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Original Research Article

In vitro Culture of Gisela 6 Semi-dwarf Rootstock

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ABSTRACT

This research was carried out to study micropropagation of Gisela 6 rootstock in order to achieve suitable media for producing 5 thousand plants. Explants were cultured in MS and WPM media containing 2 types of cytokinins BAP and TDZ at different concentrations. The results showed that the number of shoots in MS medium containing 1.2 mg L^{-1} BAP was higher than the other treatments. For rooting, the effect of basal media (MS, 1/2 MS (macroelements half) and 1/2 MS (macro and micro elements half)) and different auxins including IBA, NAA and IAA, solely or in combination with each other was investigated. The results showed that the highest percentage of rooting was due to medium 1/2 MS containing (macroelements half) 1 mg L^{-1} IBA resulted in 100% rooting. Due to low quality of roots obtained, and also the role of Fe-EDDHA and thiamine in rooting improvement, an experiment about the effects of above-mentioned elements was carried out. The results indicated that thiamine and Fe-EDDHA at concentrations of 1.6 and 150 mg L^{-1} respectively resulted in the best rooting. Finally, in acclimation phase, more than %95 survival was achieved.

Key words: Fe-EDDHA, Gisela 6 rootstock, micropropagation, 6-Benzylaminopurine, thiamine, thiadiazuron

Abbreviations: Indole-3-acetic acid(IAA), Indole-3-butyric acid(IBA), 6-Benzylaminopurine(BAP), naphthalene acetic acid(NAA), thiadiazuron (TDZ), woody plant medium (WPM), Murashige and SKoog (MS).

INTRODUCTION

Gisela 6 is a hybrid of *Prunus cerasus x Prunus canescens* and a semi-dwarf rootstock which is suitable for all kinds of sweet cherry. It is also satisfactory in a wide range of soils, especially heavy soils (Andersen et al. 1999). The average and slow growth of this rootstock has great value for establishment and development of modern intensive cherry orchards. Trees grafted on this rootstock don't have suckering problem (Long and Kaiser 2010). In Iran, conventional rootstocks like Mazzard (*Prunus avium*) and Mahlab (*P. mahlab*) are mainly used as rootstocks for sweet cherry. In recent years, there has been a growing trend toward using dwarf rootstocks in Iran. Gisela 6 rootstock is one of the sweet cherry rootstocks which will become important in the Iranian fruit industry in the near future. Producing Gisela 6 rootstock in large scale by conventional methods like cutting and layering to meet the growing internal demand for high quality, disease-free and uniform planting material, seems to be difficult. The scale and speed of production of healthy plants can be enhanced by micropropagation techniques (Aka-Kacar et al. 2010). Erbenova et al. (2001) reported 50% enhancement in multiplication rate of sweet cherry rootstocks in MS medium containing 0.5 mg L^{-1} BAP. MS and 2MS (double macro-salts) media containing 4.4 mμ BA, 0.5 mμ NAA and 0.3 mμ GA_3 were reported appropriate media for propagation of Gisela 5 (Ruzic et al. 2000). Combination of 0.5 mg L^{-1} BAP and 0.05 mg L^{-1} TDZ, and medium supplemented with 0.3 mg L^{-1} IBA were reported desirable for proliferation and rooting of *Prunus avium*, respectively (Dorkovic 2006). Tang et al. (2002) reported BAP was more effective than TDZ in shoot regeneration from leaves, and WPM was more effective than MS in caulogenesis. They also reported rooting percentage in half MS medium containing 2 mg L^{-1} IBA was higher than the same medium but containing 2 mg L^{-1} NAA. However, length and number of roots were reported greater with NAA compared to IBA. Buyukdemirci (2008) stated that MS medium containing 0.5 mg L^{-1} BAP, 0.01 mg L^{-1} IBA and 0.1 mg L^{-1} GA_3 was the best medium for proliferation. He also reported that reducing the nitrate concentration promoted more roots from the Gisela 5 explants and the best rooting was observed in the medium supplemented with 0.5–1 mg L^{-1} IBA.

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One of the main factors in micropropagation is medium, since different media have different ingredients. Particular compound, the type of culture, the verity of plant from which explants are taken and whether the explants are taken from juvenile or mature tissues can determine the effect of cytokinins. In some plants, BAP has been reported to be more effective than TDZ (Tang et al. 2002; Ruzic and Vujovic 2008); and in some other plants, TDZ has been shown to be more effective than BAP (Perez-Tornero et al. 2000; Bhagwat and Lane 2004; Matt and Jehle 2005; Espinosa et al. 2006; Canli and Tian 2008). In the rooting stage, the induction of roots on explants can be crucial part in micropropagation process based on the kind of culture, the variety of plant and the age of explant (Molassiotis et al. 2003/4; Thorpe et al. 2008). The ability of plant tissues to form adventitious roots is determined through the interaction of many exogenous and endogenous factors including hormone. In most reports, exogenous auxins such as IBA, NAA or IAA were used for rooting (Ainsley et al. 2001). The above-mentioned auxins are only required at an early stage to stimulate the emergence of newly formed roots (Dobranszki and Silva 2010). Apart from hormone, other factors which can affect rooting are the chemical ingredients of the culture medium. Iron is one of the essential microelements used in micropropagation due to its essential role in many processes like chlorophyll and DNA synthesis (Dunlap and Robacker 1988). Moreover, Fe is a constituent of peroxides, which mediates IAA catabolism (Gaspar et al. 1992). Many researchers have reported the beneficial effects of Fe-EDTA replacement with Fe-EDDHA on rooting of woody plants (Maheshwari and Seth 1965; Chopra and Rashid 1969; Rashid and Street 1973; Molassiotis et al. 2003, 2004; Antonopoulou et al. 2007). The other essential components in culture medium are vitamins (Molnar et al. 2011) required in minute amounts in plant tissue culture (Torres 1989). Among four important vitamins, thiamine is more important owing to its direct involvement in the biosynthesis of some amino acids and its role as a cofactor in carbohydrate metabolism (Al-Khayri 2001). It was also reported that thiamine brought about stimulation of adventitious rooting in *Taxus* spp (Chee 1995). No paper has reported the effects of thiamine and Fe-EDDHA on rooting of Gisela. The objectives of this research were to study a) the effects of two types of media, two types of cytokinins at four concentrations on proliferation b) the influences of different media and rooting-hormones on rooting c) the role of thiamine and Fe-EDDHA on the rooting improvement of Gisela 6.

MATERIALS AND METHODS

Plant explants were provided from controlled plants kept in greenhouse of Faculty of Agriculture of Tarbiat Modares University in spring of 2011. At first, shoots were excised into 2.5 cm-long sections; then for surface disinfection, they were agitated for 5 min in a solution containing 5 drops of Tween-20 in 100 ml of water followed by washing under running water for 1 hr. For sterilization under laminar airflow chamber, firstly explants were agitated in alcohol 70% for 30 s, then in %0.01 solution of mercuric chloride for 7 min and finally they were rinsed three times with distilled water.

MS (Murashige and SKoog 1962) and WPM media containing BAP and TDZ at four concentrations of 0.4, 0.8, 1.2 and 1.6 mg L^{-1} were investigated (Table 1).

After 30 days, at the end of the proliferation stage, the number of new shoots per explant, shoot height (cm) as well as the weight of callus (gram) were determined. Number of shoot was measured by counting newly formed shoots; and shoots height was measured by a metal ruler. For measuring the callus weight, firstly fresh cuts were made at the basal ends of the shoots and then calluses were carefully taken and weighed by scale.

For the first rooting experiment, after removal of the abnormal and lower leaves, shoots with 3-5 cm length from elongated phase were separated and then individually transferred to different media supplemented with different auxins hormone (Table 2). In the second experiment of rooting, shoots with 3-5 cm length were transferred to 1/2 MS medium (macroelements half) brought about 100 percent rooting in the previous experiment, supplemented with different concentrations of thiamine and Fe-EDDHA (Table 3). After culturing shoots in different treatments, jars containing shoots were maintained in the dark for one week. After one week, all treatments were transferred to light condition. Data including percentage of rooting, length and number of roots were recorded after four weeks. Percentage of rooting, root number and root length were determined by counting rooted explants, counting the number of formed roots and measuring by using a metal ruler, respectively. All

media supplemented with 7 g L^{-1} agar (Bacterological agar, MM0317, 'Nebotrade Ltd'), 30 g L^{-1} sucrose. The pH of media was adjusted to 5.75±3 with HCl 0.1N or NaOH 0.1N prior to sterilization by autoclaving at 121°C for 20 min. explants were maintained at 25±1 ºc and 16/8 hr photoperiod of cool-white light at 1250 lux for 30 days.

In the acclimation phase, peat and perlite at 3:2 (v/v) were used as medium. Plants were transferred to plastic tunnel with 24±1 ºc and 90% relative humidity. After one week, humidity was reduced.

The proliferation experiment was conducted as a factorial based on a completely randomized design (CRD) with 5 replications per treatment and two explants per replication. Statistical analysis of the data was carried out by using SAS software and difference among treatments means was compared by using Duncan's multiple range test at $P \leq 0.05$ level. For rooting, the experiments were performed as factorial based on a completely randomized design (CRD) with two factors and 5 replications per treatment and two explants per replication. Statistical analysis of the data was conducted using SAS 16 software and difference among treatments means was compared by using Duncan's multiple range test at $P \leq 0.05$ level.

RESULTS

Based from table 1, MS medium containing 1.2 mg L^{-1} BAP proved to be the most effective treatment with respect to shoot number. WPM containing 1.6 mg L^{-1} TDZ brought about the lowest number of shoot. In terms of shoot height, WPM medium supplemented with 1.2 mg L^{-1} TDZ showed to be the best treatment among treatments used. On the other hand, the shortest shoots were due to treatment containing BAP at concentration of 1.6 mg L^{-1}. As it can be seen from table 1, callus weight was significantly affected by interaction of medium and cytokinin and also cytokinins concentrations. The highest amount of callus was observed in MS medium containing 1.6 mg L^{-1} TDZ which did not show significant difference from 1.2 mg L^{-1} TDZ in the same medium. MS medium supplemented with various concentrations of BAP showed the lowest amount of callus.

Table 1. Effect of different treatments on shoot number, shoot length and callus weight.

Treatments	Number	Length (cm)	Callus Weight(gram)
MS+0.4 mg L^{-1} BAP	9.5±0.353 c	1.117±0.042 abcde	0.114±0.012 g
MS+0.8 BAP	11.5±0.5 b	0.98±0.02 cdef	0.157±0.0197 g
MS+1.2 BAP	13.6±0.82 a	0.994±0.083 bcdef	0.169±0.0129 g
MS+1.6 BAP	6.9±0.291 de	0.957±0.07 cdef	0.186±0.0194 g
MS+0.4 TDZ	6±0.353 ef	1.26±0.048 ab	0.485±0.03 e
MS+0.8 TDZ	4.8±0.406 fgh	0.926±0.056 def	0.929±0.0109 bcd
MS+1.2 TDZ	4±0.273 ghi	1.06±0.0397 bcde	1.198±0.021 a
MS+1.6 TDZ	3.3±0.122 hi	1.22±0.053 abc	1.259±0.039 a
WPM+0.4 BAP	4.9±0.244 fg	1.017±0.159 bcdef	0.24±0.0338 fg
WPM+0.8 BAP	6±0.316 ef	0.93±0.1 def	0.276±0.043 efg
WPM+1.2 BAP	7.6±0.678 d	0.9±0.048 ef	0.308±0.021 efg
WPM+1.6 BAP	6.2±0.681 def	0.776±0.0777 f	0.449±0.0425 ef
WPM+0.4 TDZ	3.96±0.203 ghi	1.22±0.077 abc	0.765±0.0195 d
WPM+0.8 TDZ	3.2±0.254 i	1.14±0.043 bcde	0.799±0.035 cd
WPM+1.2 TDZ	2.5±0.223 ij	1.43±0.079 a	0.98±0.204 bc
WPM+1.6 TDZ	1.5±0.158 j	1.18±0.109 abcd	1.111±0.0257 ab

Values in each column represent means + standard error. Means with the same letter are not significantly different at P<0.05.

According to table 2, there are significant differences between different treatments of rooting. The highest percentage of rooting was due to 1/2 MS medium (macroelements half) containing 1 mg L^{-1} IBA (T4) resulted in 100 percentage rooting. 1/2 MS medium (macro and microelements half) containing 1 mg L^{-1} IBA and 0.5 mg L^{-1} NAA gave the lowest percentage of rooting which was 35 percent. Statistical analysis in table 2 indicated that there are significant differences between numbers of roots in different treatments. The highest number of roots was observed in MS medium supplemented with 1 mg L^{-1} IBA, 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} IAA. An average number of 5.4 roots was observed in this treatment. The lowest root number was due to treatment number 2, in which an average number of 3.2 roots was obtained. The results obtained on the root length in table 2 pointed out significant differences between different treatments of rooting. The maximum roots length was observed in MS medium containing 1 mg L^{-1} IBA, 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} IAA (T3). An average length of 6.5 cm was obtained in this treatment. The minimum length of roots was due to 1/2 MS (all elements half) containing 1 mg L^{-1} IBA, 0.5 mg L^{-1} NAA, in which the average length of 2.15 cm was obtained.

Table 2. Effect of different medium and auxins on percentage, number and length of root.

Treatments	Percentage of rooting	Number of root	Root length
MS+ IBA 1 mg L^{-1}	50±0 ef	4.4±1.21 ab	5.2±0.51 b
MS +IBA 1 + NAA 0.5	55±3.16 de	3.2±0.37 b	2.55±0.29 cd
MS+ IBA 1 + NAA 0.5 +IAA 0.5	80±5.24 bc	5.4±0.291 a	6.5±0.35 a
1/2 MS (macroelements half)+ IBA 1	100±0 a	4.1±0.29 ab	6±0.37 ab
1/2 MS (macroelements half)+ IBA 1 + NAA 0.5	90±10 ab	5.11±0.29 ab	3.3±0.289 cd
1/2 MS (macroelements half)+ IBA 1 + NAA 0.5 +IAA 0.5	50±0 ef	4.8±0.406 ab	3.4±0.291 c
1/2 MS (all elements half)+ IBA 1	70±5 cd	3.7±0.2 ab	5±0.41 b
1/2 MS (all elements half)+ IBA 1 + NAA 0.5	35±3.16 f	4±0.44 ab	2.15±0.1 d
1/2 MS (all elements half)+ IBA 1 + NAA 0.5 +IAA 0.5	60.67±6.09 de	4.2±0.406 ab	3.15±0.15 cd

Values in each column represent means + standard error. Different letters within columns show significant differences ($p<0.05$).

According to the results obtained in table 3, different treatments of Fe-EDDHA and thiamine had significant effects on rooting percentage. As a whole, rooting percentage in most treatments was 100%. The lowest percentage of rooting was 90% due to treatments without thiamine and excessive thiamine. From table 3, it was understood that there are significant differences between numbers of roots in different levels of Fe-EDDHA and thiamine. Maximum number of roots was observed in treatment containing 1.6 mg L^{-1} thiamine and 150 mg L^{-1} Fe-EDDHA so that the average of roots number was 6.63. The lowest number of roots was due to treatment containing 0 mg L^{-1} thiamine + 200 mg L^{-1} Fe-EDDHA. The results obtained on root length in table 3 showed significant differences between different treatments used. As it can be seen from table 3, the maximum length of roots was obtained in medium containing 1.6 mg L^{-1} thiamine and 150 mg L^{-1} Fe-EDDHA. An average length of 10.4 cm was obtained in this treatment. The minimum root length was observed in treatment containing no thiamine and 100 mg L^{-1} Fe-EDDHA in which the average length of roots was 5.2 cm.

Table 3. Effect of different treatments on rooting percentage, number and length of root.

Treatments	Percentage of rooting	Number of root	Root length
Thiamine 0+ Fe-EDDHA 100	90b	4.36±0.03 ef	5.2±1.01 d
Thi 0+ Fe 150	90b	4.95±0.22 de	5.8±0.33 cd
Thi 0+ Fe 200	90b	4.25±0.15 f	6.3±0.2 bcd
Thi 1.6+ Fe 100	100a	5.28±0.19 cd	10±0.41 a
Thi 1.6+ Fe 150	100a	6.63±0.15 a	10.4±0.55 a
Thi 1.6+ Fe 200	100a	6.09±0.07 ab	10±0.57 a
Thi 2.8+ Fe 100	100a	6.13±0.25 ab	6.5±0.67 bcd
Thi 2.8+ Fe 150	100a	5.55±0.37 bcd	7±0.41 bc
Thi 2.8+ Fe 200	100a	6.2±0.08 ab	6.7±0.37 bcd
Thi 4+ Fe 100	100a	5.3±0.25 cd	7.8±0.25 b
Thi 4+ Fe 150	90b	5.85±0.48 bc	7.4±0.67 b
Thi 4+ Fe 200	90b	4.96±0.15 de	7.6±0.24 b

Values in each column represent means + standard error. Different letters within columns indicate significant differences ($p<0.05$).

DISCUSSION

Shoot branching depends upon the initiation and activity of axillary meristems, which are hormonally controlled mainly by cytokinin (Dobranszki and Silva 2010). The cytokinin BAP promotes cell division, shoot multiplication and axillary bud formation (Sutter 1996). The influence of cytokinin 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). This study showed that number of shoots increased in MS medium supplemented with BAP. As concentration of BAP increased to 1.2 mg L^{-1}, the number of shoot also increased to 13.6 shoots per explant from 9.5 and 11.5 shoots at concentrations of 0.4 and 0.8 mg L^{-1}, respectively. It sounds that there is a positive correlation between concentrations of BAP and number of shoots up to a certain concentration of BAP, so that number of shoots reaches its peak at concentration of 1.2 mg L^{-1} BAP. Among BAP treatments, the lowest number of shoot belongs to 1.6 mg L^{-1} treatment. It shows that when concentration of BAP was in excessive amount, it resulted in decrease of shoot number. One of the possible reasons can be reductive effect of higher concentrations of BAP. Apparently a certain amount of BAP is required to result in the best effect. Comparison of cytokinin type shows that BAP is more effective than TDZ in terms of shoot number in both MS and WPM media. In contrast, TDZ was more effective with respect to callus weight. It seems that TDZ concentrations used were high which exert their effects by producing more calluses compared to BAP even at 0.4 mg L^{-1} which sounds low. The other possible reason can be medium type. MS medium is the stronger medium due to its higher amount of macroelements specially NH_4NO_3. High amount of callus which is not appropriate in tissue culture was seen in media supplemented with various concentrations of TDZ. It can be explained that callus weight is significantly affected by cytokinin type, irrespective of medium type and hormone concentration, although amount of callus was significantly increased by increasing concentrations of TDZ in both MS and WPM media. Shoots height was not affected significantly by the type of medium and cytokinin, although different treatments showed significant differences from each other. In some cultures, BAP was reported to produce short rosetted shoots, or shoots which grow slowly after emergence. That can be the reason for shorter shoots in media supplemented with different concentrations of BAP. The induction of roots *in vitro* is an important step in plant micropropagation (George 1996). The ability of plant tissues to form adventitious roots depends on the interaction of many exogenous and endogenous factors such as hormone, elements and type of culture medium (Frankel and Hess 1973). 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). Different media have different effects on rooting stage as a result of different concentrations of their elements. The importance of the interaction of nutrient salts and plant growth regulators in the culture medium has long been known (De Fossard et al. 1974, Murashige and SKoog 1962). Mixtures of many different auxins have been used by some investigators (e.g., Blackmon et al. 1981, Nazary and

Yadollahi 2012), but since individual compounds may have different effects in different plants, most researchers prefer to use only one, or at most two compounds. In the current study, interaction between type of media and hormone led to different results. The highest percentage of rooting was 100% due to 1/2 MS medium (macroelements half) containing 1 mg L^{-1} IBA. One reason can be fewer chemical reactions among macroelements which were reduced by half and it is in accordance with the reports of Tang et al. (2002) and Buyukdemirci (2008). It appears that microelements have an important role in rooting so that when their concentrations in 1/2 MS were halved, the rooting percentage was not desired. On the other hand, macroelements do not seem to be effective on rooting owing to 100% rooting observed in 1/2 MS in which macroelements were halved. Macroelements are not only effective on rooting but they also caused reduction in rooting percentage. That is why MS and 1/2 MS (macro and microelements half) showed lesser rooting percentage in comparison with 1/2 MS (macroelements half). It has also been stated that IBA has better effect on promoting adventitious root formation in comparison to IAA or NAA (Stephan and Hamzah 1988, Riov 1993, De Klerk et al. 1999, Ludwig-Muller 2000, Tang et al. 2002, Nazary and Yadollahi 2012). It is more stable and less sensitive to the auxin degrading enzymes (Nordstrom et al. 1991, Epstein and Ludwig-Muller 1993, Riov 1993). IAA can be quickly metabolized by the peroxidase during the root initiation phase (Caboni et al. 1997, Nag et al. 2001). It can be the other possible reason for better effects of IBA solely in this research. In this research, when single IBA was utilized, it caused the highest rooting percentage compared to other treatments. The other reason for better effect of single IBA can be reaction between different rooting hormones so that when we combined them together, rooting percentage was not desirable. In terms of number and length of roots, MS medium containing 3 types of auxin proved to be the best treatment among different treatments used. It seems that length and number of roots was affected by interaction of medium and hormone. Reducing concentrations of macro and microelements to half of their usual concentrations can be the reason for better effect of MS medium compared to two other media. Bell amine et al. (1980) stated that different auxins can also influence the growth of the newly formed roots. Tang et al. (2002) found out better effect of NAA on number and length of root in comparison with IBA and his findings support our results. It appears that growth of roots in treatment containing 3 types of auxin was affected by above-mentioned hormones. Roots obtained from this experiment were brittle, rigid and poor in quality and quantity. For improving the quality, length and number of root, the effects of thiamine and Fe-EDDHA were studied. It has been reported that iron deficiency causes morphological and physiological changes in root, specially root tip meristems including enhancement of cell division (Hewitt and Smith 1974) inhibition of root elongation, increase in diameter of apical root zoon (Romheld and Marschner 1981, Chaney et al. 1980) and formation of rhizodermal transfer cells (Kramer et al. 1980). Thiamine as an enzyme cofactor has important effects on metabolically reactions such as glycolysis or in pentose phosphate and tricarboxilic acid cycle. In addition to its value as a nutritional compound, thiamine is also the secondary messenger in activation of proteins. The results of this experiment showed treatments containing 1.6 mg L^{-1} thiamine and 150 mg L^{-1} Fe-EDDHA gave the best results in terms of root length and root number. It appears that thiamine at concentration of 1.6 mg L^{-1} has good interaction with iron at concentration of 150 mg L^{-1}. Roots in this treatment were thin and flexible and also emerged from the basal end led to better acclimation and lesser loss in hardening phase under controlled conditions. Having such roots also resulted in better growth of plants in greenhouse. According to table 3, percentage of rooting in treatments supplemented with 4 mg L^{-1} thiamine and Fe-EDDHA at concentrations of 150 and 200 mg L^{-1} was 90%. One of the possible reasons can be excessive amount of thiamine (4 mg L^{-1}) which may affect availability of other microelements or interfere in their actions. In treatments without thiamine and different concentrations of Fe-EDDHA, percentage of rooting was 90%. It was also observed that roots in treatments lack of thiamine were rigid and brittle in comparison to other treatments. It seems that vitamin can directly affect the quality of roots so that roots growth is influenced by lack of thiamine. As a whole, addition of thiamine to 1/2 MS medium (just macroelements half) had great effect on root number and length, but enhancing its concentration to 4 mg L^{-1} had reductive effect on rooting percentage. On the other hand, lack of thiamine resulted in thick, inflexible and brittle roots caused plants not to grow well in greenhouse and acclimation stage. Apart from thiamine, comparison of number and length of roots in 1/2 MS medium (macroelements half) supplemented with two different types of irons Fe-EDTA and Fe-EDDHA showed

the better effect of Fe-EDDHA on improvement of above-mentioned parameters. It can be concluded that positive interaction between thiamine and Fe-EDDHA improved the rooting stage of Gisela 6.

CONCLUSIONS

The results of this study indicate that BAP at concentration of 1.2 mg L^{-1} brought about the highest number of shoots in proliferation phase. In rooting stage, 1/2 MS medium in which macroelements were reduced by half in combination with 1 mg L^{-1} IBA solely resulted in desirable results. Our results also showed that thiamine and Fe-EDDHA had significant improvement in both quantity and quality of roots. It can be concluded that the best rooting medium for Gisela 6 is 1/2 MS (macroelements half) supplemented with 1 mg L^{-1} IBA, 150 mg L^{-1} Fe-EDDHA and 1.6 mg L^{-1} thiamine. And finally, using this protocol, we have been able to produce 5 thousand high quality and uniform plants which can be continued to supply the growing demand for high performance Gisela 6 rootstock.

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