*Pak. J. Bot.*, 45(5): 1801-1806, 2013.

# EFFECT OF IBA ON ROOTING FROM SOFTWOOD CUTTINGS OF 'TETRAPLOID LOCUST' AND ASSOCIATED BIOCHEMICAL CHANGES

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**Abstract**

'Tetraploid Locust' is one of the most popular and commercially valuable cultivars. An attempt was performed to explore propagation techniques by softwood cuttings for nursery industry. Results showed that cuttings pretreated with Indole-3-butyric acid (IBA) 1400 mg/L for 2 h soaked method got the best rooting traits (rooting percentage is 77.63%, root number is 8.73 and root length is 3.23 cm). Therefore pre-treatment with IBA 1400 mg/L was recommended and used in further experiments for biochemical research. The adventitious rooting was obtained in three distinct phases i.e. induction (0-15 d), initiation (15-20 d) and expression (20-30 d). Indole-3-acetc acid oxidase (IAAox) activity with IBA treatment increased slightly as compared to the control. IAAox activity increased at induction and expression phases and decreased at initiation phase. Peroxidase (POD) activity with IBA treatment increased from the induction up to the expression phase. Polyphenol Oxidase (PPO) activity increased both in IBA treatment and control at induction phase but declined at initiation and expression phases. Content of Indole-3-acetc acid (IAA) and abscisic acid (ABA) had much different during rooting. IAA content with IBA treatment increased steadily as compared to control. ABA content of the control increased steadily as compared to IBA treatment. IAAox activity and IAA content might be playing vital roles in rooting.

## Introduction

'Tetraploid Locust' is popular and well-known ornamental plants. In recent years, the demand for some excellent cultivars has been increasing. Hence, the propagation method is worth for special attention.

'Tetraploid Locust' is tetraploid somatic (4n), which are introduced from diploid somatic (2n) of 'Diploid Locust'. They are generally propagated by grafting. The major shortcoming of this method is it has a longer period to maturity. It is necessary to culture as a rootstock for 1-2 years before grafting. Then it can be transplanted 2-3 years later. Seed propagation is another way to propagate 'Tetraploid Locust', but the offspring cannot keep the exact superior characteristics of the parent plants. Thus the stand yields low productivity results. *In vitro* propagation is a prospective method that can reproduce numerous clonal plants. However, it is dependent on good material and technical conditions. It's difficult to promote this method in most places (Yang *et al.*, 2007). Thus, cutting propagation, which has been widely used in other plants, has been taken into consideration. There is no latent root primordial in cuttings of 'Tetraploid Locust'. Adventitious roots develop from induced root primordial (Liang *et al.*, 2006). As a result, cuttings from 'Tetraploid Locust' are often difficult to root.

Adventitious rooting formation from the stem cutting is influenced by a number of external and internal factors (Gyana, 2006). It is well established that exogenous auxins play a central role in the determination of rooting capacity, whereas most of the commercial propagation is done by rooting with IBA (Kotis *et al.*, 2009). For example, plum cuttings with 2000 mg/L or 3000 mg/L IBA treatment gave the best rooting response (Sharma & Aier, 1989) and 1000 mg/L IBA significantly stimulated rooting of 'Domat' olive (Murat & Elmas, 2008). In some cases, high content of IAA are associated with the promotion of adventitious rooting. A higher concentration of IAA stimulates the formation of root primordial. After IAA accumulates to a certain concentration, it stimulates rooting. In some cases, root development also occurred by decreasing IAA content just at induction phase (Zhang *et al.*, 2004; Khalid *et al*., 2011). These confusing reports indicate necessity for further study. Since IAAox and POD participate in IAA catabolism, they can modify the hormonal balance of plants. On the other hand, it has been proposed that IAAox are similar to POD to help regulate IAA content (Mohamed & Splittstoesser, 1990). PPO is another oxidase that is closely related to the concentration of IAA (Syed *et al*., 2013; Waqas *et al*., 2011). The important role of PPO is due to its effect on phenolic metabolism. In addition, PPO can produce a rooting co-factor with IAA which is conducive to rooting.

The study focused on cutting propagation. The goals of this work were (a) to define the optimal IBA concentration for root formation by softwood cuttings and (b) to monitor the variation of IAAox, POD, PPO activity and lastly (c) to quantify the IAA and ABA content in the base of these cuttings during the rooting process.

## Materials and Methods

To take softwood cuttings of 'Tetraploid Locust' just as the wood began to harden off. Softwood cuttings measuring 12-15cm in length and 0.5-1 cm in diameter were obtained from triennial cutting orchard of the testing field. The testing filed was located at the teaching and experimental nursery garden of Northwest Agriculture and Forestry University, Yangling, Shaanxi, China.

Each cutting retained a compound leaf. Each compound leaf had two leaflets. The top cut of the cuttings was perpendicular which was 1 cm from top bud. The lower cut of the cuttings was 45° located at 0.5cm from the lower bud. Treat them with root promoting auxins at the basal end (2-3 cm) for 2 h. The cuttings received distilled water (control) or treatments of IBA (800, 1000, 1200 1400, 1600, 1800 and 2000 mg/L). The treatments were based on the results from our preliminary tests in the laboratory.

There were three replicates of 80 cuttings each. They were planted immediately in nursery beds with sand under the natural conditions. Intermittent mist was supplied for 10 s at 3 min intervals. Root-taking condition of the groups should be tested at $45^{th}$ d from planting. Tested quantity, length of the roots with EPSON PERFECTTONTM 4990 PHOTO root scanner, and carried out variance analysis with average value of the groups.

Samples were picked respectively on 0 d 10 d 15 d 20 d 25 d and 30 d from planting, which was on the basis of the preliminary morphological and anatomical observation. Five cuttings were selected randomly from each group every time. Clean them with clear water and rapidly take out skin layer within 1 cm range of rooting zone. After freezing in liquid nitrogen store them in ultra-low-temperature refrigerator. IAAox, POD and PPO activities were measured following the methods of Li and Gao. Content of IAA and ABA was performed by indirect enzyme-linked immunosorbent assay (ELISA) as described by Guo *et al*., in detail (2009). ELISA kits were purchased from China Agricultural University. Universal Microplate Spectrophotometer of ELx800™ type (BioTek, USA) was applied to test hormone content of each sample.

Variance analysis was adopted to analyze different IBA concentration influence on cutting adventitious roots. Based on this analysis, significance difference was analyzed by Duncan's multiple comparison tests, among which percentage was transferred by arc-sine. The whole data processing was completed via SPSS16.0 for Windows software.

## Results and Discussion

**IBA treatments on rooting:** Rooting traits was presented in Table 1. Root initiation was developed after 15 d in most of the softwood cuttings with IBA treatment, but none in the control. Cuttings pretreated with IBA showed significant difference $p<0.05$ in rooting performance. Cuttings with IBA 1400 mg/L showed a maximum percentage of rooting (77.63%) and number of roots (8.70). It was followed by a dose of IBA 1200 mg/L, which the corresponding values were 60.93% and 7.17. The highest length of roots was obtained with IBA 1200 mg/L. There was no significant difference between IBA treatments of 1200 mg/L and 1400 mg/L on roots length. Therefore with the protocol frame established, a concentration of 1400 mg/L should be used on 'Tetraploid Locust' softwood cuttings. This concentration was also used further for biochemical studies.

**Table 1. Effect of IBA concentrations on some morphological traits in softwood cuttings of 'Tetraploid Locust'.**

| Treatments (mg/L) | % Of rooting (Mean ± SE)* | No. of roots/cutting (Mean ± SE)* | Av. Root length/cm (Mean ± SE)* |
|---|---|---|---|
| Control($H_2O$) | 0 | 0 | 0 |
| IBA800 | 28.68 ± 0.15d | 2.83 ± 0.21d | 1.58 ± 0.06c |
| IBA1000 | 46.54 ± 0.84c | 5.83 ± 0.78c | 2.39 ± 0.16b |
| IBA1200 | 60.93 ± 0.34b | 7.17 ± 0.37b | 3.42 ± 0.41a |
| IBA1400 | 77.63 ± 0.24a | 8.70 ± 0.25a | 3.23 ± 0.08a |
| IBA1600 | 47.13 ± 0.64c | 5.43 ± 0.44c | 2.27 ± 0.13b |
| IBA1800 | 17.9 ± 0.46e | 3.13 ± 0.67d | 0.89 ± 0.28d |
| IBA2000 | 9.40 ± 0.31f | 1.83 ± 0.49e | 0.42 ± 0.33e |

*Each value = treatment mean of three replicates (each replicate contains 80 cuttings). Each value = treatment mean ± SE (standard error). Mean having the same letter in a column were not significantly different by Duncan's multiple comparison test ($p<0.05$)

The present study showed that the application of auxin significantly improved rooting traits. The same results have been reported on many plants capable of rooting (Zeng *et al*., 2005). The effect varied with auxin concentration and type. IBA 1400 mg/L produced the best rooting traits than others on 'Tetraploid Locust'. Li *et al*., (2004) studied the effect of auxin and cytokinin on the formation of callus and root primordial. He concluded that plant hormones influencing cell division and differentiation are essential for cuttings rooting. IBA had a better effect than NAA. Higher or lower concentration of auxin did not substantially produce a better rooting result. In this study, cuttings pretreated with IBA 1400 mg/L rooted significantly better. IBA 1000 mg/L and IBA 1600 mg/L showed no significant difference. The inhibitory effect caused by high or low exogenous auxin also occurred in other plants such as olive cultivar 'Moraiolo'(Ansar *et al*., 2009).

**Change of IAAox activity:** Biochemical processes play a major role in the rooting process of plants. The biochemical studies of 'Tetraploid Locust' were conducted from 10 to 30 d at every four days intervals during the rooting process. Generally, the adventitious rooting occurred in three distinct phases i.e. induction, initiation and expression, as proposed for poplar and *Camellia sinensis* (Gyana, 2006). The results (Fig. 1-a) showed that IAAox activity of IBA-treated cuttings increased at induction (0-15 d) and expression phase (20-30 d), decreased at initiation phase (15-20 d). IAAox activity was higher in the control as compared to IBA treatment. The increasing IAAox activity at induction phase appears to be responsible for the better development of adventitious roots with IBA treatment, possibly because of the decreasing level of IAA content (Gaspar *et al*., 1992). It agreed with the results of Dhawan & Nanda (1982), Kakkar & Rai (1986). At initiation phase, IAAox activity was decreased slowly and IAA content was increased, which helped to form roots (Nag *et al.*, 2001).

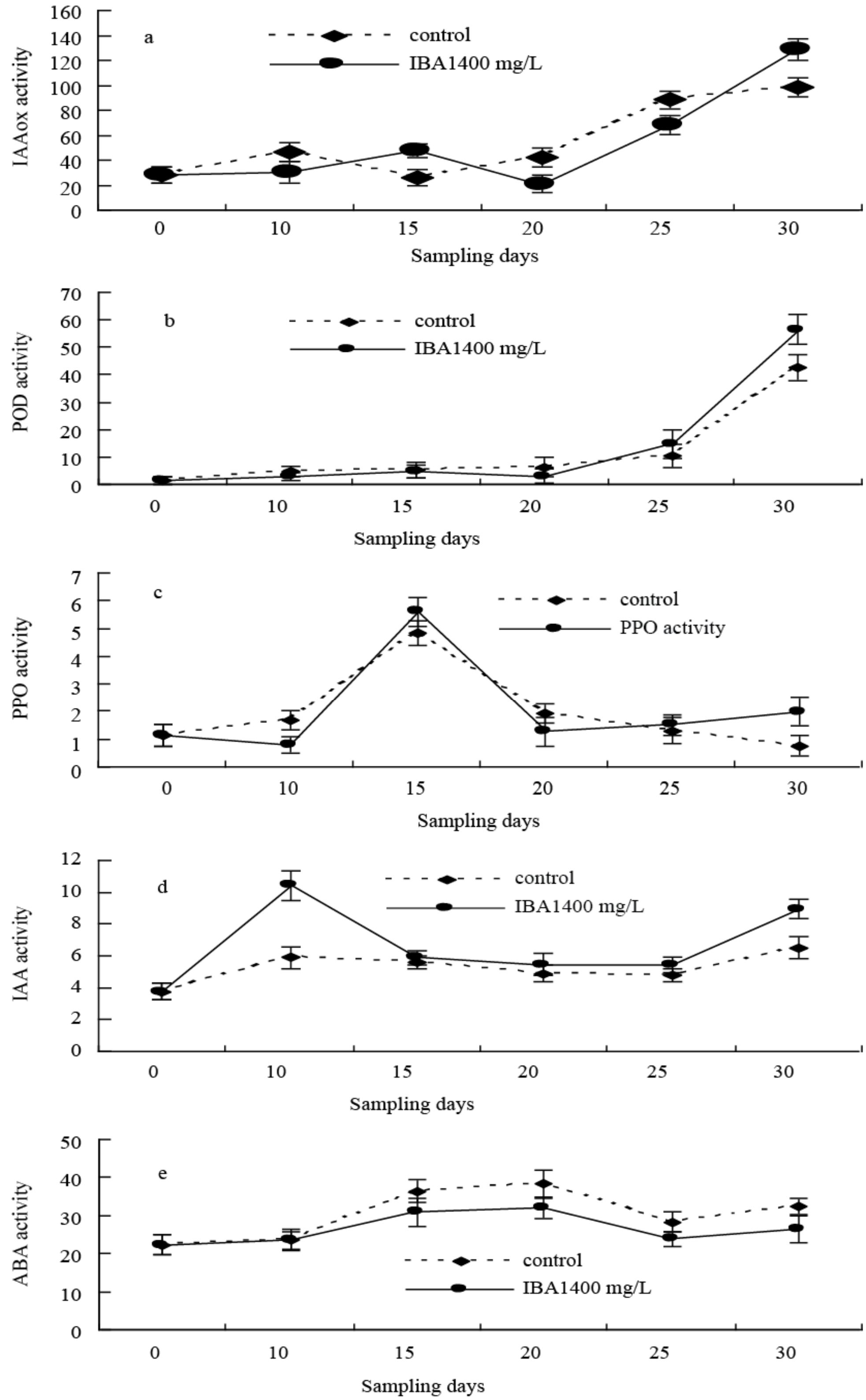


Fig. 1. Change of IAAox, POD, PPO activity and IAA, ABA content in different time periods of root development from softwood cuttings of 'Tetraploid Locust' pretreated with or without IBA (1400 mg/L). (a) IAAox activity: Enzyme activity (µg IAA/mg protein/h) was expressed as the amount of IAA (in µg) digested in ml of enzyme solution in hour. (b) POD activity: One unit of POD activity is equivalent to a $A_{470}$ of 1.0 for 1 mg protein in 10 min. (c) PPO activity: The activity was determined in terms of enzyme activity/mg protein/min. (d) IAA content and (e) ABA content: The content was determined by µg IAA or ABA/g fresh weight.

**Change of POD activity:** POD activity was measured because it is considered that an early increase in the POD activity accounted for the subsequent decrease in the IAA content (Li *et al.*, 2009). The results (Fig. 1-b) indicated that POD activity with IBA treatment was the same as the control. Both in IBA treatment and control cuttings, POD activity increased slowly up to expression phase and increased clearly at expression phase. POD activity with IBA treatment was higher than control. The increase of POD activity observed in cuttings at induction and initiation phases can serve as a good marker for rooting ability (Nordstrom & Eliasson, 1991). POD is involved in the metabolism of auxin and the lignification of cell walls. The higher value of POD activity is correlated with higher level of metabolism, increased respiration and the formation phenolic compounds in formation of lignin. The formation of lignin is crucial for the formation of vascular cells in callus, cell walls and the lignification of roots during the rooting process. In this study, POD activity of IBA-treated cuttings was significantly higher than the control at expression phase, which was important for root elongation.

**Change of PPO activity:** PPO is a copper-containing enzyme which catalyzes the oxidation of various phenolic substances. Phenolic substances play a vital role in the formation and development of adventitious roots. One of the important functions of PPO is to catalyze the condensation between phenolic substances and IAA to form "IAA-phenol-acid complex". It is a cofactor of growing roots and acts as a stimulant of adventitious root formation (Li, 2000). The present study (Fig. 1-c) showed PPO activity increased both in IBA-treated and control cuttings at induction phase but declined slowly at initiation and expression phases. The results agreed with the findings of Cai and Zeng (2008). However, in this study, PPO activity with IBA treatment was higher than control. It was in line with Li *et al.,* (2000). Li's study suggests that cuttings treated by IBA stimulate the activity of cambium cells, so that cambium cells produce a large amount of IAA. Then they promote the PPO in plants to change towards the way beneficial for forming and developing adventitious roots. In addition, PPO activity with IBA treatment showed more activity in cuttings of ‘Tetraploid Locust’. They inferred that plenty “IAA-phenol-acid-complex” was conducive in the growth of root primordial and inducement of adventitious roots and thus greatly improved the rooting percentage. It consisted with Upadhyaya & Davis (1986) and Huang *et al*., (2002).

**Change of IAA content:** Auxin has the biggest influence on rooting of cuttings among many plant hormones. This study also determined the IAA content with sampling days (Fig. 1-d). Results showed that whether cuttings were treated by IBA or not, IAA content showed the same trend. It increased first, decreased then and increased once again. This was consisted with Nojiri *et al.,* (1996), while contrary to Venis *et al.,* (1996). It indicated that changes were much different between species. The difference could be either subtle or significant. In ‘Tetraploid Locust’ study, IAA content with IBA treatment was much higher than control. This indicated IBA treatment had the capability of regulating metabolism level of IAA. IAA level in treated cuttings increased greatly in the first 10 d, because of slow degrading speed of IAAox and POD to degrade IAA. Moreover, IAA content in ‘Tetraploid Locust’ cuttings was fairly high. During 10-15 d, IAA content decreased, which was beneficial to induce adventitious roots. At expression phase (20-30 d), IAA content raised slowly, because rooted cuttings can synthesize new IAA to ensure normal growth of plants.

An important physiological function of IAA is promoting the formation of adventitious roots. IAAox can oxidize IAA. Activation of IAAox in plants may make it difficult to establish roots, since the ability to degrade IAA is strong and basipetally transport auxin is also scarce, yielding negative effect on rooting. On the contrary, plants that root easily are low in IAAox activity. The ability to degrade IAA is low and more IAA can be transported to the root formation sites. It is good for inducing roots. It also can be seen that the highest peaks of IAAox in ‘Tetraploid Locust’ cuttings corresponded to the highest content of IAA. Excessive accumulation of IAA was not conducive to root primordial establishment. Hence it has been suggested high content of IAA and high activity of IAAox was the main reason of inhibiting rooting ability of ‘Tetraploid Locust’.

**Change of ABA content:** ABA is a rooting inhibitor. There have been different reports on the relationship between ABA content and the formation of root primordial in recent years. ABA content keeps declining in the early period of *Liriodendron Chinese*. It meets the minimum when the adventitious roots formation (Zhang *et al.*, 2004). While, few reports say ABA content keeps declining throughout the rooting. For example, ABA content of peach keeps declining in the whole rooting process. In this study (Fig. 1-e), ABA content showed a slightly increasing tendency. After 10 d, ABA content of ‘Tetraploid Locust’ in control was apparently higher than IBA treatment. According to that, we can draw the conclusion that metabolism in ‘Tetraploid Locust’ was active and it's good at synthesizing various types of hormones. IBA treatment can inhibit the synthesizing of ABA. ABA was mainly formed by roots and was sent to aboveground organs via fluid circulation to inhibit IAA transportation and the growth of the plant (Bahrun *et al.*, 2002; Boyer & Westgate, 2004). In the formative period of root primordial, ABA content of ‘Tetraploid Locust’ kept rising which was helpful to balance excessive IAA. It also helped the IAA/ABA ratio to gain balance and promoted rooting. The ratio of IAA/ABA can serve as a good marker for rooting ability in cuttings of *Populus davidiana* (Chen *et al.*, 1994), *Betula platyphylla* (Zhang *et al.*, 2001), *Populus alba*, *Populus tomentosa* and *Pinus bungeana* (Guo *et al.*, 2004).

## Conclusion

'Tetraploid Locust' is a cross-bred genus, while not a natural selection species. Thus it is difficult to root. Since appropriate auxin treatment could produce good rooting response, softwood cutting propagation had proven to be an effective strategy for production of 'Tetraploid Locust'. And IBA 1400 mg/L was recommended in its softwood cuttings. In addition, the adventitious rooting was obtained in three distinct phases i.e. induction (0-15 d), initiation (15-20 d) and expression (20-30 d). Lastly, when exogenously IBA 1400 mg/L was applied, IAAox activity, POD activity, PPO activity and endogenous hormones (IAA and ABA content) had a strong relationship with rooting response. High activity of IAAox and content of IAA in the induction phase were main causes in decreasing root response.

## Acknowledgements

This work was supported by the National Natural Science Foundation of China (30972352).

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(Received for publication 6 December 2010)