STUDIES CONCERNING THE UTILISATION OF "IN VITRO" MICROPROPAGATION IN THE CONSERVATION PROCESS OF SOME VALUABLE GENOTYPES OF PEPPER - *CAPSICUM ANUUM L.*

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Abstract. *Plant tissue cultures offer a powerful tool which might accelerate the production of pepper plants with desire quality traits and resistance to biotic and abiotic stresses. The success of these efforts depends on an efficient "in vitro" plant regeneration system and genetic stability of regenerated plants.*

The present study aimed at establishing a rapid and efficient method for the micropropagation of some valuable genotypes of pepper. Many concentrations and combinations of growth regulators were used to define an efficient regeneration medium. The best reaction - direct bud formation was observed on explants cultured on MS medium added with BAP. The combination of BAP and NAA increased the percentage of organogenesis and the development of the explants. While, in MS medium, added with NAA alone we obtained the development of the embryos to a complete plant and no adventitious buds were observed.

Rezumat. *Cultura de celule şi ţesuturi „in vitro" reprezintă în momentul actual o unealtă puternică care poate determina, accelerarea producerii de noi genotipuri de ardei cu trăsături calitative superioare sau cu rezistenţă la factorii de stres abiotice şi biotici, Succesul acestor eforturi depinde de dezvoltarea unui sistem de regenerare "in vitro" eficient care să permită obţinerea unui număr suficient de plante, în strictă dependenţă de obiectivul propus spre rezolvare, dar în acelaşi timp să menţină stabilitatea genetică a plantelor regenerate.*

Studiul de faţă işi propune determinarea stabilirea unei metode eficiente şi rapide de micropropagare a unor genotipuiri valoroase de ardei. În scopul identificării mediului cel mai efficient au fost testate numeroase concentraţii şi combinaţii de fitohormoni. Cele mai bune rezultate obţinute pentru inducerea lăstarilor a fost obţinut pe varianta de mediu Murashige Skoog, 1962 suplimentată cu BAP. Combinarea BAP-ului cu NAA a determinat o creştere a procentului de organogeneză şi dezvoltare a lăstarilor. Mediul MS suplimentat cu NAA singur nu a permis obţinerea de plante noi ci doar evoluţia explantului iniţial către plantă complet dezvoltată cu rădăcini.

INTRODUCTION

Plant genetic resources in agri-horticultural crops are of immense value to mankind. The plant breeders require large resources of genetic variation (genepools) for crop improvement. The bigger variation is, the better are the chances of finding particular characters, such as resistance genes for diseases, pests and nematodes or for adaptation to wider ecological amplitudes and stress conditions.

However, in the wake of spread of high yielding varieties, this genetic variability is gradually getting eroded. This situation leads to increasing demands for suitable actions in order to conserve the existing germplasm resources. Even more, the big proportion of the genetic erosion from now a day, impose immediate measures that should conserve the germplasm in such a manner that there are minimal losses or changes in genetic variability of the population

Recent years have witnessed significant advancement in researches demonstrating increasing applications of *in-vitro* techniques in the genetic conservation of germplasm resources. *In-vitro* techniques now provide suitable approaches, which can lead to the safe conservation of germplasm.

In the same time these techniques offers the possibility of a rapid multiplication of the valuable breeding material with the sureness of the preservation of the initial donor material. The multiplication technique in vitro allows the rapid multiplication of the plants as well as the individual clonation of the selected exemplars, which are presenting excellent qualities impossible to maintain through sexual multiplication. By the means of these techniques the process of multiplication can be associated with obtaining of virus – free plants.

Until now was reported: organogenesis from many explants (Agrawal eat al., 1989, Szasz et al., 1995), somatic embryogenesis from anther and immature zygotic embryos (Binzel et al., 1996) and even GMO (through Agrobacterium tumefaciens) – Kang et al., 1998. But until now there were no reports concerning the regeneration protocols for plant regeneration to Romanian pepper genotypes.

MATERIAL AND METHODS

The biological material is represented by seeds belonging to three genotypes – CERES, FERRARI F_1 and FIESTA F_1 , originated from the Vegetable Research and Development Station Bacau seed collection. The seeds were surface sterilized by immersion in mercuric chloride solution (HgCl2) 0.1% for 10 minutes, followed by repeated washing with sterile distilled water. The sterile seeds were cultivated on a basic medium Murashige Skoog, 1962 without hormones.

After 12-15 days the seeds germinated and the plants were used as donor source of explants – figure 1. The apexes were cultivated on 5 medium variants (table 1) derived from Murashige Skoog, 1962 and supplemented with 3% sucrose and solidified with 0.8% agar. The pH of the medium was adjusted at 5.8 before autoclaving at 121^0C for 20 minutes. The cultures were then incubated under controlled conditions (temperature 24^0C, light intensity of 2000 lux, 16-h photoperiod and 80% air humidity).

Figure 1 – Germinated seeds used as donor source for explants

The morphogenetic response of pepper explants to hormones types and concentrations in nutritive mediums was expressed as a number of explants which formed adventitious buds and where shoots differentiated.

Table 1

Variants of nutritive medium with different hormonal factors utilized for "in vitro" regeneration

Components	A_1	A_2	A_3	A_4	A_5
Macroelements	MS	MS	MS	MS	MS
Microelements	MS	MS	MS	MS	MS
Vitamins	MS	MS	MS	MS	MS
BAP	5.0	2.0	-	-	-
KIN	-	-	5.0	2.0	-
NAA	1.0	-	1.0	-	2.0
IBA	-	1.0	-	1.0	-
Sucrose	30 g/l	30 g/l	30 g/l	30 g/l	30 g/l
Agar	8 g/l	8 g/l	8 g/l	8 g/l	8 g/l
pH	5,8	5,8	5,8	5,8	5,8

After the development of the shoots the cultures were transferred to differentiation mediums that contained BAP and NAA in lower concentrations as well as GA3.

Well developed shoots were transferred on the rooting mediums that contained only NAA or without growth regulators.

After 2 weeks, the rooted plants were acclimatized and planted in a potting mixture of sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for acclimatization during 2 weeks before transfer to green house.

RESULTS AND DISCUSSIONS

The data were taken every week after the inoculation of the explants on the cultivation medium. Over the first four weeks of culture, the ability of shoots regeneration was very low. Depending on the genotype, the number of explants with shoots amounted to 0.3-5.0 on average (table 2).

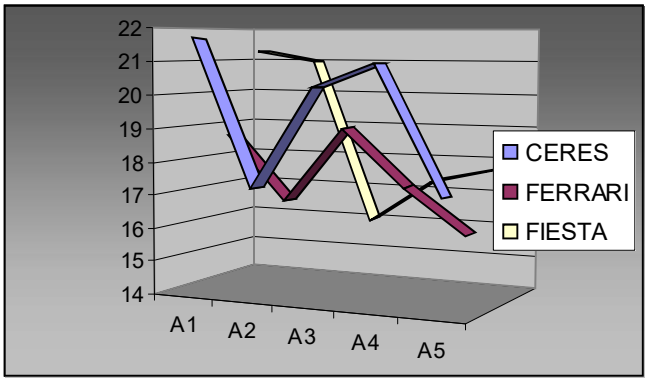
Table 2

The regeneration percent at pepper genotypes

Variant	CERES		FERRARI		FIESTA	
	Number	%	Number	%	Number	%
A1	21.7	90.4	18.7	77.8	21.3	88.9
A2	17.3	72.2	16.7	69.6	21.0	87.5
A3	20.3	84.6	19.0	79.2	16.0	66.7
A4	21.0	87.5	17.3	72.2	17.3	72.2
A5	17.3	72.2	16.0	66.7	17.7	73.6

* number is the means for 3 repetition, each repetition comprised 25 explants

The graphic 1 presents a schematic representation of the genotype behavior on the tested variants.



Graphic 1 - The genotype response on the tested nutritive variants

Direct bud formation was observed only on the variants A1 and A2, characterised by the presence of BAP. The combination of BAP and NAA increased the percentage of organogenesis and the development of the explants. Optimum values for bud induction from zygotic embryos were obtained in MS medium, supplemented with 5 mg/l of BAP and 1 mg/l of NAA (Table 1). One hundred percent of explants cultured on this medium turned green and showed a good differentiation: cotyledons spread and became large, hypocotyls reached an average of 1 cm to 1.5 cm in length. Since 6 days of culture we observed the emergence of some leaves and globular structures on the embryo explants without any intervening callus (Fig. 2). Continuous and asynchronic growth of buds was formed and the responses to organogenesis varied between cultured explants (from 3 to 19) with a mean rate of 7.5 per organogenic explant.

Figure 2: Globular structures and shoots developed on the apex basis

The substitution of NAA with IBA in combination with BAP initiated callus formation without regeneration. The regeneration medium MS with KIN was also not effective. The shoots, thus formed, were then transferred on a differentiation medium containing BAP, NAA and GA3 that allowed its further development and also the development of new shoots (fig. 3). The adition of GA3 in the medium improved the development of the shoots.

Full grown shoots were cultivated on rooting medium for the development of the roots. The most efficient medium was the medium containing NAA in concentration of 1 mg/l (fig. 4).

Figure 3 – New shoots developed at the basis of the initial explant

Figure 4 – Well developed plants with roots

After 2 weeks, the rooted plants were acclimatized – figure 5 and planted in a potting mixture of sterilized sand + vermiculite (1:1 ratio) in plastic cups, hardened in a mist chamber (80% relative humidity) for 2 weeks before transfer to green house.

Figure 5: Plants on acclimatization stage

Figure 6: Rooted plants prepared for planting

Figure 7 – Plants on potting mixture

CONCLUSIONS

In vitro plant regeneration depends on the genotype and explant source. Exogenous growth regulators are important for the expression of this capacity.

The results obtained, indicates the fact that bud induction is strongly depending on the cytokinins, more precise on the presence of the BAP in the medium. The combination with NAA improves the regeneration respond of the explants. When replacing the NAA with IBA the formation of the buds was inhibited.

If the cytokinins is represented by KIN, the induction and elongation of the adventitious buds is very low or even absent.

On A1 and A2 variants the shoot regeneration did not involved callus and by this it decreases the probability of somaclonal variation.

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