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Effects of water stress on berberine, jatrorrhizine and palmatine contents in amur corktree seedlings

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Abstract: Amur corktree (*Phellodendron amurense*) is one of the important associated species in broadleaved-Korean pine forests, and in the pharmaceutical resource plants of famous Chinese traditional medicine, named cortex phellodendri. Berberine, jatrorrhizine and palmatine are the main alkaloids to which high attention is paid by the researchers. In the present study, water stress treatments with four conditions, that is, mild drought, severe drought, waterlogging, and control (soil water potentials were controlled in the ranges of – 40– – 20 KPa, – 80– – 60 KPa, ＜ – 80 KPa and – 20–0 KPa), were performed using the technique of root-sphere osmotic irrigation in a soil pond. The changes in the main medicinal compositions of berberine, jatrorrhizine and palmatine contents under different water conditions were discussed. As for the annual growing of amur corktree seedlings, mild drought was generally beneficial to the synthesis and accumulation of the three above-mentioned alkaloid contents. The three alkaloid contents did not show great changes under severe drought whereas those contents had significantly reduced under waterlogging compared with controls. Meanwhile, the growth of amur corktree seedlings was inhibited by the treatments of drought and waterlogging. The height, diameter and biomass of amur corktree seedlings were significantly lower than those of the control seedlings, which meant that the three alkaloid contents in a single seedling still kept the highest in control seedlings. Stem cortex was the medical part in Chinese traditional medicine and also kept the most abundant of the three alkaloid contents in amur corktree. Results indicated that short periods of mild drought could improve the berberine contents in the stem cortex, which might have reference value for the cultivation of amur corktree seedlings to obtain alkaloids.

Key Words: water stress; amur corktree (*Phellodendron amurense*); berberine; jatrorrhizine; palmatine

As an important environmental factor for plant survival, water can function on plants in different states, quantities, and durations. Meanwhile, different species or the same species in different developmental stages may have different demands of water[1−5]. Until now, many researches have focused on the relationship between water stress and plant primary metabolism, for example, water effect on the quantities and qualities of agricultural and economical crops[6−11]. As secondary metabolites, alkaloids are the adaptive results of plants during the long process of plant evolution. Plant heredity controls the biosynthesis of alkaloids, whereas some environmental factors (including biological and abiological factors) could induce, promote or inhibit alkaloid production. Actually, the alkaloids could vary in quantity and quality when responding to environmental stress[12−20]. Therefore, to explore environmental regulations in plant alkaloids, the interactions between plants and environments can be thoroughly analyzed from the angles of secondary metabolites and provide some reasonable guidance for the cultivation of important alkaloid plants.

Amur corktree (*Phellodendron amurense*) is an important associated species in the broadleaved Korean pine forest, in Northeast China, and also a pharmaceutical resource plant for its phloem, a Chinese traditional medicine, named cortex phellodendri. Now berberine, jatrorrhizine, palmatine, magnoflorine and phellodendrine have been separated and identified from cortex phellodendri, and more attention has been paid, by the researchers, to berberine, jatrorrhizine and palmatine. However, there still lack of fundamental researches on the effects of soil water on the main medicinal components. In the present study, to explore influences of cultivating condi-

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tions on medicinal compositions and provide some basic information for illuminating the relationship between plant secondary metabolism and environments, the variations in the main medicinal compositions, berberine, jatrorrhizine and palmatine contents under different water conditions in amur corktree seedlings, through soil water controls, have been discussed.

1 Materials and methods

1.1 Amur corktree seedling cultivation and water controls

Experiments were preformed in the green house, Jilin Normal University, Jilin, China (43º9’N, 124º20’E; elevation 169 m). Amur corktree seeds were sown in the green house after three months of sand-burying at 0–5 ℃ in the refrigerator, and then transplanted for the water stress treatment.

Water control of amur corktree seedlings was done with the technique of root-sphere osmotic irrigation in a soil pool according to the methods of Cui *et al*[21]. The seedlings of amur corktree were transplanted into soil pools of 4 m in length, 1.2 m in width and 0.35m in depth, which were divided into 4 subpools. Spacing of seedlings was 15 cm between and within rows, and there were about 200 plants in each pool. Water treatments were set forth with 4 conditions: mild drought, severe drought, waterlogging and control, and soil water potentials were controlled in the ranges of －40–－20 KPa, －80–－60 KPa, ＜－80 KPa and －20–0 KPa, respectively. Plastic cloth was laid under the soil in the waterlogging pool, which made the soil surface able to slightly retain water. Severe drought treatment was in fact out of the range of tension measurement, and temporary slight wilting at noon was seen as the criteria for water control. Five repeats per treatment (five subpools) were selected randomly. Time for the water control was for 120 d.

1.2 Sampling and determination of alkaloid contents

Water treatment was started from June 10, 2004 to October 8, 2004, and samples were collected between 8:00 am and 9:00 am at intervals of 20 d and lasted for seven times. One seedling in a good growing condition was randomly selected from each subpool, respectively. After plant height and stem diameter at the base were measured, the root, stem xylem, cortex and leave were separated and dried at 80℃ to constant weight and then weighed, and alkaloid contents were determined (with five repeats).

The contents of berberine, jatrorrhizine and palmatine were determined on a high-performance liquid chromatography system (Waters, USA) consisting of 717 autosampler and 2487 UV detector following Qin Yanjie *et al*’s method. The alkaloids were ultrasonically extracted by 60% ethanol for 60 min. The analysis was preformed on a C18 column (KYA HIQ sil, Japan) with the mobile phase of acetonitrile aqueous solution (ACN/water 1:1, 1000 ml of solution containing 3.4 g of potassium dihydrophospate and 1.7 g of Sodium dodecyl sulfate) at a flow rate of 1.0 ml/min, an injection volume of 10 µl and a detective wavelength of 345 nm. Berberine and jatrorrhizine were bought from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Palmatine was bought from Sigma-Adrich Company.

2 Results and discussion

2.1 Effect of water stress on berberine contents in amur corktree seedlings

Berberine content was quite different in different parts of the amur corktree seedlings. It was the highest in the stem cortex (the part used as a Chinese traditional medicine), and the maximum was 36.6 times and 1.8 times that in stem xylem and roots, respectively, whereas berberine content in leaves was only 0.07% in the stem cortex. Berberine contents in stem xylem and leaves were so low that even if they were changed under water stress the absolute value was still very small, which meant little when compared with that in the stem xylem, and hence no more discussion was made (the same was the case with jatrorrhizine and palmatine). On the whole, berberine contents in stem xylem and roots of amur corktree seedlings increased with plant development, whereas they decreased in the roots at the end of the treatment (on October 8, amur corktree seedlings were at the end of the growing season) (Fig.1).

From the point of view of stem cortex with the highest content of berberine, berberine content was the highest under mild drought from the 20th day to the end of the treatment, showing a significant difference (P < 0.01) from others after 80 days of treatment. Berberine contents were similar under severe drought and waterlogging, lower than those in the control since the 60th day of the treatment, and close to those in the control at the end of the treatment (on October 8). Similar to stem cortex, berberine contents in roots were the highest under mild drought stress, and those under severe drought and waterlogging were close to or lower than those in the control (Fig.1).

Stem cortex and roots are the main parts containing berberine, and hence in general, mild drought may be beneficial to the biosynthesis and accumulation of berberine in amur corktree seedlings, whereas berberine contents under severe drought and waterlogging are close to or lower than those in the control in amur corktree seedlings.

2.2 Effect of water stress on jatrorrhizine contents in amur corktree seedlings

Jatrorrhizine content in amur corktree seedlings was far lower than the berberine content, which was about 3% of berberine content. Jatrorrhizine content was the highest in the stem cortex too; the maximum was 22.3 and 4.3 times that in stem xylem and roots, but no jatrorrhizine was detected in the leaves. Jatrorrhizine content rapidly increased in stem cortex with plant development, showing a single-peak curve and

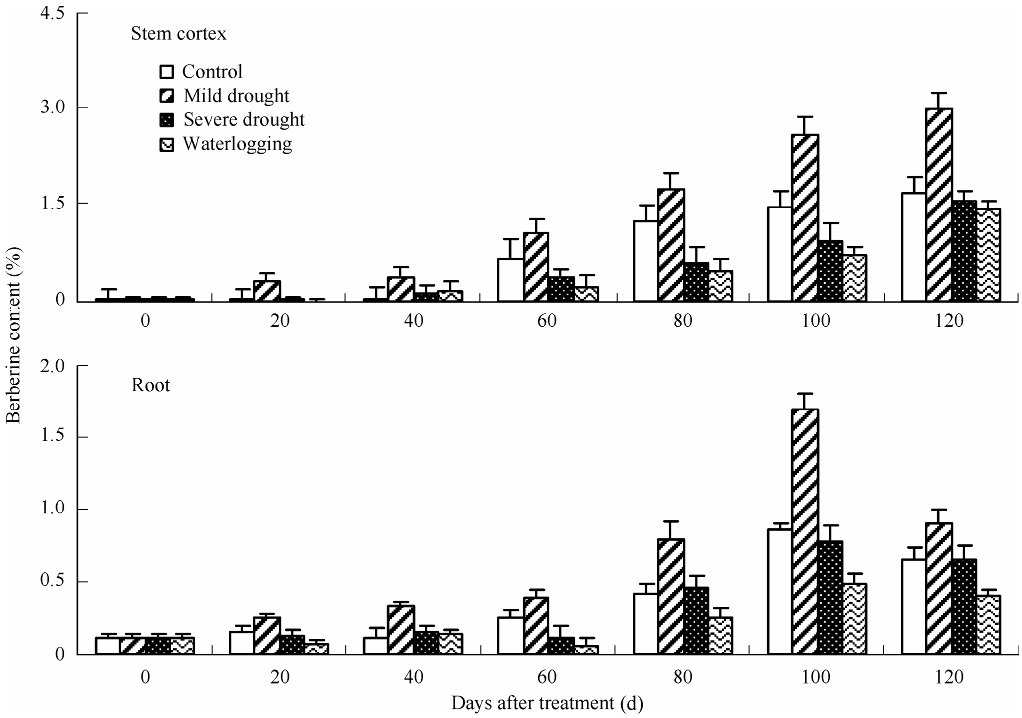

Fig. 1 Changes of berberine content of amur corktree seedlings under water stress

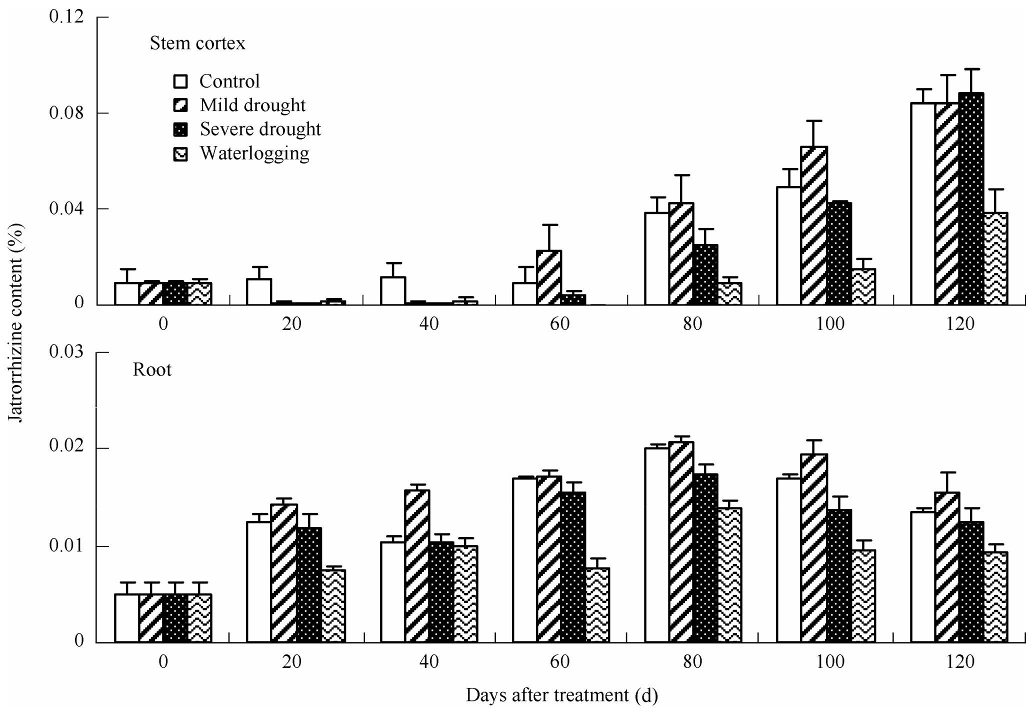

Fig. 2 Changes of jatrorrhizine content of amur corktree seedlings under water stress

arriving at the highest level on the 80th day (Fig. 2).

Jatrorrhizine content in the stem cortex of amur corktree seedlings tended to be higher after 60 days under mild drought treatment than in the control treatment, but the differences were not significant ($P > 0.05$), whereas jatrorrhizine content under severe drought treatment was lower than or close to that in the control, showing no significant difference yet ($P > 0.05$). Jatrorrhizine content was significantly lower than that in the control after 60 days waterlogging treatment ($P < 0.01$). Jatrorrhizine content in roots was remarkably higher than that in the control under 40 days of mild drought treatment, but close to that in the control under other sampling times of treatment. Jatrorrhizine content in amur corktree roots under severe drought had a lower trend at the end of the treatment than in the control treatment, but without significant difference ($P > 0.05$). Jatrorrhizine content under waterlogging was apparently lower than in the control at the end of the treatment ($P < 0.01$) (Fig. 2).

2.3 Effect of water stress on palmatine contents in amur corktree seedlings

Palmatine contents in amur corktree seedlings were about 10% of the berberine contents. Among the different parts palmatine content was also the highest in stem cortex, which was 20.1 and 20.5 times that in xylem and roots, respectively, and that in the leaves was only 0.18% of that in the stem cortex. Palmatine contents in the stem cortex and roots basically increased with the plant developmental process, whereas they changed little in the roots at the end of the treatment (from 100 days to 120 days) (Fig. 3).

Palmatine contents in stem cortex under 60–100 days of mild drought treatment and 80–100 days of severe drought treatment were all higher than those in the control ($P < 0.01$), but close to those in the control under other periods of treatment. Palmatine content in stem cortex under waterlogging after 80 days of treatment was significantly lower than that in the control and that under drought treatment ($P < 0.01$). Palmatine contents in roots of amur corktree seedlings were higher under 60 days and 80 days of mild drought treatment than those in the control ($P < 0.01$), whereas there was no significant difference among other treatment periods ($P > 0.05$) (Fig. 3).

At the end of the growing season, drought treatment was beneficial to the synthesis and accumulation of palmatine in the stem cortex of amur corktree seedlings, but waterlogging was not (Fig. 3).

2.4 Effect of water stress on biomass and alkaloid yield in amur corktree seedlings

Table 1 shows the measuring results for the last sampling (on October 8 and treated for 120 days). Soil water had a great effect on amur corktree seedlings. Both drought and waterlogging treatment severely inhibited the growth of amur corktree seedlings, and the plant height, stem diameter and biomass were all remarkably lower than those in the control.

Mild drought treatment tended to improve the alkaloid contents in amur corktree seedlings especially the contents of berberine and palmatine in the stem cortex, but because the biomass of seedlings under treatment were less than half of that in the control, three alkaloid yields per plant were the highest in the control, whereas under severe drought and waterlogging they were even lower (Table 1).

3 Conclusions

In conclusion, for the current-year amur corktree seedlings, mild drought (water potential – 80– – 60 KPa) was more beneficial for the production and accumulation of berberine, jatrorrhizine and palmatine in the stem cortex. There were no significant differences in the three alkaloid contents between

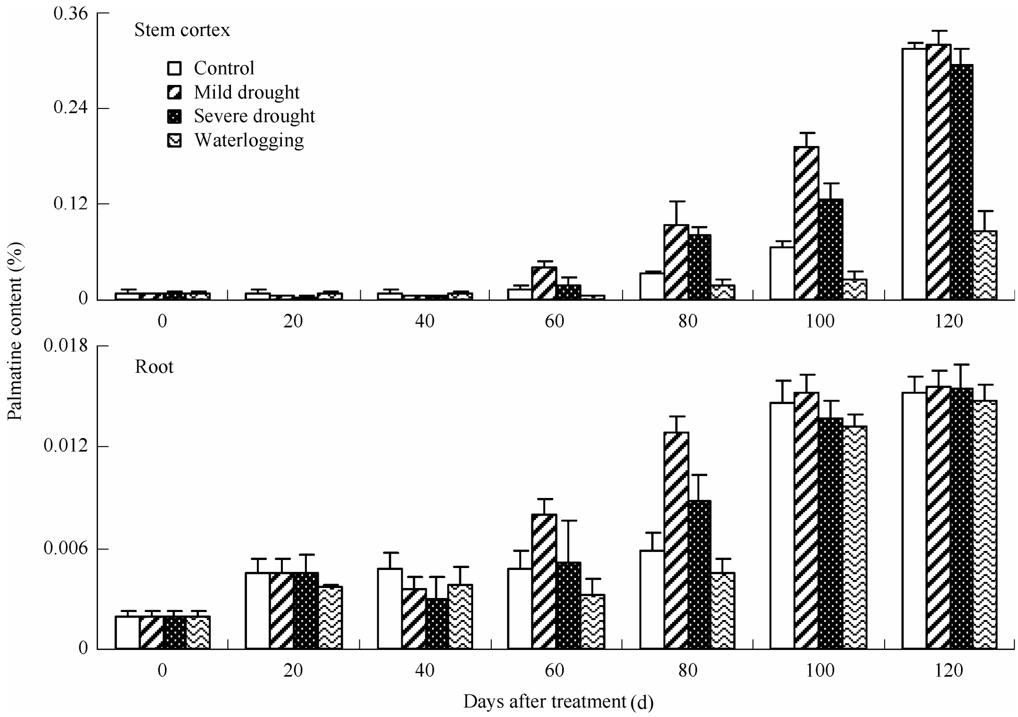

Fig. 3 Changes of palmatine content of amur corktree seedlings under water stress

Table 1 Biomass and alkaloid production of amur corktree seedlings

	Height (cm)	Stem diameter (mm)	Biomass (g/plant)	Berberine (mg/plant)	Palmatine (mg/plant)	Jatrorrhizine (mg/plant)
Control	53.6a	7.7a	18.96a	76.34a	2.22a	5.85a
Mild drought	35.5b	5.5b	8.87b	69.76a	1.56b	4.16b
Severe drought	29.0c	4.8c	4.90c	24.00b	0.80c	2.10c
Waterlogging	18.9d	4.5c	2.61d	11.10c	0.28d	0.53d

Different letters in the same columns denote significant differences ($p<0.05$)

severe drought (water potential < –80 KPa) and the control (water potential –40– –20 KPa), whereas waterlogging (water potential –20 – 0 KPa) basically resulted in the decrease of the three alkaloid contents in amur corktree seedlings (especially in stem cortex). Meanwhile, drought or waterlogging treatment resulted in growth inhibition in amur corktree seedlings, where plant height, stem diameter and biomass were all significantly lower than those in the control. Therefore, the alkaloid yield per plant was still the highest in the control.

Generally, plant secondary metabolism and its metabolites result from the response and adaptation to different environmental stresses during the long process of evolution, and hence the production of secondary metabolites closely relate to environmental factors (including biotic and abiotic factors). The alkaloids of secondary metabolites play crucial roles in the plants chemical defense against insects and herbivores[23]. The alkaloid contents in the stem cortex of amur corktree seedlings increase under mild drought, which means that there may be some correlation between alkaloids and abiotic environments, but this correlation may not be so direct as that between biology and environment, and needs more exploration to elucidate. On the other hand, many scholars have thought that secondary metabolites are produced at the cost of plant growth. When the stresses are high enough to affect plant survival, plants should decrease the secondary metabolites to succeed in their main goal of keeping growth[19]. Under the assumption that there is some relation between alkaloid metabolites and abiotic environments, it can be explained that the decrease of alkaloid contents in amur corktree seedlings for severe drought and waterlogging seriously influence the regular growth of amur corktree seedlings.

Stem cortex is the medicinal part of cortex phellodendri, a Chinese traditional medicine, and also contains the highest contents of the three alkaloids in amur corktree seedlings. The three alkaloids in the stem cortex gradually increase with plant development.

Although the effects of water treatments were remarkable at the end of the period (100d of treatment), mild drought showed enhancement on the production and accumulation of the three alkaloids at 60 days of treatment (Fig. 1, Fig. 2 and Fig. 3), which means that short periods of mild drought treatment might favor the alkaloid contents in amur corktree seedlings. Thus, with the aim of gaining alkaloids in the cultivation of amur corktree seedlings, the higher alkaloid yield per plant than the normal seedlings could be obtained through providing normal water for amur corktree seedlings to gain an optimal biomass production and then improve the alkaloid contents through mild drought treatment at the proper time. Of course, this hypothesis needs more experiments to prove.

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