

Effects of Myo-Inositol and Thiamine on Micropropagation of GF677 (Peach × Almond Hybrid)

Sadegh Sepahvand (Corresponding author)

Graduate student, Azad University of Tehran, Science and Research Branch, Tehran, Iran

Tel: 98-912-683-1371 E-mail: Sa.sepah@gmail.com

Ali Ebadi

Professor, University College of Agriculture & Natural Resources of Tehran, Karaj, Iran

Tel: 98-912-567-7521 E-mail: Ebadi@ut.ac.ir

Kazem Kamali

Assistant Professor, Department of Environmental Engineering

Faculty of Natural Resources and Desert Studies, Yazd University, Yazd, Iran

Tel: 98-913-352-5822 E-mail: kamali100in@yahoo.com

Saeid Ali Ghaemmaghami

Instructor, Agricultural Institute, Iranian Research Organization for Science & Technology (IROST)

Tehran, Iran

Tel: 98-912-148-8705 E-mail: Ghaemmaghami@irost.org

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Abstract

GF677 is a vigorous rootstock, used for both peach and almond worldwide. Nowadays, it is propagated is mainly through micro propagation. Widening and undesirable growth of leaves as well as poor rooting are major problems during its in vitro culture. In this study, effects of myo-inositol at 10 and 100 mg/l In MS medium were studied at explants establishment and shoot growth stages. Thiamine was also used in LS medium at 0, 1.6, 2.8 and 4 mg/l at rooting stage. This experiment was conducted in Randomized Complete Design with 10 replications. Myo-inositol at 10mg/l could prevent undesirable widening of leaves as well as rosetting of shoots. Thiamine at 1.6 mg/l was highly effective in rooting of shoots.

Keywords: Myo-Inositol, Thiamine, In vitro propagation, Leaf widening, Rooting, GF677

1. Introduction

Gf677 is a major rootstock for both peach and almond, widely used in many countries. It was discovered in 1940 (Stylianidas et al., 1988). At first, it was noticed due to its tolerance to calcareous soils and later by developing tissue culture techniques, it became a dominant rootstock for peach in France (Rom and Carlson, 1987). Gf677 is a vigorous rootstock (Loreti et al., 1985; Maragoni et al., 1985), which is tolerant to chlorosis induced by high soil lime (Syrgiannidis, 1985; Bindra, 1975). Grafted tree on this rootstock is vigorous and productive (Maragoni et al., 1985; Minguzzi, 1989). GF677 is also tolerant to drought and has moderate resistance to some diseases (Rom and Carlson, 1987). Furthermore, it is compatible with most almond and peach cultivars (Grassely, 1987). Sexual propagation of this rootstock resulted in segregation and producing of non-uniform seedlings. To prevent this problem, it is propagated by asexual methods (Rom and Carlson, 1987).

Asexual propagation of GF677 is difficult and its micropropagation is encountered by vitrification, shoot rosetting and low rooting (Tsipouridis et al., 2003). Miller et al. (1982) used different media with and without vitamin in the rooting stage. Results showed that vitamin had significant effect on rooting ability of shoots. Effects of myo-inositol and cytokinin on rooting of GF677 explants were studied. Results showed that myo-inositol accelerates BA transport to different parts of plant. Kas et al. (2004) compared MS and ½ MS for in vitro propagation of GF677 and concluded that ½ MS medium was more convenient and prevented abnormal shoot growth which was observed in MS medium. Tsipouridis et al. (2003) compared different media for in vitro propagation of GF677 and eventually introduced ½ MS and Knop as more suitable media compared to MS medium.

In this study, effects of myo-inositol were studied on prevention of rosetting and leaf expansion during explants establishment, shoot proliferation and shoot growth as well as the effect of thiamine on rooting.

2. Methods and Materials

2.1 Plant materials

Explants were collected at mid May 2010. Shoots with length of 15-20 cm were cut off and transferred to laboratory. Shoots were washed with tap water, and then were cut off to pieces of three cm length, each including one bud. Explants were sterilized with 0.1 % mercury chloride for 3.5min. Later, they were washed twice with double distilled water containing citric acid 700 mg/l (Fig. 1).

2.2 Growth conditions

Explants were cultured in different media and then grown in growth room with light intensity of 2500-3000 lux, photoperiod of 16/8 hours light/dark, relative humidity of 45 % and constant temperature of 25±1 °C. McCarthy glasses were used for the establishment stage of explants.

MS and modified MS (with 10 mg/l myo-inositol) containing 0.1 mg/l BA, 7 g/l agar and 30 g/l sucrose (pH was adjusted on 5.7) were used.

Subculture was carried out after one month, and then explants were transferred to proliferation medium. After proliferation, small shoots were transferred to MS media containing 10 or 100 mg/l myo-inositol as well as agar 7 mg/l, sucrose 30 g/l and BA 0.1 mg/l (pH was adjusted on 5.7), in which shoot growth was completed within three weeks. Shoots with 5-6 cm length were transferred to LS rooting media (Fig. 2), in which, IBA was used at concentrations of 0, 1, 1.3, 1.6 and 1.9 mg/l, thiamine at concentrations of 0, 1.6, 2.8 and 4 mg/l, Fe-EDDHA at 100 mg/l, agar at 7 g/l and sucrose at 30 g/l (pH was adjusted on 5.7). Cultures were kept in dark for seven days and then transferred to light (2000-2500 lux). During rooting period, temperature was set at 23±1 °C. Rooting was initiated after eight days and completed after six weeks. Rooting percentage, root number and root length were recorded. This experiment was conducted as factorial in Randomized complete Design with 20 replications. Data was analyzed with SAS software.

3. Results and Discussion

3.1 Effect of myo-inositol on shoot growth

Different media have different effects on shoot growth due to their different concentrations of nutrients. At establishment stage, there is a problem in which leaves get large and wide due to high carbon sources in MS medium, which ends in dwarf and rosette shoots. In this work, decreasing the level of myo-inositol from 100 mg/l to 10 mg/l resulted in normal shoot growth. Mean comparison showed that the highest shoot length (4.6 cm) was obtained in modified MS with only 10 mg/l myo-inositol (Fig. 3). This treatment increased shoot growth, leaving no sign of rosetting (Fig. 4).

3.2 Effect of thiamine on rooting

Thiamine and IBA had significant effects on rooting percentage in such a way that even lower concentrations of thiamine caused increment of rooting in all IBA levels. Rooting percentage was also influenced by interaction between thiamine and IBA (Table 1). The lowest percent of rooting occurred in LS medium without thiamine. However, the highest rooting percentage were obtained in LS medium with thiamine at 1.6 mg/l, IBA at 1, 1.3 and 1.6 mg/l or thiamine at 2.8 mg/l, IBA at 1.3 and 1.6 mg/l (Fig. 5).

Number of roots/shoot was also affected by thiamine, IBA and their interaction. Thiamine at 1.6 and 2.8 mg/l produced the highest number of roots. However, its application at 4 mg/l or even control had the lowest number of roots. IBA at 1.3 or 1.6 mg/l both produced the highest number of roots. The lowest number of roots was obtained in control.

Length of roots was also affected by thiamine in which application of thiamine at 1.6 mg/l produced the longest root and control had the shortest root. IBA at 1.3 and 1.6 mg/l produced the longest roots, but its application at 1 and 1.9 mg/l significantly resulted in shorter roots. The highest root length was observed in treatment with no IBA application and lowest root length were obtained with IBA application at 1.9 mg/l, indicating the negative effect of IBA on root length.

Application of thiamine in LS medium had a great effect on root number and length, but increasing its amount to 4 mg/l had reductive effect on root number and there were no significant effect between thiamine at 4 mg/l and control. Thiamine as an enzyme cofactor has important effect on metabolically reactions such as glycolysis or in pentose phosphate and tricarboxylic acid cycle. In addition to thiamine value as a nutritional compound, it is also secondary messenger in activation of proteins with low molecular weight. Thiamine also causes increment in expression of genes involved in producing defective enzymes which increase plant resistance to plant pathogens (Hwanlee and Soonok, 2005). Interaction between IBA and thiamine (Fig. 6) showed that in LS medium the highest root number was obtained with thiamine at 1.6 mg/l and IBA at 1.3 mg/l. IBA at 1.9 mg/l together with different levels of thiamine had reductive effect on root number. With no application of thiamine, root number/shoot correlated with IBA levels and highest root number was obtained with IBA at 1.9 mg/l. Thiamine and IBA interaction showed that the highest root length belonged to control, but its lowest amount resulted with IBA at 1.9 mg/l and with no thiamine (Fig. 7).

Results of this experiment showed that myo-inositol application at 10 mg/l could be a solution to shoot rosetting and abnormal largening of leaves. In addition, application of thiamine at 1.6 mg/l and IBA at 1.3 mg/l resulted in the highest rooting of GF677 in vitro conditions. Therefore, the modified MS medium was best for establishment of GF677 explants and also for normal shoot growing. However, for rooting, LS medium with 1.6 mg/l thiamine and 1.3 mg/l IBA were proved to be best options.

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Table 1. The Analysis of variance of rooting percentage, root number and root length under effect of thiamine and IBA application

Source of Variation	df	Mean Squares		
		Root percentage	Root number	Root length
Thiamine	3	3.830**	71.424**	67.697**
IBA	4	5.1663**	88.725**	108.424**
Thiamine × IBA	12	0.4112**	13.711**	5.339**
Error	380	0.1255	3.526	0.7462

** : significant difference at %1 probability level.



Figure 1. Establishment of GF677 explants



Figure 2. GF677 shoots at rooting medium

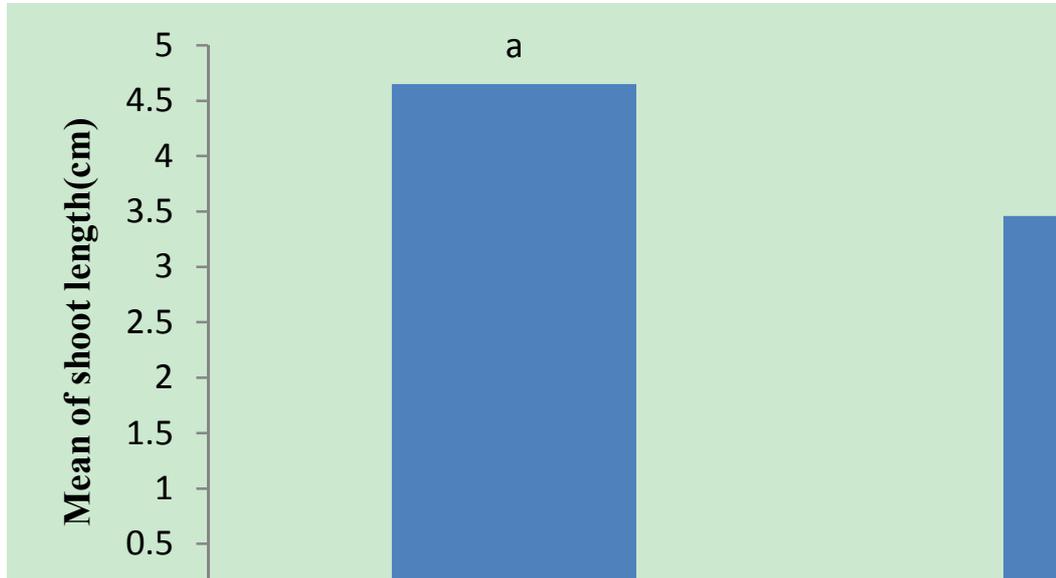


Figure 3. The Effect of myo-inositol on shoot growth of GF677.



Figure 4. The Effect of myo-inositol on shoot length

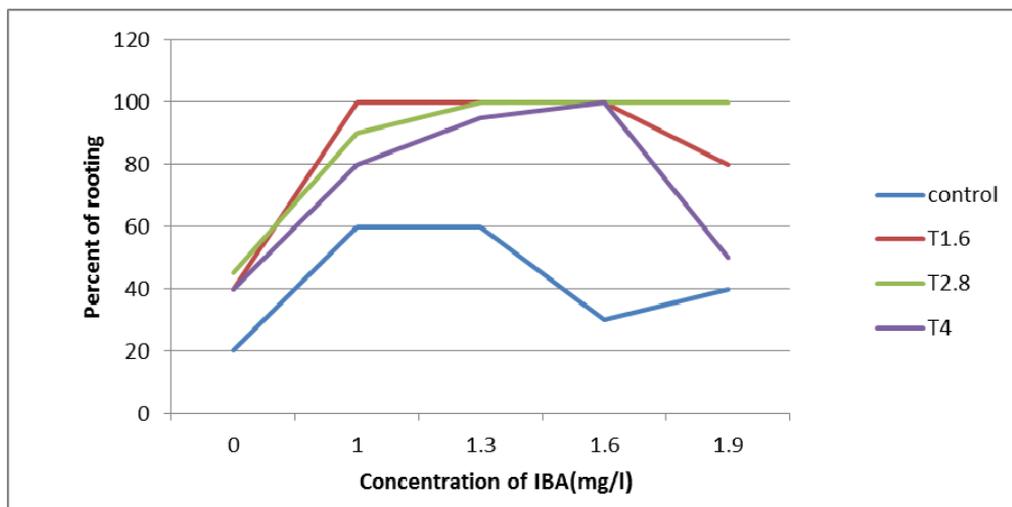


Figure 5. The Effect of different levels of thiamine and IBA on percent of rooting

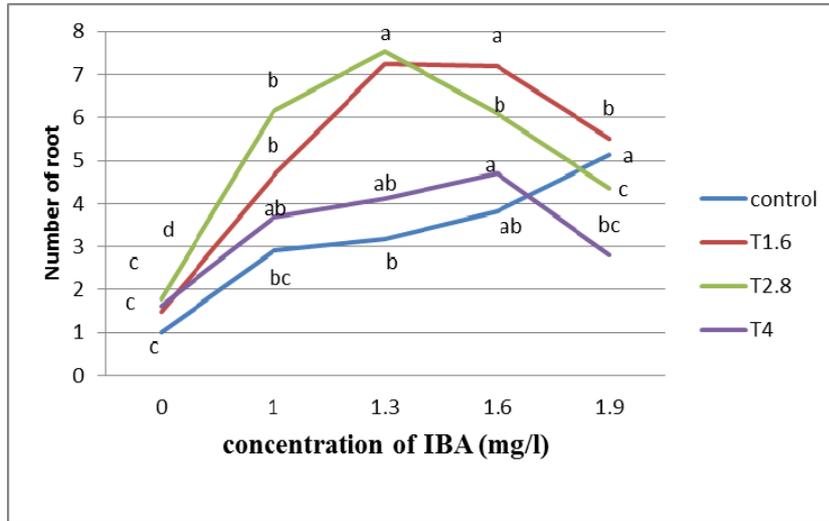


Figure 6. The Effect of thiamine and IBA on root number of GF677

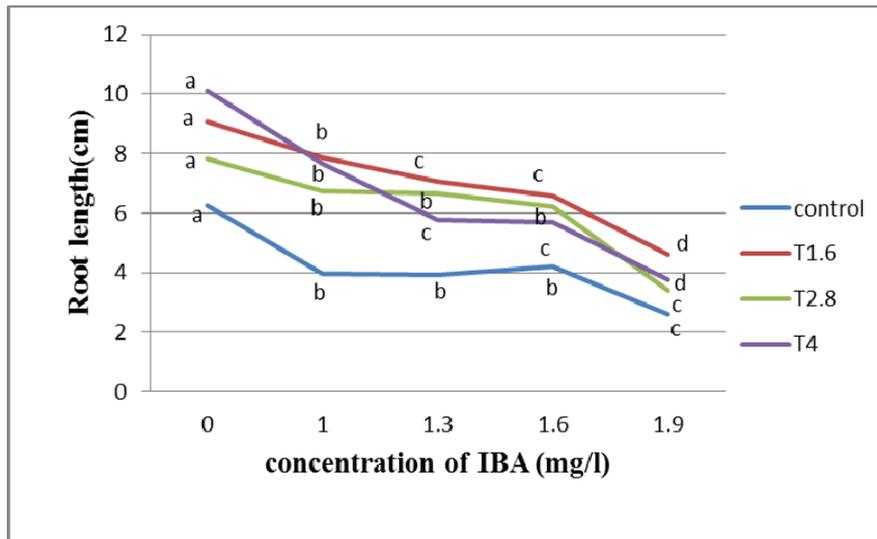


Figure 7. The Effect of thiamine and IBA on root length of GF677