Indole-3-butyric acid on rooting and endogenous plant hormones in tetraploid and diploid *Robinia pseudoacacia* hardwood cuttings

Efectos del ácido indol-3-butirico en el enraizamiento y hormonas vegetales endógenas en cortes leñosos de *Robinia pseudoacacia* tetraploide y diploide

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Abstract. *Robinia pseudoacacia* (locust hereafter) is an ornamental tree with various uses. Both homologous tetraploid (tetraploid hereafter) and diploid cultivars are commercially available. The tetraploids have advantageous traits, but poor germinability, and cuttings are difficult to root. Since auxin applications can promote rooting, we evaluated the effects of dipping cuttings in various indole-3-butyric acid (IBA) concentrations for four hours on rooting parameters. IBA significantly affected all measured parameters of both tetraploid and diploids cuttings. Tetraploid cuttings produced no roots without IBA. The effects were strongest at 1000 and 800 mg/L IBA for tetraploids and diploids, respectively. Values for tetraploids and diploids respectively, were (1) 75.6 and 100% for rooting percentages, (2) 11.74 and 13.5 for average root numbers, and (3) 5.24 and 5.5 cm for average root lengths per rooted cutting. We also examined the content of endogenous hormones in cuttings treated with (1) IBA at optimal concentrations or (2) water (controls). Diploids seemed to require high indole-3-acetic acid (IAA) levels during root primordia initiation, but IAA contents were substantially and consistently higher in tetraploids. Trends in IAA levels were opposite to those of abscisic acid (ABA) in tetraploids; however, this did not happen in diploids. Root induction was negatively or positively related to IAA content and IAA/ABA ratio in tetraploids or diplods, respectively. Rooting was associated with high ABA contents in tetraploids, but low ABA contents in diploids. Zeatin riboside contents were proportional to numbers of roots per cuttings and root length. The results demonstrate clear physiological rooting-related differences between tetraploid and diploid locust cuttings, indicating that high IAA contents constrain rooting in tetraploids.

Keywords: Abscisic acid; Indole-3-acetic acid; Indole-3-butyric acid; *Robinia pseudoacacia*; Zeatin riboside.

Resumen. *Robinia pseudoacacia* es un árbol ornamental con varios usos. Tanto los cultivares tetraploide homólogo como diploide están disponibles comercialmente. Los tetraploides tienen características ventajosas, pero baja capacidad de germinación, y las estacas son difíciles de enraizar. Desde que las aplicaciones de auxina pueden promover el enraizamiento, evaluamos los efectos de sumergir las estacas en varias concentraciones de ácido indol-3-butírico (AIB) durante 4 horas y ver los efectos sobre el enraizamiento. Las concentraciones estudiadas de este ácido afectaron significativamente todos los parámetros tanto en las estacas del tetraploide como en las del diploide. Las estacas del tetraploide no produjeron enraizamiento sin AIB. Los mayores efectos fueron a 1000 y 800 mg/l de AIB para las estacas del tetraploide y diploide, respectivamente. Los valores para los tetraploides y diploides, respectivamente, fueron (1) 75,6 y 100% para los porcentajes de enraizamiento, (2) 11,74 y 13,5 para el número de raíces promedio, y (3) 5,24 y 5,5 cm para longitud radical promedio por estaca enraizada. También examinamos el contenido de hormonas endógenas en estacas tratadas con (1) AIB a concentraciones óptimas y (2) agua (controles). Los diploides parecieron requerir altos niveles de ácido indol-3-acètico (AIA) durante la iniciación de primordios radicales, pero los contenidos de AIA fueron substancialmente (y consistentemente) más altos en los tetraploides. Las tendencias en los niveles de AIA fueron opuestas a las del ácido abscísico (ABA) en los tetraploides. Sin embargo, esto no sucedió en los diploides. La inducción radical estuvo negativamente o positivamente relacionada con el contenido de AIA y la relación AIA/ABA en los tetraploides o diploides, respectivamente. El enraizamiento estuvo asociado con altos niveles de ABA en los tetraploides, y bajos niveles de esta hormona en los diploides. Los contenidos de ribosido de zeatina fueron proporcionales al número de raíces por estaca y la longitud radical. Los resultados claramente demuestran diferencias fisiológicas relacionadas con el enraizamiento entre las estacas tetraploides y diploides de *R. pseudoacacia*, indicando que altos contenidos de AIA limitan el enraizamiento en los tetraploides.

Palabras clave: Ácido abscísico; Ácido indol-3-acético; Ácido indol-3-butírico; *Robinia pseudoacacia*; Ribosido de zeatina.

INTRODUCTION

Robinia pseudoacacia [locust (one individual of *Robinia pseudoacacia)* hereafter] is an attractive ornamental tree that also has various commercial uses. Homologous tetraploid (tetraploid hereafter) locust cultivars, generated by artificially-inducing chromosome doubling in diploid locust cells, have several desirable traits. They include strong tolerance of low temperatures, drought, nutrient deficiency, dust and salinity. However, since homologous tetraploids have four sets of ho-mologous chromosomes that cannot be stably separated and ordered in metaphase of meiosis, high genetic variations are frequently observed in their offspring (Luo et al. 1985; Chen et al. 2005). Furthermore, embryos of homologous tetraploids have poor germinability, and their seedling cultivation peri-ods are long. This is because tetraploid locust is mainly repro-duced by grafting. However, this is severely constrained by low propagation coefficients.

Propagation by cuttings generally has high propagation coefficients, and can be applied to large-scale plantations. This is why the method is widely used for asexual propagation of many kinds of trees. However, tetraploid locust is difficult to root. We have previously shown that the survival rate of tet-raploid cuttings is much lower than that of diploid cuttings under similar experimental conditions. Thus, it is important to examine the effects of genetic variation on asexual propa-gation in this species to identify the rooting constraints of tetraploids.

Previous studies on asexual propagation of tetraploid lo-cust have usually focused on cutting (Hu et al., 2004) or tissue cultivation (Wang et al., 2002) techniques. However, propa-gation efficiencies, and all stages of plant development, are strongly affected by the concentrations and ratios of endog-enous (and exogenous, if applied) phytohormones (Li et al., 2005). Thus, information on the concentrations (and ratios) of hormones in tetraploid and diploid cultivars, and the ef-fects of endogenous hormone applications, could facilitate improvement of their propagation. The relationships between activities of several key hormone-metabolizing enzymes, and enzymes influenced by hormones (indoleacetic acid oxi-dase, polyphenol oxidase and peroxidase), and rooting ability in tetraploid locust have been reported by Ma et al. (2007). However, no rooting studies have previously examined rela-tionships between levels of endogenous plant hormones, [e.g. the auxin IAA, abscisic acid (ABA) and the cytokinin zeatin riboside (ZR)], and the rooting ability of tetraploid and dip-loid locusts during root-induction. Therefore, the objectives of this study were (a) to investigate the effect of a synthetic auxin (indole-3-butyric acid, IBA) at various concentrations on root formation in cuttings (diploid and tetraploid) of the species, and (b) to establish the relationship (if any) between rooting responses and levels of endogenous plant hormones (IAA, ABA and ZR) during root-induction in tetraploid and diploid locusts. These hormones were selected because they are all known to influence rooting processes.

MATERIALS AND METHODS

Plant material and collection site. Cuttings and sample material of tetraploid and diploid locusts were collected for laboratory analysis from a 3-year-old, field-grown mother stock at the nursery of the College of Forestry (108° 07' E, 34° 12' N, altitude 445 m), North-West Agriculture and Forestry University, Yang Ling, China. Annual precipitation was 660 mm, there were 220 frost-free days, the mean an-nual temperature was 12.9 °C, and mean temperature during February, March and April was 2.8, 7.9, 14.4 °C, respec-tively, in 2010.

Rooting experiment. On the morning of February 23, 2010, cuttings (length approximately 15 cm, diameter 10-12 mm) were taken from the sub-terminal part of 40-50 cm long shoots of selected plants. Sets of three replicates of 80 cut-tings were bundled. Thereafter, the basal inch of the bundles was dipped into solutions with various concentrations of IBA (400, 600, 800, 1000 and 1200 mg/L) during 4 h. Controls were dipped in water for 4 h. The basal 5 cm of each cut-ting was then placed in sand in trays in a glasshouse that was equipped with an automatic mist system. Day/night air tem-peratures in the glasshouse were maintained at 15/25 °C, with 70-100% relative humidity. During the rooting period, mist was supplied intermittently for 10 s at 5 min intervals be-fore visible callus developed, and for 15 s at 30 min intervals thereafter.

Thus, the rooting experiments had a complete randomized design with three replicates, each consisting of 80 cuttings. The rooting percentage, average number of roots per rooted cutting, root length and average root diameter were deter-mined using an EPSON PERFECTTON™ 4990 PHOTO root scanner, 2 months after planting.

Measurement of endogenous plant hormones. Five cut-tings were randomly selected from the sets treated with IBA at each concentration (and controls) at 0, 15, 20, 25, 30 and 35 days after planting. The selected cuttings were rinsed with water, and the skin layer (within 1cm range) was rapidly tak-en out. After being quickly frozen in liquid nitrogen, these samples were stored in an ultra-low-temperature refrigerator. Enzyme-linked immunosorbent assays (ELISA) were then used to determine contents (in triplicate) of endogenous plant hormones (IAA, ABA and ZR) using ELISA kits. These kits were purchased from the Chinese Agricultural University. An ELx800™ Microplate spectrophotometer (BioTek, USA) was also used, following the manufacturers' recommenda-tions. Finally, contents of the hormones in each sample were estimated (in ng/g FW) from logit ELISA calibration curves.

Statistics. The effects of the hormone treatments on the measured rooting parameters were evaluated by Analysis of Variance (ANOVA) using SPASS 16.0 for Windows software. Rooting percentages were arcsine-transformed to meet normality requirements. The significance of differences between treatment-related mean values was assessed by the Least Significant Difference (LSD) test.

RESULTS

Effects of IBA concentration on rooting of tetraploid and diploid locusts. The concentration of the IBA solution used to dip the cuttings significantly influenced all measured root traits (rooting percentage, average number of roots per rooted cutting, root length and average root diameter) of the diploids. The result was the same in the tetraploids but for root diameter (Table 1). The IBA concentration also influenced more strongly the tetraploid than the diploid cuttings (rooting in the water-treated controls was 58.39% in the diploids, and 0% in the tetraploids). Briefly, values for all of the rooting parameters increased with increasing IBA concentrations up to 1000 mg/L for tetraploids, and 800

mg/L for diploids. However, further concentration increases resulted in a substantial decline of the study root traits. Root length was maximal at 600 mg/L, and dipping in 800 and 1000 mg/L IBA resulted in 100% rooting in the diploids. Between-concentration differences in all these parameters were also significant, except for root length and diameter in the tetraploids. Average root numbers of diploid and tetraploid cuttings peaked at 11.74 ± 0.80 and 13.48 ± 0.53, respectively, and root lengths at 7.55 ± 0.61 and 5.24 ± 0.83 cm, respectively.

Changes in the levels of endogenous plant hormones in tetraploid and diploid locusts. Figure 1 describes the morphological and anatomical observations used to distinguish the three main phases of root development. Timing for the three main phases of root development (callus induction, root primordial initiation and expression) differed considerably between the IBA-treated tetraploid and diploid cuttings. They occurred 0-20 d, 20-30 d, 30-35 d after planting, respectively, in the tetraploid and 0-15 d, 15-20 d, 20-35 d after planting, respectively, in the diploids. As described above, IBA concentrations of 1000 mg/L and 800 mg/L optimally

Table 1. Mean (± SE) root number, root length, root diameter and rooting percentage of tetraploid or diploid Robinia pseudoacacia cuttings (three replicates of 80 cuttings each) treated with IBA at the indicated concentration (or water).
Tabla 1. Promedio (± EE) del número, longitud y diámetro radicales, y porcentaje de enraizamiento de estacas de Robinia pseudoacacia tetraploide o diploide (tres réplicas de 80 estacas cada una) tratadas con IBA a la concentración indicada (o agua).

| Cultivar	IBA Concentration (mg/L)	Average root number (piece)	Average root length (cm)	Average root diameter (mm)	Rooting percentage (%)
Tetraploid locust	Control (H_2O)	0C	0B	0B	0D
	IBA400 mg/L	5.24 ± 0.71 B	4.3 ± 0.88 A	1.87 ± 0.25 A	40.4 ± 0.47 C
	IBA600 mg/L	4.55 ± 0.59 B	4.11 ± 0.75 A	1.86 ± 0.24 A	48.16 ± 0.33 C
	IBA800 mg/L	10.31 ± 0.87 A	4.15 ± 0.66 A	1.88 ± 0.42 A	61.5 ± 0.34 B
	IBA1000 mg/L	11.74 ± 0.80 A	5.24 ± 0.83 A	2.11 ± 0.41 A	75.56 ± 0.43 A
	IBA1200 mg/L	5.12 ± 0.59 B	4.76 ± 0.72 A	1.92 ± 0.32 A	60.2 ± 0.41 B
	F	42.081	7.213	6.598	227.454
	p	0.0001	0.0025	0.0036	0.0001
Diploid locust	Control (H_2O)	9.15 ± 0.55 B	4.52 ± 0.43 B	1.57 ± 0.07 AB	58.39 ± 0.93 E
	IBA400 mg/L	7.36 ± 0.59 B	4.7 ± 0.63 B	1.53 ± 0.08 AB	74.51 ± 0.64 D
	IBA600 mg/L	7.38 ± 0.67 B	7.55 ± 0.61 A	1.14 ± 0.17 B	92.98 ± 0.73 B
	IBA800 mg/L	13.48 ± 0.53 A	5.5 ± 0.50 AB	1.78 ± 0.16 A	100 ± 0 A
	IBA1000 mg/L	9.12 ± 0.51 B	7.25 ± 0.39 A	1.33 ± 0.14 AB	100 ± 0 A
	IBA1200 mg/L	8.46 ± 0.86 B	6.89 ± 0.40 A	1.38 ± 0.1 AB	88.24 ± 0.99 C
	F	12.848	7.002	3.09	560.105
	p	0.0002	0.0028	0.0508	0.0001

For each parameter, means within columns with different letters are significantly different at the p<0.01 probability level according to LSD test. F: F test value; p: probability level.
Para cada parámetro, los promedios con letras diferentes dentro de cada columna son significativamente diferentes a p<0.01 de acuerdo a la prueba de LSD. F: valor de la prueba de F; p: nivel de probabilidad.

Fig. 1 Root development of *Robinia pseudoacacia* through hardwood cuttings. (a) Just born callus in tetraploid locust after 20 d from planting. (b) Root primordial initiation from callus after 30 d from planting in tetraploid locust (40×). (c) Root expression from callus after 35 d from planting in tetraploid locust. (d) Tetraploid locust plant from hardwood cutting after two months from planting. (e) Just burn callus in diploid locust after 15 d from planting. (f) Root primordial initiation from callus after 20 d from planting in diploid locust (40×). (g) Root expression from callus after 25 d from planting in diploid locust. (h) Diploid locust plant from hardwood cutting after two months from planting.

Fig. 1. Desarrollo de raíces de *Robinia pseudoacacia* a partir de estacas leñosas. (a) Callo recién nacido en *R. pseudoacacia* tetraploide después de 20 días desde la plantación. (b) Iniciación de primordios radicales desde callos después de 30 días desde la plantación en *R. pseudoacacia* tetraploide (40X). (c) expresión radical desde el callo después de 35 días desde la plantación en *R. pseudoacacia* tetraploide. (d) Planta de *R. pseudoacacia* tetraploide a partir de estacas leñosas después de dos meses de la plantación. (e) Callo recién nacido en *R. pseudoacacia* diploide después de 15 días desde la plantación. (f) Iniciación de primordios radicales a partir de callos después de 20 días desde la plantación en *R. pseudoacacia* diploide (40X). (g) expresión radical desde el callo después de 25 días desde la plantación en *R. pseudoacacia* diploide. (h) Planta de *R. pseudoacacia* diploide a partir de estacas leñosas después de dos meses desde la plantación.
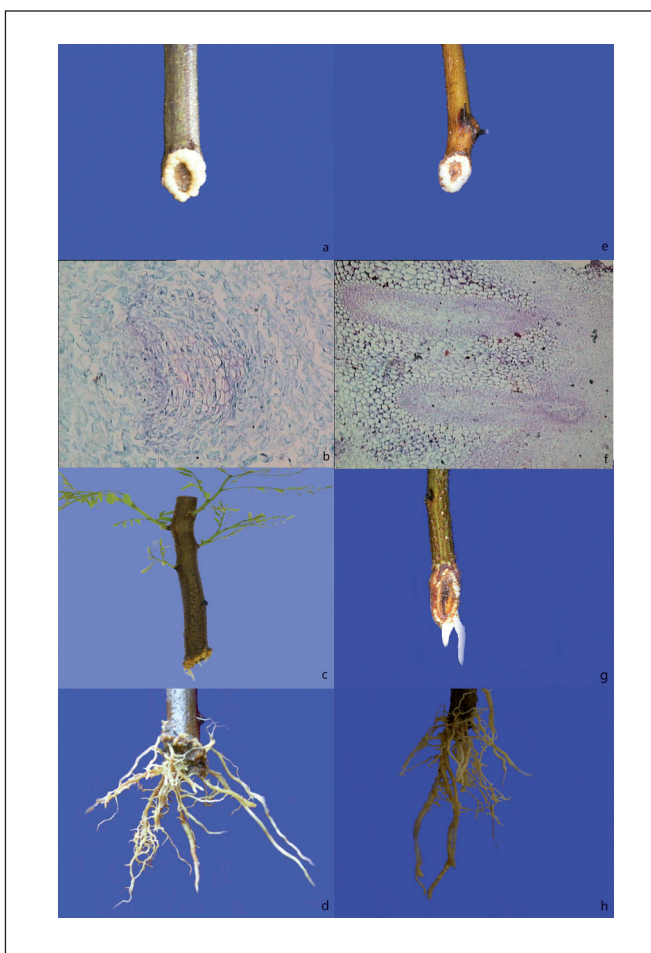
promoted root formation and development in the tetraploids and diploids, respectively; therefore, these concentrations, and the water-treated controls, were used for further endogenous plant hormones studies.

Changes in IAA contents. For diploids, the trends of IAA contents during root-induction were consistent with those observed for tetraploids (Fig. 2-a). During the callus induction phase (0-15 d), IAA contents of controls and IBA-treated cuttings increased by 17.8% and 47.2%, respectively. During the root primordial initiation phase (15-20 d), they reached the lowest levels (24.5% and 66.3% of the initial content, respectively), and during the expression phase (20-35 d), their IAA contents gradually increased. In addition, the IAA content of the diploids was consistently lower than that of their tetraploid counterparts in all three phases (Fig. 2-a). Further, the IAA content was consistently lower in IBA-treated tetraploids than in their controls, while the reverse pattern was observed for the diploids. Similarly, during the callus induction and root primordial initiation phases, the range of IAA contents was greater among the tetraploid than diploid controls. However, the range was greater for the diploids among the IBA-treated cuttings. The differences between the IAA contents of the tetraploids and diploids were extremely significant (F=16.239, p=0.0001). Thus high IAA contents may be a major cause of the lower rooting percentages of the tetraploids.

Changes in ABA contents. As shown in Fig. 2-b, there were substantial differences in ABA contents, and their trends, between the tetraploids and diploids. In the tetraploids, they decreased during the callus induction phase, but increased during the root primordial initiation phase, peaking 30 d after planting. There were significant differences in contents between the controls and IBA-treated tetraploids (F=13.50, p=0.0213). During the expression phase, the ABA contents of the controls and ABA-treated tetraploids gradually declined (unlike their IAA contents, which increased, as mentioned above). In addition, the ABA content was consistently higher in the IBA-treated tetraploids than in the controls.

In contrast to the tetraploids, ABA contents continuously decreased during the callus induction and root primordial initiation phases in the diploids, and low ABA contents favored root primordial initiation in these cuttings. During the expression phase, ABA contents gradually rose in the diploids, and these contents were significantly lower than in the tetraploids (F=9.419, p= 0.0004). The results strongly indicate that the IAA/ABA ratio, rather than the concentration of either hormone alone, was a key determinant of the cuttings' rooting capacity.

Changes in ZR contents. ZR contents increased during the callus induction phase, and decreased during the root pri-

Fig. 2. Changes in (a) IAA content, (b) ABA content, (c) ZR content, (d) IAA/ABA ratio, (e) IAA/ZR ratio and (f) ABA/ZR ratio during rooting of the *Robinia pseudoacacia* cuttings.

Fig. 2. Cambios en los contenidos de (a) IAA, (b) ABA, y (c) ZR, y en las relaciones (d) IAA/ABA, (e) IAA/ZR y (f) ABA/ZR durante el enraizamiento de las estacas de *Robinia pseudoacacia*.
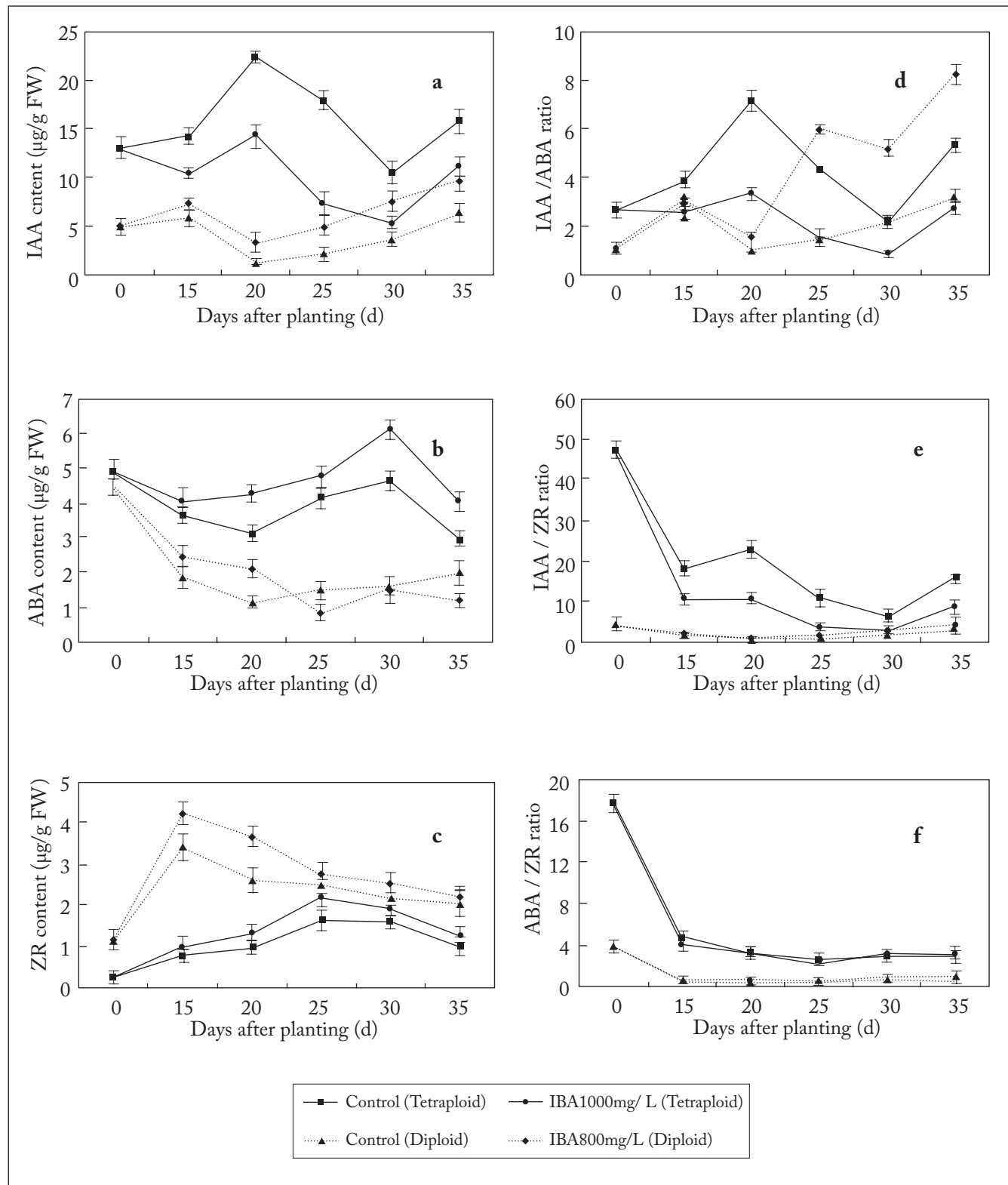
mordial initiation and expression phases (Fig. 2-c) in both tetraploids and diploids. Also, ZR contents were lower in tetraploids than in diploids, and lower in the controls than in IBA-treated cuttings. In tetraploids, ZR contents peaked 25 d after planting, and there was no significant difference in peak values between the control and the IBA-treated cuttings (F=0.86, p=0.4065).

In diploids, ZR contents were maximal 15 d after planting, and were higher in IBA-treated cuttings (4.23 μg/g FW) than in the controls (3.41 μg/g FW), although there were no significant differences in their peak values (F=1.20, p=0.3357). Further variance analysis suggested that there were significant differences between the control peak values and those of the treatments in tetraploid and diploid locusts (control: F=7.99, p=0.0475; treatment: F=8.19, p=0.0459). The main action of ZR (a cytokinin) is the promotion of cell division and expansion. Thereafter, our results indicate that the ZR contents of the tetraploids and diploids were positively correlated with rooting number and root length.

Changes in IAA/ABA ratios. Similar trends in IAA/ABA ratios to those observed in IAA contents during the three phases were detected in both tetraploids and diploids (Fig. 2-d). In the tetraploids, the ratio varied less among IBA-treated cuttings (2.67-2.75) than among the controls (2.67-5.32). During the root primordial initiation phase, the ratio was negatively associated with rooting ability. The results further indicated that high IAA contents inhibited rooting in the tetraploids.

In the diploids, there were no significant differences in the IAA/ABA ratio (F=3.45, p=0.1367) between the IBA-treated cuttings and the controls during the callus induction and expression phases. During the root primordial initiation phase, the IAA/ABA ratio was positively associated with rooting ability.

Changes in IAA/ZR ratios. The IAA/ZR ratio was consistently and significantly (F=3.832, p=0.0256) higher in the tetraploids than in diploids (Fig. 2-e). In addition, the range of IAA/ZR ratios was lower in IBA-treated tetraploids than in their controls, while the opposite was true for the diploids. Further, the IAA/ZR ratio declined in both tetraploids and diploids until 30 d after planting. However, the ZR ratio decreased much more rapidly in the tetraploids, and although reductions in ZR contents contributed to this decline, it was largely due to changes in their IAA contents. These results suggest that abnormal IAA/ZR ratios, mainly due to alterations in IAA contents, also affected rooting in the tetraploids.

Changes in ABA/ZR ratios. In the tetraploids and diploids the ABA/ZR ratio declined during the rooting process (Fig. 2-f), rapidly during the callus induction phase, and less quickly during the root primordial initiation and expression phases. It was also higher in the tetraploids (range, 2.17-4.63) than in the diploids (0.30-0.97). No significant differences in ABA/ZR ratios were found between tetraploids and diploids locusts (F=2.141, p=0.1269).

DISCUSSION

Adventitious rooting formation in stem cuttings is influenced by diverse endogenous factors (Gyana, 2006). In addition, equally diverse exogenous factors can affect rooting parameters. For example, auxin applications can increase the rooting capacity of many plants (Marks & Simpson, 2000; Husen & Mishra 2001; Husen 2003; Kiran 2008). Therefore, auxin (generally IBA) is widely used in commercial propagation (Kotis et al., 2009). This study showed that IBA treatment increased rooting percentages and promoted root development more in tetraploid than diploid locusts. In addition, rooting took longer in the tetraploids (which were generated by doubling the chromosome number of diploid locust cells) than the diploids. Duplicating genes may have three possible fates: retention of their original functions, silencing or differentiation and implementation of new functions. Hence, duplication of an entire genome may have complex results, expressed as changes in genetic traits, due to the interactive effects of widely varying changes in the activities and functions of numerous genes (Wendel, 2000).

Particularly significant changes in gene activities following duplication for rooting may be those affecting auxin concentrations, or its ratios with other hormones, since auxin plays major roles in root initiation. Basipetally transported IAA often promotes the induction of root primordial and increases the number of cells per root primodium (Haissig, 1972). It also stimulates RNA and protein synthesis (Scott, 1972). Thus, for instance, Caballero (1979) found that it is a limiting factor for root initiation, and increases levels of rooting promoters in 'Ascolano', 'Gordal' and 'Swan Hill' olive cultivars. However, rooting controls are more complex than these observations may suggest, since reductions in auxin levels have been associated with adventitious rooting potential in some species or organs of some species [e.g. buds and leaves of olive cv. Raseei (Ayoub & Qrunfleh, 2006), *Chrysanthmum* spp. (Stoltz, 1968) and peach cvs. Crest May and Arm King (Tsipouridis et al., 2006)]. Similarly, during the root primordial initiation phase in our tetraploid locust cuttings (which had substantially higher IAA levels than the diploids), IAA contents steadily declined. This indicates that high rather than low auxin levels constrained rooting in those cuttings. This disagrees with the common opinion that IAA is the primary trigger of root initiation. This finding is also consistent with conclusions of Song (2001), that the super-selective oxidase system can reduce endogenous IAA contents to levels that promote root primordia initiation in chimeric *Populus tomentosa* cuttings. In some cases, root de-

velopment was associated with reductions in IAA contents during the induction phase. This result contrasts with findings of Zhang & Guo (2006), who found that primordial initiation was generally associated with high IAA contents in *Paulownia fortune*.

There are also conflicting reports about the relationship between ABA content and root primordial initiation. In some cases ABA has no apparent inhibitory effect on rooting of cuttings (Kracke & Cristoferi, 1983; Kelen & Ozkan. 2003). For example, grape is difficult to root although its ABA content is higher than that of readily-rooting plants (Theophilus et al., 2010). However, ABA can promote rooting of other plants (Hartung & Turner, 1980). Our results are consistent with the latter case, since high ABA contents provided an appropriate IAA/ABA ratio for rooting in the tetraploids. ZR is a cytokinin that plays major developmental roles by promoting cell division and expansion. Thus, the ZR content was closely related to the quantity and length of roots formed by both diploids and tetraploids, but it was not the key factor in root induction.

In conclusion, although a high auxin/cytokinin ratio is traditionally believed to promote rooting in cuttings, numerous studies (including ours) indicate that effects of hormones can vary, even within the same species. Furthermore, different types of endogenous plant hormones interact physiologically both synergistically and antagonistically, so the ratios of plant hormones are often key determinants of root formation and development. The changes in IAA/ABA, IAA/ZR and ABA/ZR ratios were more pronounced in tetraploid than in diploid locusts; the IAA/ABA and IAA/ZR ratios were higher than the ABA/ZR ratio, and these changes in ratios appear to have been crucial for rooting in the tetraploids. The results clearly indicate the importance of plant hormones (especially auxin), and their ratios, for rooting in cuttings.

ACKNOWLEDGEMENTS

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