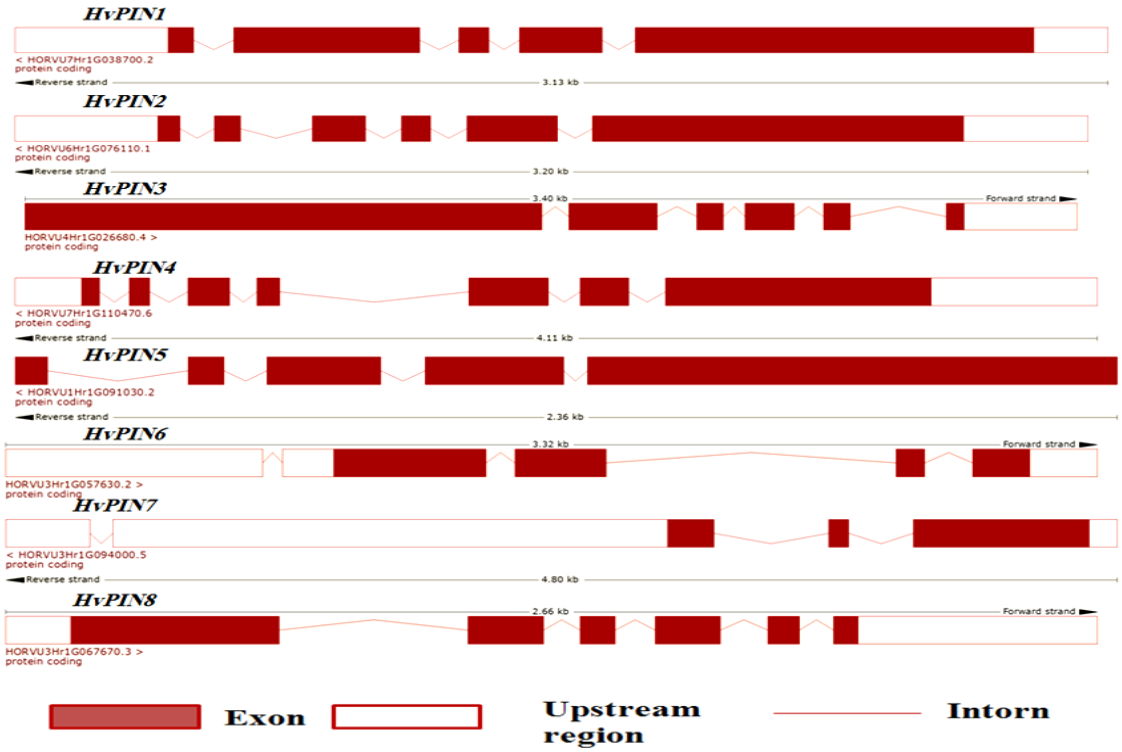

Figure 4. Gene structure of HvPIN genes

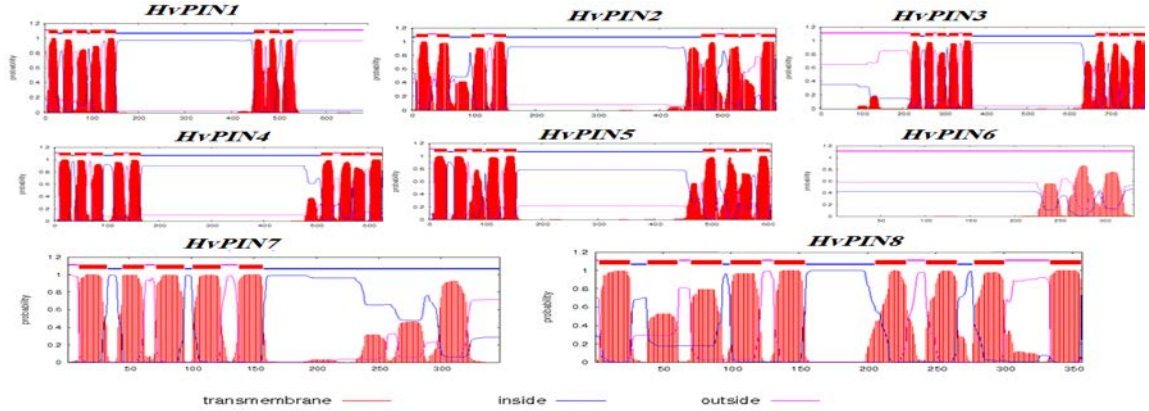

Figure 5. Transmembrane topology analysis of barley HvPIN proteins. The protein transmembrane topology was predicted by using the TMHHM Server v2.0 (Krogh et al. 2001). Red peaks represent the predicted transmembrane helices.

3.2. Rapid decrease in the concentration of free IAA in response to salinity stress

The concentration of free IAA was determined by immunological ELISA assay at different time points of salinity stress (1, 2, 4, and 8 days). After one day of salinity stress, the concentration of free IAA was significantly decreased ($P < 0.01$) (Figure 6). Free IAA concentration in barley plants under salinity stress was decreased by 25% of its concentration under control growth conditions (57 ng g^{-1} FW and 76.5 ng g^{-1} FW, respectively). After 2, 4, and 8 days of salinity treatment barley plants showed no difference in the concentration of free IAA compared with the control plants. Moreover, barley plants under control conditions did not show any significant change in the level of free IAA at different time points of their growth.

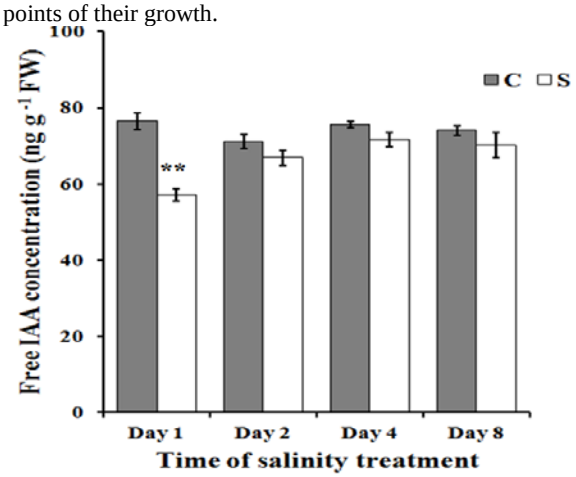

Figure 6. Concentration of free IAA in the expanding leaves of salinity-treated barley plants (S) and the control plants (C). Bars represent standard errors of the means. * $P< 0.05$, ** $P< 0.01$, and *** $P< 0.001$. Values are the means of three biological replicates.

3.3. differential transcription expression profile of HvYUC and HvPIN genes in barley plants under salinity stress

HvYUC genes showed differential transcription profiles in barley plants under salinity stress (Figure 7A). The transcription of *HvYUC2* and *HvYUC4* was not affected by salinity stress. The transcription expression of *HvYUC6* was down-regulated after 8 days of salinity stress but did not change after 1, 2, and 4 days of salinity stress. The transcription of *HvYUC7* was down-regulated after 1 day of salinity stress but did not change after 2, 4, and 8 days of salinity stress. Moreover, the transcription of *HvYUC2, 4, 6,* and *7* in the control barley plants did not change after different growth times (1, 2, 4, and 8 days).

The transcription of the majority of the tested PIN genes was not affected by salinity stress (Figure 7B). The transcription of *HvPIN4* was significantly up-regulated after 1 and 2 days of salinity stress. The transcription of *HvPIN2*, *HvPIN7* and *HvPIN8* was not altered in barley plants after different time points of salinity stress. Moreover, the transcription of *HvPIN4, 7* and *8* in the control barley plants was not altered after different growth times (1, 2, 4 and 8 days).

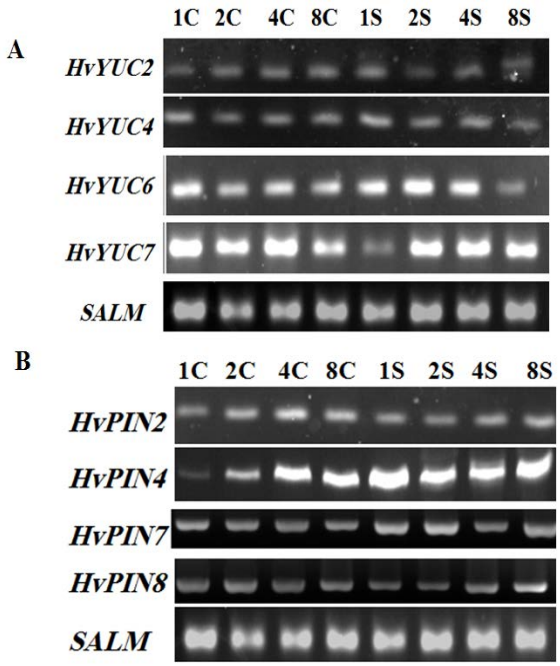

Figure 7. Transcription profile of HvYUC genes (A) and HvPIN genes (B) under control (C) and salinity stress (S) conditions after 1, 2, 4 and 8 days of salinity stress

4. Discussion

In this study, free IAA was quantified in barley seedlings under 100 mM salinity stress conditions compared with the control conditions, where no salinity stress was applied. A rapid decease in IAA concentration was observed in barley seedlings after 1 day of applying salinity stress. In other reports, the level of endogenous IAA was shown to be differentially altered in response to different abiotic stresses (Sharma *et al.*, 2015). In fact, salinity stress resulted in a significant decrease in IAA concentration in different plant species such as rice, tomato, poplar, and creeping bentgrass (Nilsen and Orcutt 1996; Dunlap and Binzel 1996; Junghans *et al.*, 2006; Krishnan and Merewitz 2015). The rapid decrease in IAA concentration in the expanding barley leaves under salinity stress shown here might be the cause of growth inhibition at this phase of salinity stress. Indeed, salinity stress was shown to stimulate a quiescent state in the early phase of its progression (Julkowska and Testerink 2015).

The rate-limiting step of IAA biosynthesis is the conversion of (indole-3-pyruvic acid) IPA into IAA: this step is catalyzed by members of the YUC gene family (Mashiguchi *et al.*, 2011; Zhao 2014). YUC genes are widely conserved in many plant species such as maize, rice, Arabidopsis, and Marchantia (Kasahara 2016). In this study, 6 *OsYUC* and 8 *HvYUC* genes were identified. Most of the *HvYUC* gene family members were phylogenetically related and clustered in one clade. This was the case in rice (Yamamoto *et al.*, 2007). Moreover, all eight proteins of the *HvYUC* genes contain the conserved domain CzcO (predicted flavoprotein CzcO associated with the cation diffusion facilitator CzcD). This match was shown for YUC genes in various plants species (Yamamoto *et al.*, 2007; Zheng *et al.*, 2016; Wang *et al.*, 2017). In the

present study, the transcription profile of *HvYUC* genes in the expanding barley leaves under salinity stress showed a significant down-regulation of *HvYUC7* after 1 day of salinity stress. Indeed, a significant reduction in the concentration of the free IAA was also shown after 1 day of salinity stress. This might suggest a critical role of *HvYUC7* in the biosynthesis of IAA in barley plants in response to salinity stress.

Auxin transport is crucial for the normal functioning of auxin under different environmental conditions (Forestan and Varotto 2012; Korver *et al.,* 2018). Members of the PIN gene family have a rate-limiting function as auxin efflux carriers. Moreover, they are crucial players in the maintenance of a steady-state of auxin levels, which is important for optimum and harmonized growth and developmental responses (Petrásek *et al.,* 2006; Křeček *et al.,* 2009). In the present study, auxin transport members of the PIN gene family were identified in barley. Eight different *HvPIN* genes were identified. Most *HvPIN* genes were shown to have the membrane transport domain, which is characteristic of auxin efflux carrier proteins (Křeček *et al.,* 2009; Zhou and Luo 2018). Low salinity stress inhibited the transcription of *AtPIN2* (Zhao *et al*., 2011). In Arabidopsis, salinity stress down-regulated the expression of PIN genes in the root tissues. This resulted in the inhibition of root meristem growth (Liu *et al.,* 2015). In soybean (*Glyine max*) plants, *GmPIN* genes responded differentially to the different abiotic stresses (Wang *et al.,* 2015). The results of quantitative PCR revealed that the number of down-regulated *GmPIN* genes under salinity stress was larger than that of the up-regulated genes. In watermelon (*Citrullus lanatus*), the transcription of PIN genes was differentially altered in response to different abiotic stresses (Yu *et al.,* 2017). The transcription of *ClPIN2* and *ClPIN10* was significantly down-regulated in the shoot tissue after 24 hours of salinity stress. In the root tissue, the transcription of *ClPIN2* and *ClPIN10* was not changed, but the transcription of the other *ClPIN* genes was up-regulated. Moreover, differential regulation of PIN genes was shown in cotton (*Gossypium hirsutum*) plants in response to drought and salinity stress and in leaf and root (He *et al.,* 2017). In the present study, the transcription of *HvPIN4* was up-regulated in the expanding barley leaves after 1 and 2 days of salinity stress. At 4 and 8 days of salinity stress the expression of this gene was high and the same in the two treatment groups. This increase in the expression of *HvPIN4* in the control group might be related to the progress in plant's development. Meaning, at these stages of development *HvPIN4* is switched on. The transcription profile of the other *HvPIN* genes was not altered in response to salinity stress. This indicates a regulatory role of *HvPIN4* in the expanding leaves of barley under salinity stress.

The regulation of auxin homeostasis is a complex process that requires the action of many gene families for biosynthesis, conjugation/deconjugation, transport and signaling. The maintenance of auxin homoeostasis is crucial for normal plant development, patterning, and growth. Plants under osmotic stress from drought or salinity show a significant decrease in auxin, and this results in growth inhibition as an acclimation strategy (Naser and Shani 2016). Therefore, dissection of the molecular and biochemical bases of auxin action in plants under salinity stress will help in the optimum utilization of this important growth regulator for the development of salinity-tolerant plants.

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Authors' contributions

AH and KA designed the study; AS conducted lab experiments; AH analyzed the data; AH wrote the manuscript, and KA and AH reviewed the manuscript.

Conflict of interest

Authors declare no conflict of interest.

References

Abogadallah G, Serag M and Quick W. 2010. Fine and coarse regulation of reactive oxygen species in the salt tolerant mutants of barnyard grass and their wild-type parents under salt stress. *Physiol Plant.,* **138**: 60-73.

Balzan S, Johal G and Carraro N. 2014. The role of auxin transporters in monocots development. *Front. Plant Sci.,* **5**: 393-404.

Bennett MD and Leitch, IJ. 2012. Plant DNA *C*-values database (release 6.0, Dec. 2012) http://www.kew.org/cvalues/.

Bielach A, Hrtyan M and Tognetti V. 2017. Plants under stress: Involvement of auxin and cytokinin. *Int J Mol Sci*., **18**: 1427-1450.

Distelfed A, Avni R and Fischer AM. 2014. Senescence, nutrient remobilization, and yield in wheat and barley. *J Exp Bot.,* **65**: 3783-3798.

Dunlap JR and Binzel ML. 1996. NaCl reduces indole-3-acetic acid levels in the roots of tomato plants independent of stress induced abscisic acid. *Plant Physiol.,* **112**: 379-384.

Forestan C and Varotto S. 2012. The role of PIN auxin efflux carriers in polar auxin transport and accumulation and their effect on shaping maize development. *Mol Plant,* **5**: 787-798.

Ghanem M, Albacete A, Martínez-Andújar C, et al. 2008. Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). *J Exp Bot.,* **59**: 3039-3050.

He P, Zhao P, Wang L, et al. 2017. The PIN gene family in cotton (*Gossypium hirsutum*): genome-wide identification and gene expression analyses during root development and abiotic stress responses. *BMC Genomics,* **18**: 507-517.

Hiei Y, Ishida Y and Komari T. 2014. Progress of cereal transformation technology mediated by *Agrobacterium tumefaciens*. *Front Plant Sci*., **5**: 628-638.

Hua W, Zhu J, Shang Y, et al. 2015. Identification of suitable reference genes for barley gene expression under abiotic stresses and hormonal treatments. *Plant Mol Biol Report,* **33**: 1002-1012.

Ingvordsen C, Backes G, Lyngkjær M, et al. 2015. Significant decrease in yield under future climate conditions: Stability and production of 138 spring barley accessions. E*ur J Agron.,* **63**: 105-113.

Javid M, Sorooshzadeh A, Sanavy S and Allahdad I . 2011. The role of phytohormones in alleviating salt stress in crop plants. *Aust J Crop Sc.,* **5**: 726-734.

Julkowska M and Testerink C. 2015. Tuning plant signaling and growth to survive salt. *Trends Plant Sci*., **20**: 586-594.

Junghans U, Polle A, Düchting P, et al. 2006. Adaptation to high salinity in poplar involves changes in xylem anatomy and auxin physiology. *Plant Cell Environ.,* **29**: 1519-1531.

Kasahara H. 2016. Current aspects of auxin biosynthesis in plants. *Biosci Biotechnol Biochem.,* **80**: 34-42.

Kaya C, Tuna AL and Yokaş I. 2009. The role of plant hormones in plants under salinity stress. *In*: Ashraf M, Ozturk M, Athar H (Eds) **Salinity and Water Stress**. Tasks for vegetation sciences. Vol. 44. Springer,Dordrecht, pp 45-50.

Korasick D, Enders T and Strader L. 2013. Auxin biosynthesis and storage forms. *J Exp Bot.,* **64**: 2541-2555.

Korver R, Koevoets I and Testerink C. 2018. Out of shape during stress: A key role for auxin. *Trends Plant Sci.*, **2**: 783-793.

Kramer EM and Ackelsberg EM. 2015. Auxin metabolism rates and implications for plant development. *Front Plant Sci.*, **6**: 150-158.

Křeček P, Skůpa P, Naramoto S, et al. 2009. The PIN-FORMED (PIN) protein family of auxin transporters. *Genome Biol.,* **10**:249-259.

Krishnan S and Merewitz E. 2015. Phytohormone responses and cell viability during salinity stress in two creeping bentgrass cultivars differing in salt tolerance. *Am Soc Hortic Sci.,* **140**: 346-355.

Krogh A, Larsson B, von Heijne G and Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol.,* **305**: 567-580.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol*., **35**: 1547-1549.

Liu W, Li RJ, Han TT, et al. 2015. Salt stress reduces root meristem size by nitric oxide-mediated modulation of auxin accumulation and signaling in Arabidopsis. *Plant Physiol.,***168**: 343-356.

Ludwig-Müller J. 2011. Auxin conjugates: their role for plant development and in the evolution of land plants. *J Exp Bot.,* **62**: 1757-1773.

Mano Y and Nemoto K. 2012. The pathway of auxin biosynthesis in plants. *J Exp Bot.,* **63**: 2853-2872.

Marti M, Florez-Sarasa I, Camejo D, et al. 2011. Response of mitochondrial thioredoxin PsTrxo1, antioxidant enzymes, and respiration to salinity in pea (*Pisum sativum* L.) leaves. *J Exp Bot*., **62**: 3863–3874.

Mashiguchi K, Tanaka K, Sakai T, et al. 2011. The main auxin biosynthesis pathway in Arabidopsis. *PNAS*, **45**: 18512-18517.

Munns R and Gilliham M. 2015. Salinity tolerance of crops – what is the cost? *New Phytol*., **208**: 668-673.

Munns R and Tester M. 2008. Mechanisms of salinity tolerance. *Annu Rev Plant Biol*., **59**: 651-681.

Naser V and Shani E. 2016. Auxin response under osmotic stress. *Plant Mol Biol.,* **91**: 661-672.

Nilsen E and Orcutt DM. 1996. **The Physiology of Plants under stress - Abiotic Factors**. Wiley, New York.

Peleg Z and Blumwald E. 2011. Hormone balance and abiotic stress tolerance in crop plants. *Curr Opin Plant Biol*., **14**: 290-295.

Petrásek J, Mravec J, Bouchard R, et al. 2006. PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science.* **312**: 858-560.

Prasch CM and Sonnewald U. 2015. Signaling events in plants: Stress factors in combination change the picture. *Environ Exp Bot.,* **114**:4-14.

Ryu H and Cho YG. 2015. Plant hormone,s in salt stress tolerance. *J Plant Biol.,* **58**: 147-155.

Sharma E, Sharma R and Borah P. 2015. Emerging roles of auxin in abiotic stress responses. *In*: Pandey GK (Ed) **Elucidation of Abiotic Stress Signaling in Plants**. Springer, New York, pp 299-328.

Sudhakar C, Lakshmi A and Giridarakumar S. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci*., **161**: 613–619.

Vanneste S and Friml J. 2009. Auxin: a trigger for change in plant development. *Cell,* **136**: 1005-1056.

Wang W, Gu L, Ye S, et al. 2017. Genome-wide analysis and transcriptomic profiling of the auxin biosynthesis, transport and signaling family genes in moso bamboo (*Phyllostachys heterocycla*). *BMC Genomics,* **18**: 870-885.

Wang Y, Chai C, Valliyodan B, Maupin C, Annen B and Nguyen H T. 2015. Genome-wide analysis and expression profiling of the PIN auxin transporter gene family in soybean (*Glycine max*). *BMC Genomics*, **16**: 951-963.

Wani S, Kumar V, Shriram V and Sahd S. 2016. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J*., **4**: 162-176.

Woodward AW and Bartel B. 2005. Auxin: regulation, action, and interaction. *Ann Bot.,* **95**: 707-735.

Yamamoto Y, Kamiya N, Morinaka Y, Matsuoka M and Sazuka T. 2007. Auxin biosynthesis by the YUCCA genes in rice. *Plant Physiol.,* **143**: 1362-1371.

Yu C, Dong W, Zhan Y, Huang Z, Li Z, Kim S and Zhang C. 2017. Genome-wide identification and expression analysis of ClLAX, ClPIN and ClABCB genes families in *Citrullus lanatus* under various abiotic stresses and grafting. *BMC Genetics*, **18**: 33-47.

Yurekli F, Porgali B and Turkan I. 2004. Variations in abscisic acid, indole-3-acetic acid, gibberellic acid and zeatin concentrations in two bean species subjected to salt stress. *Acta Biol Cracov Bot.,* **46**: 201-212.

Zhao Y. 2012. Biosynthesis: A simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. *Mol Plant,* **5**: 334-338.

Zhao Y. 2014. **Auxin biosynthesis**. The Arabidopsis book. American Society of Plant Biologists. e0173. doi: 10.1199/tab.0173.

Zhao Y, Wang T and Zhang W, Li X. 2011. SOS3 mediates lateral root development under low salt stress through regulation of auxin redistribution and maxima in Arabidopsis. *New Phytol.,***189**: 1122-1134.

Zheng L, Zhang L, Duan K, et al. 2016. YUCCA type auxin biosynthesis genes encoding flavin monooxygenases in melon: Genome-wide identification and developmental expression analysis. *S Afr J Bot.,* **102**: 142-152.

Zhou JJ and Luo J. 2018. The PIN-FORMED auxin efflux carriers in plants. *Int J Mol Sci.,* **9**: 2759-2779.

Zörb C, Geilfus CM, Mühling KH and Ludwig-Müller J .2013. The influence of salt stress on ABA and auxin concentrations in two maize cultivars differing in salt resistance. *J Plant Physiol.,* **170**: 220-224.