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The influence of season collection of explants on micropropagation of peach rootstock GF-677

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Abstract

The influence of season on the rate of multiplication on in vitro culture of peach rootstock GF- 677 was investigated on Murashige and Skoog (MS) media, supplemented with GA3 0.1 mg/L and IAA 0.1mg/l. Benzyladenine (BAP) at concentrations 1mg/l was used in the multiplication stage and 1mg/l IBA in the stage of rooting. Shoot-tip and nodal segment explants were collected from 5 years old rootstock GF-677 (*Prunus persica x Prunus amygdalus*) in February 24th (from dormant shoots that have been sprouted in climatic room), March 22th, April 20th, May 18th and September 15th during the 2009 growing season and have been sterilized by sodium hypochlorite (NaOCl) 10% for 20 min. The data on the effect of the season collection of the explants on number of shoots per explants, the mean shoot length and the percentage of rooted shoots were recorded six weeks after culture. In vitro performance of explants indicated a positive correlation between shoot proliferation and season collection The highest number of shoots per explants (3,5) was obtained on explants collected in March 22th (3,5), which was on a par with explants collected in February 24th (from shoots that have been sprouted in climatic room). Moreover, the highest shoot length was observed on explants collected on February and March (1,53cm and 1,505cm respectively). The percentage of rooted shoots from explants sampled on February was not markedly greater than those sampled on March. The number of shoots per explants, the shoot length and the percentage of rooted shoots on explants sampled in April, May and September were significantly lower than those sampled in February and March. The amount of chlorophyll a + b of the shoots coming from explants collected in March was markedly greater than those collected in February, April, May and September.

Key words: Peach, in vitro, explants, chlorophyll, rootstock

1. Introduction

Micropropagation is being used extensively for the rapid clonal propagation of many fruits, nuts and ornamental trees, since it enables rapid propagation and hastens the availability of new cultivars [11]. GF677 rootstock (Prunus amygdalus x Prunus persica) is an important rootstock for peach. The peach is an important fruit tree for Albania. Prunus genus is one of the major difficulties with respect to in vitro propagation from mature tissues. Micropagation from shoot cuttings of plum cv, Kantimirovskaja has been reported by Leontiev- Orlov et al [4]. A protocol for in vitro propagation of Prunus domestica was developed by H.Y Yan et al [10], as well.

The first investigation about GF677 micropropagation was carried out by Kester [2] and Tabachnik and Kester [9].

Later, many researchers have achieved propagation in vitro of this peach rootstock and have identified the appropriate substrate and the suitable concentration of phytohormones on its proliferation and rooting [1, 3, 7].

The aim of this study was to investigate whether in vitro shoot formation of peach rootstock GF- 677 depended on the season when the explants were collected.

Effect of season of explants collection, have been demonstrated for numerous plant species including Strawberry (*Fragaria xananassa*) [8], Lotus (*Nelumbo nucifera Gaertn*) [6] Jackfruit (*Artocarpus heterophyll Us Lam*) [5].

The present study was the first attempt for micropropagation of rootstock GF-677 (*Prunus persica x Prunus amygdalus*) using explants sampled on different dates of current season's growth. This experiment is carried out in the in vitro laboratory of the Agricultural Technology Transfer Center of Vlora and the Researche Institute of Fruit culture, Roma Italy.

2. Materials and Methods

Single-node and shoot apical explants (1-2cm) produced from tender shoots, born in 1-year branches of 5 years-old rootstock GF-677 (Prunus persica x Prunus amygdalus) trees were prepared aseptically,

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disinfected for 20 min in a 10% solution of NaOCl and were washed with sterilized distilled water thrice to remove all the traces of disinfectant. These explants were then vertically cultured in culture tubes with 10 ml MS medium supplemented with cystine 0.5 mg/l, pantotheneic acid calcium 10 mg/l, thiamine HCl 0.5mg/l, glycine 2mg/l, myoinositol 100mg/l, GA3 0.1mg/l, IAA 0.1mg/l and sacharose 30mg/l. The pH was adjusted to 5.6, before adding 6.0 g/l Oxoid agar No3. Benzyladenine (BAP) at concentrations 1mg/l was used in the multiplication stage and 1mg/l IBA in the stage of rooting.

To investigate the influence of season on the rate of multiplication on in vitro culture of peach rootstock GF- 677, shoot-tip and nodal segment of rootstock GF-677 (Prunus persica x Prunus amygdalus) were collected and cultured on different dates during 2009 season's growth, as it is shown below:

February 24th (from dormant buds that have been sprouted in climatic room),

March 22th,

April 20th,

May 18th

September 15th

The data on the effect of season of explants collection, on number and length of the shoots per explants, were recorded six weeks after culture, while the number and length of the roots per shot and the concentration of chlorophyll a+b were recorded six weeks after the planting of the shoots for rooting.

By using the spectrometric method through field portable determinants (Chlorophyll content meter (CM-200) is calculated the amount of chlorophyll (Ca + Cb) and the ratio Ca / Cb. Calculation is done in mg / g dry weight of the leaf.

Experiment with peach rootstock explants were arranged in a randomized complete block design with 30 explants per treatment and four replications. The dates were analyzed for statistical significance by analysis of variance with mean separation by Duncan's multiple range tests.

3. Results and discussion

The mean shoot number and mean shoot length per explants of different dates collection are summarized in Table 1. The highest shoot number was observed on explants collected during early spring season (3,475), which was on a par with shoot number per explants collected during late winter season (3, 38).The lowest shoot number per explants was observed during spring and late spring season (2, 7 and 2, 45), which was on a par with shoot number per explants collected during early autumn season (2, 3). High levels of growth promoting substances and low growth inhibitors in actively growing shoots during early spring may be responsible for high explants proliferation during late winter and early spring. The mean shoot length was not markedly different between different dates of explants collection (Table 1).

Table 1: Mean shoot number per culture and mean shoot length in different dates of explant collection**.**

Date of explants collection	**Shoot number**	**Shoot length (cm)**
February 24th (from dormant buds sprouted in climatic room)	3.38a	1.53a
March 22th	3.475a	1.505a
April 20th	2.7b	1.38a
May 18th	2.45.b	1.41 a
September 15th	2.3b	1.40 a

** Separation by Duncan's multiple range tests, at P< 0.05*

+Mean of four replications

Percentage of rooted cuttings, primary root number and the mean root length per shoots formed from proliferation of explants collected in different date explants are summarized in table 2. As shown in table 2, the statistical analysis of the data obtained after the rooting of the shoots revealed significant differences between the shoot's rooting capacity during February and March as compared to April, May and September. The mean root length and root number per shoot was not markedly different between dates of collection.

The data presented in table3 show that the concentration of chlorophyll A and B, b was higher during early spring season (March 22th) and lower on the autumn season (September15th) which was on a par with late spring and late winter season (April 20th, May 18th and February 24th). The reason why it happens is maybe because the content of plant donor elements (Mg, N), that constitute the structure of chlorophyll, is higher in the early spring.

Table 2: Percentage of rooting shoots, mean number of roots per shoot and mean root length in different dates of explants collection

Date of explants collection	Percentage of rooting	Root number	Root length(cm)
February 24th, (from dormant buds sprouted in climatic room	53.5 a	2.0a*+	1.5
March 22th	49.7a	2,2a	1.9a
April 20th	36.4b	1,7a	1.4a
May 18th	26 c	1,9a	1.6a
September 15th	38.6b	1,8a	1.5a

** Separation by Duncan's multiple range tests, at P< 0.05*

+Mean of four replications

Table 3: The concentration of chlorophyll A and B in different dates of explants collection

Date of explants collection	Ca	Cb	Ca+Cb	Ca/Cb
February24th (from dormant buds sprouted in climatic room	2.25a	1.31a	3.5 a *+	1.71°
March 22th	2.64b	1.47b	4.11a	1.80°
April 20th	2.30a	1.32a	3.62a	1.74°
May 18th	2.18a	1.21a	3.39a	1.80°
September15th	2.13a	1.16c	3.29a	1.84°

** Separation by Duncan's multiple range tests, at P< 0.05*

+Mean of four replications

Our results indicate that the suitable time of vegetation for multiplication in vitro of GF 677, drawn from several indicators as a rate of proliferation. Percentage of rooting shoots, shoot length, number of average root per shoot was the March and February as the period where plants have a large amount phytohormones which preserve important role in differentiation and regenerative potentials that affect in proliferation and rhizogenesis.

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