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Genetics and Selection

In vitro Morphogenesis of Loquat (*Eriobotria japonica* L.)

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ABSTRACT. Using the method of micro propagation, conditions of mass obtaining of somatic clones in *in vitro* culture have been established for loquat (*Eriobotria japonica* L.) and the effect of phytohormones on shoot rooting investigated. Different concentrations of synthetic cytokinin - benzylaminopurine (BAP) and auxin-1-naphthaleneacetic acid (1-NAA) and their different combinations were tested. Cytokinin at low concentration (8 mkM) stimulated apical morphogenesis, which was less expressed when adding higher concentrations of BAP – 12-26 mkM or 20-24 mkM to the cultivation medium .

It has been revealed that introduction of auxin together with cytokinin into the cultivation medium caused an intensive callogenesis in the basal part of the main shoot. Callogenesis was more active in the case of 3mkM concentration of 1-naphthaleneacetic acid as compared to 1mkM concentration of the same growth regulator. The latter concentration of 1-naphthaleneacetic acid turned out to be most favorable for the expression of morphogenetic potential of the callus. As to the influence of phytohormones on the rooting of regenerated shoots, indolebutyric acid (IBA) at 5mkM proved to be the most effective of all growth regulators used. The index of rooting of regenerated shoots attained 80-90% in this variant. *© 2009 Bull. Georg. Natl. Acad. Sci.*

Key words: *loquat, clonal micropropagation, morphogenetic potential, benzylamionopurine (BAP), naphthaleneacetic acid (NAA), indolebutyric acid (IBA).*

The micropropagation technique is one of the most significant methods of propagation of economic crops, as it allows mass obtaining of clonal regenerates, thus supplying horticulture with planting material of valuable crop plants [1].

Peculiarities of introduction into culture of loquat (*Eriobotria japonica* L.) have been studied and the morphogenetic potential of clonal micropropagation of the crop has been established.

loquat (*Eriobotrya japonica* L.) is a fruit tree in the family Rosaceae, indigenous to south-eastern Asia. At present the species is distributed in China, India, Japan, USA, Mediterranean countries, western Georgia and other subtropical countries.

Eriobotrya japonica is an evergreen woody plant - large shrub or small tree, with a rounded crown, short trunk and woolly new twigs. The tree can grow to 3-12 m height, but is often smaller, about 3-4 m. Fruit is used as food. Loquat is often grown as an ornamental tree [2].

Explants for the cultures of loquat were taken from adult fruit-trees. Auxiliary and apical buds of sterile cultures and seeds, separated from mature fruits for mass clonal propagation, were applied.

Surface sterilization of apical and dormant buds of vegetating shoots of loquat tree was performed in order to get aseptic culture. Aqueous solutions of Diocide at three different concentrations – 0.1%, 0.2% and 0.5% – were used for this purpose. Time of exposition was 20 minutes. Experiments have shown that 0.2% solution of Diocide was the most efficient, but the output of sterile material was lower than needed. Diocide at 0.1% concentration did not provide satisfactory sterilization, but at 0.5% concentration it caused intoxication of tissues. Due to this we

considered it to be reasonable to use the embryos as initial aseptic material. Sterilization of seeds was performed by means of burning on the flame of spirit-lamp [3-5].

At the first stage of micropropagation the basic Gambourg medium (B5) was supplemented with benzylaminopurine (BAP) at 5mkM concentration. At the next stages of cultivation combinations of different concentrations of benzylaminopurine (BAP) and 1-naphthaleneacetic acid (1-NAA) were added to the medium according to the variant of the experiment.

Subcultivation was performed on every 25^{th} or 30^{th} day. Explant was incubated to light in phytotrone: illumination was 2-3 kLux, photoperiod 16/8 hours, temperature 27 ± 1^{o} C.

The results of the experiment revealed a high germination capacity of seeds of loquat. At the initial stage of cultivation embryo development proceeded at the expense of nutrients accumulated in the seed-lobes. At the next stage formation of shoots and buds was dependent on the ratio and concentration of phytohormones in the cultivation medium. The process of growth was heavily delayed on a medium free of phytohormones. The explant gradually became weaker and finally perished.

Explants have revealed high potential of regeneration of meristemic shoots on the tested medium (Table), but they responded differently to various concentrations of phytohormones in the nutrition medium.

In the case of low content of cytokinins in the medium – 8 mkM – stimulation of apical morphogenesis was observed. Shoot height and average number of internodes totaled 75.1 mm and 10.8 units respectively. Average number of apical buds amounted to 4.0 and that of adventitious buds – 3.6 units (Table).

Increase of BAP concentration in cultivation medium up to 12-16 mkM caused retardation of apical shoots and formation of morphogenic callus of medium size at the basal part of the explant. Later meristemic nodes were formed on the callus which finally developed into green adventitious shoots. Application of cytokinin at higher concentrations (20-24 mkM) caused an obvious delay of apical dominance of shoots and intensive formation of morphogenetic nodes, though development of adventitious shoots from those nodes was hindered. The formed regenerated plants were characterized by thickened organs, namely shoots and leaves.

Regeneration of micro shoots was activated by introducing into the cultivation medium of auxins together with cytokinins. Application of naphthaleneacetic acid at 3mkM concentration caused more intensive formation of callus at the basal part of the main shoot at the addition of the same growth regulator at 1mkM concentration. Morphogenetic potential of the formed callus was realized at both concentrations of synthetic auxin NAA used, but NAA at 1mkM concentration turned out more favorable for the process of realization of morphogenesis potential (Table). As NAA at 3mkM concentration induced more intensive callogenesis than morphogenesis, the coefficient (rate) of propagation in this case was lower as compared to the variant where 1mkM concentration of NAA was applied.

The next stage of micropropagation was rooting. To form roots 30-40 mm high shoots were transferred to the cultivation media containing indolebutyric (IBA) acid and naphthaleneacetic acid (NAA) at 3-5 mkM concentration. All cultivation media, applied in our experiments, provided rooting, but indolebutyric acid (IBA) at 5mkM concentration proved to be the most efficient, giving 80-90% index of rooting. The rooted regenerated plants were then transferred to unsterile conditions. Acclimation of regenerates was performed in conditions of glasshouse. Duration of acclimation was 10-15 days and rate of acclimation amounted to 70-80%. After acclimation the regenerated plants grew well in open ground.

Thus, as a result of our experiments conditions of mass obtaining of somatic clones of loquat *in vitro* culture have been established. Optimum concentrations of

Table

Effect of growth regulators on the induction of microclones (n=50)

Phytohormone, mkmM		Average length of shoots, mm	Average number of internodes	Average number of apical buds	Average number of adventitious buds
BAP	NAA				
8	1	75.1	10.8	4.0	3.6
12	1	31.2	6.1	2.6	13.2
16	1	14.8	3.6	1.0	21.0
8	3	51.5	7.6	3.4	5.0
12	3	22.0	3.5	1.9	16.1
16	3	10.5	2.2	0.9	15.9

phytohormones cytokinin (benzylaminopurine (BAP)) and auxin (naphthaleneacetic acid (NAA)), ensuring the realization of morphogenetic potential of the callus, have been revealed. The effect of phytohormones - naphthaleneacetic acid (NAA) and indolebutyric acid (IBA) on the process of shoot rooting was studied.

გენეტიკა და სელექცია

იაპონური მუშმულას (*Eriobotria japonica* L.) მორფოგენეზი *in vitro* არეში

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ნაშრომში ქსოვილური კულტურის ტექნიკის გამოყენებით შემუშავებულია იაპონური მუშმულას (*Eriobotria japonica* L.) *in vitro* კულტურაში სომატური კლონების მასობრივი მიღების პირობები და შესწავლილია ჰორმონების ზეგავლენა ყლორტების დაფესვიანებაზე. გამოცადეს სინთეზური ციტოკინინის - ბენზილამინოპურინის (ბაპ) და აუქსინის – ნაფტილ-ძმარმჟავას (ნძმ) განსხვავებული კონცენტრაციები და მათი სხვადასხვა თანაფარდობა. ციტოკინინის დაბალი კონცენტრაცია (8 მკმ) ასტიმულირებდა აპიკალურ მორფოგენეზს, რომელიც შესამჩნევად სუსტდებოდა არეში ციტოკინინის უფრო მაღალი (12-16 მკმ) ან (20-24 მკმ) კონცენტრაციით შეტანისას. გამოვლინდა, რომ საკვებ არეში ციტოკინინთან ერთად აუქსინის - ნაფტილძმარმჟავას შეტანა იწვევდა ინტენსიურ კალუსოგენეზს ძირითადი ყლორტის ბაზალურ ნაწილში, რომელიც უფრო აქტიური იყო ნძმ-ს 3 მკმ კონცენტრაციისას, ვიდრე ამავე ზრდის რეგულატორის 1 მკმ კონცენტრაციის გამოყენების შემთხვევაში. ნაფტილძმარმჟავას ეს უკანასკნელი კონცენტრაცია უფრო ხელსაყრელი აღმოჩნდა კალუსის მორფოგენეტიკური პოტენციალის გამოსავლენად. რაც შეეხება ფიტოჰორმონების გავლენას რეგენერირებული ყლორტების დაფესვიანებაზე, ამ თვალსაზრისით გამოყენებული ზრდის რეგულატორებიდან ინდოლილერბომჟავა (იემ) 5 მკმ კონცენტრაციით ყველაზე ეფექტური აღმოჩნდა. მისი გამოყენების შემთხვევაში დაფესვიანების ინდექსი შეადგენდა 80-90%.

REFERENCES

1. *N.V.Kataeva, R.G.Butenko* (1983), Mikroklonal'noe razmnozhenie rastenii, M. (in Russian).
2. *L. A. Gogolashvili* (1976), J. Subtropikuli kulturebi, 3-4 [Subtropical Cultures] (in Georgian).
3. *O. L. Gambourg et al.* **(**1968), Can. J. Biochem., **5:** 46.
4. *A.B.Burgutin* (1991), In: Biologiya kul'tiviruemykh kletok i biotekhnologiya rastenii. M. (in Russian).
5. *A.L.Loginova* (2001), Tezisy dokladov: Issledovaniya molodykh botanikov Sibiri: 34-35. Novosibirsk (in Russian).

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