RESEARCH ARTICLE **(Open Access)**

# Callus Induction and Adventitious Shoot Regeneration from Different Explants of Rootstocks GF-677 (*Prunus amygdalus x P. persica*)

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**Abstract**

GF-677 is one of the most suitable rootstocks for almond and peach used in calcareous soils to overcome lime induced chlorosis. The objective of this study is the *in vitro* cultivation of the peach rootstock GF-677, in determining the protocol for inoculation and for micropropagation in the medium, influencing the development and the outgrowth of the rootstock. The present study focused on the effect of different explants of this rootstock on regenerative potential tissue culture techniques. The explants used in the research were taken from vegetative buds (organized tissues) and internode stem segments (non-organized tissues) sampled in March during the 2010-2012 growing season. Organogenesis and proliferation was obtained on MS [10] medium supplemented with BAP 0.35mg/l and $GA_3$ 0.1mg/l. Each treatment includes 4 replications. Results were obtained in the inoculation phase after 40 days. For internodes-explants, percentage of callusogenesis resulted 58%, the number of shoots 1.43, length of shoots 0.87cm, number of leaves 2.34. Best results were taken apex growth- explants, the number of shoots 2.26, length of shoots 1.52 cm and number of leaves 3.53.

**Keywords:** culture *in vitro*, rootstock, micropropagation, organogenesis.

## 1. Introduction

The Prunus genus is one of the most difficulty with respect to *in vitro* organogenesis from mature tissues. *In vitro* culture of GF-677 is usually based on using MS, WPM, TK and other media m which result in low rates of shoot proliferation and/or some problems such as vitrification (during tissue culture) and also low rooting rate GF-677 is one of the most suitable rootstocks for almond and peach used in calcareous soils to overcome lime induced chlorosis. Afterwards [13] leaded the first studies of peach rootstock micropropagation from apical buds. In Italy [15] achieved peach rootstock GF-677 *in vitro* micropropagation.

This rootstock presents advantages in the micropropagation *in vitro* [8]. The traditional methods not satisfy market demand for plum and peach rootstocks. Thanks to the culture *in vitro* is possible for the rapid clonal propagation of this rootstocks. The rootstock GxN22, GF-677, Mr.S 2/5, Marianna and Mirabolano were tested in two culture media MS combined with four BAP concentration (0.1:0.3:0.5 and 0.7mg/l).

The purpose of this work was to determine the best explants for the in vitro multiplication of rootstocks GF-677 (*Prunus amygagdalus x P. persica*) from vegetative buds (organized tissues) and internode stem segments (non-organized tissues).

## 2. Material and Methods

The study was conducted in the laboratory *in vitro* at the Centre of Agricultural Technology Transfer (ATTC) Vlore. As starting material were used explants' taken by the vegetative organs of GF-677 obtained in the Screen House, plants "parent" certified. Explants 1– apical bud (0.8mm) (organized tissues); Explants 2 – internod stem segments (1.5cm) (non- organized tissues). For each variant was tested repeat x 4 with 10 plants. The data were taken after 40 days of explants inoculation.

***Indicators in the study:***

- Direct and indirect Organogenesis.
- The number of shoots obtained for inoculation

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(Accepted for publication March 25, 2015)
***ISSN**: 2218-2020,* 

- The length of the of shoots
- The number of leaves of each of shoots

An analysis of variance was performed on the data, and the significant differences among treatment means were calculated by the Lsd test at $P < 0.05$ Analysis of Variance (ANOVA).

Tissue cultures pass through these stages:

***Phase 0. Taking explants:***

Explants are taken into the certified "parent" plants' Screen House of the fruits trees species in March, 2010-2012.

***Disinfection and sterilization***

-Rinse of explants by running water.

-Sinking in 70% ethyl alcohol and rinse with distilled water.

-Sodium hypochlorite pest control with 0.5 % NaOCl for 20 min.

-Three times rinse with distilled water, sterilized.

***Phase 1. The explants' inoculation***

After the disinfection, we did the inoculation on the nutritive ground. The leafy outgrowths are cut into pedicle pieces with one knot and dive to their base on the nutritive ground. The shoots are taken by 5-6 cm half ligneous slips. After cleaning and rid of outside wrappers not damaging the shoot, the shoots are placed in sterile tubes as ground substrate inoculation [10] stock solution prepared with solvents under the macroelements (x 10), microelements (x100) of Fe-EDTA (x100), vitamins of group B (x100), with hormonal composition IBA(0.1 mg/l), giberiline GA3 (0.1mg/l), BAP (0.35mg/l), ANA(0.01mg/l), sucrose (30g/l), agar (7g/l), pH=5.7-5.8. Explants are ready for propagation after 4 weeks. They are taken from vegetative room and transferred to room on the ground proliferation of boxing laminar, by sterilized pliers and lancets. In this way is stimulated the development of new buds of phase of inoculation.

***Conditions of culture***

The explants at all stages are held in the vegetative room of plant growth in controlled optimal conditions $T = 22^{0}$ -$24^{0}$ C, photoperiod 16 hours of light, radiant luminous intensity 3000 lux

## 3. Results and Discussion

For explants taken as internode or apical bud has been noted that their development is oriented according to the nature of the corresponding explants report cytokinin/auxin[11]. Morphology of developing explants passes in the following steps: • Bulge of explants and its initial development. • The appearance of visible fructification on the explants' surface. • Development of new bodies. After 4-5 weeks have been seen multiple joints. The presence of ANA and BAP induces new forms and development of newly formed buds. On the surface of each callus have been shown 4-5 well developed shoots. The ratio cytokine / auxin favor the formation of pedicles and leaves in rosettes. Calluses pass after three weeks in fresh ground for further development in which every 15-20 days the seedlings are selected, split and transplanted in fresh breeding ground that is realized through subcultures that makes possible the renewal of nutrient components and little plants' proliferation *in vitro* that grow in the vegetative chamber [12]

***Callusing phase and the formation of buds***

Explants of internod stem segment (non-organized tissues), move on phase of callus - genesis using the MS ground in the presence of a high ratio cytokine / auxine (BAP 0.35 mg/l; ANA 0.01mg/l). In this case we have transition to the indirect organogenesis. [4]. Morphogenesis of explants developed at this stage of development goes through three stages associated with cell division and growth.

The culture of internode explants

- Increasing the amount of radical explants and formation of massive callus.
- Formation of the meristemoids on the surface of callus.
- The formation of new bodies from meristemoids.

After 30-40 days are noticed morphological differences related to the cytological developments of the stages in which pass the explants.

•*The first stage* is the formation of callus. After staying in the vegetative room 3-4 weeks, is noticed that in the unorganized explants has been shown friable calluses composed by parenchimatic cells in various forms, vacuoles and undifferentiated nucleus. Callus is formed by cambium cells. But important in the formation of callus are vascular syphedicle cells xylem and phloem.[5].

• *The second stage* of organogenesis is the formation of a compact callus. Organization of regular parenchimatic cell structure is more succinct and specific. Explants in indirect organogenesis since pass through the callus-genesis phase, appeared new forms, the meristemoids on the callus surface . After 4-5 weeks of culture building on callus appear multiple joints. ANA auxine in the feeding ground induces new

forms, and developing buds [1]. • *The third stage* is the differentiation of large cells on the outside and new cells in the inner part of callus. The formation of new bodies in the initial phase is realized in favor of increasing the number and size of meristemoids cells.

• A characteristic of peach explants is the differentiation inside the cell that is expressed in differentiating their color. The tissue forming zone represents initial pro cambium. The structure has two areas of cell growth in its base and top. These areas with small cells represent the base meristemoid and the top of the structure, which provide the beginnings of primary roots, shoots and primary little leafs. In more grown explants distinct the differentiation of tissues. In the focus is the conductor tissue and in the leafy beginning is distinguished the parenchimatic tissue [7].

***Phase of the length of buds***

Nearly after 4 weeks, on the surface of each callus have been shown 1-3 well developed shoots each of 0,6-2,2 cm length. High rapport Cytokine / auxine regulate and promote the formation of pedicles and leaves in rosette. In order further development of buds, the calluses pass after 3-4 weeks on new nutritive ground of callus genesis. Potential embryogene has been reduced by the length of time of culture, but this has been fixed over by passing the callus on the fresh and proper ground. The explants pass on fresh terrain of callus genesis for the development of new buds.

***The culture of apical buds***

The explants-apical leaf bud are organized structure as merystema and move on without going directly organogenesis callus stage. The development of buds begins soon on the ground with relatively high cytokine/auxine [9] and is represented by the appearance of green structures equipped with primary leafs.

**Figure 1.** Developed direct organogeneses

***Proliferation***

By separating the buds from a callus new shoots by micro fission passing the little shoots in a nutrient fresh ground which is rich with salts, vitamins, and hormones. After three months of culture *in vitro* the number of shoots was increased. From morphological point of view the new shoots are formed with uniform leaves, small round and dense for GF-677.

The mean shoot number and mean shoot length per explants of different explants are summarized in Table 1 and 2. The mean shoot length and the mean shoot number was markedly different between different of explants.

**Table 1.** The shoot number of different explants

| Explants | Year I | Year II | Year III | Means |
|---|---|---|---|---|
| Buds | 2.2 | 2.7 | 1.9 | 2.2666b |
| Internodes | 1.8 | 1.4 | 1.1 | 1.4333a* |
| Means | 2 | 2.05 | 1.5 | 1.85 |

DMV = 2.792013 , @ =0.05 according Tukey –Kramer Test.

**Table 2.** The shoot length (cm) of different explants

| Explants | Year I | Year II | Year III | Means |
|---|---|---|---|---|
| Buds | 1.17 | 1.74 | 1.65 | 1.52b |
| Internodes | 0.93 | 1.1 | 0.58 | 0.87a* |
| Means | 1.05 | 1.42 | 1.115 | 1.195 |

DMV = 2.792013, alfa @ =0.05 according Tukey-Kramer Test.

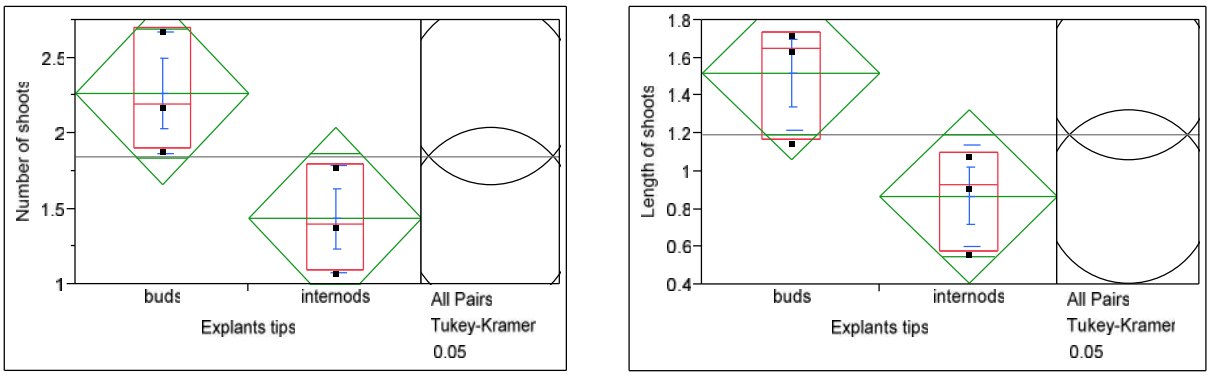


**Figure 2.** The diagram of boxplots (variances, standard deviation and the mean) for the number and length of shoots for explants

## 4. Conclusions

By comparing the two types of explants used in micropropagation turns out that the culture of apical buds has a great advantage compared to the disorganized structures (internodes stem explants). Apical buds as organized structures are developed after 10 days and have directly undergone through the organogenesis.

- The internods as disorganized structures have indirectly undergone the organogenesis- callus geneses, which is represented by beige inflated structure.
- Organized structures as leaf buds give a greater number of sprouts compared to unorganized explants.
- Micropropagation of the peach rootstock GF-677 via the *in vitro* culture is an efficient fast.

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