

Effects of plant growth regulators on growth and carbohydrate accumulation in shoots and roots of two almond rootstock seedlings

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Effects of plant growth regulators on growth and carbohydrate accumulation in shoots and roots of two almond rootstock seedlings.

Abstract — Introduction. *Prunus amygdalus* and *P. webbii* can be used as rootstocks for almond cultivars due to their adaptability to severe environmental conditions. However, the time required for seedlings to reach a suitable size for transplanting may take 1 to 3 years. Our study tested the effects of foliar application of growth regulators on the vegetative growth and carbohydrate accumulation in shoots and roots of these species. **Materials and methods.** Six-week-old seedlings were treated with gibberellic acid (GA_3) (100 mg L^{-1}) alone or with GA_3 followed by ethephon [(100 and 200) mg L^{-1}], or chlormequat chloride (CCC) [(500 and 1000) mg L^{-1}], or paclobutrazol (PBZ) [(500 and 1000) mg L^{-1}]. **Results and discussion.** Most levels of plant growth regulators significantly enhanced seedling growth. However, GA_3 alone was most effective on stem height, leaf area, and shoot fresh and dry weights of both almond species. The thickest stems of *P. amygdalus* and *P. webbii* were obtained from the application of $100 \text{ mg GA}_3 \text{ L}^{-1}$ followed by application of (1000 and 500) mg PBZ L^{-1} , respectively. In both species, PBZ significantly increased leaf chlorophyll content compared with the controls as well as with the other treatments. Application of GA_3 alone on *P. webbii* and of GA_3 followed by 100 mg ethephon L^{-1} on *P. amygdalus* showed the highest root number, and root fresh and dry weights. High levels of soluble sugars and starch in the shoots and roots of both species were observed when GA_3 application was followed by PBZ. **Conclusion.** These results demonstrated that application of plant growth regulators to the seedlings might be a useful way of enhancing growth of *P. amygdalus* and *P. webbii* and reducing the time and cost of seedling production.

Iran Islamic Republic / *Prunus amygdalus* / *Prunus webbii* / rootstocks / seedlings / growth / plant growth substances / gibberellic acid / ethephon / chlormequat / paclobutrazol

Effets des régulateurs de croissance végétaux sur la croissance et l'accumulation d'hydrate de carbone dans les tiges et racines de deux porte-greffes d'amandier.

Résumé — Introduction. *Prunus amygdalus* et *P. webbii* peuvent être utilisés comme porte-greffes de cultivars d'amandier du fait de leur adaptabilité à de sévères conditions environnementales. Cependant, le temps requis pour que les plantes atteignent une taille apte à la transplantation peut être de 1 à 3 ans. Notre étude a analysé les effets de l'application foliaire de régulateurs de croissance sur la croissance végétative et l'accumulation d'hydrate de carbone dans les tiges et les racines de cette espèce. **Matériel et méthodes.** Des plants de 6 semaines ont été traités avec de l'acide gibbéralique (GA_3) (100 mg L^{-1}) seul ou avec GA_3 suivi de l'application d'éthéphon [(100 et 200) mg L^{-1}], ou de chlorure de chlorocholine (CCC) [(500 et 1000) mg L^{-1}], ou de paclobutrazol (PBZ) [(500 et 1000) mg L^{-1}]. **Résultats et discussion.** La plupart des doses de régulateurs de croissance ont augmenté de manière significative la croissance des plantes. Cependant, GA_3 utilisé seul a été le plus efficace pour augmenter la hauteur des tiges, la surface foliaire et les poids frais et secs des tiges des deux espèces d'amandier étudiées. Les tiges les plus épaisses de *P. amygdalus* et de *P. webbii* ont été obtenues à partir de l'application de $100 \text{ mg GA}_3 \text{ L}^{-1}$ suivie de l'application de (1000 et 500) mg PBZ L^{-1} , respectivement. Pour les deux espèces, l'application de PBZ a augmenté de manière significative la teneur en chlorophylle des feuilles comparée à celle des plantes témoin et à celle des plantes des autres traitements. L'application de GA_3 seul sur *P. webbii* et de GA_3 suivi de 100 mg éthéphon L^{-1} sur *P. amygdalus* a donné le nombre de racines le plus élevé, ainsi que les poids frais et secs de racines les plus importants. On a observé des niveaux élevés de sucres et d'amidon solubles dans les tiges et les racines des deux espèces quand l'application de GA_3 a été suivie de celle de PBZ. **Conclusion.** Ces résultats ont démontré que l'application de régulateurs de croissance végétaux pourrait être efficace pour augmenter la croissance de *P. amygdalus* et de *P. webbii* et réduire la période et le coût de production de ces porte-greffes.

Iran République islamique / *Prunus amygdalus* / *Prunus webbii* / porte greffe / plantule / croissance / substance de croissance végétale / acide gibbéralique / éthéphon / chlorméquat / paclobutrazol

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Received 12 March 2008
Accepted 20 May 2008

Fruits, 2008, vol. 63, p. 363–370
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DOI: 10.1051/fruits:2008032
www.fruits-journal.org

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1. Introduction

Iran is an important center for wild and domesticated almond trees. Bitter almond (*Prunus amygdalus* var. *amara*) seedlings are the primary rootstock for almond in Iran. However, *P. webbii*, a wild almond species used in semi-desert areas to control soil erosion, is also used as an almond rootstock due to its adaptability to severe environmental conditions [1, 2]. It takes 1 to 3 years for a seedling of bitter or wild almond to reach the suitable size for budding. Therefore, increasing the growth rate can shorten this time.

Plant growth regulators are widely used for modifying plant growth and development in many plants [3]. Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol], a member of the triazole plant growth inhibitor group, is a broad-spectrum gibberellin biosynthesis inhibitor [4]. The primary action of PBZ is to inhibit ent-kaurene oxidase, which catalyzes the sequential oxidations from ent-kaurene to ent-kaurenoic acid in the early sequence of gibberellin biosynthesis [5], resulting in reduced plant height [6, 7], increased chlorophyll content [8], and enhanced carbohydrate accumulation in shoots and roots [9, 10].

Chlormequate chloride (CCC) [(2-chloroethyl) trimethyl ammonium chloride], a gibberellin biosynthesis inhibitor, has been used on cereals since the mid-1960s [11]. CCC is a favorable choice for general application since it is easily broken down in the environment [12]. Chlormequate chloride disrupts gibberellin biosynthesis prior to the steps at which PBZ acts, inhibiting cyclization of geranylgeranyl diphosphate to copalyl diphosphate [4]. Aphalo *et al.* [13] found that application of CCC to containerized silver birch seedlings partially inhibited their height, growth and dry mass accumulation without negative effects on root growth in the following spring. They concluded that CCC could be a useful tool in nursery management. Studies also demonstrated that CCC reduced tree vigor and stem length and increased shoot diameter [14, 15].

Ethephon, which decomposes into gaseous ethylene, chloride and phosphate ions,

is known to cause changes in vascular anatomy [16]. Research has found that ethephon increased xylem wall thickness [17, 18], inhibited cell elongation and promoted lateral expansion [19]. Neel reported that application of ethylene gas to the lower halves of young tree trunks enclosed in plexiglass tubes causes extreme thickening of the trunk [20].

The objective of our study was to study the effects of ethephon, CCC and PBZ applied after gibberellin acid (GA₃) treatment on growth, rooting and allocation of assimilates of two almond rootstock seedlings.

2. Materials and methods

Viable dehulled nuts of *P. amygdalus* var. *amara* and sound nuts of *P. webbii* were selected using a flotation technique [21]. The sound nuts were scarified mechanically and then soaked in tap water for 48 h. The nuts were mixed with moist peat moss [3 peat moss: 1 seed (V/V)] and stratified by keeping them at (5 ± 1) °C for 30 d. After stratification, nuts were sown directly in 5-kg black plastic bags filled with a 1:1:1 (V/V/V) mixture of sand, leaf mold and loam soil in early spring. The plastic bags were then kept in an outdoor nursery area and irrigation was done. The experiment was arranged in a completely randomized design with eight treatments, four replications and four plants per replication (plastic bag).

Six-week-old seedlings were sprayed with 100 mg·L⁻¹ GA₃. The control plants were treated with distilled water. Four weeks after GA₃ treatment, seedlings were then sprayed with (100 or 200) mg ethephon·L⁻¹ or (500 or 1000) mg CCC·L⁻¹ or (500 or 1000) mg PBZ·L⁻¹. Twelve weeks later, plants were removed from the containers. The media were carefully washed from the roots. The length and diameter of stems, leaf size, chlorophyll content, fresh and dry weight of shoots, root number, root diameter, and fresh and dry weight of roots were determined. Leaf chlorophyll content was measured photometrically in 80% acetone [22]. Leaf size and root number and diameter were measured using the Delta-T SCAN

image analysis system (Windias software). Shoot and root samples were lyophilized, ground and stored at -20°C until sugar analysis. Soluble sugars were extracted with 80% ethyl alcohol, and starch was extracted with perchloric acid. The extracted soluble sugars and starch were quantified with anthrone using a spectrophotometer at 630 nm [23].

All data were subjected to analysis of variance (ANOVA) and means were compared using the least significant difference (LSD) test at $P < 0.05$, using MSTATC (Michigan State Univ., MI, USA) software.

3. Results and discussion

3.1. Effect of GA₃, ethephon, CCC and PBZ on seedling height

Twelve weeks after the application of GA₃ and its combination with other chemicals, seedling height of both *P. amygdalus* and *P. webbii* was increased compared with control (*table I*). Nevertheless, this increase was not statistically significant for GA₃ + 200 mg ethephon·L⁻¹ or GA₃ + 1000 mg PBZ·L⁻¹ in *P. amygdalus* (*table I*). The tallest seedlings of *P. amygdalus* and *P. webbii* were obtained from the application of GA₃ alone. However, application of ethephon, CCC or PBZ following GA₃ reduced the increasing effect of GA₃ on plant height (*table I*). This result indicates an inhibitory effect of ethephon, CCC and PBZ on plant height. The effect of GA₃ on stem elongation was consistent with previous findings with pecan [24] and wild species of pistachio [25].

3.2. Effect of GA₃, ethephon, CCC and PBZ on stem diameter

Stem diameter was also increased by GA₃ alone in both species (*table I*). In *P. amygdalus*, application of ethephon, CCC and PBZ following GA₃ showed a further increase in stem diameter, with the most effect caused by 1000 mg PBZ·L⁻¹ (154% of control). In *P. webbii*, application of CCC and PBZ following GA₃ also showed a fur-

ther increase in stem diameter, with the thickest stem (2.36 mm) caused by 500 mg PBZ·L⁻¹ (*table I*). Rahemi and Baninasab [25] reported similar findings with GA₃ in two wild species of pistachio. They concluded that GA₃ significantly increased stem diameter of some selected *P. mutica* and *P. khinjuk* seedlings. The increasing effect of PBZ on stem diameter is in agreement with that reported by Costa *et al.* [26] in pear. It has been reported that CCC increased shoot diameter of mango [14]. Ethephon also increased seedling diameter only in *P. amygdalus* compared with GA₃ alone (*table I*). Neel [20] reported that ethylene increased eucalyptus seedling trunk diameters. He demonstrated ethylene thickened *Eucalyptus fasciculosa* stems by stimulating phloem development. Experiments have demonstrated a potential role of ethylene in secondary growth in stem tissues [16, 18]. Application of ethrel has been shown to stimulate cambial cell division in both gymnosperm and angiosperm trees [16, 18]. Gurusinghe and Shackel [16] reported similar findings with almond trees. They concluded that ethephon induced biochemical and anatomical changes that could increase the amount of cell wall material in the sheared area of the cambial zone.

3.3. Effect of GA₃, ethephon, CCC and PBZ on leaf size, chlorophyll content and shoot mass

Application of GA₃ alone significantly increased leaf size in both *P. amygdalus* and *P. webbii* (*table I*), confirming the results reported by Marcelle and Oben [27]. However, application of GA₃ followed by (100 or 200) mg ethephon·L⁻¹ or 1000 mg PBZ·L⁻¹ reduced leaf size in *P. webbii*.

The leaf chlorophyll content was significantly increased when (500 or 1000) mg PBZ·L⁻¹ were applied after GA₃ application in both species compared with the other treatments (*table I*). Previous studies have shown that chlorophyll content was affected by PBZ treatment [8, 28]. The increase in chlorophyll content in seedling leaves treated with PBZ may be attributed to the increase in endogenous cytokinin content.

Table I.
Effects of plant growth regulators on shoot growth and chlorophyll content of *Prunus amygdalus* and *P. webbii* seedlings.

Treatment	Dose (mg L ⁻¹)	Stern height (cm)	Stern diameter (mm)	Leaf size (cm ²)	Chlorophyll (mg g ⁻¹ dry weight)	Shoot fresh weight (g)	Shoot dry weight (g)	
	amygdalus	webbii	amygdalus	webbii	amygdalus	webbii	amygdalus	webbii
Control								
GA3	100	16.5 d	9.2 c	3.33 e	1.41 e	4.75 bc	2.32 b	2.84 bc
GA3	100	28.4 a	18.9 a	3.91 d	1.88 d	5.65 a	2.79 a	2.73 bc
+ ethephon	100	22.5 bc	14.3 b	4.49 c	1.92 cd	5.22 ab	1.83 cde	2.93 b
+ ethephon	200	20.4 bcd	13.3 b	4.72 bc	2.05 bcd	5.17 ab	1.60 e	2.78 bc
GA3	100	23.1 b	15.1 b	4.63 bc	2.28 ab	4.73 bc	2.21 bc	3.96 bc
+ CCC	500	23.7 b	14.3 b	4.91 ab	2.13 abc	4.47 c	2.07 bcd	2.63 c
+ CCC	1000							
GA3	100	22.8 bc	15.4 b	4.87 ab	2.36 a	4.66 bc	1.98 bcde	1.95 de
+ PBZ	500	19.0 ccd	14.8 b	5.09 a	2.19 ab	4.53 c	1.67 de	2.56 c
+ PBZ	1000							

Means in each column followed by the same letters are not significantly ($P < 0.05$) different, using the LSD test.

GA₃: gibberellic acid, CCC: chlormequat chloride, PBZ: paclobutrazol.

Table II.Effects of plant growth regulators on root growth of *Prunus amygdalus* and *P. webbii* seedlings.

Treatment	Dose (mg·L ⁻¹)	Root number		Root diameter (mm)		Root fresh weight (g)		Root dry weight (g)	
		<i>amygdalus</i>	<i>webbii</i>	<i>amygdalus</i>	<i>webbii</i>	<i>amygdalus</i>	<i>webbii</i>	<i>amygdalus</i>	<i>webbii</i>
Control		33132.7 c	6257.6 cd	1.21 d	1.48 d	4.16 de	0.64 b	0.60 cd	0.06 b
GA ₃	100	37475.5 bc	10881.0 a	1.68 ab	2.06 a	4.40 cde	0.94 a	0.59 cd	0.10 a
GA ₃	100								
+ ethephon	100	50572.2 a	7013.4 bcd	1.19 d	1.57 cd	8.62 a	0.75 ab	1.19 a	0.07 b
+ ethephon	200	41146.5 bc	8462.5 bc	1.07 d	1.68 bcd	5.35 c	0.83 ab	0.71 c	0.08 ab
GA ₃	100								
+ CCC	500	39964.0 bc	8775.9 ab	1.28 cd	1.82 abc	4.98 cd	0.84 ab	0.70 c	0.08 ab
+ CCC	1000	43621.3 ab	8507.2 bc	1.33 bcd	1.63 bcd	7.06 b	0.77 ab	0.95 b	0.08 ab
GA ₃	100								
+ PBZ	500	35746.2 bc	6021.7 d	1.63 abc	1.71 bcd	4.53 cde	0.75 ab	0.57 cd	0.08 ab
+ PBZ	1000	33825.0 c	5773.5 d	1.72 a	1.93 ab	3.45 e	0.69 b	0.48 d	0.07 b

Means in each column followed by the same letters are not significantly ($P < 0.05$) different, using the LSD test.GA₃: gibberellic acid, CCC: chlormequat chloride, PBZ: paclobutrazol.

It has been proposed that PBZ stimulates cytokinin synthesis that, in turn, enhances chloroplast differentiation and chlorophyll biosynthesis, and prevents chlorophyll degradation [29–31].

Application of most plant growth regulators significantly increased shoot fresh and dry weights in both species compared with control. The largest increase was found when GA₃ was applied alone (table I). Addition of the ethephon, CCC and PBZ to GA₃ reduced the effect of GA₃. A similar result was reported from other research [25, 28].

3.4. Effect of GA₃, ethephon, CCC and PBZ on root quality

Root quality is an important index of seedling quality and a critical factor for transplanting. Spraying *P. amygdalus* seedlings with GA₃ plus 100 mg ethephon·L⁻¹ or 1000 mg CCC·L⁻¹ increased root number as well as root fresh and dry weight compared with control (table II). Spraying *P. webbii* seedlings with GA₃ alone or GA₃ plus 500 mg CCC·L⁻¹ increased root number compared with control, whereas only GA₃ alone increased root fresh and dry weight (table II). Studies have

shown that GA₃ may inhibit or promote root growth in different species [32, 33]. Our results agree with those of Campbell and Pan *et al.* [34, 35].

In both species, application of GA₃ alone or followed by 1000 mg PBZ·L⁻¹, and also in *P. amygdalus* GA₃ + 500 mg PBZ·L⁻¹ and in *P. webbii* GA₃ + 500 mg CCC·L⁻¹, significantly increased root diameter compared with control (table II). This is similar to the results of Davis *et al.* [6], who reported plants treated with PBZ had thicker roots than untreated controls.

3.5. Effect of GA₃, ethephon, CCC and PBZ on shoot and root soluble sugar and starch content

Application of PBZ in both species' seedlings, and GA₃ plus 100 mg ethephon·L⁻¹ in *P. amygdalus* and GA₃ + 500 mg CCC·L⁻¹ in *P. webbii*, significantly increased shoot soluble sugars compared with controls (table III). Spraying of both species' seedlings with PBZ also significantly increased root soluble sugars compared with control (table III). Increased soluble sugar content in all plant parts following foliar application of PBZ was

Table III.

Effects of plant growth regulators on carbohydrate content ($\text{mg}\cdot\text{g}^{-1}$ dry weight) of shoots and roots of *Prunus amygdalus* and *P. webbii* seedlings.

Treatment	Dose ($\text{mg}\cdot\text{L}^{-1}$)	Shoot soluble sugars		Root soluble sugars		Shoot starch		Root starch	
		<i>amygdalous</i>	<i>webbii</i>	<i>amygdalous</i>	<i>webbii</i>	<i>amygdalous</i>	<i>webbii</i>	<i>amygdalous</i>	<i>webbii</i>
Control		97.1 c	66.4 c	12.7 bc	16.2 bcd	56.6 d	23.6 c	106.7 e	64.0 f
GA3	100	101.4 bc	68.8 bc	10.2 d	12.4 d	61.2 cd	24.0 c	109.2 e	60.3 f
GA3	100								
	+ ethephon	100	104.5 ab	70.3 bc	12.0 cd	16.9 abc	63.2 cd	24.8 c	121.3 d
	+ ethephon	200	100.8 bc	71.6 bc	13.2 bc	14.8 cd	61.7 cd	24.2 c	128.7 d
GA3	100								
	+ CCC	500	103.4 abc	74.1 b	14.3 b	17.4 abc	69.4 bc	27.2 bc	173.2 c
	+ CCC	1000	100.9 bc	73.0 bc	14.6 b	18.0 abc	71.3 b	26.8 bc	168.1 c
GA3	100								
	+ PBZ	500	107.1 ab	81.4 a	18.1 a	19.4 ab	82.2 a	30.5 ab	202.9 b
	+ PBZ	1000	109.7 a	84.2 a	17.4 a	20.7 a	90.4 a	32.4 a	116.1 a

Means in each column followed by the same letters are not significantly ($P < 0.05$) different, using the LSD test.

GA₃: gibberellic acid, CCC: chlormequat chloride, PBZ: paclobutrazol.

also found by Mehouachi *et al.* [9]; however, Yim *et al.* did not observe any significant effect of PBZ on stem soluble sugar content of rice seedlings [28]. Shoots of *P. amygdalus* treated with (500 and 1000) mg PBZ·L⁻¹ showed a 34% and 48% increase in starch content, respectively (table III). The highest shoot starch content in *P. webbii* was observed with the application of (500 and 1000) mg PBZ·L⁻¹ (table III). Mehouachi *et al.* reported similar findings with citrus rootstock seedlings [9]. In earlier work, apple trees treated with PBZ had increased starch content attributed to a reduced amylase activity [36]. Correlations between stem diameter and shoot soluble sugars ($r = 0.73$ and $r = 0.76$, respectively, in *P. amygdalus* and *P. webbii* seedlings) and also shoot starch ($r = 0.75$ and $r = 0.70$, respectively, in *P. amygdalus* and *P. webbii* seedlings) were identified. These correlations suggest that stem diameter is related to the available amounts of soluble sugars and starch in stem tissues.

Roots, whether treated or not, had higher starch content than shoots (table III). In both *P. amygdalus* and *P. webbii*, roots of seed-

lings treated with PBZ had markedly higher starch compared with controls and the other treatments (table III). A correlation was also found between stem diameter and root starch ($r = 0.78$, $r = 0.72$, respectively, in *P. amygdalus* and *P. webbii* seedlings).

4. Conclusions

Our results suggest that spraying of almond seedlings with plant growth regulators might be a useful method of enhancing growth of almond rootstocks to shorten the necessary time for seedlings to reach the transplanting size.

Acknowledgement

We thank Mrs. M. Ghomsheh and Mr. B. Modarres for their valuable technical assistant throughout this experiment. The authors thank Dr. J. Arvin for the gift of PBZ. This work was supported by the Isfahan University of Technology Research Council, Iran.

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Efectos de los reguladores de crecimiento vegetal sobre el crecimiento y la acumulación de hidrato de carbono en los tallos y en las raíces de dos porta injertos de almendro.

Resumen — Introducción. *Prunus amygdalus* y *P. webbii* pueden emplearse como porta injertos de cultivares de almendro gracias a su adaptabilidad a condiciones medioambientales severas. Sin embargo, el tiempo necesario para que las plantas alcancen un tamaño apto para el transplante puede ser de 1 a 3 años. Nuestro estudio analizó los efectos de la aplicación foliar de reguladores de crecimiento sobre el crecimiento vegetativo y la acumulación de hidrato de carbono en los tallos y en las raíces de esta especie. **Material y métodos.** Se sometieron a tratamiento plantas de 6 semanas con ácido giberélico (GA_3) ($100 \text{ mg} \cdot \text{L}^{-1}$) sólo o con GA_3 seguido de la aplicación de etefón [$(100 \text{ y } 200) \text{ mg} \cdot \text{L}^{-1}$], o de clormequat (CCC) [$(500 \text{ y } 1000) \text{ mg} \cdot \text{L}^{-1}$], o de paclobutrazol (PBZ) [$(500 \text{ Y } 1000) \text{ mg} \cdot \text{L}^{-1}$]. **Resultados y discusión.** La mayoría de las dosis de reguladores de crecimiento incrementó de modo importante el crecimiento de las plantas. No obstante, GA_3 empleado sólo fue el más eficaz para aumentar la altura de los tallos, la superficie foliar así como los pesos en fresco y en seco de los tallos de ambas especies de almendro estudiadas. Los tallos más gruesos de *P. amygdalus* y de *P. webbii* se obtuvieron a partir de la aplicación de $100 \text{ mg} \text{ GA}_3 \cdot \text{L}^{-1}$ seguido de la aplicación de $(1000 \text{ y } 500) \text{ mg PBZ} \cdot \text{L}^{-1}$, respectivamente. Para ambas especies, la aplicación de PBZ incrementó significativamente el contenido de clorofila de las hojas en comparación con aquél de las plantas piloto y con el de las plantas de otros tratamientos. La aplicación de GA_3 solo en *P. webbii* y de GA_3 seguido de $100 \text{ mg etefón} \cdot \text{L}^{-1}$ en *P. amygdalus* dio el número de raíces más elevado, así como los pesos en fresco y en seco de raíces más importante. Se observaron niveles elevados de los azúcares y de almidón solubles en los tallos y en las raíces de ambas especies tras la aplicación de GA_3 seguido de la de PBZ. **Conclusión.** Estos resultados demostraron que la aplicación de reguladores de crecimiento vegetales podrían ser eficaces para aumentar el crecimiento de *P. amygdalus* y de *P. webbii* así como reducir el periodo y el coste de producción de estos porta injertos.

Iran República Islámica / *Prunus amygdalus* / *Prunus webbii* / portainjertos / plántulas / crecimiento / sustancias de crecimiento vegetal / ácido giberelico / etefon / chlormequat / paclobutrazol