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THE EFFECT OF PLANT GROWTH REGULATORS ON CALLUS INDUCTION AND REGENERATION OF GF677 ROOTSTOCK

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ABSTRACT
GF677 is one of the most suitable rootstocks for almond and peach used in calcareous soils to overcome lime-induced chlorosis. This hybrid produces strong roots and has a good potential for pests and diseases. Propagation by tissue culture technique is the most important one in woody plants. It is very important and necessary to develop a micro propagation system for GF677 rootstock. In the current research, *in vitro* optimization of tissue culture and mass production of almond was investigated. In this idea, explants of actively growing shoots were collected and sterilized, then transferred to MS medium with different concentrations and combinations of plant growth regulators. The Experiment was done in completely randomized blocks design, with 7 treatment and 30 replications. After 4 weeks, calli induction, proliferation, shoot length and number of shoot per explants were measured. Results showed the best medium for shoot initiation and proliferation was MS + 0.01 mg/l IBA (Indol-3- Butyric Acid) +2 mg/l BA (Benzyl Adenine). Autumn was the best season for collecting explants. The shoots were transferred to root induction medium with different concentration of plant growth regulators. The best root induction medium was MS+0.5 mg/l IBA (Indol Butyric Acid).

KEY WORDS: *In vitro*, GF677 rootstock, Micro propagation, Tissue culture, Shoot proliferation.

INTRODUCTION

GF677 rootstocks an interspecific hybrid (Peach × Almond) is propagated asexually as clone. It is specially used on alkaline soils because of its resistance to lime induced iron chlorosis (El Gharabi & Jadiri, 1994). This hybrid can grow well on soils with low fertility status (Charrera *et al*., 1998). It is difficult to be multiplied on mass scale through cuttings because of very low rooting ability (Ammer, 1999). The first report about micropropagation of this rootstock was obtained by Kester (1970) and Tabachnik and Kester (1977). Then Fasolo *et al*.(1970) could root explants in the soil.Jona and Gribado (1990) successfully measured the amount of ethylene in the medium and final research was accomplished by Dimassi-Theriou (1995) for rooting of GF677. He compared different culture media and results on the rooting of these rootstocks depend on the type of medium culture (Dimassi-Theriou, 1995). Thus, the plant tissue culture techniques are more valuable for the clonal propagation of GF677. Therefore, the aim of this study was to determine the conditions needed to optimize micropropagation methods for GF677 rootstock from buds explants by *in vitro* culture. Yet, there have been no useful protocol on optimization of propagation of GF677 rootstock through tissue culture.

MATERIAL AND METHODS

Plant materials

Actively growing shoots of the GF677 rootstock were collected in four seasons from trees growing at the Agricultural and Natural Resource Research Center of Razavi Khorasan in Iran. After removing the leaves, the shoots were cut into segments 1-2cm in length, and then washed with running water for 1 hour. After that, the scions were surfaced sterilized in 0.02% (w/v) mercuric chloride for 3 min, and then rinsed with 70% ethanol for 2min. Later, the segments were sterilized by immersion in a 30% (w/v) calcium hypochlorite solution, containing 0.05% (v/v) Tween 20 for 15 min; finally they were rinsed four times with sterile distilled water.

Micropropagation

Shoot tips with 0.5 to 1cm long containing a single bud were established in tubes containing 20 ml of Murashig and Skoog (1962) medium (MS), supplemented with 30 gl^{-1} sucrose,7 gl^{-1} agar (Agar-Agar, Sigma) and different concentrations and types of plant growth regulators (PGRs) (Tab. 3).The pH was adjusted to 5.8 prior to autoclaving at 120 ºC for 20 min. The culture were maintained at 25±2 ºC with 16 h photoperiod (35 µmol $m^{-2}s^{-1}$), provided by white fluorescent lamps. Proliferating axillaries shoots were sub cultured once every 3 week. In the second stage, proliferating shoots were separated, and transferred to a medium containing the same composition. The MS medium without PGRs was used as control. Each treatment was replicated 30 times. Shoot induction, number of shoot per explants, length of shoot per explants, callusing and rate of infection were reported.

Rooting treatment

Four weeks old micro propagated shoot were transferred to 20 ml tubes containing a basal medium of MS with different type and concentration of plant growth regulators (Tab. 1). The MS medium without PGRs served as a control. Each treatment was replicates for 10 times. Rate of root induction, number of shoots and length of roots were records after 4 weeks.

Hardening off

Plantlets with roots' length between 0.5 and 1 cm length were selected for establishing in green house. Explants were removed from culture and the roots were gently washed in distilled water to remove any residual medium. Then, shoots were potted in a 1:2:1 mixture of perlite, sand and soil, respectively. Plantlets were covered with clear borosilicate beaker to maintain a 90±5% relative humidity, for 4 weeks before transferring into the growth room. Relative humidity was slowly decreased by gradually removing beakers. Plantlets were acclimatized after 3 weeks in a green house at 25±2 ºC under natural daylight conditions.

Experimental design and statistical analysis

A completely randomized blocks design was used for the study with data from each experiment being analyzed separately. In micropropagation the treatments were replicated 30 times, and in rooting induction, treatments were replicated 10 times, with each replicate comprising one explant.

The Duncan's test was adjusted at p= 0.01 probability level to separate mean differences when significant treatment effects were detected.

TABLE 1. Composition of PGRs used during rooting experiment.

Treatment Codes	Medium	Composition
E	MS	(1 mg/l) IBA
A	MS	(0/5 mg/l) NAA
N	MS	(0/5 mg/l) IAA + (1 mg/l) BA
B	MS	(1 mg/l) NAA
L	MS	(0/5 mg/l) NAA + (0/5 mg/l) IBA
D	MS	(0/5 mg/l) IBA
T	MS	(0/5 mg/l) IAA
Control	MS	_____

RESULTS AND DISCUSSION

The best multiple shoot initiation was obtained on the MS medium supplemented with BA at 2 mgl^{-1} and IBA at 0.01 mgl^{-1} (Tab. 1 and 3) with a shoot number of 3.15 per explants, on the 4^{th} week of culture (Tab. 3). The higher length of shoots, with a shoot length 1.21cm and the most callus initiation with a 9.5 were observed on MS medium supplemented with the same PGRs as the above (Tab. 3). Conclusions show that BA treatments caused better shoot proliferation and better shoot multiplication (Fig. 1, 2, 3). The results indicated that between the different seasons, autumn was the best time for collecting explants from trees on the field (Tab. 4). There were significant differences between rate of contaminations and date of collecting the explants (Table 2). The best root formations were observed in MS medium supplemented with 0.5 mg l^{-1} IBA (Tab. 6). The maximum number of root induction was 7, the mean value for roots per seedling was 1.92 and for root length was 2.8 cm data obtained on MS medium supplemented with 0.5 mg l^{-1} and 1.0 mg l^{-1} IBA, for the first two (treatments D and E, respectively) and with MS medium + 0/01 mgl^{-1} IBA+2.0 mgl^{-1} BA for the last expriment (Tab. 3). No root development was observed in control groups. The present results are in agreement with Tabachnik and Kester (1977) observations. They reported that the use of BA in shoot multiplication was absolutely necessary.

Hisajima (1982) reported that the best result for proliferation of the almond was obtains from MS medium supplemented with 0.2 mgl^{-1} BA + 0.005 mgl^{-1} IBA. Isikalan *et al*. (2008) determined that the best multiple shoot initiation for almond was obtained on the MS medium supplemented with BA 2 mgl^{-1}, with a shoot number of 5.7±1.04 mgl^{-1} per explants. Increasing BA concentration from 1mgl^{-1} to 3mgl^{-1} may significantly reduce the length of shoots, as observed by Shekafandeh and Khush-khui (2008) in guava (*Psidium guajava*, Myrtaceae). Ahmad *et al*.(2004) reported that IBA is the preferable auxin for *in vitro* rooting of peach rootstock GF677 and the maximum number of root and root length were obtained when applying 0.4 mgl^{-1} IBA. Besides plant grow regulation factors, the year's seasons have been observed to influence in different degrees the micropropagation of temperate cultures. Shahrzad and Emam (2000), for instance, reported that summer and the early autumn were the best time for collecting *Populous euphratica* explants in order to shoot proliferation. Nasiri (2000) show that mid autumn is the best time for taking explants from *Olea europaea* trees.

The present experiments demonstrated that shoot multiplication could be achieved on MS medium supplemented with 2 mgl^{-1} BA and 0.01 mgl^{-1} IBA. BA treatments resulted in a better elongation of GF677 rootstock scion. However, MS medium with 0.5 mgl^{-1} IBA was also effective for root induction and root elongation. After 3 weeks rooted shoots of GF677 rootstock acclimatized and then were successfully transferred to natural condition. Channuntapipat *et al*.(2003) showed that the maximum rooting of shoots for some almond hybrid rootstocks occurred on half strength MS medium with 2.4 mgl^{-1} IBA. Ainsley *et al*. (2001) determined that IBA and NAA are the most suitable auxin for rooting seedlings of 'Ne plus ultra' and 'Non pariel' almond cultivars *in vitro* conditions. The type and concentration of auxin during rooting period strongly influenced the quality of root system during rooting period. The application of NAA resulted in poor rooting of almond shoots. This might be explained by the NAA resistance to degradation by the auxin-oxidase enzyme (Smulders *et al*.,1990). Nissen and Sutter (1996) have shown that, in tissue culture media IAA is photo-oxidized rapidly (50% in 24h) while the IBA oxidized slowly (10%), while NAA is very stable.

CONCLUSION

The results obtained in the present research can be used as guidelines for improving propagation of GF677 as the most suitable rootstock for almond and peach. In addition, the result demonstrated which is the optimized stage for root induction. Since in micropropagation rooting of micro cutting is often a challenging step, losses at this stage have vast economic consequences. In conventional propagation via cuttings many woody plants are also recalcitrant to root. Thus, a research on root formation is highly important from the practical point of view. It can be conclude that proliferation and multiplication of GF677 rootstock by tissue culture methods is a fast, economic and valuable.

TABLE 2. Summary of treatment interaction as determined analysis of variation for shoot proliferation.

Source of variation	df	Rate of contamination	Callus initiation	Shoot length (cm)	Shoot number	Shoot induction
Date of collecting explants	3	107753/96**	0/86**	1/44**	8/95**	1.14**
PGRs treatment	6	554/56**	1/10**	1/78**	12/89**	1.05**
Date of collecting PGRs × explants treatment	18	149/80ns	0/07ns	0/14ns	0/97ns	0/06ns
Error	812	2032/84	0/14	0/11	0/78	0/11

*$p \leq 0.05$; **$p \leq 0.01$; **ns** no significant

TABLE 3. Effect of different PGRs treatment on shoot proliferation.

Treatment codes	Composition of PGRs treatment	Mean rate contamination	Mean callusing	Mean shoot length (cm)	Mean shoot number	Mean Shoot induction
H	IBA(0/01mg/l)+BA(2 mg/l)	42/91 a	9/5 a	1/21 a	3/15 a	9 a
N	BA(1mg/l)+IAA(0/5m g/l)	42/91 a	7 ab	0/77 cb	2/57 b	6/25 b
J	TDZ(4mg/l)	39/58 a	5/75 b	0/84 b	1/93 c	6 bc
K	IBA(0/01mg/l)+BAP(1 /27mg/l)	37/50 a	5/5 b	0/6 cd	1/52 cd	4/5 bc
F	IBA(0/01mg/l)+BAP(0 /68mg/l)	37/91 a	5/75 b	0/73 cb	1/57 cd	4/5 bc
C	IAA(0/1mg/l)+BA(1/5 mg/l)	40/41 a	4/75 b	0/75 cb	1/67 cd	3/8 c
Control	_____	40/41 a	0.0 d	0.0 d	0.0 d	0.0d

- Values with the same letters in the same column are not significantly different ($p \leq 0.01$) according to Duncan's test.

FIG. 3. Seedlings on MS medium with IBA (0.5 mgl^{-1})

TABLE 4. Effect of different date of collecting explants on shoot proliferation

Date of collecting explants	Mean rate contamination	Mean callusing	Mean shoot length (cm)	Mean shoot number	Mean Shoot induction
May	b36/19	ab 7	b0/81	b2/43	b5/83
August	b39/76	cb 5/6	c0/72	c1/95	bc4
November	c15	a9/3	a0/98	a3/57	a8/5
February	a70	c3/8	c0/67	c1/90	c3/75

- Values with the same letters in the same column are not significantly different (p=0.01) according to Duncan's test.

TABLE 5.Summary of treatment determined analysis of variance for shoot proliferation.

Source of variation	df	Root length(cm)	Root number	Root induction
Treatment	8	2/103**	3/07**	0/9**
Error	81	0/239	0/362	0/128

*$p\leq0.05$; **$p\leq0.01$

TABLE 6. Effect of different PGRs treatment on rooting stage

Treatment	Control	T	N	L	B	A	E	D
Mean root induction	0c	0c	1cb	2b	3b	3b	6a	7a
Mean root number	0c	0c	1/2b	1/2b	1/37ab	1/3b	1/92a	2/11a
Mean root length(cm)	0c	0c	2/8a	0/85b	0/79b	0/93b	1/2a	1/7a

- Values with the same letters in the same column are not significantly different (p=0.01) according to Duncan's test.

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REFERENCES

Ahmad, T, Ur-Rahman, H, Laghari, MH (2004) Effect of different auxin on *in vitro* rooting of peach rootstock GF_{677}. Sarhad Journal of Agriculture, 20(3): 373-375.

Ainsley, PJ, Collins, GG, Sedgley, M (2001) *In vitro* rooting of almonds (*Prunus dulcis* Mill), *In vitro* Cellular and Developmental Biology-plant, 37(6)778-785.

Ammer, M. (1999) Performance of Hansen, GF655 and GF677 peach rootstocks for rooting with the use of IBA under greenhouse condition. M. Sc. Thesis, Univ. Arid Agri. Rawalpindi, Pakistan.65 pp.

Channunapipat, C, Sedgley, M, Collins, G (2003) Micropropagation of almond cultivars "Nonpareil" and "Ne plus ultra" and the hybrid rootstock , "Titan × Nemgard". Science Horticulture, 98:473-484.

Charrera, M., G.A. Parasi and R. Monet (1998) Rootstock influence on the performance of the peach variety "*Catherine".Acta Hort.,* 465: 573-577.

Dimassi–Theriou, K. (1995) *In Vitro* rooting of roostock GF-677 (*Prunus amygdalus*×*Prunus persica*) as influenced of culture–tube sealing material. *J. Hort .Sci*.70:105-108.

El.Ghardi ,A. and Jraidi, B. (1994) Performance of rootstocks of almond, peach and peach × almond hybrids with regard to iron chlorosis. Acta Hort., 373:91-97.

Fasolo, F., Malavasi, F. and Ranieri, R. (1987) Preliminary investigation on *in vivo* rooting of micropropagation of GF677, peach rootstock. Acta Horticulturae, 212: 181-287.

Hisajima, S (1982) Multiple shoot formation from almond seeds and an excised single shoot. Agricultural Biology and Chemistry, 46:1091-1093.

Isikalan, C, Adiyaman Akbas, F, Namli, S, Tikat, E, Basaran, D (2008) *In vitro* micropropagation of almond (*Amygdalus communis* L.CV Nonpareil). African Journal of Biotechnology, 7(12):1875-1880.

Kester, D.E. (1970) Growth *in vitro* of tissue of almond, almond hybrids and some other prunus.Hort.Science, 5(4): 349.

Murashige, T, Skoog, F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plant, 15:473-497.

Nasiri, M (2000) Micropropagation of olive (*Olea europeae*). Iranian Rangeland and Forest Plant Breeding and Genetic Research Journal. 230:37-79.

Nissen, SJ, Sutter, EG (1990) Stability of IAA and IBA in nutrient medium of several tissue culture procedures.Hort. Sci. 800-802.

Shahrzad, SH, Emam, M (2000) Micropropagation of *populus euphratica* using tissue culture. Iranian Rangeland and Forest Plant Breeding and Genetic Research Journal. 230:11-36.

Shekafandeh, A, Khush-Khui, M (2008) Effect of bud position and culture medium on prolifiratin from nodal culture of two guava cultivars. Asian Journal of Plant Science, 7:177-182.

Smulders, MJM, Van Devan, ETWM, Croes, AF, Wullems, GJ (1990) Metabolism of 1-naphthaleneacetic acid in explants of *tobacco*: evidence for release of free hormone from conjugates. Journal of Plant Growth Regulators, 9:27-34.

Tabachnik, L, Kester, DE (1977) Shoot culture for almond and almond –peach hybride clones *in vitro*. Horticultural Science, 12:545-547.