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Effect of different concentrations of indole butyric acid, putrescine and hydrogen peroxide on stem cuttings of the rootstock GF677(*Prunus amygdalus* × *Prunus persica*) according to the cutting season

Efecto de diferentes concentraciones de ácido indol butírico, putrescina y peróxido de hidrógeno en el enraizamiento de esquejes de tallo de durazno GF677 (*Prunus amygdalus* × *Prunus persica*) según las temporadas de corte

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ABSTRACT

The rootstock GF677 is an interspecific hybrid with an important economic and horticultural value. In this research, the effect of indole butyric acid (IBA) in combination with putrescine (Put) and hydrogen peroxide (H_2O_2) on rooting of GF677 semi-hardwood stem cuttings in three cutting seasons (July, March and October) was investigated. Treatments as IBA (0, 1000, 2000 and 3000 mg L^{-1}), Put (0, 800, 1600 and 3200 mg L^{-1}) and H_2O_2 (1.5, 3 and 6% w/v) were included. The results showed that in July cuttings, the highest levels of callogenesis were observed in IBA treated cuttings in both concentrations of 1000 and 2000 mg L^{-1}. The rooting was very low in July cuttings, while the highest percentage of rooting (14%) was observed in the combination of 2000 mg L^{-1} IBA+ 3% H_2O_2. In March, the cuttings treated by 1000 mg L^{-1} IBA+800 mg L^{-1} Put and 1000 mg L^{-1} IBA+1600 mg L^{-1} Put revealed the highest percentages of callus formation 83.31 and 83.33%, respectively. In these cuttings, the highest percentage of rooting (63.88%) was gained at 2000 mg L^{-1} IBA+3200 mg L^{-1} Put. The application of 1000 mg L^{-1} IBA+800 mg L^{-1} Put increased root fresh weight. In cuttings prepared in October, only 800 mg L^{-1} Put caused callus formation in more than 55% of the cuttings. The rooting of cuttings at this time was as low as the July cuttings, whereas the highest rooting percentage was observed in cuttings treated with IBA at a concentration of 1000 mg L^{-1}. Overall, the experiment showed that the season of the cutting and the treatments with IBA+Put or H_2O_2 could improve rooting properties of the rootstock GF677.

Keywords:
Adventitious rooting
Carbohydrate
Co-factors
Semi-hardwood cuttings
Woody cuttings

RESUMEN

El portainjerto GF677 es un híbrido interespecífico con un importante valor económico y hortícola. En este estudio, se investigó el efecto del ácido indol butírico (IBA) en combinación con putrescina (Put) y peróxido de hidrógeno (H_2O_2) en el enraizamiento de esquejes de tallos de madera semidura GF677 en tres temporadas de corte (julio, marzo y octubre). Los tratamientos incluyeron IBA (0, 1000, 2000 y 3000 mg L^{-1}), Put (0, 800, 1600 y 3200 mg L^{-1}) y H_2O_2 (1.5, 3 y 6% p/v). Los resultados mostraron que en los esquejes de julio, la callogénesis más alta se observó en los esquejes tratados con IBA en ambas concentraciones de 1000 y 2000 mg L^{-1}. El enraizamiento fue muy bajo en los esquejes de julio y el mayor porcentaje de enraizamiento (14%) se observó en la combinación de IBA 2000 mg L^{-1}+H_2O_2 3%. En los esquejes de marzo, el mayor porcentaje de formación de callos se alcanzó en los tratados con IBA 1000 mg L^{-1}+Put 800 mg L^{-1} e IBA 1000 mg L^{-1}+Put 1600 mg L^{-1}, 83.31 y 83.33%, respectivamente. En estos esquejes, el mayor porcentaje de enraizamiento (63.88%) se obtuvo con IBA 2000 mg L^{-1}+Put 3200 mg L^{-1}. La aplicación de IBA 1000 mg L^{-1}+Put 800 mg L^{-1} aumentó el peso fresco de la raíz. En esquejes preparados en octubre, el Put solo a una concentración de 800 mg L^{-1} provocó la formación de callos en más del 55% de los esquejes. El enraizamiento de los esquejes en este momento era tan bajo como los esquejes de julio y el mayor porcentaje de enraizamiento se observó en los esquejes tratados con IBA a una concentración de 1000 mg L^{-1}. En general, el experimento mostró que la temporada de corte y el tratamiento con IBA+Put o H_2O_2 podría mejorar las propiedades de enraizamiento de los esquejes de GF677.

Palabras clave:
Enraizamiento adventicio
Carbohidratos
Co-factores
Esquejes de madera semidura
Esquejes leñosos

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Vegetative propagation is typically an efficient method of maintaining specific traits of plant genetic resources. Considerable amount of superior quality planting materials can be produced through clonal propagation within short time (Baul *et al.,* 2010; Hartmann *et al.,* 2011; Wetzstein *et al.,* 2018). Adventitious rooting, as one of the most important methods of plant vegetative propagation, is a requirement for successful production of practical clones. It is also one of the most efficient methods for fast commercial production of horticultural plants worldwide (Shi-Weng *et al.,* 2009b). Hardwood cuttings are one of the least expensive and easiest methods of vegetative propagation. They are not easily perishable, may be shipped safely over long distances and require little or no special equipment during rooting (Hartmann *et al.,* 2011).

The GF677 (*Prunus amygdalus* × *Prunus persica*) is a valuable rootstock, which has a wide range of compatibility with various cultivars and species (Karimi and Yadollahi, 2012; Gainza *et al.,* 2015). It is tolerant against donor Fe deficiency and suitable for semi-drought conditions, calcareous and poor fertility soils (Legua *et al.,* 2012; Bagheri *et al*., 2016; Ranjbar *et al.,* 2019). Therefore, to find the appropriate methods to improve the GF677 propagation is highly important (Tsipouridis and Thomidis, 2004; Tsipouridis *et al.,* 2005; Karimi and Yadollahi, 2012; Sarikhani *et al.,* 2017).

Environmental and endogenous factors such as temperature, light conditions, plant growth regulators (PGRs), carbohydrate, mineral elements and other molecules, may act as signals and induce groups of cells to alter the result in adventitious rooting (Shi-Weng *et al.,* 2009a; Hartmann *et al.,* 2011). In addition to the genotype of the plant, the nutritional status, the phenological stage, the environmental factors, and the climatic conditions cause seasonal variations in the rooting capability of the woody cuttings (Hartmann *et al.,* 2011). Application of plant growth regulators (PGRs) is one of the most effective procedures to increase root initiation, rooting percentages, and quality and uniformity of roots. The most widely used PGRs for this purpose is indole butyric acid (IBA) (Ozelbaykal and Gezerel, 2005; Asl moshtaghi and Shahsavar, 2011; Nazary and Yadollahi, 2012; Nag *et al.,* 2013; Caplan *et al.,* 2018). Furthermore, it has been reported that polyamines (PAs) are able to promote root formation and root development in difficult-to-root plants; particularly, Put has displayed a better response in comparison with other PAs (Rey et al., 1994; Wu et al., 2010; Sivanandhan et al., 2011; Silvestri et al., 2018). Rey *et al.* (1994) found a powerful enhancing effect of PAs on microshoots rooting of hazelnut. PAs improved rooting when microshoots were treated with IBA in a synergistic mode; probably generated a better induction of roots, while PAs had only a limited beneficial impact on rooting when applied without IBA. Additionally, Put in combination with IBA promoted early rooting and increased the rooting percentage in hazelnut (Cristofori *et al.,* 2010), olive (Rugini *et al*., 2016) and in *Ficus* (Ghasemi and Kosh-Khui, 2019). Some studies demonstrated a possible improvement of the auxin stimulation on adventitious root formation when it is mixed with co-factors such as phenolics (Bartolini and Tattini, 1986; De-Klerk *et al.,* 1999), flavonoids (Lewis *et al.,* 2011) and hydrogen peroxide (H_2O_2) (Sebastiani and Tognetti, 2004; Shi-Weng *et al*., 2009a and b). Moreover, there is a connection between PAs effects on rooting enhancement of cuttings and increase of peroxidase activity at the basal end of cuttings (Rugini *et al.,* 1997).

The relationship between tissue carbohydrate contents and rooting has remained controversial for many years (Tsipouridis and Thomidis, 2004). Most of the tree species can be rooted with leafy cuttings rather than leafless cuttings (Ky-Dembele *et al*., 2011). Indeed, further carbohydrate substances, seasons and cutting date are critically important elements. For instance, some cuttings as quince can root at any season of the year, whereas cuttings obtained from another species such as cherry and olive root are only successful at a certain time of the year (Hartmann and Loreti, 1965; Hartmann *et al.,* 2011). Ucler and Parlak (2004) examined the influence of IBA and cutting date on rooting of semi-hardwood cuttings of kiwifruit and reported that the cuttings which were taken in August had better rooting compared to those taken in July. In addition, they concluded that cutting date had a significant influence on rooting potential.

In this context, the aim of this research was to evaluate the effects of IBA, Put and H_2O_2 in different concentrations and seasons, separately or in combination with each other, on rooting of GF677 rootstock.

MATERIALS AND METHODS

Location and plant material

This experiment was conducted in the research greenhouse of Bu-Ali Sina University, Hamedan, Iran (1741.5 masl, 34°47′N, 48°30′E), as a completely randomized design with three replications and 12 cuttings in each replication. Uniform cuttings of GF677 (*Prunus amygdalus* × *Prunus persica*) were collected from Paradise Nursery in Hamedan province and Sanaz Nursery in Zanjan province. At first, the semi-hardwood cuttings with about 30 cm length were immersed in Put or H_2O_2 solutions for 30 s, and then treated with IBA for 10 s. Subsequently, cuttings were planted diagonally in a medium involving sand and perlite with a ratio of 80 and 20%, respectively. Furthermore, the rooting bed was covered with covering film to maintain the moisture content. The research included three experiments as following:

First experiment

The first experiment was done on July 6th, and semi-hardwood leafy cuttings were taken from the middle third of the current season branches of the 3-year-old GF677 donor plants. The cuttings were treated with Put and H_2O_2 in seven levels including distilled water (control), Put at three concentrations of 800, 1600 and 3200 mg L^{-1} and H_2O_2 at three concentrations of 1.5, 3 and 6 % w/v, as one factor. Another factor was IBA in four concentrations of 0, 1000, 2000 and 3000 mg L^{-1}.

Second experiment

The experiment was prepared on March 6th and hardwood cuttings without leaf were taken from the current season branches of the GF677 donor plants. Similar to the first experiment, the cuttings were treated with IBA, Put and H_2O_2.

Third experiment

This experiment was performed on October 16th and semi-hardwood leafy cuttings were taken from the current season branches of the GF677 donor plants. Based on results of first and second experiments, the desired levels of IBA (0, 1000 and 2000 mg L^{-1}), Put (800 and 1600 mg L^{-1}) and H_2O_2 (1.5 and 3 % w/v) were used at this stage.

In all three experiments, cuttings were taken in early morning. Immediately after treatment, the cuttings were cultured on the medium mentioned previously. The moisture content was monitored daily. After three months, cuttings were removed from the culture medium and some traits were analyzed such as callus proliferation, rooting of cuttings, fresh and dry weight of rooted and/or callused cuttings.

Statistical analysis

All data were normalized using arc-sin transformation method, and later statistically analyzed based on full factorial experiment in a completely randomized design by using the SAS software (version 9.1). Analysis of variance and Duncan's multiple-range tests ($P \leq 0.05$) were performed to assess possible significant differences among treatments.

RESULTS AND DISCUSSION

First experiment

The analysis of variances ($P \leq 0.01$) showed significant effects of IBA, Put and H_2O_2 and their interactions on callus proliferation percentage (Data of analysis of variance not shown). The callus proliferation was from 0.00 to 42.33% of treated cuttings taken in July. The highest percentage of callus proliferation (42.33%) was observed in cuttings treated with IBA 1000 mg L^{-1}, which showed no significant differences with some other treatments. However, the lowest percentage (0.00%) corresponded to Put treated cuttings. Furthermore, no callus proliferations were noticed using 3000 mg L^{-1} IBA+1600 mg L^{-1} Put (Table 1).

The effect of IBA, Put and H_2O_2 and their interactions on callus fresh weight were significant at $P \leq 0.01$. Application of IBA at 1000 mg L^{-1} led to the highest callus fresh weight in comparison with other treatments. The lowest callus fresh weight was obtained by Put at 800 mg L^{-1}, 1600 mg L^{-1}, 3200 mg L^{-1}, and also 3000 mg L^{-1} IBA+1600 mg L^{-1} Put with no significant difference from control (Table 1).

Regarding rooting response, the application of IBA, Put and H_2O_2 and their interactions were significant ($P \leq 0.01$) on rooting percentage. Rooting percentage in this experiment was ranged between 0.00 to 13.83%. The highest rooting percentage (13.83%) was obtained by the application of 2000 mg L^{-1} IBA+ 3% H_2O_2. It did not have a significant difference when the treatments: 3000 mg L^{-1} IBA+3200 mg L^{-1} Put, 3000 mg L^{-1} IBA+1.5% H_2O_2 and

1000 mg L^{-1} IBA+1.5% H_2O_2 were used. The results of this study showed that the effect of cuttings preparation time as well as auxin, Put and H_2O_2 treatments were effective on callus formation and rooting of GF677 cuttings. Auxin has been proven to be an important factor in the rooting of many cuttings. Therefore, the internal auxin level is vital in callus production and rooting of cuttings in various plants. In the case of high levels of endogenous auxin, rooting can easily occur in many cases (Hartmann *et al.,* 2011). In contrast, in cases where external auxin treatment is required, the use of IBA as a commercial auxin to enhance rooting of many plants has been demonstrated (Shiozaki *et al.,* 2013). The lowest rooting percentage was gained in all treatments without IBA such as those of control and different concentrations of Put-H_2O_2 (Table 1).

Table 1- Effect of IBA, Put and H_2O_2 on callus proliferation and rooting of GF677 cuttings taken in July.

IBA concentration (mg L^{-1})	Put-H_2O_2 concentration (mg L^{-1} - %)		Callus proliferation (%)	Callus fresh weight (mg)	Rooting (%)
0		0	14.33 a-f	5.67 f-j	0.00 e
		800	0.00 g	0.00 j	0.00 e
	Put	1600	0.00 g	0.00 j	0.00 e
		3200	0.00 g	0.00 j	0.00 e
		1.5	28.16 a-d	12.03 c-h	0.00 e
	H_2O_2	3	37.33 ab	7.30 d-i	0.00 e
		6	17.00 a-f	3.43 g-j	0.00 e
1000		0	42.33 a	62.43 a	8.33 abc
		800	17.33 a-f	7.00 f-j	0.00 e
	Put	1600	5.50 e-g	0.57 ij	2.83 de
		3200	14.16 b-g	5.00 g-j	5.50 bcd
		1.5	31.16 a-c	14.17 c-h	8.33 abc
	H_2O_2	3	30.83 a-c	6.50 e-i	0.00 e
		6	8.33 c-g	16.57 c-h	0.00 e
2000		0	42.33 a	47.07 ab	5.50 bcd
		800	19.66 a-f	24.73 b-d	8.33 abc
	Put	1600	2.83 fg	1.17 ij	5.66 bcd
		3200	11.50 b-g	27.33 bc	5.66 bcd
		1.5	14.33 a-f	5.10 f-j	0.00 e
	H_2O_2	3	25.83 a-d	33.00 bc	13.83 a
		6	8.33 c-g	13.23 c-h	0.00 e
3000		0	19.83 a-f	27.00 b-d	5.66 bcd
		800	5.66 d-g	5.47 h-j	0.00 e
	Put	1600	0.00 g	0.00 j	5.50 bcd
		3200	14.16 b-g	9.00 e-i	11.50 ab
		1.5	5.66 d-g	21.80 b-f	8.66 abc
	H_2O_2	3	17.00 a-f	23.00 b-e	5.66 cd
		6	19.66 a-e	19.10 c-g	5.50 bcd

IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide.
Means with similar letters within each column are not significantly different according to Duncan's test ($P \leq 0.05$).

The effect of IBA treatment on root length ($P \leq 0.05$) was significant. The amount of Put at 1600 and 3200 mg L^{-1}, and also H_2O_2 at 1.5 and 3% had no significant effects on root length in comparison with IBA in all concentrations. The highest root length (5.1 mm) was observed in cuttings treated with 2000 mg L^{-1} IBA with no significant difference between 1000 mg L^{-1} and 3000 mg L^{-1} IBA (Figure 1).

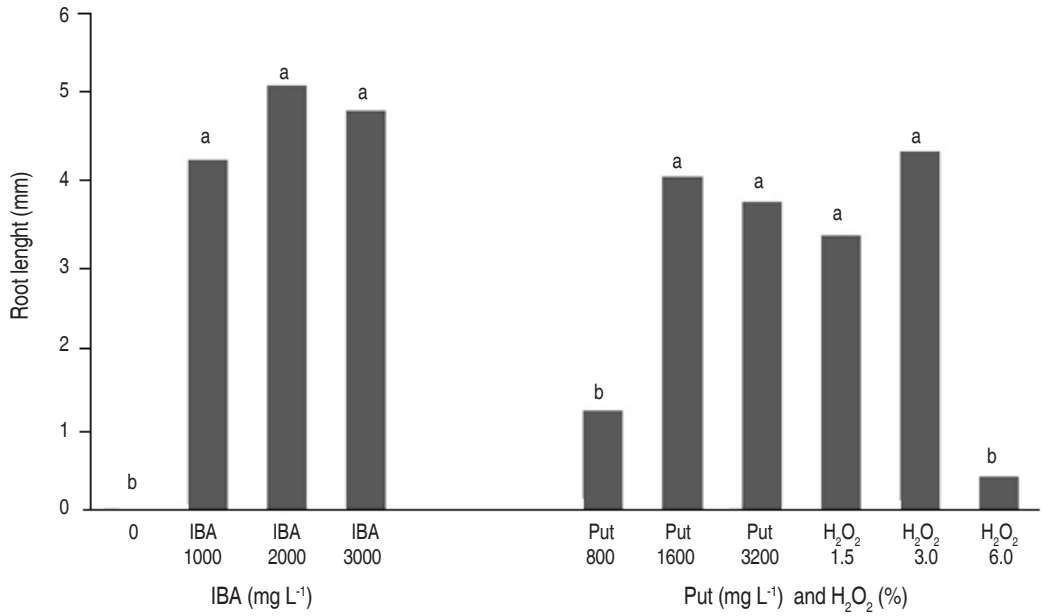

Figure 1. Effect of IBA, Put and H_2O_2 on root length of GF677 cuttings in July. In each group, columns with similar letters have no significant difference according to Duncan's test ($P \leq 0.05$). IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide.

Second experiment

Analysis of variance showed significant effects of IBA, Put and H_2O_2 treatments and their interactions on percentage of callus proliferation ($P \leq 0.01$). The highest callus proliferation was obtained at 1000 mg L^{-1} IBA+1600 mg L^{-1} Put, with no significant difference at 1000 mg L^{-1} IBA+800 mg L^{-1} Put, 1000 mg L^{-1} IBA+3200 mg L^{-1} Put, 1000 mg L^{-1} IBA+ 3% H_2O_2, 2000 mg L^{-1} IBA+800 mg L^{-1} Put and 3000 mg L^{-1} IBA+800 mg L^{-1} Put. The callus proliferation percentages for control and IBA 2000 mg L^{-1} treatments were zero. Also, the IBA at 3000 mg L^{-1} had no significant difference with regard to control (Table 2). These results showed that rooting was gained after callus production. Callus formation of GF677 was coincided with data that was obtained from rooting percentage and number of roots. Researchers have suggested that basal callus formation generally tracks rooting of GF677 with young hardwood trees (Tsipouridis and Thomidis., 2004; Tsipouridis *et al.,* 2005; Karimi and Yadollahi, 2012). Other authors have claimed that callus formation might help the rooting of some plant species with hardwood cuttings (Lodama *et al.,* 2016; Zhou *et al.,* 2018).

Application of IBA, Put and H_2O_2 and their interactions on rooting percentage were significant ($P \leq 0.01$) in cuttings taken in March. The highest rooting percentage (63.88%) was attained from cuttings treated by 2000 mg L^{-1} IBA+3200 mg L^{-1} Put (Figure 2), which did not have a significant difference with the treatments:1000 mg L^{-1} IBA+1600 mg L^{-1} Put (58.32%), 2000 mg L^{-1} IBA+ 800 mg L^{-1} Put (58.31%), 3000 mg L^{-1} IBA+3% H_2O_2 (52.77%), different levels of IBA with 800 mg L^{-1} Put, 2000 mg L^{-1} IBA+3% H_2O_2 (47.21%) (Figure 3), and 3200 mg L^{-1} Put+1000 mg L^{-1} IBA (47.20%). The lowest rooting percentage was resulted from control and treatments without IBA (Table 2).

The application of IBA, Put and H_2O_2 treatments and their interactions on root length were significant at ($P \leq 0.01$). The highest root length was obtained at 2000 mg L^{-1} IBA+1600 mg L^{-1} Put, 1000 mg L^{-1}

IBA+1600 mg L^{-1} Put and 1000 mg L^{-1} IBA+800 mg L^{-1} Put. The root length for control and Put treatments with 1600 mg L^{-1} and 3200 mg L^{-1} was zero and had no significant difference with 800 mg L^{-1} Put, H_2O_2 (1.5 and 3%), IBA (2000 and 3000 mg L^{-1}) treatments (Table 2).

Table 2- The interaction effect of IBA, Put and H_2O_2 on callus proliferation and rooting of GF677 hardwood cuttings in March.

IBA concentration (mg L^{-1})	Put-H_2O_2 concentration (mg L^{-1} - %)		Callus proliferation (%)	Rooting (%)	Root number	Root length (mm)	Root fresh weight (mg)	Root dry weight (mg)
0		0	0.00 j	0.00 g	0.00 l	0.0 f	0.00 n	0.00 k
		800	22.21 g-i	2.77 fg	0.66 jkl	3.3 ef	0.06 n	0.03 jk
	Put	1600	38.88 e-g	0.00 g	0.00 l	0.0 f	0.00 n	0.00 k
		3200	27.77 gh	0.00 g	0.00 l	0.0 f	0.00 n	0.00 k
		1.5	41.65 d-g	2.77 fg	2.33 j-l	10.0 d-f	0.56 mn	0.13 i-k
	H_2O_2	3	49.98 b-f	2.77 fg	0.33 kl	3.3 ef	0.40 mn	0.10 jk
		6	58.31 b-e	8.33 fg	3.33 j	15.0 b-d	3.06 k-m	0.93 f-j
1000		0	24.99 g-i	27.77 de	23.00 d-g	16.6 a-d	12.66 d-h	3.33 b-e
		800	83.31 a	58.31 ab	36.66 a-d	28.3 a	29.80 a	6.16 ab
	Put	1600	83.33 a	58.32 ab	26.00 c-f	28.3 a	27.63 ab	7.16 a
		3200	63.88 a-d	47.20 a-c	31.00 a-d	20.0 a-d	18.80 c-e	4.23 a-d
		1.5	44.43 c-g	30.54 de	17.33 e-i	21.6 a-d	9.40 g-i	2.10 c-f
	H_2O_2	3	69.41 ab	41.65 b-d	18.66 e-h	15.0 b-d	12.00 e-i	2.66 c-f
		6	58.31 b-e	27.77 de	8.66 i	18.3 a-d	4.56 j-l	0.73 f-j
2000		0	0.00 j	5.55 fg	3.00 j	10.0 d-f	1.30 m-n	0.33 g-k
		800	66.64 a-c	58.31 ab	44.66 ab	25.0 a-c	19.56 b-d	4.63 a-c
	Put	1600	55.53 b-e	36.09 c-e	29.00 b-e	28.3 a	14.66 d-g	2.33 c-f
		3200	55.53 b-e	63.88 a	46.33 a	21.6 a-d	26.29 a-c	6.83 a
		1.5	30.54 f-h	27.76 de	15.66 f-i	26.6 ab	8.30 h-j	2.10 d-g
	H_2O_2	3	55.54 b-e	47.21 a-c	34.66 a-d	20.0 a-d	16.90 d-f	3.63 a-d
		6	38.87 e-g	19.43 ef	11.00 hi	13.3 c-e	6.60 i-k	1.43 e-i
3000		0	5.55 ij	5.55 fg	2.00 j-l	10.0 d-f	0.80 mn	0.20 h-k
		800	66.64 a-c	55.54 ab	38.00 a-c	16.6 a-d	14.73 d-g	4.03 a-d
	Put	1600	24.99 g-i	27.76 de	14.66 g-i	15.0 b-d	10.60 f-i	2.50 c-f
		3200	30.55 f-h	30.54 de	38.00 a-c	25.0 a-c	14.66 d-g	3.86 a-d
		1.5	13.88 h-j	19.44 ef	12.00 hi	23.3 a-c	3.93 j-l	1.10 e-h
	H_2O_2	3	55.54 b-e	52.77 ab	33.00 a-d	25.0 a-c	12.43 d-h	2.86 c-e
		6	16.66 h-j	5.55 fg	2.66 jk	13.3 c-e	1.50 l-n	0.26 h-k

IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide.
Means with similar letters within each column have no significant differences based on Duncan's test ($P \leq 0.05$).

Figure 2. GF677 semi-hardwood cuttings three months after treatment of 2000 mg L^{-1} IBA+3200 mg L^{-1} Put in March.

Figure 3. GF677 semi-hardwood cuttings three months after treatment of 2000 mg L^{-1} IBA+3% H_2O_2 in March.

The application of IBA, Put and H_2O_2 treatments and their interactions on root number were highly significant ($P \leq 0.01$). The highest root number (46.3) was observed in 2000 mg L^{-1} IBA+3200 mg L^{-1} Put, 2000 mg L^{-1} IBA+800 mg L^{-1} Put, 3000 mg L^{-1} IBA+800 mg L^{-1} Put, 1000 mg L^{-1} IBA+800 mg L^{-1} Put and 1000 mg L^{-1} IBA+3200 mg L^{-1} Put treatments. The lowest number of roots was recorded for control and 1600 and 3200 mg L^{-1} Put, while they did not have significant differences with 1.5 and 3% H_2O_2 and 3000 mg L^{-1} IBA treatments (Table 2). The study showed that Put had some effects on root formation and its growth. Data on length and dry weight of roots illustrated that application of Put in the rooting of GF677 resulted in better quality roots compared with IBA treated cuttings. Moreover, concentrations of 800 and 1600 mg L^{-1} Put were better than its higher concentration (3200 mg L^{-1}) on the above-mentioned traits. PAs have been showed to increase root elongation and growth by increasing cell division. The amount of Put increases during elongation in the differentiation zone, indicating that PAs are involved in root development (Tang and Newton, 2005). In fact, application of PAs increases the synthesis of internal PAs in plant tissue (Vondrakova *et al.,* 2015). Increasingly, PAs are associated with increased mitotic activation and increased primary and lateral roots. The presence and involvement of genes are also related to the synthesis of Pas, which play a role in root development in the presence of PAs (Mahdavian *et al.,* 2020). Supplementary using of PAs as a contemporary group of plant hormones considerably enhanced the number of rooting saplings of GF677.

This study revealed that Put by-itself did not have much effect on rooting of GF677 cuttings; however, a greatest effect was seen when IBA was used. IBA generates the root induction and Put stimulates the root growth, increasing synergism between both. Similar results also were obtained by Karimi and Yadollahi (2012). They showed that the highest weight of dry roots was attained with 2 mM Put treatments, and the lowest weight of roots was observed with treatment of 3000 mg L^{-1} IBA. The rate of saplings along callus was substantially more exclusive in 2 and 4 mM Put and 1500 mg L^{-1} IBA treatment and the lowest number of the saplings with callus was noticed with a treatment of 3000 mg L^{-1} IBA (Karimi and Yadollahi, 2012).

Third experiment

The application of IBA, Put and H_2O_2 and their interactions on callus proliferation percentage were significant ($P \leq 0.01$). The highest percentage of callus proliferation was observed under the treatments: 800 mg L^{-1} Put (55.55%), 1000 mg L^{-1} IBA+800 mg L^{-1} Put (41.65%), 3% H_2O_2 (36.1%), 1000 mg L^{-1} IBA (30.54%) and 2000 mg L^{-1} IBA (24.99%) (Table 3).

Table 3- The interaction effect of IBA, Put and H_2O_2 on rooting and callus proliferation of GF677 semi hardwood cuttings in October.

IBA concentration (mg L^{-1})	Put-H_2O_2 concentration (mg L^{-1} - %)		Callus proliferation (%)	Root number	Callus fresh weight (mg)	Callus dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
0 (control)		0	22.21 c-g	1.66 de	9.95 c	3.52 bc	3.10 cd	0.68 de
	Put	800	55.55 a	6.33 a-d	35.31 b	8.67 b	16.96 ab	5.62 b
		1600	25.00 c-f	1.66 de	29.09 bc	6.07 bc	1.10 cd	0.27 de
	H_2O_2	1.5	27.76 b-e	0.0 e	24.50 bc	5.46 bc	0.00 d	0.00 e
		3	36.10 bc	0.33 e	29.01bc	6.74 bc	0.75 c	0.23 de
1000		0	30.54 b-d	8.33 ab	25.88 bc	4.62 bc	4.45 c	1.44 cd
	Put	800	41.65 b	2.33 c-e	74.60 a	15.25 a	2.25 cd	0.82 de
		1600	19.44 d-g	2.66 c-e	34.80 b	6.39 bc	2.96 cd	0.89 de
	H_2O_2	1.5	8.33 gh	1.00 e	8.21 c	1.63 c	0.65 cd	0.16 e
		3	13.88 e-h	3.33 c-e	16.55bc	3.93 bc	2.58 cd	0.95 de
2000		0	24.99 c-f	9.66 a	31.95 b	5.93 bc	30.38 a	10.99 a
	Put	800	13.88 e-h	3.66 b-e	15.12 bc	2.99 bc	4.63 c	1.51 cd
		1600	11.11 f-h	7.33 a-c	15.88 bc	3.48 bc	15.01 b	3.41 bc
	H_2O_2	1.5	2.77 h	0.0 e	9.51 c	1.83 c	0.00 d	0.00 e
		3	19.44 d-g	3.00 c-e	21.67 bc	4.36 bc	2.08 cd	0.68 de

IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide.
Means with similar letters within each column have no significant differences based on Duncan's test ($P \leq 0.05$).

Data analysis showed significant effects of IBA ($P \leq 0.05$), Put and H_2O_2 ($P \leq 0.01$) and their interactions ($P \leq 0.01$) on callus fresh weight. The highest callus fresh weight was observed at 1000 mg L^{-1} IBA+800 mg L^{-1} Put (74.6 mg) and the lowest amount of callus fresh weight was recorded for control (9.95 mg), 2000 mg L^{-1} IBA+1.5% H_2O_2 (9.51 mg) and 1000 mg L^{-1} IBA+1.5% H_2O_2 (8.21 mg) (Table 3).

The effect of IBA ($P \leq 0.05$), Put and H_2O_2 ($P \leq 0.01$) and their interactions ($P \leq 0.01$) on callus dry weight was significant. Highest callus dry weight (15.25 mg) was obtained in cutting treated by 1000 mg L^{-1} IBA+800 mg L^{-1} Put. Also, the least callus dry weight observed from 2000 mg L^{-1} IBA+1.5% H_2O_2 (1.83 mg), and 1000 mg L^{-1} IBA+1.5% H_2O_2 (1.63 mg), while they did not have a significant difference with control (Table 3).

The IBA treatment had no significant difference with the interaction treatments on rooting percentage. There were no significant differences among different amounts of IBA, Put and H_2O_2 with control. The rooting percentage in 1.5% H_2O_2 treatment (0.92) decreased in comparison with control (Table 4). The activity of the enzymes at the rooting zone of stem cuttings may facilitate easy and rapid cell differentiation toward rooting. Peroxidase is an enzyme that initially increases its activity in root initiation and root development and has a positive effect on rooting. In addition, it is used as a marker in rooting to improve and enhance rooting (Hartmann *et al.,* 2011). H_2O_2 is a co-enzyme that catalyzes the oxidation of a variety of organic compounds. Numerous rooting studies have shown that H_2O_2 plays an essential role in rooting of cuttings (Sebastiani and Tognetti, 2004; Shi-Weng *et al.,* 2009a and b). Shi-Weng *et al.* (2009a) demonstrated that H_2O_2 may act as a signal molecule, involved in the auxin-induced formation of adventitious root formation. Their results showed that higher concentrations of H_2O_2 were required during the induction of adventitious root formation. Moreover, applying of IBA with H_2O_2 significantly refined the rooting of saplings compare to untreated ones; but the rooting percentage was low compared when only IBA was used. According to Sebastiani and Tognetti (2004), IBA+H_2O_2 caused considerable higher root number comparing with only IBA treatment on olive cultivars. The effect of Put and H_2O_2 treatments on root length was significant at ($P \leq 0.01$), while IBA treatment and their interactions were not significant. The highest root length was observed with 800 mg L^{-1} Put treatment (23.4 mm) and the least root length was obtained at 3% H_2O_2 treatment with (4.8 mm) (Table 4).

Table 4- Effect of IBA, Put and H_2O_2 on root length and rooting of GF677 stem cuttings in October.

Treatments		Rooting (%)	Root length (mm)
IBA concentration (mg L^{-1})			
	0 (Control)	4.34 a	9.3 a
	1000	8.26 a	10.4 a
	2000	7.21 a	11.6 a
Put-H_2O_2 concentration (mg L^{-1} - %)			
Put	800	6.47 a	23.4 a
	1600	8.21 a	9.4 abc
H_2O_2	1.5	0.92 b	5.5 c
	3	6.47 a	4.8 bc

IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide. Means with similar letters within each column have no significant differences based on Duncan's test ($P \leq 0.05$).

Analysis of variance showed significant effect of Put and H_2O_2 ($P \leq 0.01$), IBA ($P \leq 0.05$) and their interactions ($P \leq 0.05$) on root number. The Highest root number was obtained at 2000 mg L^{-1} IBA only, 1000 mg L^{-1} IBA only and 2000 mg L^{-1} IBA+1600 mg L^{-1} Put. The least root number resulted under treatments of: 1.5 and 3% H_2O_2, 1000 mg L^{-1} IBA+1.5% H_2O_2 and 2000 mg L^{-1} IBA+1.5% H_2O_2, which had no difference with control, 1600 mg L^{-1} Put, 1000 mg L^{-1} IBA+800 mg L^{-1} Put, 1000 mg L^{-1} IBA+1600 mg L^{-1} Put, 1000 mg L^{-1} IBA+3% H_2O_2, 2000 mg L^{-1} IBA+800 mg L^{-1} Put and 2000 mg L^{-1} IBA+3% H_2O_2 treatments (Table 3).

The application of IBA, Put and H_2O_2 treatments and their interactions on fresh and dry weight of roots were highly significant ($P \leq 0.01$). The most root fresh weight (30.38 mg) was observed by 2000 mg L^{-1} IBA treatment, whereas the least root fresh weight was obtained using the treatments: 1.5% H_2O_2 (0 mg), 2000 mg L^{-1} IBA+ 1.5% H_2O_2 (0 mg), 1600 mg L^{-1} Put (1.1 mg), 1000 mg L^{-1}

IBA+1.5% H_2O_2 (0.65 mg) and 3% H_2O_2 (0.75 mg) (Table 3). The effects of IBA, Put and H_2O_2 treatments and their interactions on root dry weight were significant at ($P \leq 0.01$). The highest root dry weight resulted using 2000 mg L^{-1} IBA. Moreover, root dry weight in 1.5% H_2O_2 and 2000 mg L^{-1} IBA+1.5% H_2O_2 were zero, which had no significant difference with control and other treatments (Table 3). In the present study, the use of the only auxin (IBA) produced callus and rooting in the three times of cutting collection. The need of using auxin for rooting of GF677 cuttings has been reported in previous studies (Tsipouridis and Thomidis. 2004; Tsipouridis *et al.,* 2005; Karimi and Yadollahi, 2012). Based on Tsipouridis *et al.* (2005) the appropriate concentration is between 500 and 2500 mg L^{-1} IBA. Similar results were obtained by Karimi and Yadollahi (2012) who reported rooting of 57% of GF677 cuttings when treated by 1500 mg L^{-1} IBA using a quick-dip method. The rooting decreased at 3000 mg L^{-1} IBA. High concentrations of IBA may have toxic effects on cuttings, thereby reducing the rooting (Karimi and Yadollahi, 2012). In addition, in this study, it was observed that time of cuttings preparation was very effective on callus formation and rooting of GF677 cuttings. Little rooting was observed in cuttings prepared in July and October. In contrast, rooting was much higher in those cuttings that were taken in March. The rooting success of peach cuttings has been reported to be affected by the date of collection of cuttings (Tworkoski and Takeda, 2007). The effect of cuttings collection date on the rooting of many cuttings, including olives (Hartmann and Loreti, 1965; Khajehpour *et al.,* 2014) and peach (Tofanelli *et al.,* 2003), has been demonstrated. It seems that several factors related to the time of preparation of cuttings can be effective on rooting of cuttings (Hartmann *et al.,* 2011). Khajehpour *et al.* (2014) observed no rooting on the cuttings collected in October, but the cuttings collected in August rooted well. They also reported better rooting in cuttings taken in March compared to those taken in late summer or early autumn (Khajehpour *et al.,* 2014). However, conflicting results have been reported by other researchers. Tofanelli *et al.* (2003) for peach cuttings reported that the most favorable time for collecting of cuttings is between October 25 and November 13.

CONCLUSION

The best results of callus formation and rooting were observed in cuttings prepared in March. The current experiment showed that the application of IBA, Put and H_2O_2 in comparison with control, increased the rooting of hard and semi-hardwood cuttings of GF677 rootstock. Treatment of cuttings by only IBA was more effective than only Put or H_2O_2. Additionally, IBA at concentrations of 1000 and 2000 mg L^{-1} indicated better influence on rooting. Also, the use of inexpensive chemicals such as Put or H_2O_2 together with IBA had a positive effect on increasing the rooting of GF677 cuttings, and the use of Put had a greater effect on root growth. The high concentration of only Put at 3200 mg L^{-1} and H_2O_2 at 6% caused the least effect on measured traits while these dosages combined with IBA showed a favorable effect on mentioned factors.

REFERENCES

Asl moshtaghi E and Shahsavar AR. 2011. The effects of IBA and H_2O_2 on rooting of 2 olive cultivars. Journal of Chemical Health Risks 1(1): 35-38. https://doi.org/10.22034/JCHR.2011.543971

Bagheri S, Davoodi D, Amiri ME, Bayanati M and Entesari M. 2016. Effect of different culture media on the micropropagation of GF677 (*Prunus amygdalus × P. Persica)*. Journal of Horticultural Science 30(4): 616-623. https://doi.org/10.22067/jhorts4.v0i0.32259

Bartolini G and Tattini M.1986. Effects of phenolic acids and auxin on rooting *Olea europaea* L. cuttings. HortScience 21, 2–262.

Baul TK, Mezbahuddin M, Hosssain MM and Mohiuddin M. 2010. Vegetative propagation of *Holarrhena pubescens* a wild tropical medicinal plant: effect of indole-3-butyric acid (IBA) on stem cuttings. Forestry Studies in China 12(4): 228–235. https://doi.org/10.1007/s11632-010-0409-3

Caplan D, Stemeroff J, Dixon M and Zheng Y. 2018. Vegetative propagation of cannabis by stem cuttings: effects of leaf number, cutting position, rooting hormone, and leaf tip removal. Canadian Journal of Plant Science 98: 1126-1132. https://doi.org/10.1139/cjps-2018-0038

Cristofori V, Rouphael Y and Rugini E. 2010. Collection time, cutting age, IBA and putrescine effects on root formation in *Corylus avellana* L. *cuttings*. Scientia Horticulturae 124(2): 189–194. https://doi.org/10.1016/j.scienta.2009.12.034

De-Klerk GJ, Krieken WVD and Jong J. 1999. Review the formation of adventitious roots: new concepts, new possibilities. *In Vitro* Cellular & Developmental Biology. 35: 189–199. https://doi.org/10.1007/s11627-999-0076-z

Gainza F, Opazo I, Guajardo V, Meza P, Ortiz M, Pinochet J and Munoz C. 2015. Rootstock breeding in *Prunus* species: ongoing efforts and new challenges. Chilean Journal of Agricultural research 75 (Suppl.1). https://doi.org/10.4067/S0718-58392015000300002

Ghasemi M and Kosh-Khui M. 2019. The effect of cutting type, leaf area, leaf number, putrescine and indole-3-butyric acid on the rooting of *Ficus* cuttings (*Ficus elastic Roxb. ex Hornem*). Advances in Horticultural Science 33(1): 3-11. https://doi.org/10.13128/ahs-22935

Hartmann HP and Loreti F. 1965. Seasonal variation in the rooting of olive cuttings. Journal of the American Society for Horticultural Science 87: 194–198.

Hartmann HP, Kester DE, Davies FT and Geneve RL. 2011. Plant Propagation: Principles and Practices. 7th ed. Prentice-Hall, New Jersey, press. 915 p.

Karimi S and Yadollahi A. 2012. Using putrescine to increase the rooting ability of hardwood cuttings of the peach*x*almond hybrid GF677. Journal of Agrobiology. 29(2):63-69. https://doi.org/10.2478/v10146-012-0010-6

Khajehpour G, Jameizadeh V and Khajehpour N. 2014. Effect of different concentrations of IBA (indole butyric acid) hormone and cutting season on the rooting of the cuttings of olive *(Olea europaea var Manzanilla).* International Journal of Advanced Biological and Biomedical Research 2(12): 2920-2924.

Ky-Dembele C, Tigabu M, Bayala J, Savadogo P, Joseph Boussim I and Christeroden P. 2011. Clonal propagation of *Khaya senegalensis*: The effects of stem length, leaf area, auxin, smoke solution, and stock plant age. International Journal of Forestry Research. 2011(2): 10 p. https://doi.org/10.1155/2011/281269

Legua P, Pinochet J, Moreno MA, Martinez JJ and Hernandez F. 2012. *Prunus* hybrids rootstocks for flat peach. Scientia Agricola (Piracicaba, Braz.) 69(1): 13-18. https://doi.org/10.1590/S0103-90162012000100003

Lewis DR, Ramirez MV, Miller ND, Vallabhaneni P, Keith Ray W, Helm RF, Winkel BSJ and Muday GK. 2011 Auxin and ethylene induce flavonol accumulation through distinct transcriptional networks. Plant Physiology 156: 144-164. https://doi.org/10.1104/pp.111.172502

Lodama KE, Toit ES. du, Steyn JM, Araya HT, Prinsloo G, Pooly CP. du and Robbertse PJ. 2016. Improving rooting of *Lobostemon fruticosus* L. cuttings with delayed auxin treatment. South African Journal of Botany 105: 111-115. https://doi.org/10.1016/j.sajb.2016.01.005

Mahdavian M, Sarikhani H, Hadadinejad M and Dehestani A. 2020. Putrescine effect on physiological, morphological, biochemical traits of Carrizo Citrange and Volkameriana rootstocks under flooding stress. International Journal of Fruit Science 20(2): 164-177. https://doi.org/10.1080/15538362.2019.1605560

Nag S, Paul A and Choudhuri MA. 2013. Changes in peroxidase activity during adventitious root formation at the base of Mung Bean cuttings. International Journal of Scientific and Technology Research 2(5): 171-177.

Nazary R and Yadollahi A. 2012. Micropropagation of GF677 rootstock. Journal of Agricultural Science 4(5). https://doi.org/10.5539/jas.v4n5p131

Ozelbaykal S and Gezerel O. 2005. The effects of the different dose of IBA on the rooting performance in the reproduction of *(*Gmilk*)* and *(*Domat*)* olive trees by using the green twig procedure in the ecology of cukurova region. Journal of Central European Agriculture 6(4): 481-484.

Ranjbar A, Imani A, Piri-Piravt-Lou S and Abdoosi V. 2019. Effects of drought stress on almond cultivar's responses grafted on different rootstocks. Journal of Nuts 10(1): 9-24. https://doi.org/10.22034/jon.2019.664206

Rey M, Diaz-Salz C and Rodriguez R. 1994. Exogenous polyamines improve rooting of hazel microshoots. Plant Cell, Tissue and Organ Culture 36: 303–308. https://doi.org/10.1007%2FBF00046087

Rugini E, Cristofori V and Silvestri C. 2016. Genetic improvement of olive (*Olea europaea* L.) by conventional and in vitro biotechnology methods. Biotechnology Advances 34(5): 687-696. https://doi.org/10.1016/j.biotechadv.2016.03.004

Rugini E, Di-Francesco G, Muganu M, Astolfi S and Caricato G. 1997. The effects of polyamines and hydrogen peroxide on root formation in olive and the role of polyamines as an early marker for rooting ability. In: Altman A, Waisel Y. (Eds.), Biology of Root Formation and Development, Plenum Press, New York, 65–73 pp.

Sarikhani H, Ghorbanizad H and Gholami M. 2017. Effect of carbon nanotubes in micropropagation of GF677 (*Prunus amygdalus* × *Prunus persica*) rootstock. Acta Horticulturae 1155: 245-250. https://doi.org/10.17660/ActaHortic.2017.1155.35

Sebastiani L and Tognetti R. 2004. Growing season and hydrogen peroxide effects on root induction and development in *Olea europaea* L. (cvs 'Frantoio' and 'Gentile di Larino') cuttings. Scientia Horticulturae 100: 75-82. https://doi.org/10.1016/j.scienta.2003.08.008

Shiozaki Sh, Makibuchi M and Ogata T. 2013. Indole-3-acetic acid, polyamines, and phenols in hardwood cuttings of recalcitrant-to-root wild grapes native to East Asia: *Vitis davidii and Vitis kiusiana.* Journal of Botany 2013: 819531. https://doi.org/10.1155/2013/819531

Shi-Weng L, Lingii X, Shijian X, Huyuan F and Lizhe A. 2009a. Hydrogen peroxide acts as a signal molecule in the adventitious root formation of mung bean seedlings. Environmental and Experimental Botany 65: 63–71. https://doi.org/10.1016/j.envexpbot.2008.06.004

Shi-Weng L, Lingii X, Shijian X, Huyuan F and Lizhe A. 2009b. IBA-induced changes in antioxidant enzymes during adventitious rooting in mung bean seedlings: The role of H_2O_2. Environmental and Experimental Botany 66: 442–450. https://doi.org/10.1016/j.envexpbot.2009.03.005

Silvestri C. Sabbatini G, Marangelli F, Rugini E, and Cristofori V. 2018. Micropropagation and *ex vitro* rooting of wolfberry. HortScience 53(10), 1494-1499. https://doi.org/10.21273/HORTSCI13423-18

Sivanandhan G, Mariashibu TS, Arun M, Rajesh M, Kashurirengan S, Selvaraj N and Ganapathi A. 2011. The effect of polyamines on the efficiency of multiplication and rooting of *Withania somnifera* (L.) Dunal and content of some withanolides in obtained plants. Acta Physiologiae Plantarum 33: 2279-2288. https://doi.org/10.1007/s11738-011-0768-y

Tang W and Newton RJ. 2005. Polyamines promote root elongation and growth by increasing root cell division in regenerated Virginia pine *(Pinus virginiana* Mill.*).* Plant Cell Reproductive 24(10): 581-589. https://doi.org/10.1007/s00299-005-0021-5

Tofanelli M, Rodrigues J and Ono E. 2003. Rooting of peach cv. Okinawa hardwood cuttings at different stem diameters, substrates, and pots. Ciencia Rural 33: 437- 442. https://doi.org/10.1590/S0103-84782003000300007

Tsipouridis C and Thomidis T. 2004. Rooting of GF677 (almond x peach hybrid) hardwood cuttings in relation to moisture content, oxygen concentration, temperature and pH of substrate. Australian Journal of Experimental Agriculture 44(8): 801– 805. https://doi.org/10.1071/ea03059

Tsipouridis C, Thomidis T and Michailidesm Z. 2005. Influence of some external factors on the rooting of GF677, peach and nectarine shoot hardwood cuttings. Australian Journal of Experimental Agriculture 45(1): 107–113. https://doi.org/10.1071/ea03120

Tworkoski T and Takeda F. 2007. Rooting response of shoot cuttings from three peach growth habits. Scientia Horticulturae

115(1): 98–100. https://doi.org/10.1016/j.scienta.2007.08.004

Ucler A and Parlak S. 2004. Effects of IBA and cuttings dates on the rooting ability of semi-hardwood kiwifruit cuttings. Turkish Journal of Agriculture and Forestry 28(1): 195-201.

Vondrakova Z, Eliasovak M, Martincova O and Cvikrova M. 2015. Exogenous putrescine affects endogenous polyamine levels and the development of *Picea abies* somatic embryos. Plant Growth Regulation 75: 405–414. https://doi.org/10.1007/s10725-014-0001-2

Wetzstein HY, Porter JA, Janick J, Ferreira JFS and Mutui TM. 2018. Selection and clonal propagation of high artemisinin genotypes of *Artemisia annua*. Frontiers in Plant Science 9: 358. https://doi.org/10.3389/fpls.2018.00358

Wu QS, Zou YN and He XH. 2010. Exogenous putrescine, not spermine or spermidine, enhances root mycorrhizal development and plant growth of trifoliate orange *(Poncirus trifoliata)* seedlings. International Journal of Agriculture and Biology 12(4): 576– 580.

Zhou L, Li S, Huang P, Lin S, Addo-Danso ShD, Ma z and Ding G. 2018. Effects of leaf age and exogenous hormones on callus initiation, rooting formation, bud germination, and plantlet formation in Chinese fir leaf cuttings. Forests 9(8): 478. https://doi.org/10.3390/f9080478