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## Seasonal variation in rooting of the cuttings from Tetraploid Locust in relation to nutrients and endogenous plant hormones of the shoot

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**Abstract:** This study was carried out to elucidate the relationship between seasonal variation of rooting ability and inner conditions of the cuttings of Tetraploid Locust, and to determine the optimum time for rooting. It was conducted in a glasshouse at the Experimental Nursery of the College of Forestry, North-West Agriculture and Forestry University, Yang Ling, China, during 2009. Tetraploid Locust cuttings were taken from healthy trees of 3-year-old field-grown mother-stock orchard. Cuttings (15 cm in length and 10-12 mm in diameter) were collected and planted on 15 February, 15 March, 15 May, 15 June, 15 July, and 15 August. The results obtained 2 months after each planting date showed that planting the cuttings on 15 May produced significant increases in rooting percentage compared to the other planting dates. The highest total carbohydrates concentration and carbohydrate/nitrogen (C/N) ratio were recorded in the basal parts of the stem cuttings planted on 15 May, either before planting or 35 days after planting. A positive relationship of rooting percentage was found between total carbohydrate concentration and C/N ratio. No consistent relationship was established between total nitrogen and rooting percentage. Results also showed a high and a low negative relationship of the rooting percentage of the cuttings between indole-3-acetic acid (IAA) concentration and gibberellins (GAs) concentration, respectively. In addition, 35 days after planting, a positive relationship was detected between abscisic acid (ABA) concentration and the rooting percentage. From the results obtained, 15 May may be recommended as the ideal planting date for improving the rooting and the vegetative growth of Tetraploid Locust stem cuttings compared to the other investigated planting dates.

**Key words:** Carbohydrate, endogenous plant hormones, nitrogen, planting date, Tetraploid Locust

### Introduction

The propagation of Tetraploid Locust (*Robinia pseudoacacia* Linn.) cultivars requires grafting or budding on 2- or 3-year-old locust seedling rootstocks. Although grafting cultivars can be very interesting in establishing a new Tetraploid Locust orchard, the low productivity ability and

the unsatisfactory viability in some cultivars were identified as limiting factors (Sebastiani and Tognetti 2004). Any increase in propagation efficiency will, therefore, strongly enhance the commercial benefits of Tetraploid Locust.

Stem cutting is considered the simplest and most economical method of vegetative propagation

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practiced in horticultural industry for mass production within a short time (Yong Kweon and Ki Sun 1996). In general, softwood cuttings can root more easily and more quickly than hardwood cuttings (Kasim et al. 2009). The rooting potential of the softwood cuttings has been found to diminish with the increasing age of the mother shoots (Bhusal et al. 2001). Seasonal timing, the period of the year in which cuttings are taken, can play an important role in rooting. For instance, Nabali olive has the highest (55.6%) rooting percentage during September and Raseei olive yields the highest (92.6%) rooting percentage during February (Ayoub and Qrunfleh 2006). Optimum times for collecting the Tetraploid Locust cuttings have not been determined.

Information for critical propagation dates, based on the physiological conditions of cuttings, is needed to help industry make management decisions for initiating and terminating propagation (Tsipouridis et al. 2006). Total carbohydrate and total nitrogen levels, and C/N ratio (Druege et al. 2004; Rapaka et al. 2005) have been reported to influence the adventitious rooting of plant species. Generally, nitrogen has been negatively correlated to rooting. High nitrogen supply to stock plants, meeting or even surpassing the level necessary for maximum growth, has often been observed to decrease subsequent rooting of cuttings. Such effects have been discussed repeatedly in relation to decreased carbohydrate levels and C/N ratio, which was first suggested by Kraus and Kraybill (1918), as important for root formation (Druege et al. 2000). Many studies reported that the effect of nitrogen on rooting was related to the carbohydrates concentration in cuttings (Kim et al. 1977; Hambrick et al. 1985).

The importance of root promoting substances for adventitious root formation has been extensively studied, but few reports are available concerning changes in endogenous auxin in cuttings (Ayoub and Qrunfleh 2006). Many studies showed that rooting response also correlates with the interactions between endogenous plant hormones (Guo et al. 2004; Tsipouridis et al. 2006). Auxin is believed to play a central role in the formation of adventitious root (Weigel et al. 1984). The accumulation of auxin at the basal part of the cutting is well documented (Ann-Caroline and Lennart 1991). Yoshitaka (1996)

found that the IAA concentration peaked when the root primordial cells were forming and roots were developing, which was in accordance with the result of Machida and Fujii (1967). The effect of ABA on adventitious root formation was utilized for exogenous application rather than measuring endogenous ABA concentration in relation to rooting. In some studies, ABA application has been found to promote rooting (Basu et al. 1968; Blazich 1977). In other studies, however, it has been found to inhibit rooting in begonia leaves (Heide 1968).

GAs is a family of naturally occurring tetracyclic diterpenoids, some of which have intrinsic biological activities that affect many aspects of plant development (Aleman et al. 2008). Bioactive GAs are known to regulate seed germination and storage mobilization, stem elongation, flower initiation, pollen and fruit growth, as well as root development. Gibberellic acid ( $GA_3$ ) is the only gibberellin that has been extensively studied in this respect (Hansen 1988). An inverse relationship between the endogenous  $GA_3$  level and the rooting ability was found in Jonathan apples (Takeno et al. 1983) and Nabali olive (Ayoub and Qrunfleh 2006). However, the literature about the correlations of rooting with the levels of tissue nutrients and endogenous plant hormones is still confusing (Guo et al. 2009), indicating the necessity for further study.

Therefore, the present study was carried out to elucidate the relationship between different collecting seasons of stem cuttings and seasonal variation of rooting ability, as well as the inner conditions of stock plant, such as carbohydrate, nitrogen, and endogenous plant hormone (IAA, ABA and GAs) levels and C/N ratio in cuttings of Tetraploid Locust, and to determine the time required for the rooting of stem cuttings.

## Materials and methods

### Plant material

During this study, cuttings and sample material of Tetraploid Locust for laboratory analysis were taken from a 3-year-old field-grown mother stock orchard at the nursery of College of Forestry, North-West Agriculture and Forestry University, Yang Ling, China. Samples were taken at 6 dates in 2009: 15

February, 15 March, 15 May, 15 June, 15 July, and 15 August. On each date, cuttings about 15 cm in length and 10-12 mm in diameter were taken from the sub-terminal part of shoots with 40-50 cm length. Fresh samples were taken before planting from the basal 2 cm of cuttings. Samples were used for the estimation of various chemical parameters.

### Rooting experiment

Cuttings were soaked in cold water for 24 h to leach out rooting inhibitors. Then the basal 2.5 cm length of the cuttings was dipped in 1000 mg L<sup>-1</sup> IBA solution for 2 h. The cuttings were subsequently placed in a bench in a glasshouse equipped with an automatic mist system, and a 5 cm length of the basal part was buried in sand. Air temperature in the glasshouse was maintained at 18-28 °C, with 70%-100% relative humidity. During the rooting period, intermittent mist was supplied for 10 s at 3 min intervals before the callus appearance and 30 s at 30 min intervals after the callus appearance.

The rooting experiments for all sampling dates were arranged in a complete randomized design with 4 replicates and each replicate consisted of 80 cuttings. Rooting percentage, average number of roots per rooted cutting, root length, and average root diameter were calculated with EPSON PERFECTON™ 4990 PHOTO root scanner 2 months after planting.

### Estimation of chemical composition

Chemical analysis was done in both samples before planting and 35 days after planting at each date. The determination of chemical characteristics was carried out in order to find any possible relationship between the internal chemical components and rooting ability of Tetraploid Locust cuttings at different preparation times. Therefore, samples were chemically analyzed to determine IAA, ABA, and GAs concentration ( $\mu\text{g g}^{-1}$  fresh weight) with Indirect Enzyme-Linked Immunosorbent Assay (ELISA) method as described by Guo et al. (2009).

In addition, chemical analysis of dried samples of the basal 2 cm of cuttings was conducted to determine the total carbohydrates concentration (% of dry matter) as described by Yong Kweon and Ki Sun (1996). The total nitrogen concentration (% of dry matter) was also measured using the Kjeldahl method as described by Hambrick et al. (1991)

with a Technicon auto-analyzer. The C/N ratio was calculated by dividing the respective values of total carbohydrate by total nitrogen.

### Statistics

The results were statistically analyzed using SPSS 16.0. Analysis of variance (ANOVA) was performed to determine significant differences. Mean separation was carried out by the Least Significant Difference test (LSD). Data of rooting percentage were arcsine transformed and the transformed dates were statistically analyzed.

## Results

### Seasonal changes in rooting

The relationship between different cutting collection dates and rooting ability of Tetraploid Locust is presented in Table 1 and Figure 1. In general, significant differences were recorded between the effects of the collection date on the rooting percentage and the average number of roots per cutting. No significant differences were found among the root lengths or the average root diameters of the cuttings collected from March to July.

Stem cuttings collected on 15 May had a significantly higher rooting percentage compared to those collected on the other dates, and followed by those collected in June. On the other hand, the lowest rooting percentage was obtained when the stem cuttings were collected in August. Concerning the cutting collection dates, it was clear that cuttings prepared on 15 February yielded the largest number of roots per cutting, significantly higher than those of cuttings collected in other months. Root length showed a similar pattern to average root diameter.

### Chemical composition of the basal part of cuttings

#### *Seasonal variations in total carbohydrates and nitrogen concentration, C/N ratio*

Results in Table 2 showed the seasonal changes in total carbohydrates and total nitrogen levels as well as C/N ratio before planting and 35 days after planting of the cuttings. It was clear that the bases of Tetraploid Locust cuttings taken on 15 May had the highest carbohydrate value (both before and 35 days after planting), significantly higher than those of the cuttings taken at the other dates, followed by

Table 1. Effect of different cutting collection dates on the rooting percentage, root length, number of roots per rooted cutting, and average root diameter of Tetraploid Locust.

Cutting collection dates	Rooting percentage (%)	Root length (cm)	Number of roots per rooted cutting	Average root diameter (mm)
15 February	55.56 c	5.39 a	7.7 4a	2.11 a
15 March	34.22 e	5.02 ab	7.33 ab	1.87 ab
15 May	81.05 a	4.51 ab	6.52 bc	1.82 ab
15 June	63.59 b	4.83 ab	6.02 c	1.94 ab
15 July	47.64 d	4.75 ab	4.38 d	1.83 ab
15 August	19.13 f	2.26 b	3.06 e	1.76 b

Means having different letters in a column are significantly different ( $P < 0.05$ ), according to the Least Significant Difference test.



Figure 1. Tetraploid Locust cuttings after rooting on different planting dates (a) 15 February, (b) 15 March, (c) 15 May, (d) 15 June, (e) 15 July and (f) 15 August.

cuttings taken on 15 June. The lowest carbohydrates concentration was determined in cuttings taken on 15 August. A positive correlation (Figure 2a) was found between the total carbohydrates concentration and rooting percentage of Tetraploid Locust cuttings both before ( $r = 0.98$ ) and 35 days after planting ( $r = 0.91$ ).

As for the total nitrogen concentration, cuttings taken on 15 March had the highest nitrogen concentration before planting. However, 35 days after planting, cuttings taken on 15 February and 15 June gave the highest values of nitrogen concentration. In addition, a negative ( $r = -0.53$ ) correlation and a positive ( $r = 0.44$ ) correlation (Figure 2b) were found between nitrogen concentration (before and 35 days after planting) and the rooting percentage of Tetraploid Locust cuttings, respectively.

The total carbohydrates and nitrogen concentration in the basal parts of the cuttings were decreased 35 days after planting compared with the values recorded before planting. The data also showed that C/N ratio followed a similar trend to that of the total carbohydrates concentration and rooting percentage both before and after planting. This finding is supported by the positive correlation (Figure 2c) found between C/N ratio and rooting percentage before ( $r = 0.97$ ) and 35 days after planting ( $r = 0.88$ ).

#### Seasonal variations in plant hormone concentration

Results showed significant differences in endogenous IAA concentration of Tetraploid Locust cuttings

Table 2. Effect of different cutting collection dates on the total carbohydrates concentration, total nitrogen concentration, the C/N ratio, and the endogenous hormone IAA, ABA, and GAs concentration in the basal part of Tetraploid Locust cuttings.

Chemical composition of basal part of cuttings						
Before planting						
Cutting collection dates	Total carbohydrates concentration (% of dry matter)	Total nitrogen concentration (% of dry matter)	C/N ratio	Endogenous hormone concentration ( $\mu\text{g g}^{-1}\text{FW}$ )		
				IAA	ABA	GAs
15 February	24.53 bc	2.46	9.97 bc	14.27 d	10.63 b	3.97 d
15 March	21.68 cd	2.61	8.31 cd	27.51 b	8.51 c	5.11 cd
15 May	32.82 a	2.26	14.52 a	11.98 d	14.86 a	7.34 b
15 June	27.72 b	2.43	11.41 b	19.23 c	7.32 cd	5.58 c
15 July	22.84 c	2.18	10.48 bc	25.63 b	10.45 b	4.02 d
15 August	17.91 d	2.49	7.19 d	34.56 a	5.51 d	10.6 a
35 days after planting						
Cutting collection dates	Total carbohydrates concentration (% of dry matter)	Total nitrogen concentration (% of dry matter)	C/N ratio	Endogenous hormone concentration ( $\mu\text{g g}^{-1}\text{FW}$ )		
				IAA	ABA	GAs
15 February	22.31 ab	2.26	9.87 ab	17.41 c	8.75 bc	6.82 b
15 March	19.04 bc	2.15	8.86 bc	20.44 ab	8.52 c	5.33 c
15 May	24.78 a	2.18	11.37 a	8.02 e	11.38 a	4.59 cd
15 June	20.89 b	2.26	9.25 b	11.56 d	9.74 abc	5.47 c
15 July	22.52 ab	2.20	10.22 ab	18.09 bc	10.5 3ab	4.18 d
15 August	16.45 c	2.17	7.58 c	22.13 a	5.27 d	10.05 a

Means having different letters in a column are significantly different ( $P < 0.05$ ), according to the Least Significant Difference test.

collected on all sampling dates (before or 35 days after planting) (Table 2). IAA concentration in cuttings before planting was higher than that in those after planting except in cuttings collected in February. Rooting percentage was significantly affected by the IAA concentration that varied in cuttings collected on different sampling dates. Either before or 35 days after planting, IAA concentration was the highest in cuttings collected in August and the least in those gathered in May compared to other sampling dates. The increase in IAA concentration in Tetraploid Locust cuttings from May to August coincided with a decrease in rooting percentage from

81.05% in the cuttings collected in May to 19.13% in those collected in August. This finding is supported by a high negative (before planting  $r = -0.93$ , 35 days after planting  $r = -0.95$ ) correlation found between IAA concentration and the rooting percentage of the cuttings (Figure 2d).

ABA concentration in Tetraploid Locust cuttings (before or 35 days after planting) was the highest in the cuttings collected in May and the lowest in the cuttings collected in August (Table 2). The ABA concentration in the cuttings collected in May was significantly higher than that in the cuttings collected in the other months. However, insignificant

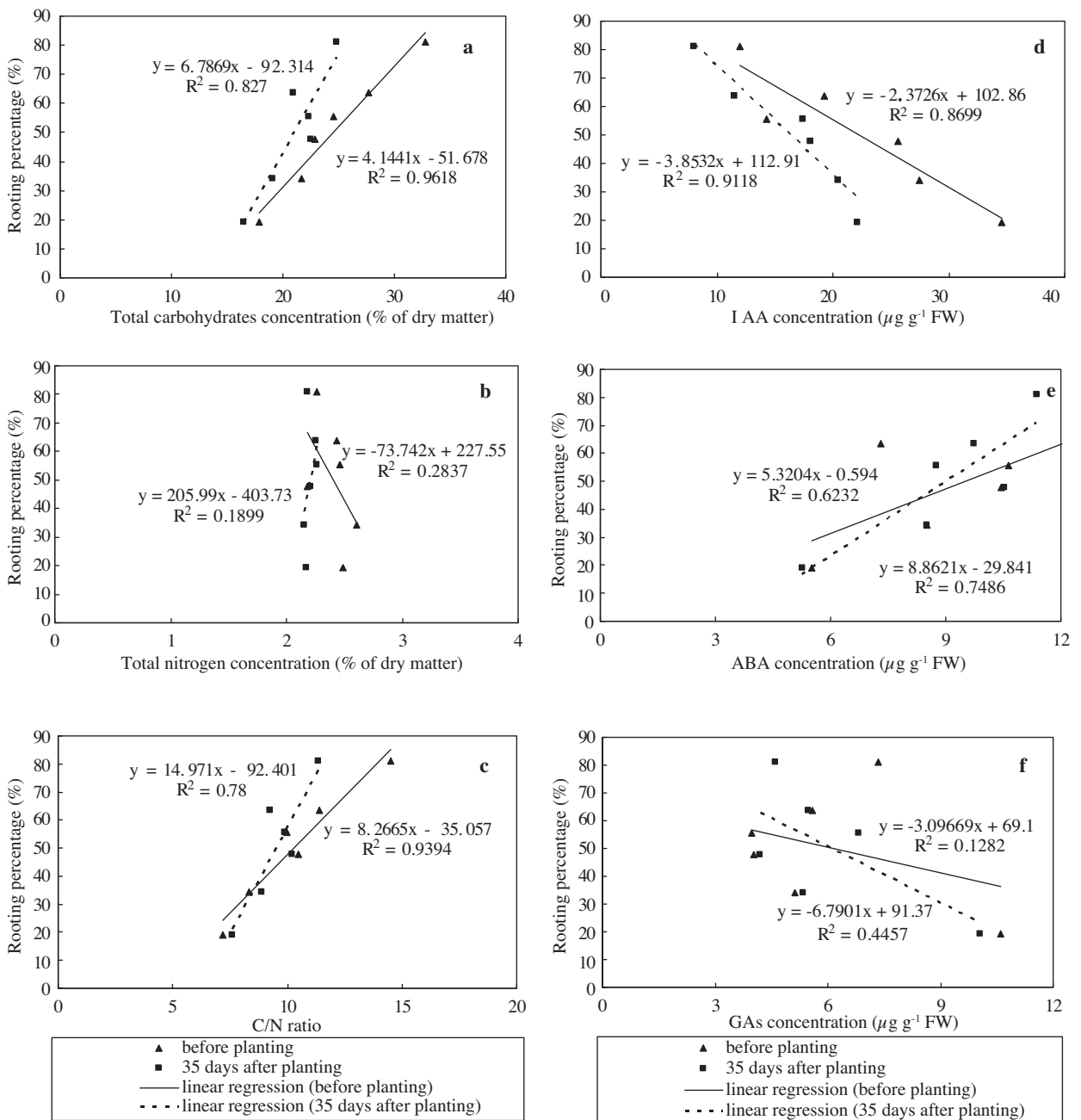


Figure 2. Relationship between (a) Total carbohydrates concentration (b) Total nitrogen concentration (c) C/N ratio (d) IAA concentration (e) ABA concentration (f) GAs concentration and the rooting percentage in Tetraploid Locust cuttings.

differences in ABA concentration were recorded between February and July, and June and August before planting. Insignificant differences were also found between June and July, and February and June 35 days after planting. Moreover, a positive correlation (before planting  $r = 0.79$ , 35 days after planting  $r =$

0.87) was found between ABA concentration and rooting percentage of the cuttings (Figure 2e).

In this study, no significant differences in GAs concentration were recorded between cuttings collected in February, March, and July before planting (Table 2). Also insignificant differences in

GAs concentrations were found between cuttings collected in March, May and June, and May and July 35 days after planting. A negative correlation (before planting  $r = -0.36$ , 35 days after planting  $r = -0.67$ ) was found between GAs concentrations and rooting percentage (Figure 2f).

## Discussion

Successful cutting propagation has been associated with the ideal collection date of cuttings (Sharma and Aier 1989; Howard 1996; Rosier et al. 2004). Rooting percentage of Tetraploid Locust cuttings was high when the cuttings were taken from newly formed vigorously growing shoots in May and June after the stop of new shoot development (Table 1). Similar results have been reported by Hartmann and Loreti (1965) on leafy olive, Yong Kweon and Ki Sun (1996) on *Abeliophyllum distichum*, Agnihotri and Ansari (2000) on *Bambusa vulgaris* var. *striata* and *Dendrocalamus strictus*, DengXiong et al. (2000) on *Quisqualis indica*, Hussein (2003) on *Beaumontia grandiflora* Tworokski, and Takeda (2007) on peach. The concentration of the total carbohydrates (Table 2) in Tetraploid Locust cuttings confirmed these findings since the trend of the total carbohydrates was positively related (Figure 2a) to the rooting percentage. On one hand, the reduction in the rooting percentage that was observed in the July cuttings may be attributed to that mother plants tended to flower at this time, causing the utilization of auxin for floral bud development and the depletion of the carbohydrate reserves (Yong Kweon and Ki Sun 1996; Hussein 2003; Hussein 2008). On the other hand, the lower rooting percentage of cuttings collected in July in comparison with that of the March cuttings may be due to the low temperature of the rooting medium and to the decrease in the photosynthetic rate, which leads to less carbohydrate production.

The high carbohydrates concentration and C/N ratio during the growing season coincided with the high rooting percentage in Tetraploid Locust cuttings (Table 2). The values of C/N ratio were also positively related to the rooting percentage around the year, as previously mentioned in Figure 2c. New shoot growth of Tetraploid Locust stopped in July, followed by the accumulation of carbohydrates because photosynthetic products were not exhausted

by new shoot growth, and then the carbohydrates were converted into fructose and glucose from July to March of the next year (Yong Kweon and Ki Sun 1996). This result is in line with Yong Kweon and Ki Sun (1996) as they concluded that carbohydrate concentration of *Abeliophyllum distichum* hardwood cuttings was maintained at minimum levels from January to February, and then increased during the shoot growing period. In addition, Kasim et al. (2009) working on bitter almond found that high rooting ability was accompanied by their high C/N ratio during the growth season. Moreover, Hussein (2008) reported that the C/N ratio may be an important factor influencing the root ability of *Thunbergia grandiflora* cuttings since the values of C/N ratio were positively related to the rooting percentage around the year. Moreover, EI-Boraie's work (1998) on *Gardenia jasminoides*, Mahros's work (2000) on *Bougainvillea glabra* var. *sanderiana*, *B. glabra* var. *variegata* and *B. spectabilis* "Snow White", and Hussein's work (2003) on *Beaumontia grandiflora* confirmed this conclusion. The data in Table 2 also showed that the total carbohydrate content in the basal parts of the Tetraploid Locust cuttings was decreased 35 days after planting compared with the values recorded before planting. This is in agreement with the findings of Hussein (2003) on *Beaumontia grandiflora* and Hussein (2008) on *Thunbergia grandiflora*.

The relationship between nitrogen and adventitious root formation of cuttings has remained controversial. Appropriate nitrogen concentration is necessary for root initiation because nitrogen is essential in synthesis of nucleic acid and protein, which are necessary for root differentiation (Kim et al. 1977; Hambrick et al. 1985). Breen and Muraoka (1973) suggested that seasonal variations of nutrients in plants occur according to the movement of nutrients connected with sink and source relationship, which depends on growth rate. Yong Kweon and Ki Sun (1996) found that nitrogen concentration of softwood and leaf in the late stage was lower than that in the early stage of growth. Therefore, the high nitrogen concentration repressed rooting of softwood cutting in white forsythia. The work of Kasim et al. (2009) on bitter almond showed a negative relationship between nitrogen concentration and rooting percentage. In this study, the total nitrogen concentration of



Tetraploid Locust cuttings barely changed (Table 2). Therefore the range for nitrogen concentration was very narrow to get a strong correlation (Figure 2b) between rooting percentage and total nitrogen concentration, possibly because all the parent trees were adequately and equally fed with nitrogen at the nursery.

Many researchers reported that there was a positive correlation between the endogenous IAA concentration and the rooting ability of cuttings (Arden 1971; Gaspar and Hofinger 1988; Shin et al. 1988; Geneve 1991). IAA promoted the induction of root primordial and increased the number of cells per root primodium by basipetal auxin transport (Haissig 1972). In addition, auxin is important for RNA and protein synthesis (Scott 1972). Caballero (1979) found that auxin was a limiting factor of root initiation and was able to increase the level of rooting promoters in the olive cultivars Ascolano, Gordal, and Swan Hill. In this work, IAA concentration was negatively correlated with rooting percentage in cuttings collected on different dates (Figure 2d). Similar results were obtained in various plant species, indicating that auxin levels did not seem to be a limiting factor of root initiation. The work of Ayoub and Qrunfleh (2006) (in buds and leaves of the Raseei olive cultivar), Stoltz (1968) (in *Chrysanthemum* spp.) and Tsipouridis et al. (2006) (in Crest May and Arm King) revealed that IAA concentration dropped with time and had a negative relationship with rooting potential, which appeared to be conflicting with the popular opinion that IAA is the primary trigger for root initiation. We agree with the explanation given by Tsipouridis et al. (2006) that there are possibly other simulating factors affecting rooting activities.

There have been 2 postulated roles for endogenous ABA in rooting. In general, ABA levels were positively correlated with rooting, particularly in seasonal variations observed in woody plants (Blakesley et al. 1991). Ayoub and Qrunfleh (2006) found that ABA concentration in Nabali olive had a promotive

effect on adventitious root formation. Basu et al. (1968) also reported a promotive effect of ABA on rooting of mango cuttings, as did Blazich (1977) on mung bean cuttings. In addition, in difficult-to-root species, higher ABA concentration than that in easy-to-root ones has been reported. Noiton et al. (1992) suggested that ABA may be one of the factors involved in the inhibition of root formation in difficult-to-root species. In Tetraploid Locust cuttings, seasonal changes in ABA concentration coincided with the rooting percentage (Figure 2e). Therefore, it is suggested that ABA concentration has a promotive influence on the rooting of cuttings. The promotive effect of ABA on rooting could be explained on the basis that ABA antagonizes the effects of gibberellins and cytokinins, both of which inhibit rooting.

As for GAs, the general trend indicated that the high GAs concentration coincided with the low rooting percentage in Tetraploid Locust cutting (Figure 2f). This result indicated that high GAs concentration had inhibitory effect on adventitious root formation. The probable inhibitory effect of GAs on rooting in 'Tetraploid Locust' is in agreement with the findings of Ayoub and Qrunfleh (2006), and Bartolini and Ministro (1982), who reported an inhibitory effect of GAs on rooting of the olive cultivars Nabali and Raseei, and Frantoio and Leccino, respectively.

From this study it is clear that the rooting behavior of Tetraploid Locust varies with the season. The optimum cutting collection time should be on 15 May. This is supported by the results that the early planting date (15 May) led to better outcomes in most of the studied characteristics in comparison to the other planting dates, which in turn were reflected in the growth and behavior of the plant.

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