EFFECT OF DIFFERENT CONCENTRATION OF BENZYLAMINOPURINE (BAP) ON IN VITRO SHOOT PROLIFERATION ALBANIAN AUTOCHTHON PLUM CV SHENGJINE

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***Abstract**: In the recent years in many countries around the world, the "in vitro" cultivation method has a wide use in studying and producing many agricultural crops, ornamental and forest plants. The solution of various problems that arise during the "in vitro" cultivation of plants as is the defining of the medium feeder, citokinins and auxines impact, selection of meristems and the optimum growth conditions, are the foundations of biotechnology today. The purpose of this study is determining of the optimal concentration of citokinins for shoot proliferation. For experimenting were taken from plum plants, cv "shengjine" and as a starting material for performing experiments are used meristems tissue during vegetative period and early branches of the plum during winter. Development of shoots in the specified medium along with other modifications requires that in the field to be added the citokinin – BAP (benzylaminopurine). M-S medium with the ½ of macro & micronutrients was proved with different doses of BAP 0.5, 1, 1.5, 2 mg / l. In the same growth conditions. For the best dose of this phytohormone was judged based on the number of shoots and their length.*

Key words: *BAP, citokinins, "invitro" cultivation, phytohormones, shoot proliferation.*

INTRODUCTION

The growth of plants depends on continuous function of the meristems. Shoot meristems are responsible for all the post-embryonic aerial organs, such as leaves, stems and flowers. It has been assumed that the phytohormone cytokine has a positive role in shoot meristem function. A severe reduction in the size of meristems in a mutant that is defective in all of its cytokine receptors has provided compelling evidence that cytokine is required for meristem activity [1].

In the recent years in many countries the method of "in vitro" cultivation has placed a wide use in studying and cultivation many agricultural crops, ornamental and forests plants. It lies in foundation of the biotechnology today. Usage of 'in vitro' cultures it's apply to the breeding and lustiness of plants, in authentic studies of physiological and genetic, in genetic development of plants, germoplasm storage and production of varying substances [4].

The purpose of this study is: a. Recognizing and adopting techniques of "in vitro" meristems growth. b. To solve various problems that arise during "In vitro" plant cultivation, as, defining the ground feeder, the impact of citokinin and auxines, meristems choice and the optimal conditions of growth. c. To increase the effectiveness of phytohormones using by adding in culture plates natural plant extracts present in plant's meristems.

FACTORS AFFECTING "IN VITRO" BREEDING OF PLANTS

Procedures of plant tissue cultures are developed to a level such that each plant species to be able to regenerate in "in vitro" by means of several methodologies. The pace of recovery of plants in the tissue cultures varies considerably from one species to another.

Various cells, tissues and organs of different plant species can be placed in a tissue culture to regenerate an entire plant [6]. Plant regeneration through “in vitro” cultures can be achieved by using one of these three methods:

1. Embryonic culture 2. Somatic embryogenesis 3.Organogenesis

A number of factors contributes to studies of the plant breeding: Explants choice, Nourishing ground composition, Medium Culture and techniques applied [2, 3].

Cytokinins (CKs), naturally occurring plant hormones that promote cell division, are essential for normal plant growth and development [9, 8]. Since Skoog and Miller demonstrated the ability of CKs to induce shoot regeneration from tissue cultures in 1957, CKs have been implicated in the control of shoot meristem activity [12], but direct evidence for this implication had been provided only recently [13]. Recently, studies have begun to indicate the importance of CK localization in the control of shoot meristem function. This review describes how spatial and temporal distribution of CK activity in the shoot meristem is established through a complex regulation of multiple steps in CK metabolism and signaling. A possible involvement of CKs in the control of meristem identity is also discussed.

In micro breeding citokinins dose determines the rates of proliferation. The objective is to obtain the largest number of branches to be able to undergo further cycles of breeding. Exceeding defined doses arise problems as apical necrosis that leads to explants drying [7, 8].

MATERIAL AND METHODS

"In vitro" cultivation of meristems for the sound plant production requires the provision of sterile working conditions, high acquisition technique, appropriate use of substances. Paramount importance in "in vitro" cultivation has sterilization of soils and plant material, container holder. To have proper sterilization it needs special environments, techniques and tools. The method consists of three important parts.

• Prepare the ground feeder, its sterilization and storage of nutrients.

• Pick up meristems (their size is important), their preparation which includes disinfection and replant them in different medium plates.

• Emplacement of cultures in an appropriate climate for shoot proliferation and replant them to the appropriate medium. Then these saplings cultivate in appropriate temperature in acclimatized green houses where they continue their development. In the experiment are used plants from cv "Shëngjine. As a starting material to perform experiments were used meristems tissues during vegetative growth and early sprig of plum in winter. On this plant material were determined laboratory conditions for seedling production, combined with the use of crude shoot extract to promote proliferation.

PREPARATION OF BIOLOGICAL MATERIAL:

From plum plants were collect meristems tissue during the vegetation period and branches snap in December, which underwent the process of isolation with a size of 0.3-0.7 cm, chemical disinfection (with sodium hypochlorite at concentrations 0.5 % and 1% of active chlorine and mercury chloride ($HgCl2$) 0.1% in different times) and washing (three times with sterilized distilled water). It followed the renewal "in vitro" in the respective mediums. Meristems tissue procedure was performed in sterile conditions.

RENEWAL CONDITIONS OF “IN VITRO” CULTURES IN PLUMS.

The initial plant material chosen after disinfection moved to two different mediums (Murashige-Skoog) M-S and Anderson prepared according to the relevant procedure in sterile glass tubes measuring 150 x 25mm , each test tube with 10 ml ground agar was settled in vegetative room temperature $24 \pm 2\ ^0$C with photoperiodicity 16 hour day/8 hour night,

lighting with white fluorescent lamp with light intensity of 2400 lux. Humidity 80% during 24 hours. To stimulate the emergence of shoots and roots in the appropriate feeding ground were used different doses of BAP and IBA. Also were proved with different doses of crude herbal extract to choose the best dose of it as in the growth of shoots and in the rooting process as well [10,11].

MEDIUM PREPARATION.

Used culture plates were based on Murashige-Skoog and Anderson which supplemented with components of micro & macro inorganic and organic compounds, phytohormones and herbal extract of plum meristems. Growth nutrients were autoclaved at temperature 120 degrees during 20min. Extraction of plant extracts from the plum meristems took place in several periods during the year, to determine best period with higher extract activity. Stimulant effect of herbal extract is provoked with bio-test[10].

Simultaneously studied its interaction with the addition of auxines and citokinins "in vitro" growth. To solve these problems by methodical and biotechnological means, were conducted experiments as following:

TYPE OF DISINFECTANT, DOSE, TIME OF TREATMENT TO GET STERILE MERISTEMS.

In the experiment were chosen plants from cv "Shëngjine" plums., where are collected their meristems in the vegetation period and also the branches in early December. After collection they were subjected to the disinfection process, with sodium hypochlorite (NaOCl) at different concentrations 0.5% and 1% of active Cl as well as mercury chloride HgCl (0.1%). Were experimented different time duration of disinfection. After disinfection plant material was rinsed with sterilized distilled water. After disinfection and sterilization process was deemed about shoot sustainability against chemical disinfectants, on their capacity to survive and to develop in the advanced preparing medium. For shoot cultivation were used several types of (Murashige- Skoog) M-S and Anderson. In each of these mediums were set 20 plants with 4 iteration using random scheme block. Selecting of optimum medium is based on the number of shoots and their length after 4 and 8 weeks. Explants were initially placed in test tube 150x25mm with 10 ml ground agar in the above conditions: temperature 24 +- 2^0C; humidity 80% day-night; light-intensity 2400 lx white fluorescent light.

THE OPTIMAL CONCENTRATION OF CITOKININS IN SHOOT PRODUCTION.

Shoot development in the relevant medium besides other modifications requires that in the medium to be added citokinina BAP. The MS medium with the ½ of macro and microelement was proven with different doses of BAP (benzo[a]pyrene). 0.5, 1,1.5, 2 mg / l, with unchanged growth conditions. For the best dose of this phytohormone was judged based on the number of shoots and their length.

RESULTS AND DISCUSSION

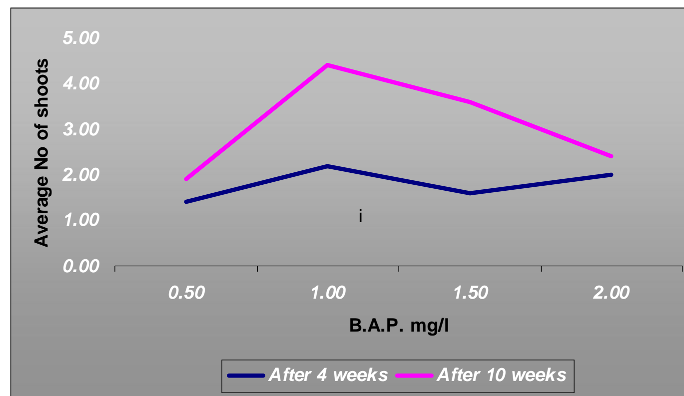
Effect of different doses of BAP in the shoot proliferation. MS medium supplemented with ½ macro- micro element and dose of 1.0 mg/l resulted the most effective in shoot proliferation, giving an average number of shoots 2.5 and their average length of 2.88, while after 10 weeks we have these results: The average shoot length of 3.85, their average number of 4.64, which is given in Table No. 1.

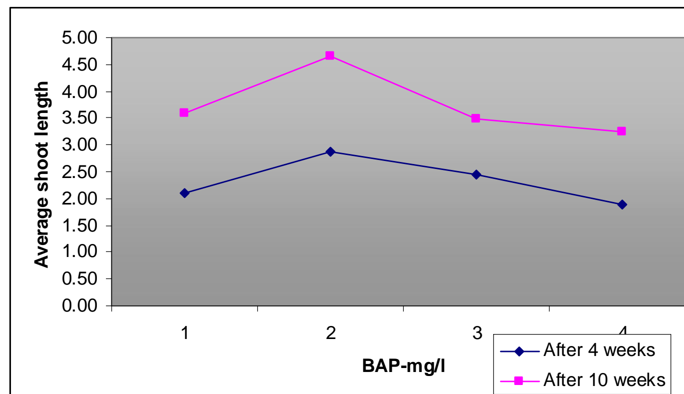
Table 1

Effect of different doses of BAP in the shoot proliferation of the cv. "Shengjine" plum.

	Variant	P 1		P 2		P 3		P 4		Average	
	BAP mg/l	Nr.	Gjat.	Nr.	Gjat.	Nr.	Gjat.	Nr.	Gjat.	Nr.	Gjat.
After 4 weeks	0.50	1.40	2.10	1.90	1.90	1.78	1.80	1.95	2.30	1.76	2.03
	1.00	2.20	2.86	2.60	2.58	2.40	3.20	2.80	2.90	2.50	2.89
	1.50	1.60	2.44	1.80	2.30	1.80	1.84	2.00	2.12	1.80	2.18
	2.00	2.00	1.90	2.00	2.14	2.20	1.64	2.10	1.30	2.08	1.75
After 10 weeks	0.50	1.90	3.60	2.10	3.88	2.30	2.40	2.30	3.10	2.15	3.25
	1.00	4.40	4.66	3.40	4.98	3.40	3.72	4.20	5.22	3.85	4.65
	1.50	3.60	3.48	3.60	3.30	3.60	3.24	3.20	3.58	3.50	3.40
	2.00	2.40	3.24	1.80	3.58	2.66	4.86	2.73	2.66	2.40	3.59

Graphic gives clearly the variation of the effect of BAP as a citokinin with visible effect in stimulating cell division and therefore increase the number of shoots for each explant. (See chart No. 1).





Further increase the dose of BAP causes reduction of the effect of shoot proliferation. Differences between variants were verified with P= 0,01 for the second variant dose1.0mg/l B.

CONCLUSIONS

Vegetative meristems used for "in vitro" breeding in cv "shëngjine" plum result as an effective initial plant material. Sterilization of plant material before planting in culture plates with 0.1% $HgCl_2$ for 3x1.5min or NaOCl at 1% active chlorine for 3x1 min +2.5 min and the highest percentage of explants, respectively 70.3% and 56%. Sterilization of the environment and work tools with alcohol 95% and the mediums in the autoclave for 20 min at 1 atm pressure and temperature 115 degrees C ensures the elimination of fungal and bacterial infection in our cultures. Nourishing medium Murashinge-Skoog modified with ½ of macro and micro elements is suitable for proliferation of the initial material placed in it. The best dose of BAP (benzo[a]pyrene) for shoot proliferation from meristems is that of 1.0 mg/l. The work done in terms of increase of planting through the "in vitro" cultures will serve not only as a way of breeding but also as a means of producing flourishing material. Phyto-sanitary status should be evaluated not only for the presence of fungi and bacterial infection but also the viruses. Evaluation of crude extract derived from the meristems requires a determination of inflammatory substances present in it by biochemical methods as chromatography or electrophoresis.

RECOMMENDATIONS

- For "in vitro" breeding of cv. Shëngjine plum recommended to use MS medium with ½ of macro and microelements at 1mg/l BAP for the shoot proliferation.
- For disinfection of plant material taken directly from the field before placed on the culture recommended to use 0.1% $HgCl_2$ for 3 times in 1.5 min or chlorine 1%, 3 times 1+2.5 min.
- Room conditions in vegetative growth, are recommended to be kept in these boundaries;

Temperature: 22 +-2c
Light: fluorescent white 2400lux
Relative humidity: day / night 80%
Photoperiod day / night: 16 / 8 hours

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