Micropropagation of GF 677 Rootstock

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Abstract

The aim of this investigation was to study micropropagation of Almond GF rootstock on *in vitro*. Explants were cultured in MS medium containing 5 concentrations of 6- Benzylaminoporine (BAP) 0.1, 0.6, 1, 1.5 and 2 mg 1^{-1} along with control treatment. Each treatment includes 5 replications. After 5 weeks of culture, the results showed that the number and length of shoots in treatment containing 1 mg 1^{-1} BAP was more than the other treatments and control. The number of shoot that was obtained in treatment of 1 mg 1^{-1} BAP was 2.98 (Table 1). The lowest number of shoot was 0.4 due to control treatment. For rooting, explants with 3-5 cm length were transferred to different rooting treatments (Table 2). The results revealed that the best rooting was obtained with treatment 3 mg 1^{-1} IBA. Number and length of roots were 2.167 and 11.53, respectively. Also results showed that putting explants in dark for 1 week is more effective than light.

Keywords: GF 677 rootstock, Micropropagation, 6-Benzylaminoporine, Tissue culture

1. Introduction

GF is a hybrid of *Prunus amygdalus* × *P. persica* and is the most commonly used rootstock for peach orchards (Antonopoulou et al., 2007). This rootstock is tolerant to Fe deficiency and specially suited to soils with poor fertility, low water availability and high CaCo3 content (Monticelli et al., 2000). It is propagated with vegetative methods (cutting and tissue culture). Due to showing a varying rooting percentage from year to year and low efficiency of cutting, tissue culture is a good and fast method for propagation of wealthy and disease-free plants of GF 667 in large quantity (Dimassi et al., 1995). Hence, a lot of researches about micropropagation of GF rootstock have been conducted (Antonopoulou et al., 2005). The first investigation about GF micropropagation was carried out by Kester (1970) and Tabachnik and Kester (Tabachnik and Kester, 1977). One of the main factor on micropropagation is hormone specially BAP. Furthermore, shoot branching depends on the initiation and activity of axillary meristems, which are hormonally controlled by cytokinin (Dobranszki and silva, 2010). The cytokinin BAP promotes cell division, shoot multiplication and axillary bud formation while inhibiting root development (Sutter, 1996). In most work on shoot multiplication of GF almond rootstock, BA was used as the cytokinin source mainly in a concentration range between 0.1 and 3 mg 1^{-1} (Kamali et al., 1990; Ahmad et al., 2003). It has been reported that 0.6 mg 1^{-1} BAP produced the highest number of shoot (Ahmad et al., 2003). In the rooting stage, the induction of roots on explants from in vitro culture is crucial part in any micropropagation process (Molassiotis et al., 2003/4). The ability of plant tissue to form adventitious roots depends on interaction of many exogenous and endogenous factors, including hormone. Most reports of adventitious root induction of woody species have involved treatments with exogenous auxins such as IBA, NAA or IAA (Ainsley et al., 2001). Exogenous auxins are only required at an early stage to stimulate emergence of new formed roots (Dobranszki and silva, 2010). It has also been reported the best hormone for rooting was IBA (0.5 mg 1^{-1}) (Vaez-Livari et al., 2005). Except hormone, other factors that can affect adventitious rooting are included photoperiod, light intensity and light quality (Rugini et al., 1993). Darkness during the last week of the rooting phase has been shown to be necessary in stimulating rooting in some woody species (Rugini et al., 1993). The positive effects of Darkness have also been reported on root number, root length (Vaez-Livari et al., 2005).

The objectives of this investigation are included study of a) the effect of Benzylaminoporine on micropropagation of GF rootstock b) the effect of different rooting-hormones on rooting of GF rootstock on *in vitro* condition c) the effect of Darkness on rooting of GF rootstock on *in vitro* condition.

2. Materials and Methods

2.1 Plant Material Sterilization

Plant explants were collected from controlled plants in greenhouse of faculty of agriculture in winter of 2010. At first, shoots were excised into 3 cm-long sections, and then for surface disinfection, they were agitated for 5 min in a solution containing 5 drops of Tween-20 in 100 ml of water, finally they were washed under running water for 1 hr. For sterilization, firstly explants were agitated in alcohol 70% for 30 sec, then in %0.01 solution of mercuric chloride for 7 min and finally they were rinsed three times with distilled water.

2.2 Basal Medium and Culture Conditions

MS (murashige and SKoog, 1962) medium containing 5 concentrations of BAP along control treatment were tested (Table 1). The media were supplemented with 30 g l^{-1} sucrose, 8 g l^{-1} agar and 50 mg 1^{-1} citric acid. The pH of media was adjusted to 5/8 before autoclaving. The explants were maintained at 25± 1 ºc and 16/8 hr photoperiod for 4 weeks. Data were recorded after 5 weeks.

For rooting, 5 cm-long shoots from previous culture were transferred to 1/2 MS medium containing 200 mg 1^{-1} Fe-EDDHA, 6 g l^{-1} agar. Treatments are mentioned in Table 2 and 3. After culturing shoots in different treatments, jars containing shoots were divided into two groups. Half of each treatment was maintained in the light and the other half was maintained in the dark for 1 week. The pH of media was adjusted to 5/8 before autoclaving. The explants were maintained at 25± 1 ºc. Data include percentage of rooting, length and number of roots were recorded after 1 month.

2.3 Data Analysis

The experiment was carried out based on completely randomized design (CRD) with 5 replications per treatment. Statistical analysis of the data was carried out by using SPSS 16 software and difference among treatment means were compared by using Least significance difference Test (LSD) (Steel et al., 1997).

For rooting, the experiment was carried out based on factorial. Statistical analysis of the data was carried out by using SPSS 16 software and difference among treatment means were compared by using Least significance difference Test (LSD) (Steel et al., 1997).

3. Results

3.1 Number of Shoots per Explant

Based from the results obtained (Table 1), there are significant differences among different treatments. Treatment containing 1 mg 1^{-1} proved to be the best treatment so that The highest number of shoot was observed in this treatment(Fig 1). On average, number of shoot in this treatment was 2.98. The lowest number of shoot was 0.4 which observed in control treatment.

3.2 Length of Shoots

According to figure 2, there are significant differences between BAP treatments. Maximum shoot length was seen in treatment containing 1 mg 1^{-1} BAP which proved to be the best treatment. An average length of 2.54 cm was obtained in this treatment. Minimum length of shoots which was 0.38 cm was due to control treatment.

3.3 Percentage of Rooting

Statistical analysis showed that there were significant differences between different treatments (Fig 3). The best results were obtained with 3 mg 1^{-1} so that percentage of rooting was 44%. The percentage of rooting in treatments number 1 and 3 were 11 and 16.5% respectively.

Statistical analysis also showed that there was no significant difference between Percentage of rooting under dark and light condition.

3.4 Number of Roots

From figure 4, it was found that there are significant differences between numbers of roots in different hormones treatments (Fig 4). Maximum number of roots was observed on treatment number 2 containing 3 mg 1^{-1} IBA, so that the number of roots was 2.167. The number of roots in treatment number 1 and 3 was 0.833 and 0.667, respectively. Dark had better effect on number of root (Fig 5). The number of root under dark condition was 1.667 compared with 0.778 in light condition.

3.5 Length of Roots

As Statistical analysis showed there are significant differences between lengths of roots in different treatments (Fig 6). Maximum root length was observed on medium containing 3 mg 1^{-1} IBA (treatment number 2). An average length of 11.35 cm was achieved in treatment number 2 (Table 2). Length of Roots in treatments number 1 and 3 were 3.5 and 5.33 cm respectively. In terms of the effect of dark and light on the length of root, Statistical analysis demonstrated that dark had better effect on length root (Fig 7). On average, the Length of roots under dark condition was 10.22 cm. by contrast; the average Length of roots under light condition was 3.36 cm (Table 3).

4. Discussion

Cytokinin stimulates the initiation and activity of axillary meristems which result in Shoot formation (Dobranszki and silva 2010). cell division, shoot multiplication and axillary bud formation can be promoted by The cytokinin BAP (Sutter 1996). The influence of cytokinins on tissue or organ cultures can be differed based on the kind of culture, the variety of plant and the age of explant (Thorpe et al., 2008). It is also reported that BAP is required at low concentrations ranging from 0.5 to 2.5 mg 1^{-1} (Thorpe et al., 2008). This study showed that number of shoot was increased as concentration of BAP increased to certain amount. As concentration of BAP increased to 1, the number of shoot also increased by 2.54 per explant from 1.93 shoot at concentration of 0.1 mg 1^{-1}. It sounds that there is a positive correlation between concentration of BAP and number of shoot to a certain concentration of BAP, so that number of shoot reaches its peak at concentration of 1 mg 1^{-1} BAP (Fig 8). At concentrations higher than 1 mg 1^{-1} BAP decrease in number of shoots can be seen. It shows that when concentration of BAP was in excessive amount, it resulted in decrease of shoot number. One of the possible reason can be reductive effect of higher concentrations of BAP. Apparently a certain amount of BAP is required to obtain the best effect. Higher concentrations of BAP brought about formation of high amount of callus which is not appropriate in tissue culture (Fig 9).

Roots formation in tissue culture can be induced by exogenous auxins such as IBA, NAA and IAA and their interaction with endogenous auxins which cannot be sufficiently synthesized by many tissues and small organs isolated *in vitro* (Thorpe et al., 2008) and they are only required at an early stage to emerge new formed roots (Dobranszki and silva, 2010). They also can influence the growth of the newly formed roots in the expressive phase of root development (Bell amine et al., 19980). Since combination of different auxins resulted in better response in olive which shows difficult rooting (Grigoriadou et al., 2002), this experiment is designed to study the effect of different auxins solely or in combination with each other. Many investigators have reported that IBA has a better effect on promoting adventitious root formation in comparison to IAA (Stephan and Hamzah, 1988; Riov, 1993; De Klerk et al., 1999; Ludwig-Muller, 2000). It is more stable and less sensitive to the auxin degrading enzymes (Nordstrom et al., 1991; Epstein and Ludwig-Muller, 1993; Riov, 1993). IAA is quickly metabolized by the peroxidase, acting as an IAA-oxidase, with the strongest activity during the root initiation phase (Caboni et al., 1997; Nag et al., 2001). It can be a possible reason for better effects of IBA in this investigation. In this study, when single IBA was applied, it resulted in more rooting percentage and also higher root length compared to two other treatments. The other reason for better effect of IBA can be more chemical reactions between different rooting hormones so that when we combined them together (treatment number 1), minimum rooting percentage was obtained. Treatment number 3 containing 2 types of hormones (IBA and NAA) brought about more rooting percentage compared to treatment number 1 (containing IBA, NAA and IAA) and treatment number 1 (single IBA) produced the highest root percentage and length of root. Apart from concentration, our results showed that when there were fewer reaction among hormones, higher root percentage was achieved. The other factor can influence the rooting is Photoperiod which determine environmental conditions of cultures in micropropagation. Antonopoulou et al (2004) reported the positive effect of a short period of darkness on the *in vitro* rooting. It has been reported that keeping cultures of *Prunus cerasifera* (Hammerschlag, 1982) and almond/peach hybrid rootstock *Titan×Nemaguard* (Channuntapipat *et al*. 2003) in the dark prior to Transferring to light condition enhanced the rooting of explants. More effective photoreceptor activation is one of the factors which can be attributed to the positive effect of dark on rooting. Phytochrome is one of the photomorphogenic photoreceptors involved in different plant growth processes (Mancinelli 1994) include control of apical dominance (Tucker 1976; Muleo and Thomas 1997), the outgrowth of axillary buds and the rooting of shoots in micropropagation (Morini and Muleo 2003). The physiologically active form of Phytochrome has a peak under red light, which represents a high fraction of the emission spectrum of the most of the fluorescent tubes emitting white light, used in micropropagation laboratories. the dark period can bring about the destruction of The active form of Phytochrome. Darkening create condition like natural soil and in the presence of auxins such as IBA induce cell division and growth and increase rooting percentage of plants which

have low rooting. On the other hand, light act as inhibitor and decrease of rooting (Mencuccini 2003). In this study, the effect of dark on rooting percentage was not significant owing to the younger age of shoots which are less responsive to the dark and auxin and it is in accordance with Modgil et al (Modgil et al., 1999). On the other hand, positive effect of dark on number and length of root in this study is in accordance with Vaez-Livari et al (2005).

In conclusion, the results of this investigation indicate that BAP in concentration of 1 proved to be the best concentrations in cases of number and length of shoot in proliferation phase. In rooting stage, 1/2 MS medium supplemented with 3 mg 1^{-1} IBA solely resulted in desirable results in terms of rooting percentage.

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Table 1. Effect of different concentrations of BAP on number of shoot and the length of shoot

Treatment	BAP (mg/l)	Number of shoot	Length of shoot (cm)
T1	control	0.4	0.384
T2	0.1	1	1.932
T3	0.6	1.65	2.422
T4	1	2.98	2.544
T5	1.5		
T6	2		

Table 2. Effect of different rooting-hormones on percentage of rooting, number and length of root of GF 677

Treatment	Type of hormone	Concentration (mg/l)	Percentage of rooting	Root number	Root length
T1	IBA + NAA + IAA	0.1 + 0.1 + 0.1	11	0.833	3.5
T2	IBA	3	44	2.167	11.2
T3	IBA + NAA	0.1 + 0.1	22	0.667	5.67

Table 3. Effect of dark and light on number and length of root of GF 677

Treatment	Number of root	Length of root
Dark	1.667	10.22
Light	0.778	3.36

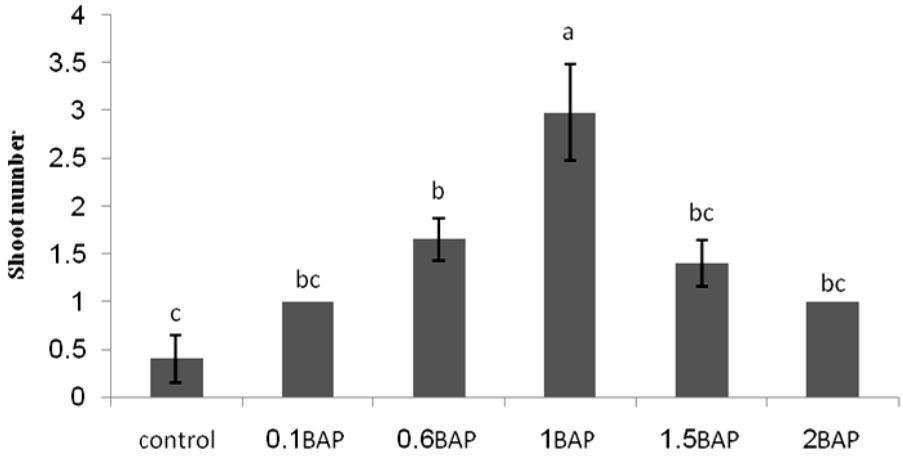

Figure 1. Different concentrations of BAP (means with the same letter are not significantly different)

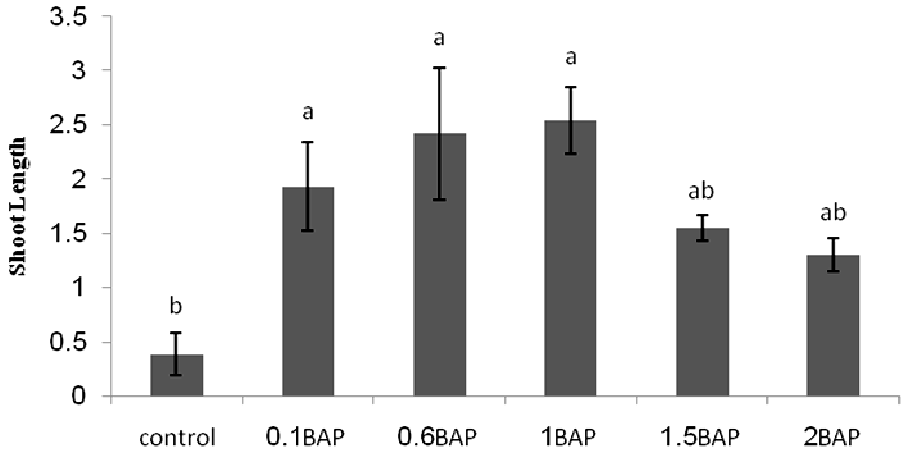

Figure 2. Different concentrations of BAP (means with the same letter are not significantly different)

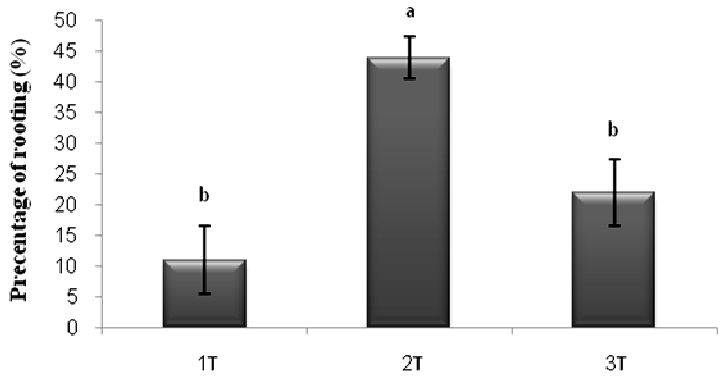

Figure 3. Different treatment of rooting (Means with the same letter are not statically different)

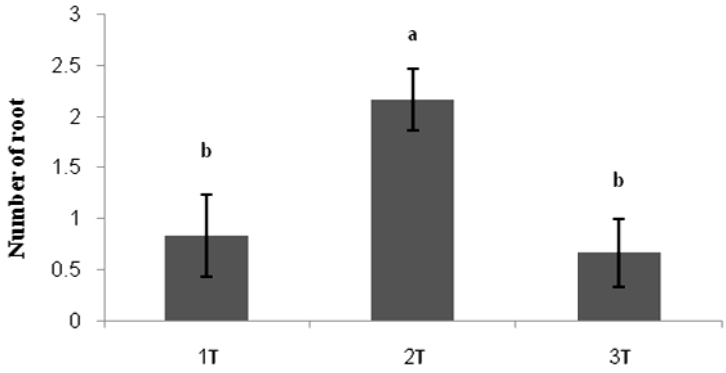

Figure 4. Effect of different rooting-hormones on number of root (means with the same letter are not significantly different)

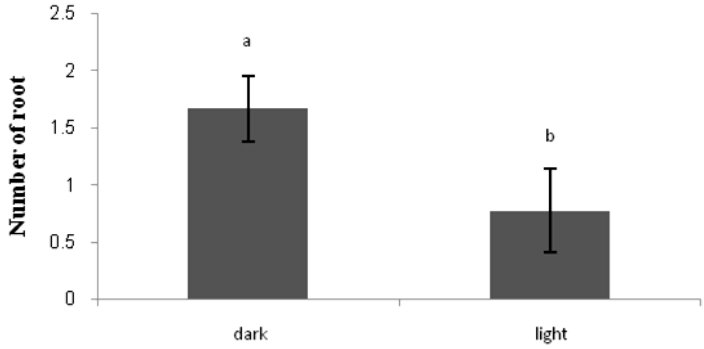

Figure 5. Effect of dark and light on number of root (means with the same letter are not significantly different)

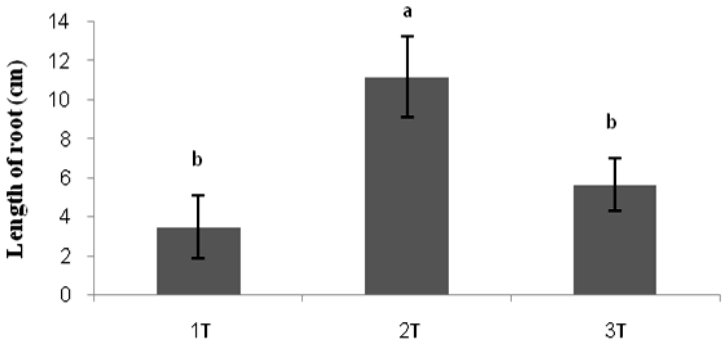

Figure 6. Effect of different rooting-hormones on length of root (means with the same letter are not significantly different)

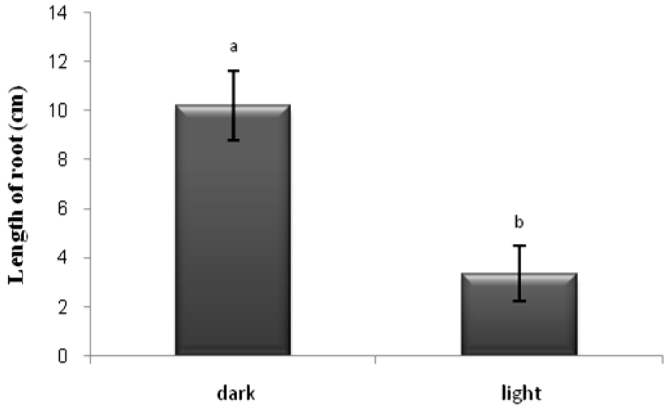

Figure 7. Effect of dark and light on root length (means with the same letter are not significantly different)

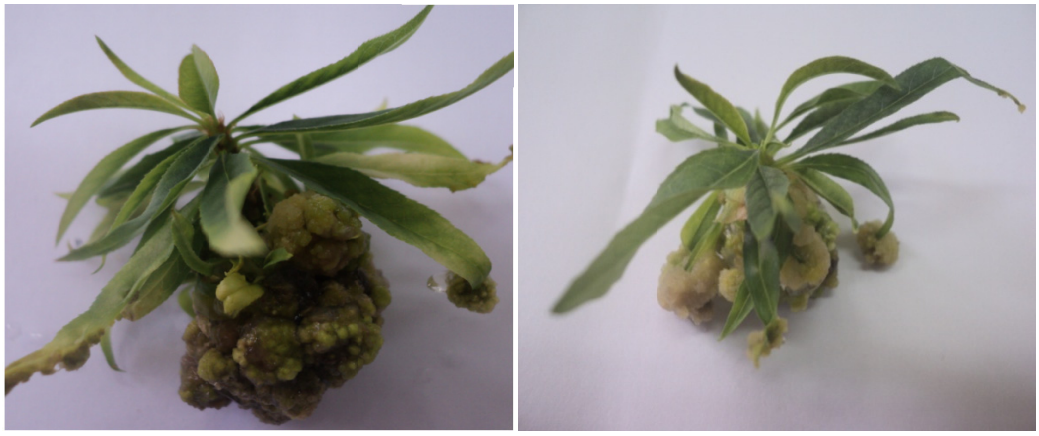

Figure 8. Amount of callus in treatment containing 1.5 (Left picture)and 2 (Right picture) mg 1^{-1} BAP

Figure 9. Effect of 1 mg/l BAP on number of shoot