International Journal of Basic and Applied Sciences. Vol. 5 No. 3. 2016. Pp. 50-54

Full Length Research Paper

The Influence of the different Factors IN Vitro Culture received Plant Regenerants of Georgian Lemon about the Appearance of Roots and Acclimatization

Nargiz Alasania, Dejan Jashi, Leila Ebralidze, Narguli Asanidze
Department of Agricultural Engineering and Technology, Batumi Shota Rustaveli State University, 6010 Batumi, Georgia.

Article history
Received: 17-06-2016
Revised: 21-06-2016
Accepted: 09-07-2016

Corresponding Author:
Nargiz Alasania
Department of Agricultural Engineering and Technology, Batumi Shota Rustaveli State University, 6010 Batumi, Georgia.

Abstract
In the article described the multiplication of Georgian lemon by method of tissue culture, getting the healthy seedling material and the acclimatization of received seedlings. The various methods for rooting the plant-regenerants and also the rooting of plant-regenerants derived from in vitro culture are discussed. It was found that 60% of rooted plants undergoing acclimatization.

Key words: *Callus, Auxins, Georgian lemon, Regenerated plant*

Introduction

The Georgian lemon Citrus limon Burm is an evergreen shrub or tree with leaves. The fruit is elliptical or wide oval forms, the pulp is yellow-green, juicy, and aromatic and quite sour [1]. The fruit has citric acid, which is used during the violation in the organism of metabolism. Citric acid and its compound dissolve uric acid and its precipitation, because advise to treat with lemon the gout. Scurvy, infectious diseases, tonsillitis, diphtheria, thrombophlebitis, malaria, tuberculosis, also are advised to treat the rheumatism disease and to accelerate wound healing.

Georgian lemon except vitamin C contains vitamins for growth**,** antirachitis vitamin [1]. From the Georgian lemon fruits are prepared citric acid, lemon oil, essential oils, there are used for the manufacture of the confectionery products, beverage, candied fruits, sweets, lemonade, etc. The fruit is preserved well and is transportable [1].

Reproduction of the lemon of tissue culture and the getting the massive amount of healthy seedling material, it is great importance for the selection practice, because in vitro culture obtained seedlings are protected from viral infections.

Materials and Methods

The object of study is Georgian lemon Citrus Limon Burm. In a primary explant used sleepy buds vegetative shoots. Surface sterilization for exemption from the microflora was performed with aqueous solution of 0.2% diotsida during 20 minutes [4]. The sterile cultures were placed on the nutrient medium of Gamborg. As hormones an added the naphthyl acetic acid(NAA), indole butyric acid(IBA) and benzylaminopurine(BAP), different concentrates and ratios [3].

The culturing explants were performed on the luminescence lighting, lighting 2-3 kilolux,Photoperiod: 16 hours light and 8 hours darkness with temperatures 27+ -1o C. On the new nutrient medium subcultivation was carried out every 20 days.The experimental study of each variant was carried out during 10-12 weeks [4]. The root plants-regenerants from in vitro medium was transferred to the non-sterile conditions. The acclimation was carried out in the greenhouse.

Results

Receiving of the plants-regenerants for the appearance of the roots was carried out on the nutrient medium Gambor(B5). Different ratios of concentrates 5-15 mkm of the BAP and 0,5-1 mkm of NAA. It should be noted, that the indicated concentrations provided the receiving of the optimum quantity of micro stalks [2].

Table 1 . Comparative Composition of Basic Media

KComponents	Murashige	Composition mg/l Gamborg [5]
I	II	III
Macro salts:		
NH_4NO_3	1650	–
KNO_3	1900	2500
KH_2PO_4	170	–
$MgSO_4.7H_2O$	370	250
$NaH_2PO_4.2H_2O$	–	150
$(NH_4)_2SO_4$	–	134
Micro salts:		
H_3BO_3	6,2	3,0
$Mn\ SO_4$	15,1	8,9
$Cu\ SO_4 .5\ H_2O$	0,025	0,025
$Zn\ SO_4$	4,8	1,1
$Na_2MoO_4.\ 2H_2O$	0,25	0,25
KI	0,83	0,75
$CoCl_2.\ 6SO_4$	0,025	0,025
$CaCl_2.6\ H_2O$	655	213
$Fe\ SO_4.7H_2O$	27,8	28
Vitamins		
Thiamine - HCl	0,1	10
Pyridoxine HCl	0,5	1
Niacin	0,5	1
Myoinositol	100	100
Glycine	2,0	–
Sucrose	3000	3000
Agar	7000	7000

In vitro culture morphogenesis process ends with the appearance of the roots of plants. It was opportunity the rooting the shoots having 3-4 and more lateral buds. For rooting used the nutrient medium of Murashige Skoog , according to the different compositions of salts and vitamins

Table 2. The influence of auxin on the quality of root shoots of lemon IZM NZM IEM

Hormones IZM	NZM	IEM	Duration of rooting (Day)	Percentage indicator of rooting	The number of developed roots on explant culture	The new bud formation
1			50	2	2	-
5			50	3	2	-
10			50	40	5	-
15			40	35	5	-
			40			
	1		40	2	3	+
	5		40	3	3	+
	10		35	70	8	-
	15		35	60	8	-
		1	**30**	**3**	**4**	+
		5	**28**	**3**	**4**	+
		10	**20**	**80**	**10**	-
		15	**20**	**70**	**9**	-

because the full composition causes excessive callusogenesis in the basal part of the bud develops callus mass, that prevents rhizogenesis and if rhizogenesis will still take place, the plants-regenerants in the conditions of acclimatization will be lost, because by the roots soaked nutritive are formed in callus and the plant will not develop normally, because not received the nutritive.

We used several methods for rooting of Georgian lemon:

1. The buds sowed on 1/2 of the agar culture medium without hormones;
2. The shoots were placed in the solution of the high concentration of auxin during 5-10 hours, aseptic conditions, after transferred on 1/2 of the culture medium without hormones;
3. To the auxins added directly the nutritive and on its sowed the shoots

Fig 1. Development of morphogenetic sprouts on ½ B_5 +10µM BAP medium

By using of the first method, it was practically impossible the rooting of microshoots of the Georgian lemon, the percentage of the rooting shoots were 2-3%, it means that it was spontaneous rooting. The influence of synthesized hormones by plant. The duration of the appearance of roots was 60-70 days.

By using of the second method, the developed shoots realized 40-60% of roots, the duration of the appearance of roots was 25-50 days.

The most effective was the third method by this method; it was possible the maximum quantity of micro shoots rooting parallel growth and development of shoots and the appearance of the de-novo buds. The intensity of the appearance of the roots, the morphology of roots and the appearance of sprays depended on the use of the nature of auxins and concentration. The concentrates of indole acetic acid(IAA) were less effective than naphthyl acetic acid(NAA) and Indole acetic acid(IAA).

IAA developed in the basal part of the micro shoots the small size of the callus tissue of the tumor type, where developed the roots system of weak, the thread shaped type. The quantity of the roots was 2-3 units. They were un sprayed and only slightly covered. For the development of the roots needed 50-60 days.

A **B**

Fig. 2. Rooted plant-regenerant A. ½MS +4µ M IEM ; B. ½MS + IEM 6µ M

Almost all used concentrations of the culture medium were the roots of microshoots but above the specified roots system not provided from the soil mineral salts normal development during the acclimatization, after the plants were dying.

The addition of NAA in the nutrient medium developed the stronger roots system. In the basal part of the micro-shoots appeared morphogenic callus tissue, where the intensity of proliferation depended on the concentration of the hormone, than the higher the concentration of hormone was, the callus was more massive. The roots developed from callus and its quantity increased 2-3 units, comparatively with variant of IAA. The adding of the quantity of roots determined the effective process of acclimatization. The development of roots was reduced to 35-40 days. The attention concentration of NAA was 4-6-8, where the percentage of appearance of roots was 60-70%. It is need to mark, that the percentage of appearance of roots decreased at high (8-10) concentration of the nutrient medium that the callus tissue under the influence of auxin transferred to the dedifferentiated division and organogenic potential overwhelmed. This regulatory may be in the case of using the IAA.

For the appearance of roots were the best IBA. The optimal concentration was 4-5mkm.The development of roots were reduced to 20-25 days. The roots were strongly developed; they were strong covered and sprayed. In parallel with the appearance of roots in the basal part were shoots.

Table 3. The influence of various concentrations of Auxins on induction of Callus

Auxin	BBMB B.A.P. M Benzylaminopurine M. K.M.		Kinetin K.M.
	_	2	4	2
1. NZM	_	+	+	+
6	_	+++	++	++
12	_	++++	++	+++
18	_	++++	++	++++
2. IZM	_	++	+	
6	_	++	++	
12	_	++++	++	
18	_	++++	++	
3. 2,4	_	+	+	
D	_	+	+	
6	_	++	+	
12	_	++	+	
18				
4. IEM	_	+	+	+
6	_	++	_	
12	_	+++	++	
18	_	++++	++	++

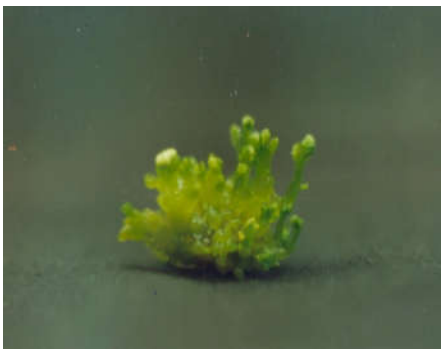

Fig. 3. Callused explant MS +5µM BAP +10 µM NZM

From the developed morphogenic callus de novo the development of adventitious shoots. In some cases, there is the activity of the lateral meristem. The development of such plants-regenerants indicates the existence of the high regenerative ability of the Georgian lemon.

The addition of the IBA concentration in the nutrient medium influenced to the morphology of leaves microshoots. Specifically, it was noticed the narrowing and lengthening of the leaves plate.

Sign was the epigenetic character, at the termination of the hormone's activities the leaf morphology returns to the normal framework. The plants-regenerants with roots transferred for the acclimation in the greenhouse, under the control temperature +-25C. As noted, from the used methods for the appearance of the roots the most effective was the addition the auxins directly to the nutrients and on its to sow the shoots, according to such rule appeared roots of the micro shoots quality were high, although the shoots placed in the solution of the high concentration of auxin during 5-10 hours and after transferred on the culture medium without hormones in the aseptic conditions, it was no less effective method, according to such rule received plants-regenerants with high efficiency was carried out the process to take root with the soil, that cannot say, agar culture medium without hormones induced plant- regenerants about the ability of the root system acclimatization.

The plant-regenerants were planted on area of 35-50 cm. and 15-20 cm. height at the ground of the cultivation boxes, where was 7-8 cm layer height, as the substrate, it was placed the sterile mixture of earth and sand with ratio of 1 to 1. The volume of nutrient of each plant was 5-3 cm, which was enough for the growth and development of plants during 40-50 days.

In the substrate, as the fertilizers, added the mixture of the solution of mineral salts and vitamins 1 for 2 according to the formula of Murashige Skog. After their release from the flask the plants planted quickly and closely, that between the roots and the

substrate is not formed a layer of air. The air prevents the absorption of the water and mineral salts by root. After planting the plants, there wereexpanded boxes, glass or polyethylene, then the plants inside themselves created the microclimate.

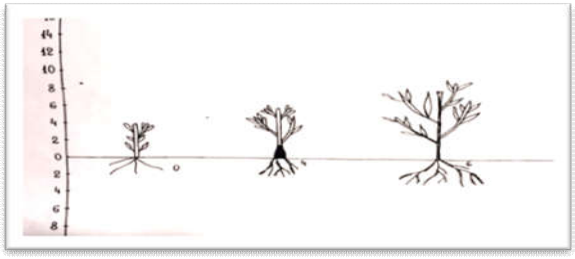

Fig. 4. The schematic image of the development of Lemon plant regenerants in non-sterile conditions during the acclimatization

In 7-8 days after planting, plant selection was carried out with the greenhouse climate, this process called the hardening process, that at first performed once in twenty four hours, on 10 minutes opening the glass or polyethylene, with the purpose of habits of the atmospheric air and humidity reduction. The duration of hardening added every day. In 10-15 days removed completely the cover (the glass or polyethylene). Parallel of the hardening the plants watered according to the formula of Murashige Skog, with the solution of mineral salts and vitamins. Additionally the plants are left during 12-15 days, and then transferred to the open ground.

In the open ground in the first half of the growth and development noted the addition in size of the leaf surface the growth of the root system. After carried out the rapid growth of the shoot parallel grew the crown and the root system of plants

References

1.G. Chkhaidze "Subtropical crops" Vol. 1, 1996.
2.Gamborg O.L. Eveleigh D.E. Culture method and defection of glucanases in cultures of wheat and barley.//Canad.J. Biochem.1968 v.46 N5.
3.Butenko R.G.cell culture. M. Nauka 1983 г.
4. Butenko R.G.Experimental morphogenesis and differentiation in cell culture of plant M. science. 1975.