IN VITRO SCREENING OF GERMINATION CAPACITY OF SEEDS AT SOME IMPORTANT VEGETABLES GENOTYPES PRESERVED IN SEED BANKS

REALIZAREA UNUI SCREENING IN VITRO PRIVIND CAPACITATEA GERMINATIVĂ A SEMINȚELOR UNOR GENOTIPURI IMPORTANTE DE LEGUME MENȚINUTE ÎN BĂNCI DE GENE

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Abstract. *Conservation of seeds in gene banks for long periods has the disadvantage of loss in germination capacity. Culture of seed or embryos in vitro is a method to overcome this limitation by forcing hormonal seed germination, resulting in direct production of plants. The experiments carried out in developing this paper targets to determine the optimum in vitro temperature and culture medium for the germination of seeds at different important genotypes of vegetables.*

Key words: vitro, cultivation, tomatoes, pepper

Rezumat. *Conservarea semințelor în bănci de gene pentru perioade lungi de timp are dezavantajul de pierdere a capacității de germinare. Cultura semințelor sau a embrionilor in vitro, este o metodă care permite depăşirea acestei limitări prin forțarea germinării semințelor prin diferite metode rezultând în producerea directă a plantelor. Experimentele realizate pentru dezvoltarea acestui deziderat s-au concentrat pentru a determina valoarea optimă a temperaturii şi mediului de cultură in vitro pentru germinarea semințelor la diferite genotipuri importante de legume.*

Cuvinte cheie: vitro, cultivare, tomate, ardei.

INTRODUCTION

The aims of applied plant science research for agriculture are to enhance crop yields, improve food quality, and preserve the environment where human beings and other organisms live. The best way for conservation of plant biodiversity and its environment, would be to achieve high crop productivity per unit area.

Genetic improvement through biotechnology needs conventional breeding because (1) the elite cultivars will be the parents of the next generation of improved genotypes, (2) field testing across locations or cropping systems and over years will be needed to determine the best selections due to the genotype-by-environment interaction.

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It is well known that conservation of seeds in gene banks for long periods has the disadvantage of loss in germination capacity. Some collected seeds although valuable due their genetic dowry, can be characterized by their low viability. The viability of the seed is affected by the conditions before harvest, the maturity, the drying conditions, as well as physical factors (temperature, humidity) during the storing (Dilday *et al.*, 1994; Blackman *et al.*, 1996). During storage, the seeds begin to deteriorate and their resistance to environmental factors during germination and growth decreases at the seedling stage (Dilday *et al.,* 1994). Culture of seed or embryos in vitro is a method to overcome this limitation by forcing hormonal seed germination, resulting in direct production of plants. This method is also a powerful tool to define crop evolution and gathering new knowledge. Such information should be incorporated into genetic enhancement programmes, especially those with an evolutionary breeding scheme. Likewise, plant ideotypes for each crop should drive the work of plant breeders.

Thus, the experiments carried out in developing this paper targets to determine the optimum "in vitro" temperature and culture medium for the germination of seeds at different important genotypes of vegetables.

MATERIAL AND METHOD

The biological material is represented by seeds belonging to two genotypes – one hybrid and one variety, for each species (tab. 1), originated from the Vegetable Research and Development Station Bacau seed collection.

Table 1

The vegetables genotypes tested concerning their germinative capacity

Nr. crt.	**Genotype**	**Specification**
	Tomatoes	
1.	Bersola F1	Hybrid
2.	Siriana	Variety
	Pepper	
3.	Apollo F1	Hybrid
4.	Dariana Bac	Variety
	Cabbage	
5.	Flavius F1	Hybrid
6.	CO-BCO 7-9	Inbred line

The seeds have lost partial or total germination but have particular relevance for breeders due to the stock of genes that contain it. The seeds were surface sterilised by immersion in mercuric chloride solution ($HgCl_2$) 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 and Quoirin – Leproive, 1977, supplemented with different plant growth regulators (tab. 2).

Table 2

Components of different nutrient media for in vitro seed germination

	GI	G2	G3	G4	G5	G6	G7	G8	G9	G10
Macro-elements	MS 1*	MS 2*	MS3*	QL	MS 1*	MS 2*	MS 3*	MS 1*	MS 2*	MS 3*
GA3	-	-	-	-	0.3	0.6	0.8	-	-	-
KIN								0.3	0.6	0.8

*MS1 - MS 100% concentration, MS2 - MS 75% concentration, MS3 - MS 50% concentration.

Under aseptic conditions, seeds were inoculated on basal medium containing 3% (w/v) sucrose and 0.8% agar. The pH was adjusted to 5.8 and autoclaved at 121°C (1.06 kg/cm^2) for 25 min.

The cultures were incubated in culture chambers in dark and then transferred in conditions with controlled light, humidity and temperature control at 25^{0}C, a 16-h photoperiod, and 5000 lx light intensity. Repeated sub cultures were done at an interval of 30 days and incubated under the same temperature as mentioned previously. The culture vessels showing signs of contamination were discarded. Day to day observation was carried out to note the responses.

For each genotype, 10 seeds were cultured and the experiment was repeated three times. The data were analyzed by ANOVA (analysis of variance), the means were compared with the control (seeds germinated in pots) using the Duncan multiple comparison test at $P < 0.05$.

RESULTS AND DISCUSSIONS

Unlike the other species addressed in this study, tomato seed germination is positively influenced by a concentration of 100% of the amount of macro and micronutrients (fig. 1, fig. 2). Furthermore, kinetin has been found to support the reaction rate of the seeds of the tomato. The best results were obtained on variants G6 - G10, with the point on the G6 version. Seed germination is achieved gradually over the course of 7-14 days, most seeds are germinated in the ninth day (tab. 3).

Fig. 1 Tomatoes seeds on culture media “in vitro”

Table 3

The seed germination of tomatoes genotypes tested in vitro culture media 14 days after inoculation

Nr. crt.	Variant	Genotype Charlotte F1	Genotype Dacia
1.	G1	55.70	55.90
2.	G2	53.19	52.60
3.	G3	50.30	49.90
4.	G4	30.90	35.30
5.	G5	59.70	59.40
6.	G6	59.20	58.90
7.	G7	57.90	56.80
8.	G8	57.60	58
9.	G9	56.80	57.10
10.	G10	55.30	57.20

Seed germination of pepper – fig. 2 ,was carried out over a period of 8-14 days with a peak on the tenth day. Control variant, represented by germination of seeds in conditions "ex vitro", on the moistened filter paper, recorded low levels of germination, significantly below those obtained on media culture "in vitro", 12.6% at Apollo F1 genotype and 21.03% at Dariana Bac genotype, , while cultivated “in vitro” condition, 79.10% and 79.7% respectively of seeds germinated and reached the seedling stage.

Fig. 2 Pepper seeds on culture media “in vitro”

Table 4

The seed germination of pepper genotypes tested in vitro culture media 14 days after inoculation

Nr. crt.	Variant	Genotype Apollo F1	Genotype Dariana Bac
1.	G1	79.10	79.70
2.	G2	77.20	78.00
3.	G3	76.16	77.70
4.	G4	49.20	42.90
5.	G5	46.50	45.30
6.	G6	67.80	68.60
7.	G7	63.20	59.10
8.	G8	70.20	69.90
9.	G9	71.60	70
10.	G10	70.90	79.10

The cabbage seeds of the tested genotypes germinated four days after of their inoculation on media variants tested. As the emergence of young plants, the cultures were transferred to the light, so in addition to pursuing the percentage of germination and seed germination dynamics over time was monitored.

The speed of germination of the hybrid Flavius F1 was higher compared to that of the inbred CO-BCO7-9 (see table above). The dynamics of seed germination has also increased in both genotypes, the percentage reached 68.9% in the first genotype (Flavius F1) and 65.7% in the case of the second (tab. 5).

Table 5

The seed germination of cabbage genotypes tested in vitro culture media 4 days after inoculation

Nr. crt.	Variant	Genotype FLAVIUS F1	Genotype CO-BCO7-9
1.	G1	67.70	65.50
2.	G2	65.19	62.00
3.	G3	68.90	65.70
4.	G4	35	23
5.	G5	69.50	45.30
6.	G6	57.58	43.60
7.	G7	52.60	59.10
8.	G8	50.20	58.20
9.	G9	69.60	58
10.	G10	50	58.10

CONCLUSIONS

The experimental results demonstrate the high efficiency of "in vitro" culture in the maintenance and recovery of seed germination at tested genotypes. This is highly important, due to the fact that in gene banks, seeds can lose vitality.

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