BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2017. Vol. **14**(4), p. 1395-1399

Propagation of Rootstocks of Gisela 5 Based On Tissue Culture Method

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http://dx.doi.org/10.13005/bbra/2584

(Received: 07 November 2017; accepted: 29 November 2017)

Cherry is one of the most common fruits in the moderate conditions. Common rootstocks like Gisela5 have been used in the gardening field in recent years. The purpose of the current study is investigating the enhancement of Gisela5 rootstock method on the basis of tissue culture. Cutting grafting was used as explant material. These explants included the following materials in the MS environment: 1mg/l IBA (Indole-3-butyric acid), 0.75 mg/l BPA, 1mg/l BPA+ 1 mg/l BPA, 0.75 mg/l BPA + 2mg/l BPA and 1mg/l BPA +2mg/l BPA. The planting was kept in the planting room with 24 ± 2 Oc temperature. The basic environment of MS completed with 0, 1,2,4,6 mg/l NAA during the rooting stage. The maximum number of branches which was 289 was achieved by means of the combination of the following values: 1 mg/l INA+ 0.75 mg/l BAP and 2mg/l IBA +1 mg/l BAP. The maximum length of the branches was 1.69 cm. varieties of concentrations of NAA were added in the rooting stage. The amount of rooting was increased by adding almost 6 mg/l of NAA into the rooting stage.

Keywords: Gisela5, Cherry, Rootstocks, Tissue culture.

Biotechnology is a kind of technology that includes varieties of sciences such as medical, engineering, life sciences and agricultural knowledge. The performance of this technology is on the basis of using the living organisms or their related productions in order to produce materials, products, services, and removing the human needs. The mentioned field is one of the main fields in the world. This kind of method must be used in both developing countries and developed countries. The most important application of biotechnology is in the agricultural section.

Micro propagation

Definition

Tissue culture is the growth of tissues or cells (tissue, fetuses, protoplast, single-celled) separate from the organism. The tissue culture technique is on the basis of totipotency of living organisms especially for plants. The mentioned technique has a vast range of applications in the agriculture industry. The plant cells have been disinfected in the appropriate environment. After that they are kept in the cultured container.

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The culture environment contains the following materials: mineral salts, vitamins, sugar and natural and artificial additives which improve the growth of plant. This kind of culture is called "tissue culture lab".

Tissue culture lab contains a vast range of cultures such as protoplast, cell, and tissue and organ plant. The culture of tissue is a pre-need of genetic engineering. This kind of method has economic advantageous. It also has more efficiency rather than the other methods.

When the traditional methods are not able to supply the demands in order to reproduction the plant materials, modern methods can produce millions plants and flowers. The method of micro propagation has been developed since many years ago.

Types of micro propagation

1. Seed culture: a seed is cultured within the glass and its related conditions. After that, it becomes a mature plant like Orchidaceae.
2. Embryo culture: in this kind of culture, the embryo is separated, and then the seed is cultured.
3. Herbal plant culture: a separated organ is able to grow in the lab environment. Different types of cultures like meristem, root, stem tip and flag are recognizable through the organ culture.
4. Callus Culture: when a cellular differentiation becomes separated, the differentiation of cell mass is produced. This event is called callus culture.
5. Cell culture: it is a kind of unique cells culture which is made by mechanical methods from a plant tissue, callus or cell's suspension.
6. Protoplast culture: Protoplast culture is the aseptic isolation & culture of protoplast in vitro with the obtaining of viable plant.

Importance of cherry

It is a kind of tree with the self- sterilization flowers. The only self-fertile cherry is Stella cherry. The sour cherry tree is completely self-fertile.

Sour cherry-cherry is a kind of fruit which is produced by the hybridization method. Its taste is the same as sour cherry. The time of its maturity is after cherry and before the sour cherry. Its size is larger than sour cherry and smaller than cherry.

History of cherry tree

This plant is derived from the region between the Black Sea and the Caspian Sea. Most of its cultured plants have been derived from Europe. Of course, some of its plants have been reformed in the growing cherry areas.

Botanical of cherry tree

Cherry is from the group of flowering plant with tall height and big leaves. This kind of tree also has shiny thin skin. Another feature of cherry tree is its color which is red or brown. Cerasus avium is its scientific name. The number of its basic chromosomes is 8. It is of the Rosaceae family and avium type. Most of these chromosomes are diploid and others are threedipoid and tetradiploid. There have been varieties of studies in this field. The purpose of this fruit's culture is oil and growth. Therefore, it is important to establish massive farm (Vister &Loni, 1996).

Growth bases of cherry like Gisela, GM 61(Damil), GM 71 (Lnmil), GM 79 (Camil), TableEdabriz, MaXMa14, Colt, Mazzard F12/1 have been applied in the field of agriculture. It is recommended that the clone types of the modern techniques are good enough in order to culture the growth bases. Gradually, the crop rocks became released because of the seed growth and other reasons such as heredity differences, strong growth and late output. Instead, using the recorded stems has been developed as the growth forces and other determined features. There are different reasons for implementing the clonal rootstocks likecontinuity of genotype, uniform population, easy production, preterm fertility because of short periods of infertility in the youth period. Other important reasons of this implication are as the following: environmental features, the fertility of fruit trees, physiological features, the quality of fruits, controlling the growth periods, the possibility of genotype growth (Yilmaz, 1992; Hartmann *et al*, 1997).

According to the statements of Fidank *et al*, 2001, there is not any problem in the process of cultivating the clone of cherry with Gisela5, MaXma 14 and Table Edabase except infection. However, vitrification is a common problem in the process of cultivating.

Kaslina *et al*, 1994, argued that 5 ml/g litre Kinetin and 1 ml/g litre IAAare the most effective growth adaptation for young trees in the laboratory proliferation of avium L Prunus.

Roozik *et al* (2000), stated that Gisela5 has the best growth in MS and MS2. Litwinczuk (2004), emphasized that 2MS environment plays

an important role in order to duplicate the Gisela5. Feucht and Dausend (1979) comprehend that the residues of prunus Avium have strong roots in the following environments: 1mg/l NAA, 1mg/l BAP, and 1mg/ lABA. Fidance *et al* (2001), added 2.55-5.10 ìM IBA to the culture environment in order to achieve 95% of Gisela5.

Methodology

In the current study, the cherries of Gisela 5 and stem plants have been used in order to achieve reliable findings. Annual seedlings have been transformed from the Fruit Research Center to the laboratory. The new seedlings are used in the propagation of cherry in the tissue culture in order to have side grafting. The end side of the plant was prepared about 1 hour under the water faucet in order to remove the phenol features. After that, the separate parts of the culture will mix with 2 drops of Tween and 100 ml water. They were washed by fungicides again. The separated cultures were sterilized during 5 minutes with the concentration 5% to 10% of sodium hypochlorite. The nutrient environment contains vitamins and micronutrient. 30 g/l of Sucrose and 7 g/l Agar were added to the culture environment. The control of propagation phase in the MS environment is through the following materials: IBA + 0.75 mg / l BAP ¡ 1 mg / l IBA + 1 mg / l BAP ¡ 2 mg / l IBA + 0.75 mg / l BAP ¡ 2 mg / l IBA + 1 mg / l BAP. In the rooting phase 0,1,2,4,6 mg/l NAA were added. The Ph was adjusted on 5.7. Almost 40 ml of nutrient materials were in 175 ml bottles. They were kept about 121 minutes under the 1.2 kg/cm2 in autoclave. The transformed separated cultures to the culture environment were kept in 2500 lux, 16 hours lightening and 8 hours darkness. The current study was on the basis of random tests by means of 5 repetitions and 10 expeditions.

Findings

Combinations of the growth system have had remarkable effects in the process of controlling these systems ($p < 0.05$) the effects of adaptations on the number of stems and branch did not have any significant value based on statistics aspects (table1).

It has been cleared that the mean of branches was 2.89 through the control plan of 1.50. The mentioned value was in per 1mg/l IBA+0.74 mg/l BAP. The length of the stem is not the same as hormone concentration. The lowest amount of exudation was 1.22 cm and its maximum value was 1.69 which was related to the 2mg/l IBA+ 1 mg/l BAP combination. The most number of branches and exudation was taken place in the following combination: 2 mg / l IBA + 1 mg / l BAP and 1 mg / l IBA + 0.75 mg / l BAP.

Table 1. The effect of different combinations for IBA and BAP which were implemented during the duplicate level on the pests of cherry- Gisela 5

Hormone Concentration (mg / l)	Number of Exiles (units)	Exit Size (cm)
1 mg/l IBA + 0.75 mg/l BAP	2.89 a ± 1.76	1.67 a ± 0.07
1 mg/l IBA + 1 mg/l BAP	2.61 a ± 0.38	1.55 a ± 0.09
2 mg/l IBA + 0.75 mg/l BAP	2.82 a ± 0.13	1.53 a ± 0.29
2 mg/l IBA + 1 mg/l BAP	2.50 a ± 0.21	1.69 a ± 0.04
Control	1.50 b ± 0.10	1.22 b ± 0.01

Table 2. The effect of different concentrations of NAA in the root making level based on Gisela5 method and spoil cherry

Hormone Concentration (mg / l)	Rootstock (%)	Root number (units)	Rooted plant Length (cm)
1 mg/l NAA	66.66	6.32 b ± 0.29	4.21 a ± 0.11
2 mg/l NAA	36.03	3.25 c ± 0.71	1.09 d ± 0.22
4 mg/l NAA	71.08	3.11 c ± 0.85	1.96 c ± 0.12
6 mg/l NAA	90.83	17.20 a ± 0.19	3.43 b ± 0.28
Kontrol	59.25	4.40 c ± 0.25	3.01 b ± 0.11

The number of roots, branches and the heights of stems were different in the following concentrations: 0, 1,2,4,6 mg/ l NAA (table2). The most amount of root making in the concentration 6 mg/l NAA was 90.83. The minimum amount of root removing was 71.8. The mean of the minimum number of roots was 3.11 in 4 mg/l NAA. The minimum amount of stem's height was determined 1.09 in 2mg/l NAA.

RESULTS AND DISCUSSION

Rouzik *et al* (2000) reported different values of concentrations as follows: 4.4 ìM BAP, 0.5 ìM NAA. 03 ìM GA3 through the blooming phase of cherry by Gisela 5. Sülüþoðlu and Çelik (2003), comprehended that 1mg/ lb BAP and 0.5MG/Lb IBA have the most important result for the exocytosis of 0.5mg/l BAP and 0.5 mg/lb IBA based on the height of the plant. According to the statements of Theiler-Hedtrich and Feuchet (1985), the best environment for the propagation is 1mg/l BAP and 0.1 MG/l. however, Silva *et al* (2003), argued that the best propagation environment is in 0.5mg/ l BAP.

The results of our research are different because of different roots. Our results are the same as Turner and Tang results. These researchers stated that using NAA is more successful than IBA. Similarly, NAA was better than IBA and IAA in the studies of Hepaksoy and Özzambak (1997). Despite the mentioned results, Zimmermann (1981) understood that the low amount of oxin will stimulate the process of root making. However, Zylka *et al* (1992) implemented the 0.5 ppm NAA, and also Anyieka *et al* (1980), implemented 0.2 ppm NAA. The results were important and even so effective.

CONCLUSION

In the present study, the enhancement of Gisela5 rootstock method on the basis of tissue culture was investigated. Cutting grafting was used as explant material. The highest number of branches was achieved when the following values: 1 mg/l INA+ 0.75 mg/l BAP and 2mg/l IBA +1 mg/l BAP were combined. The maximum length of branches was 1.69 cm. The concentration of NAA was an effective factor on root growth. The amount of rooting was increased by adding almost 6 mg/l of NAA into the rooting stage.

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