Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 2, Issue 7, July, 2021

ESTABLISHMENT OF IN VITRO MICROPROPAGATION OF FOX 11 (PYRUS COMMUNIS) ROOTSTOCK

G. G. Eshbekova
Uzbekistan, Samarkand State University, Faculty of Biology

E. K. Bozorov
SAG-Agro private company, Samarkand, Uzbekistan

K. A. Ruziev
SAG-Agro private company, Samarkand, Uzbekistan

Abstract

Pear is the most common fruit crop after apples, and is valued for its delicious taste. Pears are consumed fresh, canned, jam, juice, wine, bekmes (pear honey). In fertile lands, up to 400-500 kg of pear can be harvested from grafted trees on strong rootstocks. The aim of the present study was to improve the micropropagation protocol of Fox 11 (Pyrus communis) rootstock using shoot-tip culture. Murashige and Skoog (MS) basal medium containing 2 mg L^{-1} 6-benzylaminopurine (BAP) resulted highest percentage of shoot forming (80.3 %) and average 8 shoot per explant. On the other hand, basal medium excluding IBA resulted highest percentage of shoot forming (80.1 %) and average 8.5 shoot per explant. Root induction was best in MS medium containing 0.5 mg L^{-1} indole-3-butyric acid (IBA), 0.5 mg L^{-1} α naphthaleneacetic acid, and 10 mL L^{-1} (≈13 mg L^{-1} Fe) ethylenediamine di-2-hydroxyphenyl acetate ferric with 8.4 roots per explant. On the other hand, the longest root (6.5 cm) was obtained from increased concentration to 1 mg L^{-1} of IBA. The establishment of a well-defined micropropagation protocol will lead to further biotechnological improvement of this crop.

Introduction

Pear is the most common fruit crop after apples, and is valued for its delicious taste. Pears are consumed fresh, canned, jam, juice, wine, bekmes (pear honey). Pears grown in Uzbekistan contain 10.8-12.7% of sugar, 0.13-0.30% of acids, about 0.35% of pectin and 0.31% of ash. The climate in Uzbekistan is not conducive to pears, but we still have local varieties that are resistant to drought, heat, disease and pests. The main advantages of pears are their biological adaptability, ie resistance to heat, drought, pests, longevity (70 years and more), durability and productivity. In fertile lands, up to 400-500 kg of pear can be harvested from grafted trees on strong rootstocks. However, diseases and environmental incompatibility are major issues that arise during the cultivation of these trees[1,6,10]. In order to overcome these problems, it is necessary to produce easily propagated and adaptive rootstock of these crops[4,5,7,8,11,14].

Fox 11 (Pyrus communis) originated in the Italy and comprises many varieties with different characteristics. It has medium-low vigour slightly higher than BA29 but slightly lower than Fox16.

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 2, Issue 7, July, 2021

Affinity is very good with the most common Pyrus communis cultivars (Bartlett, Beurre Bosc, Abbe Fetel, Conference). This rootstock is suitable for lime soil with high pH.

Because of the short juvenile period in clonal rootstocks, they start bearing earlier than seedling rootstocks. Therefore, clonal rootstocks are more advantageous than those conventionally propagated [2]. Micropropagation is a useful method for clonal propagation of rootstocks [3]. In the growth of stone fruits by tissue culture, successful results can be expected by the modification of Murashige and Skoog (MS) medium [13]. The diverse concentrations of plant growth regulators (PGRs) and mineral elements affect the in vitro propagation of clonal rootstock[15]. There are limited in vitro studies on the micropropagation of Fox 11 rootstocks.

The aim of the present study was to mass propagate Fox 11 rootstock by means of shoot-tip (ST) culture. The effects of different concentrations of MS medium, as well as PGRs, were tested on in vitro culture of Fox 11 rootstock.

Material and Methods

2.1. Plant material

Fresh shoots of Fox 11 rootstocks were supplied by nursery of SAG-AGRO private company. The explants were cut into single-node segments and surface sterilized by washing under running tap water for 15 min, followed by 70% ethanol for 2 min. Then the explants were rinsed for 20 min in 15% sodium hypochlorite solution containing 1–2 drops of Tween 20. Finally, the disinfected explants were rinsed three times in sterile distilled water for 5 min each and subsequently inoculated onto the culture medium.

2.2. Medium and culture condition

MS and MS basal culture media containing macro and microelements, vitamin, ethylenediamine di-2-hydroxyphenyl acetate ferric (Fe-EDDHA), and different combinations of PGRs were used for shoot induction, multiplication, and rooting of Fox 11. All of the chemicals used in the present study were obtained from Duchefa. The pH of all the media after PGRs were added was adjusted to 5.2 using 0.1 N NaOH or 0.1 N HCl. After the media were dispersed into 2 l glass bottles, autoclaving was performed for 25 min at 121 °C and 15 psi pressure, then were poured into culture vessels. All the cultures were incubated at 25-27 °C under 16/8 h photoperiod with a light intensity of 3500 lux. The explants were transferred from the initiation media to the multiplication media after 2 weeks. Subcultures were done every 4–5 weeks.

2.3. Shoot proliferation and root induction

The sterilized shoots were excised further to approximately 0.5–1.0 cm consisting of the apical bud and 2–3 leaf sketches and transferred to MS medium containing different combinations of BAP (1, 1.5, and 2 mg L^{-1}) and GA_3 (0, 0.025, 0.05, 0.1, 0.25, and 0.5 mg L^{-1}). All the media contained 0.2 mg L^{-1} IBA. The developed shoots were subcultured every 4 weeks. The shoots after attaining a length of 2–3 cm

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 2, Issue 7, July, 2021

were inoculated into the rooting medium. Afterward, the plantlets were completely taken out of the vessels and the number of roots was counted and then they were transferred to the soil (Figure).

Figure. A view of micropropagation of Fox 11 under in vitro conditions, a, b: shooting, c: rooting, d: explant transferred to pots.

Results

Positive and noteworthy results for shoot multiplication and root induction were obtained from the in vitro clonal propagation of Fox 11 clone rootstock. The morphology of the shoots was of high quality and no defoliation or callus-like structures were observed (Figure). The statistical analysis showed that there were significant differences among the 18 MS media containing different combinations of PGRs. According to the results, the average numbers of shoots per explant in 1, 1.5, and 2 mg L^{-1} BAP

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 2, Issue 7, July, 2021

concentration were 8.0, 6.6, and 8.0, respectively, with no significant difference among the three BAP concentrations (Table 1).

Table 1. The effect of BAP concentration on shooting in the in vitro propagation of Fox 11.

BAP concentration (mg L^{-1})	Explants forming shoots (%)	Number of shoots per explant	New shoots length (cm)
1.0	72.4	8	1.3
1.5	85.2	6.6	1.0
2.0	80.3	8	0.9

The MS medium containing 1 and 2 mg L^{-1} BAP gave the best results for the formation of multiple shoots per explant. The numbers of shoots per explant at 0, 0.025, 0.05, 0.1, 0.25, and 0.5 mg L^{-1} GA_3 were 8.5, 5.9, 11.9, 9.3, 8.6, and 9.9, respectively, which were significantly different among its concentration (Table 2).

Table 2. The effect of GA_3 concentration on shooting in the in vitro propagation of Fox 11.

GA_3 concentration (mg L–1)	Explants forming shoots (%)	Number of shoots per explant	New shoots length (cm)
0	80.1	8.5	1.4
0.025	75.7	5.9	0.9
0.05	73.3	11.9	1.0
0.1	84.6	9.3	0.7
0.25	76.7	8.6	0.9
0.5	82.3	9.9	0.9

The average length of shoots per explant in 1, 1.5, and 2 mg L^{-1} BAP concentrations was 1.3, 1.0, and 0.9 cm, respectively. BAP at 1.0 mg L^{-1} was best for higher shoot length per explant according to the statistical analysis (Table 1). Furthermore, the average lengths of shoots per explant at 0, 0.025, 0.05, 0.1, 0.25, and 0.5 mg L^{-1} concentrations of GA_3 were 1.4, 0.9, 1.0, 0.7, 0.9, and 0.9 cm, respectively (Table 2).

The average root length per explant in the concentrations of 0, 0.25, 0.5, and 1 mg L^{-1} IBA was 3.2, 4.1, 4.3, and 6.1 cm, respectively. IBA at 1 mg L^{-1} gave the best results for root length. On the other hand, root lengths per plant in different concentrations of NAA (0, 0.125, 0.25, 0.5, and 1 mg L^{-1}) were 3.2, 4.1, 4.2, 5.5, and 5.8 cm, respectively. Finally, the maximum average root length per explant was obtained from a combination of IBA at 1.0 mg L^{-1} and NAA at 0.5 mg L^{-1} (Table 3).

IBA (mg L^{-1})	NAA (mg L^{-1})	Root length (cm)	Root number	Shoot length (cm)
0	0	3,2	3,4	2.08
0.25	0.125	4,1	4,7	2.75
0.25	0.25	4,2	5,6	1.66
0.5	0.25	4,32	7,3	2.00
0.5	0.5	4,5	8,4	2.00
1	0.5	6,5	5,9	2.25
1	1	5,8	4,2	2.16

ISSN: 2776-1010 Volume 2, Issue 7, July, 2021

Conclusion

Fox 11 (Pyrus communis) rootstock has medium-low vigour slightly higher than BA29 but slightly lower than Fox16. Affinity is very good with the most common Pyrus communis cultivars (Bartlett, Beurre Bosc, Abbe Fetel, Conference). This rootstock is suitable for lime soil with high pH. In Vitro method is one of the best method for propagating Fox 11 rootstock . Murashige and Skoog (MS) basal medium containing 2 mg L^{-1} 6-benzylaminopurine (BAP) resulted highest percentage of shoot forming (80.3 %) and average 8 shoot per explant. On the other hand, basal medium excluding IBA resulted highest percentage of shoot forming (80.1 %) and average 8.5 shoot per explant. Root induction was best in MS medium containing 0.5 mg L^{-1} indole-3-butyric acid (IBA), 0.5 mg L^{-1} α naphthaleneacetic acid, and 10 mL L^{-1} (≈13 mg L^{-1} Fe) ethylenediamine di-2-hydroxyphenyl acetate ferric with 8.4 roots per explant. On the other hand, the longest root (6.5 cm) was obtained from increased concentration to 1 mg L^{-1} of IBA. The establishment of a well-defined micropropagation protocol will lead to further biotechnological improvement of this crop.

References

1.Güney M. (2019) Development of an in vitro micropropagation protocol for Myrobalan 29C rootstock. Turkish Journal of Agriculture and Forestry, 43: 569-575

2.Arıcı Ş.E. (2008). Bazı sert cekirdekli meyve anaclarının doku kulturu ile coğaltılması. Suleyman Demirel Universitesi Ziraat Fakultesi Dergisi 3 (1): 19-23 (in Turkish with an abstract in English).

3.Hossini A.D., Moghadam E.G., Anahid S. (2010). Effects of media cultures and plant growth regulators in micropropagation of Gisela 6 rootstock. Annals of Biological Research 1 (2): 135-141.

4. Kumar N., and Reddy M. P. (2011) In vitro Plant Propagation: A Review. Journal of Forest Science. Vol. 27, No. 2, pp. 61-72.

5. Costa G, Grandi M (1975). The effect of various rootstocks on tree behavior and fruit weight in apricot. Istituto di Coltivazioni Arboree. Universita di Bologna 283: 55-62.

6. Dimassi-Theriou K (1995). In vitro rooting of rootstock GF-677 (P.amygdalus × P. persica) as influenced by mineral concentration of the nutrient medium and type of culture-tube sealing material. Journal of Horticultural Science 70 (1): 105-108.

7. George EF, Hall MA, De Klerk GJ (2008). Plant tissue culture procedure - background. In: George EF, Hall MA, De Klerk GJ (editors). Plant Propagation by Tissue Culture. Dordrecht, Netherlands: Springer, pp. 1-28

8. Hossini AD, Moghadam EG, Anahid S (2010). Effects of media cultures and plant growth regulators in micropropagation of Gisela 6 rootstock. Annals of Biological Research 1 (2): 135-141.

9. Sulusoglu M, Cavusoglu A (2013). Micropropagation of cherry laurel Prunus laurocerasus L. Journal of Food, Agriculture and Environment 11: 576-579.

10.Thorpe T, Stasolla C, Yeung EC, de Klerk (2008). Plant Growth Regulators. 3rd ed, Vol 1. Dordrecht, Netherlands: Springer, pp. 115-173.

11.Vujovic T, Ruzic D, Cerovic R (2012). In vitro shoot multiplication as influenced by repeated sub culturing of shoots of contemporary fruit rootstocks. Horticultural Science 39 (3): 101-107.

ISSN: 2776-1010 Volume 2, Issue 7, July, 2021

12. Fowler, M.R. 2000. Plant cell culture, laboratory techniques. In: Spier, RE. Encyclopedia of Cell Technology. New York: John Wiley & Sons, 994-1002.
13. Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-479.
14. Razdan, M.K. 1993 An Introduction to Plant Tissue Culture. Andover: Hampshire.
15. Osterc, G. and Spethmann, W. 2000. The effect of the mineral nutrition and pH of the rooting substrate on rooting and mineral content of cherry rootstock greencuttings. Phyton. 40: 153-55
16. Jones, O.P. and M.E. Hopgood. 1979. The successful propagation in vitro of two rootstocks of Prunus: the plum rootstock Pixy (P. insititia) and the cherry rootstock F12/1 (P. avium). J. Hort. Sci.
54: 63-66
17. Wang P.J. and Hu, C.Y. 1980. Regeneration of virus- free plants through in vitro culture In: Advances in Biochemical Engineering, 18, Plant Cell Cultures II. Fiechter, A. (Ed.). springer-Verlag, Heidelberg,pp. 61-99.
20. Williams, R.R., Taji, A.M. 1987. Effects of temperature, darkness and gelling agent on long-term storage of *in vitro* shoot cultures of Australian woody plant species. Plant Cell Tiss. Org. Cult. 11, 151-156.