Acta Physiol Plant (2015) 37:35
DOI 10.1007/s11738-015-1787-x

ORIGINAL PAPER

Effect of salt treatment on growth, isoenzymes and metabolites of *Andrographis paniculata* (Burm. f.) Nees

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Received: 26 June 2014 / Revised: 14 January 2015 / Accepted: 15 January 2015 / Published online: 28 January 2015
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Abstract *Andrographis paniculata* is a well-known medicinal plant having vast therapeutic potentials. Efficient use of saline land for promoting the cultivation of medicinal plants is valuable for pharmaceutical and economic benefits. Relatively little is known about the physiological and biochemical basis of salt tolerance in this plant. Here, we studied the growth, isoenzymes, malondialdehyde, proline and secondary metabolites of *A. paniculata* in response to different salt treatments with NaCl (0, 41.1, 92.4, 143.7 and 193.4 mM). The results indicated that growth traits were decreased and all the expression level of superoxide dismutase (SOD), catalase (CAT), cytochrome oxidase (CYT), peroxidase (POD), polyphenol oxidase (PPO) and esterase (EST) isoenzyme in leaves and roots was enhanced with increasing salinity. Malondialdehyde was positively correlated to salt levels. Proline decreased at first and then dramatically increased at 193.4 mM. The content of key secondary metabolites was continuously increased under less than 143.7 mM NaCl concentrations. Results suggest that *A. paniculata* has relatively salt resistance by activating enzyme expressions both in roots and leaves, elicitation of secondary metabolites and osmoprotectant, but also reasonably regulating the allocation of resources. It is preferent to using resources for defense rather than for growth in *A. paniculata* and there is metabolic shift from growth to defense under salt conditions. Induction of isoenzyme activities and production of secondary metabolites are the primary defense strategies. Additionally, moderate salt treatment could promote the accumulation of bioactive phytocompounds, which is valuable for breeding plants with high quality. Thus, the cultivation of this medicinal herb in moderate saline areas could be considered as an agricultural option. Furthermore, our upshorts can provide a reference for screening better salt-tolerant cultivars of *A. paniculata*.

Keywords *Andrographis paniculata* · Salt stress · Growth · Isoenzymes · Metabolites

Abbreviations

A. paniculata	*Andrographis paniculata*
PAGE	Polyacrylamide gel electrophoresis
UV-Vis	Ultraviolet-visible spectrophotometry
HPLC	High performance liquid chromatography
MDA	Malondialdehyde
POD	Peroxidase
SOD	Superoxide dismutase
PPO	Polyphenol oxidase
EST	Esterase
CYT	Cytochrome oxidase
CAT	Catalase
FW	Fresh weight

Introduction

Andrographis paniculata (Burm.f.) Nees, one of the most important and popular medicinal plant in China, generally known as "Chuan-Xin-Lian" has drawn much attention of researchers in recent times because of its pharmacological

Communicated by L. A. Kleczkowski.

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and pharmaceutical potentials. It exhibits anticancer, antidiabetic, anti-HIV, antibacterial, anti-malarial, anti-inflammatory, anti-angiogenic, hepatoprotective and cardioprotective properties (Subramanian et al. 2012; Chowdhury et al. 2012; Sandborn et al. 2013; Uttekar et al. 2012), which are credited to diterpene lactone compounds such as andrographolide, dehydroandrographolide and deoxyandrographolide (Zhang et al. 2013; Kumar et al. 2004). The plant has been positioned as a prioritized medicinal plant of Guangdong and Guangxi province in China with a demand of 7,500–9,000 tons in year 2011–2012 and annual growth of 12.2 %. However, poor growth and yield is more and more frequent because of various biotic and abiotic factors these years, which has hampered the profitable exploitation of this plant. *A. paniculata* cultivation is easy because of its excellent acclimation to plains, hills, dry or wet lands, seashores and even roadsides (Valdiani et al. 2012). Zhang et al. (2007) reported that the yield character and quality trait of Chuanxinlian are contradictory each other. Hence, to meet the continuously increasing demand of *A. paniculata* for traditional medical systems as well as pharmaceutical industries, it is necessary to further enlarge the planting scale and develop stress-resistent germplasm innovation for improving the yield and quality of raw medicinal materials. At present, the planting bases in China mainly located in Guangdong, Guangxi, Fujian, Hainan and Sichuan province (Shao et al. 2013), and these areas are vulnerable to storms, inundation and sea level rise, which is easy to initiate salt stress.

Soil Salinity is a very serious abiotic stress influencing agricultural production and ecological environment. Soils are known as saline when the EC is 4 dS m^{-1} or more and Nielsen et al. (2011) found that salinity, approximately 40 mM NaCl (2 dS m^{-1}), would adversely affect crop growth and productivity. Approximately 3.6×10^7 ha of lands and 7 % of arable land in China have already been salt affected (Yang 2008) and, more severely, salinization is still expending. About 10 Mha of irrigated lands are abandoned annually because of excessive salinity all over the world. Salinity induces a wide variety of plant responses such as altered gene expression, readjustment of metabolic processes, growth inhibition and so on (Munns and Tester 2008). It is reasonable to believe that two or more of these processes may be occurring simultaneously, but whether they ultimately affect crop yield or quality depends on the salinity level, natural habitat of the species, and numbers of other environmental factors. It is hardly possible to improve soil properties to enhance plant growth. Therefore, an effective way to use saline lands should be found through understanding the responses of plants to saline and cultivation of tolerant cultivars (Chawla et al. 2013). On the other hand, plants can have the capacity to accommodate extreme salinity because of special morphological and physiological adaptations or avoidance mechanisms (Uygur and Yetisir 2009). Isoenzymes are helpful biochemical markers reflecting changes in metabolic activities, corresponding to molecular events associated with growth and development or stress tolerance (Pramanik et al. 1996). Moreover, variations in enzyme activities of tissues detected by electrophoretic patterns on zymograms can easily and clearly reflect physiological activities during growth, development and differentiation of plant and as such are useful biochemical indices (Rao et al. 1992). Additionally, osmotic adjustment by producing some regulators like proline are usually connected with the stress adaptation in plants, and enhanced synthesis of secondary metabolites under stressful conditions is believed to protect cells from oxidative effects (Karray-Bouraoui et al. 2011). It is necessary to understand the ways that plants tolerate salt stress conditions for growing and developing plants on salt-affected lands. Ashraf and Orooj (2006) have reported some salt-tolerant medicinal and aromatic plants, which indicated the potential of producing medicinal plants in saline areas.

Although a wide range of physiological and biochemical adaptations to face saline conditions have been observed in numerous agronomic crop species, not much information is available in medicinal plants. Regarding *A. paniculata*, there have been no reports indicating the responses and underlying mechanisms about isoenzymes and some metabolites under salt conditions. A comprehensive determination of agro-morphological, physiological, and phytochemical behaviors of *A. paniculata* under salt stress will be helpful to achieve better understanding of the salt-tolerance mechanisms and lay a foundation for breeding better salt-tolerant and even higher quality varieties. Therefore, this work was undertaken to study the effect of salt treatment on growth, SOD, CAT, CYT, POD, PPO and EST isoenzymes, malondialdehyde, proline and secondary metabolites of *A. paniculata*, and to elucidate the mechanisms of defense strategies in terms of enzymatic and biosynthetic activities.

Materials and methods

Plant materials and treatments

Seeds of *A. paniculata* were provided by Hutchison Whampoa Guangzhou Baiyunshan Chinese Medicine Company Limited. The seeds were surface sterilized with 10 % sodium hypochlorite solution for 10 min, thoroughly rinsed with distilled water, and then soaked in petri dishes containing filter papers moistened with sterile water for germination. The petri dishes were sealed with parafilm to

prevent any water loss and then incubated under controlled growth chamber (light/dark regime of 14/10 h at 27–30 °C, relative humidity 60–75 %). The germinated seeds at two initial leaf stage were transferred into nursery seedling plates in a vinyl house under natural light conditions (the temperature was between 28 and 34 °C during the day and 25–27 °C during the night, relative humidity ranged from 42 to 64 %, the average light intensity was 396 μmol m^{-2} s^{-1}) and were maintained until eight leaf stage. Then grown in individual pots containing a 2:1 (v:v) mixture of locally available red laterite soil and peat with one plant per pot (Fig. 1). After growing the plants for 10 days, salt treatments were imposed. Experiments were conducted in the same vinyl house, and irrigated once a day with 500 mL of the Hoagland nutrient solution with different salinities (control, 4, 8, 12 and 16 dS m^{-1}) during morning hours for a period of 15 days, which was based on that 12 dS m^{-1} salinity and 15 days stress were concluded as the extreme salinity level and exposure time (Talei et al. 2013a, b, c). These salinities were applied using 41.1, 92.4, 143.7 and 193.4 mM NaCl solution added respectively to Hoagland nutrient solution. The Hoagland nutrient solution was used as the control treatment. Excess of irrigation water was allowed to freely drain from the bottom of the pots for avoiding excessive accumulation of salt in root zone (Uygur and Yetisir 2009). To prevent the seedlings' full death, after three salinity applications, the seedlings were all irrigated with 500 mL normal Hoagland nutrient solution (Talei et al. 2013b). Each treatment was replicated three times. After 15 days of treatments, plants were harvested and relevant data were collected.

Measurement of plant growth

After salt treatment, growth parameters including plant height, number of blades, shoot number and shoot length were recorded immediately, and mean ± SD values were calculated for each treatment. The sixth/fifth fully expanded leaves from the apex of the main stem were sampled for leaf area determined by the method of Bassil and Kaffka (2002). Total fresh weight of whole-plant was evaluated after being washed thoroughly with water to remove soil traces. For total dry weight determination, the whole plant was oven-dried at 40 °C until constant weight, about 72 h.

Enzyme extraction and PAGE assays

Tissue extraction of the samples was prepared for the analyses by grounding 0.5 g of fresh leaf or root material to a fine powder with liquid nitrogen and homogenizing the powder in 1.0 mL of ice cold 0.1 M Tris–HCl buffer (pH 7.8). The homogenate was centrifuged at 12,000×*g* for 20 min at 4 °C and supernatant was used for the polyacrylamide gel electrophoresis (PAGE) analyses of superoxide dismutase (SOD), catalase (CAT), cytochrome oxidase (CYT), peroxidase (POD), polyphenol oxidase (PPO) and esterase (EST) isoenzyme in leaves and roots. SOD, POD and EST isoenzymes were run on 1.0 mm thick polyacrylamide gels by 10 % separating gel and 4 % stacking gel. CYT isoenzyme was separated by 7 % separating gel and 3 % stacking gel. CAT isoenzyme was separated by 7 % separating gel and 2.5 % stacking gel. PPO isoenzyme was separated by 10 % separating gel and 3.75 % stacking gel. 20 μL of each sample was loaded. Electrophoresis was performed at 10 mA direct current and then at 25 mA constant current at 4 °C until the bromophenol blue dye front reached the bottom end of the separating gel. Staining was carried out as described by Hu and Wan (1985). After electrophoresis, the SOD zymogram was visualized by incubation for 1.0 h in a 10 mM K-phosphate buffer (pH 7.2) containing 2 mM anisidine and 0.1 mM riboflavin, under illumination. To detect CAT isoforms, the gel was rinsed with deionized water after electrophoretic run. After 10 min of soaking in 0.9 mM H_2O_2, a staining solution consisting of 2 % K-ferricyanide and 2 % ferric chloride was added. The CYT isoforms were observed by incubation at 37 °C for 10 min in a mixed solution containing 1 % (w/v) dimethyl-p-phenylenediamine, 1 % (w/v) α-naphthol dissolved in 40 % ethyl alcohol, and 0.1 M K-phosphate buffer (pH 7.4) according to the ratio of 1:1:25, in darkness. POD zymogram was

Fig. 1 Seedlings of *A. paniculata* at eight leaf stage grown in individual pots before salt treatment

visualized by incubating the gel for 5 min in a solution containing 70.4 mg ascorbic acid, 20 mL of benzidine storage solution (containing 2 g benzidine, 18 mL acitic acid and 72 mL H_2O), 20 mL of 3 % (v/v) H_2O_2 and 60 mL H_2O. PPO isozymes were detected by incubating the gel in a solution containing 20 mL of 0.06 % (w/v) α-phenylenediamine dissolved in 0.01 M oxalic acid, 60 mL of 0.05 M catechol and 20 mL of K-phosphate buffer (pH 6.8) for 30 min. For EST, the gel was incubated at 37 °C for 15 min in a solution containing 30 mg fast blue RR salt dissolved in 30 mL of 0.1 M K-phosphate buffer (pH 6.4), 2 mL of 1 % α-naphthyl acetate and 1 mL of 2 % β-naphthyl acetate both prepared with 80 % (v/v) alcohol, in darkness. Each enzyme were photographed with a digital camera.

Determination of MDA and proline

Malondialdehyde (MDA) contents were quantified as an estimate of membrane lipid peroxidation and estimated by following the procedure devised by Hameed et al. (2012). Fresh leaves (0.5 g) were homogenized in 5 mL of 1 % ice-cold trichloroacetic acid in ice-chilled mortar and pestle. The homogenate was centrifuged at 12,000×*g* for 20 min at 4 °C. Two milliliter of supernatant was mixed with 2 mL of 20 % (w/v) trichloroacetic acid containing 0.5 % 2-thiobarbituric acid heated at 95 °C for 30 min in a water bath and then cooled quickly on an ice bath. Afterwards, the mixture was centrifuged at 4 °C for 10 min at 12,000×*g* and the absorbance of the supernatant was measured at 532, 600 and 450 nm. The concentration of MDA was calculated from its extinction coefficient of 155 mM^{-1} cm^{-1}.

Proline was extracted and its content determined, as described by Nagaich et al. (2013). 0.5 g of fresh leaves was homogenized in 3 % (w/v) aqueous sulfosalicylic acid solution, centrifuged at 3,000 rpm for 20 min. The supernatant was treated with acid ninhydrin (2.5 g ninhydrin per 100 mL of a solution containing glacial acetic acid, distilled water, and 85 % ortho-phosphoric acid in the ratio 6:3:1) boiled for 1 h, and the reaction was terminated by keeping it in an ice bath for 10 min. The absorbance was then determined at 520 nm.

Diterpene lactone extraction and HPLC analysis

Harvested leaves were oven-dried at 40 °C and powdered (particle size was less than 0.5 mm) (six replicates per treatment). Leaf extracts were obtained by ultrasonic instrument for 30 min of 0.5 g of dry powder with 10 mL ethanol (85 %). The extracts were then filtered through a 0.22 μm filter paper, and stored at 4 °C until analyzed. Estimation of diterpene lactone in *A. paniculata* leaf was done using HPLC–UV. Chromatography was conducted on a Perkin Elmer 200 HPLC system using a Unimicro Technologies Ins C_{18} column (5 μm, 250 mm × 4.6 mm) with 20 μL injection and a flow rate of 0.8 mL min^{-1}. The mobile phase consisted of 0.3 % phosphoric acid in deionized water (A) and acetonitrile (B) using a gradient program of 13–23 % B in 0–10 min, 23 % B in 10–30 min, 23–35 % B in 30–35 min, 35 % B in 35–55 min and 35–80 % B in 55–60 min. The column temperature was maintained at 30 °C and absorbance was measured at 226 nm. Total lactone was the sum of each lactone compound.

Statistical analysis

Experimental data of all parameters were analyzed using one-way ANOVA method and significant differences at $p \leq 0.05$ were determined.

Results

Growth performance

Varying salt concentrations showed visible limiting effects on plant growth after 15 days salt treatment. The stunted growth was seen as shown in Fig. 2. With increasing salt level, its taproot was more and more underdeveloped and the lateral root was developed conversely, even forming tangled roots (Fig. 3). All the growth traits comprising plant height, leaf number, leaf area, shoot number, shoot length, total fresh weight, dry weight and taproot length were decreased with increasing salt concentration (Fig. 4), which indicated that the growth of *A. paniculata* was negatively correlated to the salt levels. Seedlings grown at high salt levels (≥143.7 mM NaCl) got significant reduction of those agronomical traits and even showed toxicity symptoms as growth depression, appearing scorched leaf tips and margins, leaf curling, and abscission of leaves (Fig. 2d, e). The total dry weight was significantly reduced by 50.5 % in comparison to the control at 193.4 mM NaCl concentration. There was no obvious necrotic performance observed at less than 143.7 mM NaCl conditions (Fig. 2b, c).

Isoenzyme assays

The PAGE analysis revealed the expression levels of studied enzymes including SOD, CAT, CYT, POD, PPO and EST were enhanced when increasing NaCl concentration (Fig. 5) and the activity of these enzymes changed to a different extent, also depending on the part of the plant. For all studied enzymes, there were increases in the intensity of some isoenzyme bands, denoting enzyme

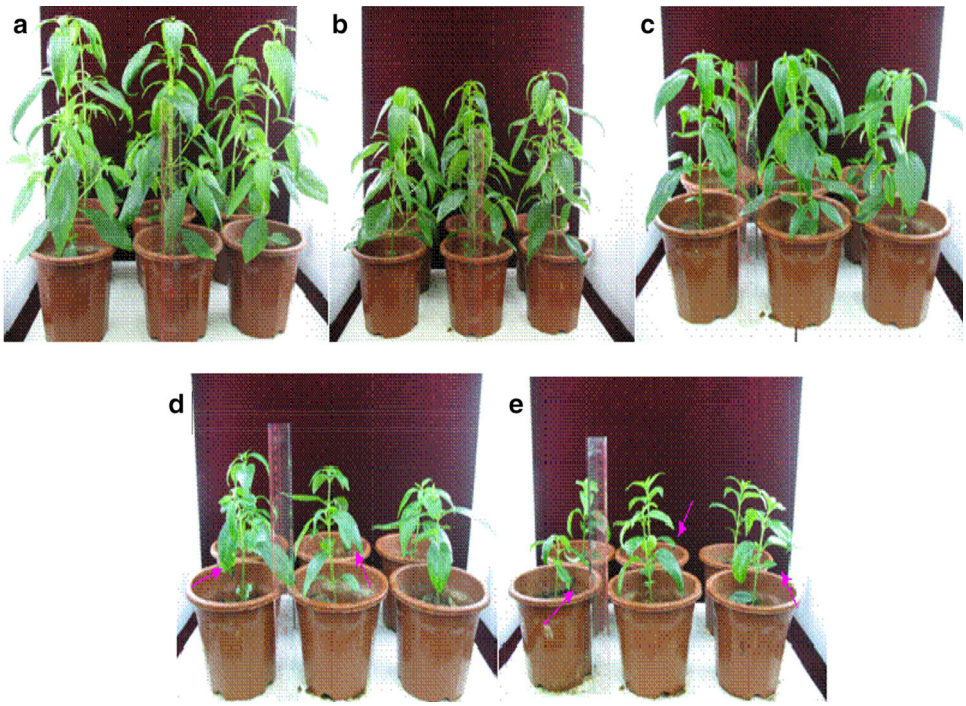


Fig. 2 Morphological observation of *A. paniculata* after 15 days of 0 mM (**a**), 41.1 mM (**b**), 92.4 mM (**c**), 143.7 mM (**d**) and 193.4 mM (**e**) NaCl treatment. *Marked areas* represent necrotic regions after 15 days of salt treatment

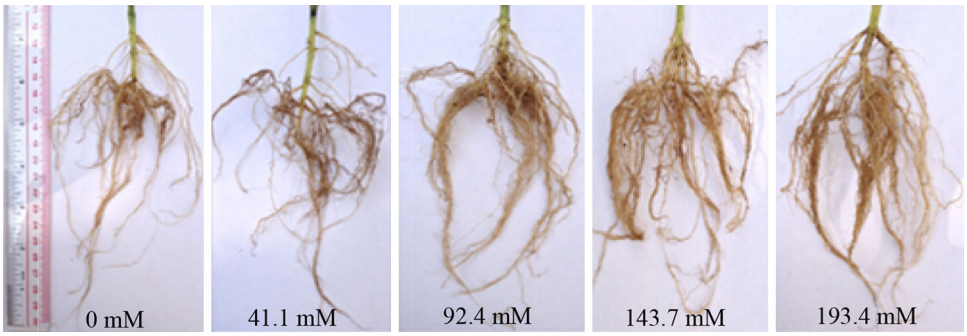


Fig. 3 Root morphological character of *A. paniculata* after 15 days of NaCl treatment

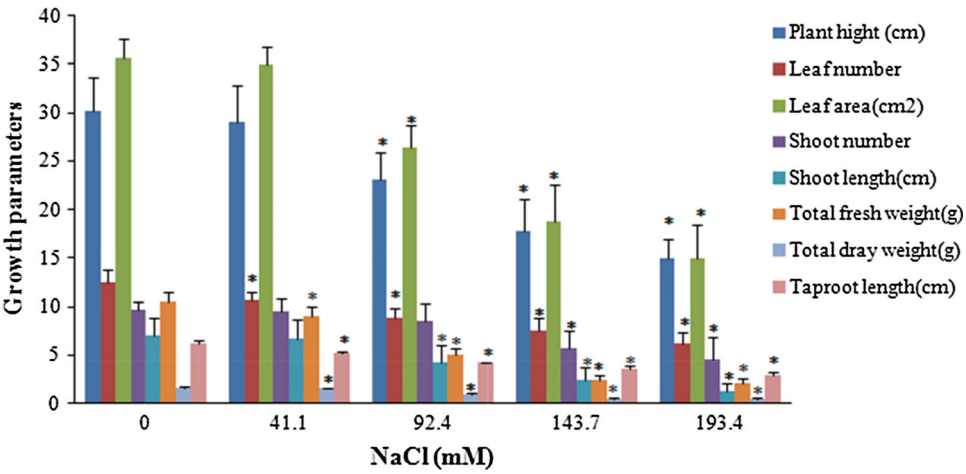


Fig. 4 Growth parameters of *A. paniculata* subjected to different levels of salt concentration. *Bars* are mean values of three independent replicates ± SD. Significantly different values at $p \leq 0.05$ are represented by *asterisk*

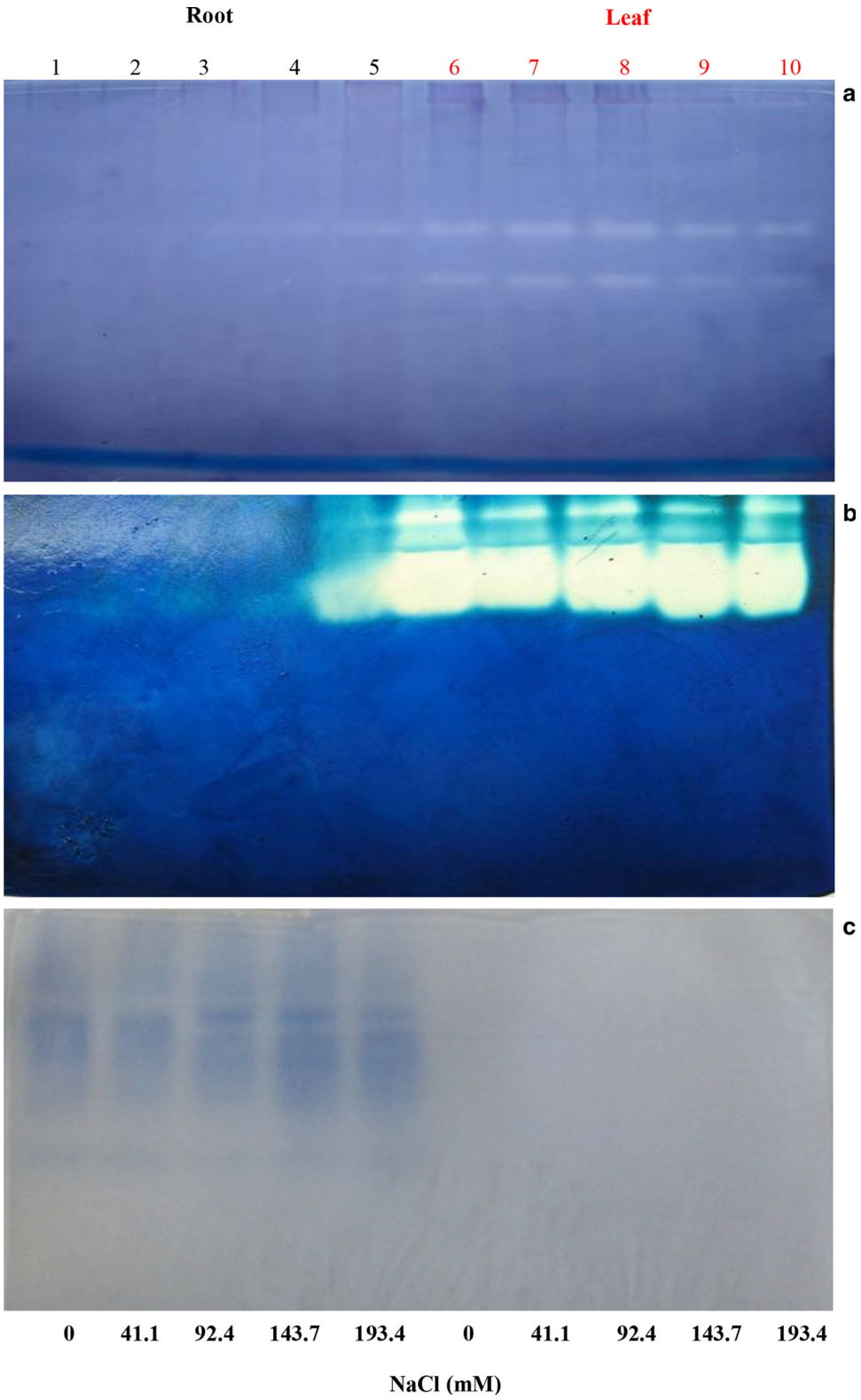
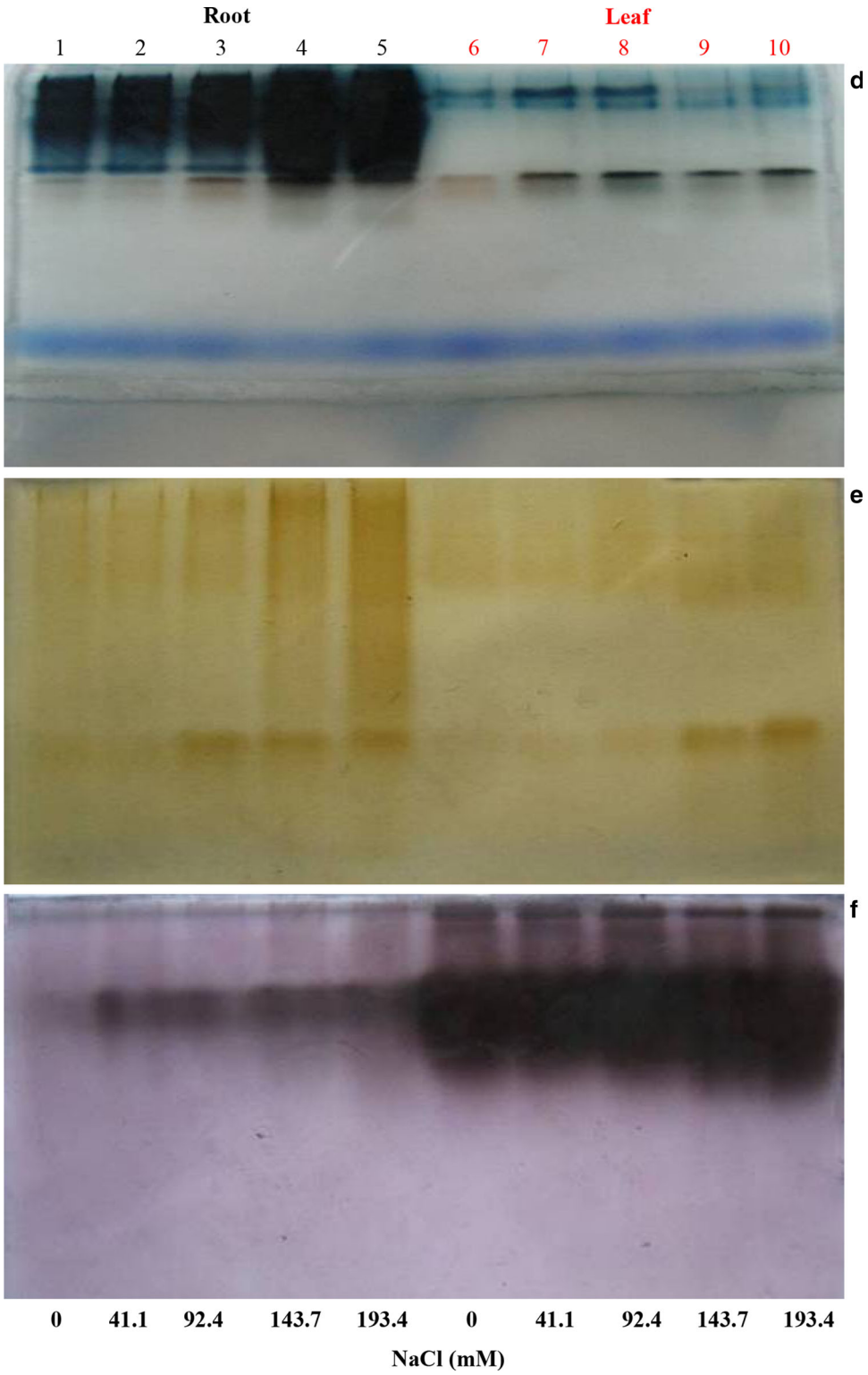


Fig. 5 Revelation of superoxide dismutase (SOD) (**a**), catalase (CAT) (**b**), cytochrome oxidase (CYT) (**c**), peroxidase (POD) (**d**), polyphenol oxidase (PPO) (**e**), and esterase (EST) (**f**) on polyacrylamide gel in roots and leaves of *A. paniculata* grown at 0, 41.1, 92.4, 143.7 and 193.4 mM NaCl conditions for 15 days (*1*, *2*, *3*, *4*, *5*—root; *6*, *7*, *8*, *9*, *10*—leaf)

activation both in roots and leaves. At low salt levels, the SOD and CAT isoenzymes in roots were not detected on the gel due to their very low activity. At the highest salt level, *A. paniculata* roots presented the highest enzyme activities, thus indicating that SOD and CAT in roots take part in detoxifying process only above a certain level of

Fig. 5 continued



toxic ion accumulation. CYT isoenzymes are active in root but not in leaf. In leaves, the band intensity changes of SOD and CAT with salt stress were not obvious, whereas a noticeable increase in band intensity of POD isoforms at less than 143.7 mM NaCl was observed. Salt treatment led to a significant increase in band intensity of PPO and EST isoenzyme both in roots and leaves. Additionally, there was a great difference in the aforementioned isoenzyme zymograms between the leaf and root. In comparison, there was more noticeable band intensity of SOD and CAT isoforms in leaf than root while CYT and PPO isoenzymes performed better in the root, which meant that leaf

exhibited a stimulation of enzyme activities with higher SOD and CAT while root experienced higher CYT and PPO activities when the plant was damaged by salt. The distinct enzyme expression patterns and levels in the leaf and root may be associated with the accumulation of different phytocompounds in the different parts of this herb that leaves of *A. paniculata* mainly contain diterpene lactones while flavonoids are accumulated in the roots (Xu and Wang 2011). Interestingly, POD and EST showed a considerable increase in roots as well as in leaves, which indicated that POD and EST isoenzyme could be used as well as bioindicators for evaluation of the stress resistance of *A. paniculata*.

Lipid peroxidation and proline

The extent of oxidative damage was estimated as the concentration of MDA, a product of membrane lipid peroxidation. Increasing supply of NaCl caused a significant increasing effect on the levels of MDA in leaves of *A. paniculata* (Fig. 6a). So under saline conditions, *A. paniculata* exhibited a significant increase in lipid peroxidation and this salt-induced effect was most pronounced by 44.8 % at 193.4 mM NaCl compared with the control.

Proline in the leaves under salt condition played an important role in the acclimation to salt stress. At first, proline levels in *A. paniculata* leaves were decreased under less than 143.7 mM NaCl concentrations. However, there was a huge increase of proline level noticed at the highest salt regime (193.4 mM NaCl) (Fig. 6b).

HPLC analysis of diterpene lactone compounds

It is agreed that diterpene lactones are the major secondary metabolites responsible for pharmacological activities of *A. paniculata*. The high-performance liquid chromatography fingerprint of diterpene lactone compounds in *A. paniculata* leaf was obtained and three key representative chemicals were identified with retention time for andrographolide (29.5 min), deoxyandrographolide (49.6 min) and dehydroandrographolide (50.7 min) (Fig. 7). All the three secondary metabolites as well as total lactone (summing the above three compounds) were increased significantly ($p \leq 0.05$) with increasing salinity at moderate salt concentrations ($\leq$143.7 mM NaCl) than control (Fig. 8). The maximum increase in the quantities of andrographolide, dehydroandrographolide and total lactone at 143.7 mM was 31.5, 39.8 and 30.8 % in comparison to the control, respectively. The maximum increase in deoxyandrographolide at 92.4 mM was 60.7 %. Andrographolide and total lactone were always increased significantly compared to the control. At 193.4 mM NaCl, all of them were relatively decreased. In addition, there were no additional ingredients generated in *A. paniculata* leaves with increasing salinity according to their fingerprints of HPLC.

Discussion

The growth of *A. paniculata* in current study was limited by increasing levels of NaCl and the negative impact was particularly marked when NaCl level was above 143.7 mM, which are in concurrence with *A. paniculata* which was categorized in the group of sensitive plants to salinity according to morphologic properties (Talei et al. 2012) and 12 ds m^{-1} salinity (about 143.7 mM NaCl) was suggested as a salt threshold of *A. paniculata* seedlings (Talei et al. 2013a, b, c). Its poor growth and yield reduction under salt conditions were probably caused by the perturbation of various physiological and biochemical processes at the cellular, tissue or whole-plant level (Sofo

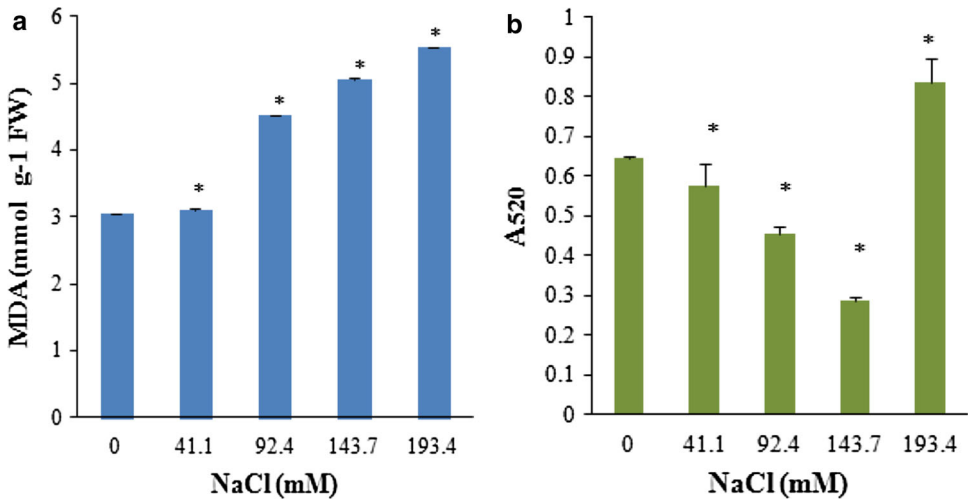


Fig. 6 Effects of salt stress on malondialdehyde (MDA) concentration (**a**) and proline content (**b**) in the leaves of *A. paniculata* subjected to different salinity conditions (0, 41.1, 92.4, 143.7 and 193.4 mM NaCl). *Bars* are mean values of three independent replicates ± SD. Significantly different values at $p \leq 0.05$ are represented by *asterisk*

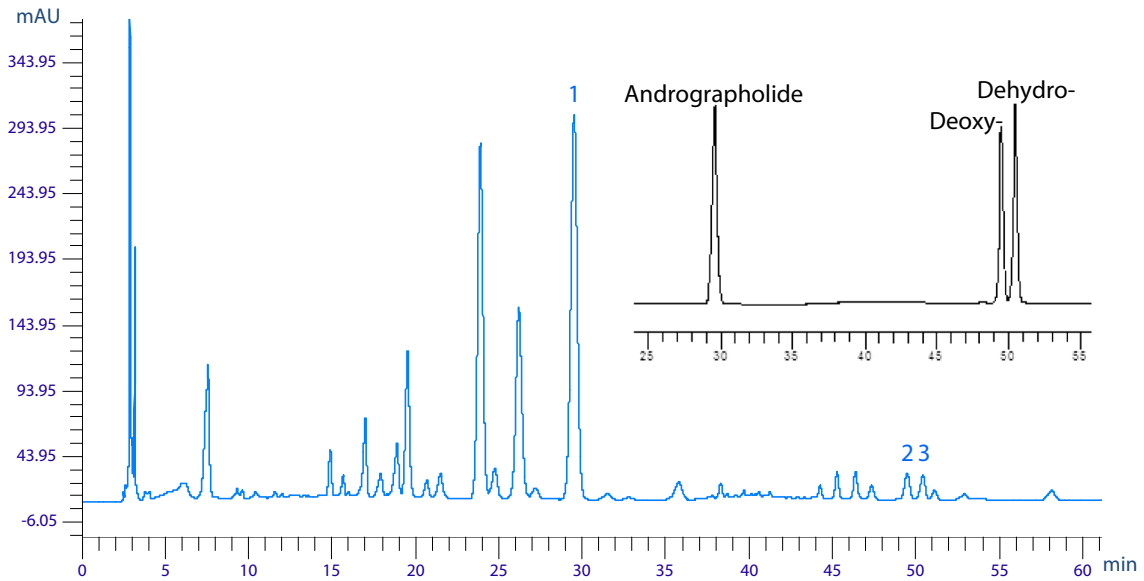


Fig. 7 HPLC fingerprint of diterpene lactone compounds in *Andrographis paniculata* leaf (*1*—andrographolide; *2*—deoxyandrographolide; *3*—dehydroandrographolide)

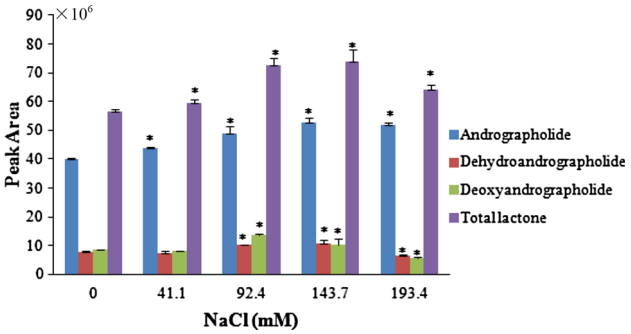


Fig. 8 The content changes of secondary metabolites in the leaves of *A. paniculata* grown at 0, 41.1, 92.4, 143.7 and 193.4 mM NaCl conditions for 15 days. *Bars* are mean values of six independent replicates ± SD. Significantly different values at $p \leq 0.05$ are represented by *asterisk*

et al. 2010). Besides, *A. paniculata* belongs to susceptible variety presenting a gradually rising level of MDA with increasing of salinity in this study. Similar results were reported by Karray-Bouraoui et al. (2011) who found that more salt sensitive provenance of *Carthamus tinctorius* L. had higher level of lipid peroxidation. MDA is a reliable indicator of membranes oxidative damages and has been largely used as a criterion to distinguish stress tolerant and sensitive cultivars (Bandurska and Gniazdowska Skoczak 2012). Increase in lipid peroxidation with higher NaCl in *A. paniculata* could be correlated with accumulation of reactive oxygen species or other secondary mechanisms such as ion toxicity and osmotic stress (Abrol et al. 2012) and initiated by enzyme lipoxygenase (Macri et al. 1994).

To cope with stress injury, plants finely regulate enzymatic defense systems to counter the deleterious effects (Hasanuzzaman et al. 2012). The combined actions of various enzymes are critical in mitigating the effects of oxidative stresses (Rasool et al. 2013). In addition, root systems are the most critical part of the plant facing with the soil-related stress factors such as salinity, so root characteristics determine, at least partially, the salinity response of plants for the injury degree or the alleviation of deleterious effect of salt stress on the shoot growth (Santa-Cruz et al. 2002), so we researched the enzyme performance in both roots and leaves. Jin et al. (2009) elucidated that the activity increase is a consequence of changed isoenzyme. Moreover, it has been reported that unchanged or enhanced enzyme activities were observed in salt-tolerant cultivars to reduce stress severity thus allowing cell growth to occur (Turhan et al. 2008). In this study, SOD, CAT, CYT, especially POD, PPO and EST in both roots and leaves displayed induction of enzyme activities in *A. paniculata* under salt conditions, suggesting that a biosynthesis stimulation of antioxidase and esterase can constitute a protective role against adverse effects. CYT, as a proton pump, has to provide large energy for all cells and take an active part in mitochondrial respiration and oxidation in the root. Radic and Pevalek-Kozlina (2010) studied esterase activity and isoesterase pattern in leaves and roots of *C. ragusina* subjected to NaCl-induced stress and implied that esterase activities and their isoenzymic patterns could serve as useful bioindicators of salinity. Our study of clear esterase isoenzymes and increasing activity also corroborates the findings of Hassanein (1999) who

demonstrated that the number of esterase isoenzymes in leaves was higher than in roots of peanut plants and the number of esterase isoenzymes increased under the influence of NaCl.

In this study, proline level was increased at 193.4 mM of NaCl, which is in agreement with the high accumulation of proline was shown in the tolerant accessions of *A. paniculata* (Talei et al. 2013a, b, c). As Mutlu and Bozcuk (2005) pointed out, proline accumulation could play a protective role against salt stress and stated by Ahmed et al. (2008), increase in proline content under extreme stress conditions could protect proteins against salt ions and inhibit the breakdown of proteins, membranes and subcellular structures. Larger amounts of NaCl acted more in inducing stress rather than balancing the ionic homeostasis (Munns and Tester 2008) so that stimulated *A. paniculata* to accumulate a mass of proline for protecting cellular functions.

Effects of salinity on medicinal plants must consider the production of bioactive secondary metabolites. Indeed, plants can utilize their secondary metabolites to withstand adverse conditions and elicitation with stressful growth environment has been widely used to enhance the yield of secondary metabolites in vitro (Vasconsuelo and Boland 2007). In present study, significant increases in andrographolide, deoxyandrographolide, dehydroandrographolide and total lactones, marker phytochemicals with important medicinal roles, were observed in *A. paniculata* under moderate salinity, suggesting that moderate salt treatment could promote the production of secondary metabolites. Similar to the results of Talei et al. (2013c), it is noteworthy that salt-tolerant *A. paniculata* were capable of accumulating anticancer phytochemicals like andrographolide. Furthermore, andrographolide was increased more significantly under salt stress than the other diterpenoids studied in this work is in agreement with that andrographolide production is not growth related but is necessarily produced under stress conditions or can be stimulated as a defense response (Gandi et al. 2012). Perhaps, the production of andrographolide requires low amount of energy.

In general, a fine regulation of enzymatic system and related metabolites occurred in *A. paniculata* subjected to salt stress. It utilized various resources for defense rather than for growth and development under salt environment, which necessarily resulted in growth depression. For survival, it could produce secondary metabolites and induce enzyme activities as primary defense strategies at low salt levels. However, it has been believed that production of secondary metabolites can consume a large chunk of energy (Caldentey and Inze 2004), which is evident from the limited growth and severe lipid peroxidation. Abrol et al. (2012) reported that induction of enzymatic defense is desirable for normal plant growth under adverse environment, but it would impede the secondary metabolite production. So at high NaCl concentration (193.4 mM), a metabolic shift from production of phytochemicals to higher enzyme activities and accumulation of proline has to be formed. Meanwhile, the enzyme actions in roots and leaves need to coordinate mutually, which conform to the theory of optimal defense suggesting allocation of resources based on the value of particular tissue and the attack (Orians and Ward 2010). Nevertheless, further study is needed for the roles of different enzymes, relevant phytochemicals of each part in *A. paniculata* and the relationships between them.

Conclusion

Based on our comparative analysis, *Andrographis paniculata* showed tolerance to medium salinity, although stunned growth was observed at higher salinity levels. The enzymatic and biosynthetic activities belong to the most important mechanisms of its stress tolerance. Salt-induced enhancement of antioxidative enzymes and esterase in roots and leaves and induction of secondary metabolites seems to be efficient ways for maintaining its growth and survival. Moreover, proline accumulation at high salinity showed considerable resistance to salinity.

A moderate salt treatment that does not noticeably affect biomass production can enhance the bioactive phytochemical yields in *A. paniculata*. Hence, the cultivation of this medicinal plant in moderate saline lands could be feasible, which is useful for expanding its ecological adaptability as well as increasing yield by enlarging planting areas. Our findings can be a baseline necessary to conduct further studies related to breeding *A. paniculata* cultivars with better stress tolerance and quality.

Author contribution statement Yan-hua Shao designed and carried out the experiment, performed data analysis, and wrote the manuscript. Jun-li Gao, Xiang-wei Wu and Qian Li participated in taking data on growth parameters. Jian-gang Wang provided the necessary reagents. Prof. Ping Ding supervised the research and reviewed the manuscript. Prof. Xiao-ping Lai obtained the funds. All the authors approved the final draft for submission and take full responsibility for the content. All the authors have contributed their efforts to this work, but also abide and satisfy the conditions of authorship.

Acknowledgments This work is supported by the key projects in the National Science & Technology Pillar Program during the Twelfth Five-year Plan Period Foundation of China (Grant No. 2011BAI01B01). The authors offer grateful thanks to School of Chinese Pharmaceutical Science, Guangzhou University of Chinese Medicine for the experimental sites, as well as to the Hutchison Whampoa Guangzhou Baiyunshan Chinese Medicine Company

Limited for the access to *A. paniculata* seed collection. We are thankful to Prof. Ding and Prof. Lai for helping in supervising and to relevant co-workers for their kind help in undertaking this study.

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