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IN VITRO MICROPROPAGATION OF POTATO (*Solanum tuberosum* L). CULTIVARS

SUMMARY

In vitro micropropagation is an alternative to conventional (vegetative) propagation of potatoes whereas aseptically meristem cultures were used which gave pathogen free plants. Different sterilization protocols were used for disinfecting the isolates potato sprouts from two potato genotypes named Excuisita and Bergerac. After 35-40 days of culture shoot height, number of shoots per explants, and number of roots were measured. It was found that the suitable sterilization protocol giving high percentages of survived individuals was that of 1% $HgCl_2$. The sterilized sprouts were cut to isolate apical meristems which were cultured on shoot induction medium containing solidified MS media with vitamins and exogenous plant growth regulators and incubated at optimized culture conditions in room culture. The cultivar Bergerac showed greater ability for *in vitro* propagation with 6.3 shoots per explants but Excuisita plantlets presented higher shoot length (72.5 mm). The aim of the study was the presentation of suitable protocol for *in vitro* induction of potato plantlets stocks free of pathogens.

Key words: *In vitro, meristem cultures, cultivar, MS media, shoot length.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a very important crop in agricultural production of our country and around the world. According to FAO data it is grown in 180 countries worldwide but mostly in Asia, then in Europe; South and Central America. The beginning of potato cultivation in Albania dates 100 years. Today in the Republic of Albania potatoes are propagated on over 9500 hectares and almost every year the area expand with an average yield of 20t/ha.

Micro propagation is the alternative to conventional propagation of potatoes. *In vitro* propagation methods using nodal cuttings, meristem tips and micro tubers are more reliable to maintain genetic integrity of the multiplied clones since differentiation and the subsequent organogenesis/embryo genesis with the accompanying genetic changes have been reported (Wang and Hu 1982). The potential value of tissue culture in potatoes production has been used for disease free seed production in many countries. Seed production technique of

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potato can be designed with *in vitro* multiplication through either plantlet regeneration or micro tuber production (Hossain M.J.2005).

The main goal of this research was to set up a culture of meristem tips cultures as initial explants of two potato cultivars Excuisita and Bergerac in *in vitro* conditions. Explants development, organogenesis stage of explants on MS media supplemented with growth regulators and possibilities for minituber producton from potatoes plants were followed during this experimental work.

MATERIAL AND METHODS

The experiment was conducted at the Institute of Plant Genetic Resources, (Agriculture University of Tirana). Potato cultivars Excuisita and Bergerac were used as plant material for evaluation on their response to *in vitro* regeneration. Clean tubers were treated with 2ppm GA_3 for rapid sprouting. One week old sprouts were used as initial explants.

Sterilization of initial explants-sprouts:

The sprouts of about 0.5-1cm were surface sterilized by washing under flow of tab water for 15-30 minutes. In this experiment four different sterilization protocols were used.

After washing the sprouts are surface sterilized by dipping in:

-70 % C_2H_5OH for 30 seconds

-mercuric chloride ($HgCl_2$) solution 0.1%, 0.5% and 1%

-washed several times with autoclaved distilled water

Another chemical solution used for sprouts sterilization was sodium hypochlorite (10%). All the glassware and instruments were thoroughly washed and dried at 80°C. Distilled water and glassware used for explants were autoclaved for 20 minutes.

In vitro culture conditions:

The initial explants were cultured in Murashige and Skoog (Murashige and Skoog 1962) solid medium (pH=5.8) in test tubes supplemented with plant hormone. MS media was prepared with 3% sucrose, 0.6% agar, 2ppm Ca pantothenic acid and 0.25ppm GA_3.

The cultures were placed in culture growth room under the following conditions:

-temperature 25 ±1°C

-relative humidity 50%

-photoperiod 16/8 hour light/dark and

-illumination of 50µmol·m^2·s^{-1}.

The observations were recorded regularly till to 30 days for the non-growing cultures, infected cultures and healthy cultures. Data recorded during proliferation and multiplication phases, of biometric parameters were statistically elaborated using ANOVA analyses.

RESULTS AND DISCUSSION

The influence of gibberellin acid on stimulation of sprout formation was positive. The treatment with 2ppm GA_3 was efficient for the two potato cultivars. All treated tubers resulted with *de novo* a sprout, wish shows effect of 100% in sprouts formation. A higher number of sprouts are formed from the cultivar Excuisita with an average of 8.21 sprouts per tuber (Figure.1).

Table 1. The effect of GA_3 treatments on *in vitro* sprout formation in potatoes tubers *in vivo*

Cultivar treated with 2ppm GA_3	*Number of treated tubers*	*Nr. of sprouts obtained per tuber*	*Sprout formation %*	*Length of sprouts ± mm*	*Sprout colour*
Excuisita	20	8.21±0.5	100	9.07±1.0	Green
Bergerac	20	7.78±0.5	100	7.33±0.5	Pink

Figure 1. The effect of GA_3 for rapid sprout formation

Effect of sterilization treatment:

Results shows that between two chemical solutions $HgCl_2$ was found more effective than NaOCl. All the explants of two potato cultivars of our study, treated with sodium hypochlorite solution, resulted infected (100%). While mercuric solution gave positive results in Excuisita and Bergerac explants with a high survival rate of 57 %.

Our results were higher from those reported from other authors (Kanwal *et al*. 2006, Badoni *et al*.2010, Liljana *et.al*. 2012). During the first phase of in vitro propagation (proliferation), explants inoculated on MS Strong media supplemented with pyhtohormones, reacted positively giving high results on germination (Figure 1).

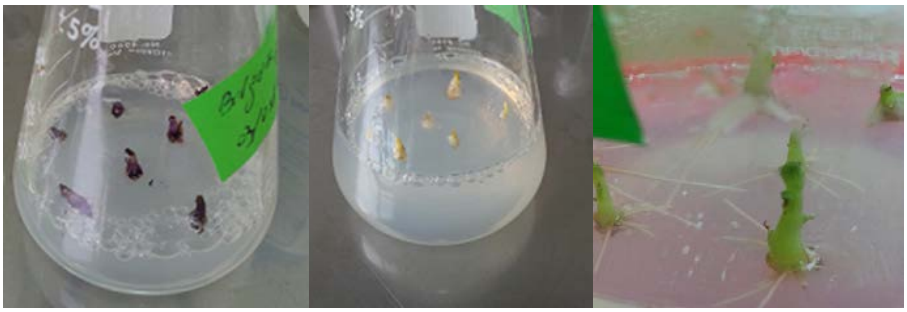


Figure 2. Explants proliferation in MS media

Between two cultivars used, Excuisita explants gave 96.34% of germination on agar media 0.6% and Bergerac cultivar 85.5% of explants germination. The germinated shoot tips produced plantlets with normal morphological development.

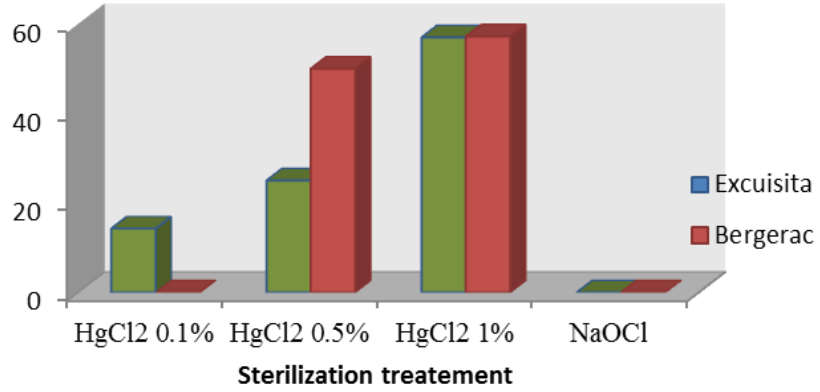
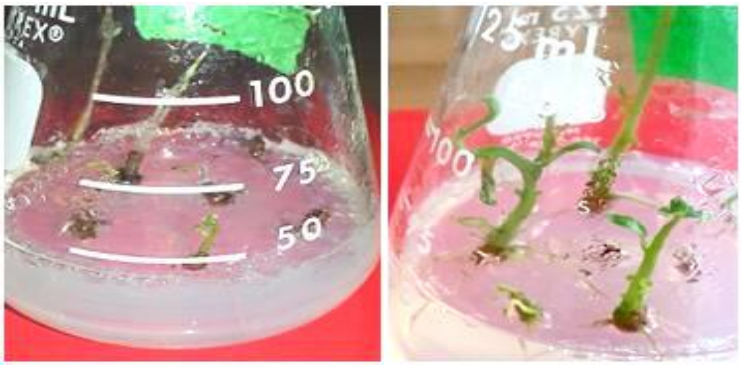


Figure 3. Effect of sterilizaton treatment on potatoes explants

The effect of MS media on *in vitro* shoots, roots and leafs formation from sprouts explants of two potato cultivars is shown in table 2.

Table 2. Biometric parameters measured of Excuisita and Bergerac potatoes cultivars on MS media

Cultivar on MS media	*Nr. of explants*	*Shoot length/mm*	*Nr. of shoots /explants*	*Nr. of roots /explants*	*Nr. of leaves/ explants*	*% rooting*
Exciusita	34	72.2±0.5	3.7±0.7	4.6±0.8	4.0±0.5	32.35
Bergerac	34	54.7±0.7	6.3±0.9	3.8±0.9	3.7±0.7	100



(a) (b)

Figure 4. Bergerac (a) and Excuisita (b) explants during proliferation phase

The combination of 0.25ppm GA_3 with BAP showed good results for improving shoot height during sub culturing phase. The results show that cultivar Excuisita has maximum potential for creating new plantlets with higher values on shoot height (±72.2mm) and leaf number in compare of Bergerac cultivar (±54.76mm). ANOVA analysis proved that the differences between two potatoes cultivars related shoot height trait were significantly different at the P0.05 and P0.01 level of the probability (Figure 5).

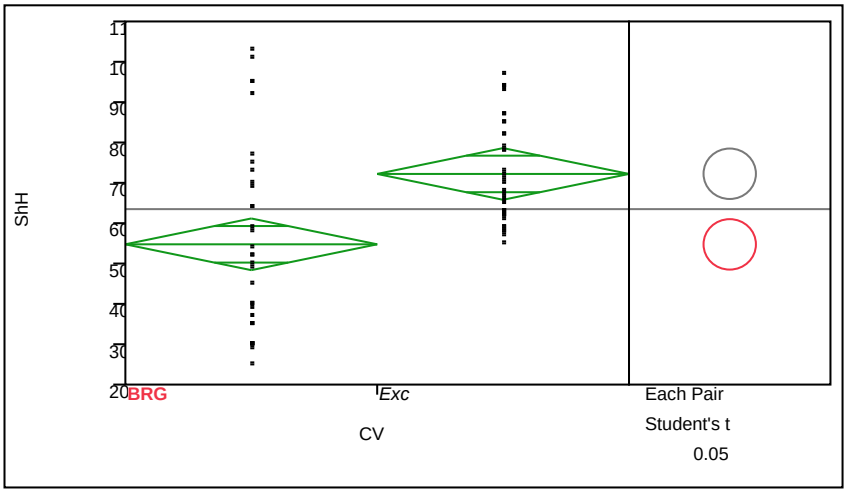


Figure 5. Comparison of shoot height data among two potatoes cultivars

Our results are comparable with those reported from Badoni *et al*.2009 and even better than the results of Liljana *et al*.2012. The percentage of rooting differs for the two potatoes cultivars where the maximum results (100%) are obtained for Bergerac *in vitro* plantlets.

Figure 6. Potatoes plantlets (Excuisita & Bergerac) *in vitro* development

The combination of GA_3 with BAP was effective in improving other parameters of our study, as number of shoots/explants. Bergerac cultivar was the one with higher values on number of shoots per explants created (±6.37) in compare with 3.75 shoots/explants of Excuisita *in vitro* new plantlets. Our results goes within the limits of those reported from Badoni *et al.* 2009 (2.7 till 9.4 shoots). Our potatoes cultivar used in this study showed not significant differences for two other quantitative parameters as the number of leaves and number of roots created. This was proved statistically using anova analysis as it is shown (Figure 7).

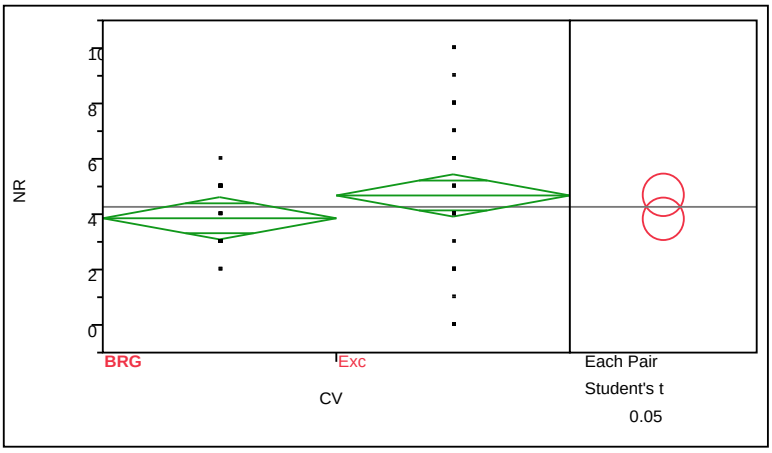


Figure 7. Comparison of ± number of root data among two potato cultivars

Earlier studies on in vitro potatoes regeneration have shown that the culture media, growth regulators and carbohydrate concentration affect micro tuberisation under *in vitro* conditions. El –Sawy *et al.* 2007 reported that sucrose is an important factor for micro tuber formation. Regarding this author the highest tuber formation was achieved when 12% sucrose was added to culture media. High sucrose concentrations may act as inducing signal leading to starch accumulation, so to increase the present of micro tuberisation the concentration

of sucrose must be higher. None of the cultivars used in our study was able for micro tuber formation on MS media supplemented with 3% sucrose. Same results are suggested from Hoque *et al*.2010.

Plantlets of two potatoes cultivars reacted differently on acclimatization process. Only the Excuisita *in vitro* plants (Figure 8) survived after acclimatization on mixed pre-prepared composition (soil and sand).

Figure 8. Excuisita *in vitro* plant acclimatization process

Three to four water sprays were given daily with a sprayer to keep the soil moist and maintain humidity for initial one week. Once the plants established and start growing normal irrigation was followed.

Minituber is an intermediate stage of potato seed production between laboratory micro propagation and field multiplication. We achieved the formation of minitubers with an average weight of 10-12 g from Excuisita plants (Figure 8).

Figure 9. Excuisita plant producing minitubers.

CONCLUSIONS

Results obtained in the present study, suggest that the type of method used for explants disinfections, was the right one, indicating a high survival rate. Also the type of explant and nutrinet medium used for the *in vitro* establishment and proliferation of *S.tuberosum L.* germplasm, effected the rate of proliferation giving satisfactory results for our two *potatoes* resources. The multiplication rate of two *potatoes cultivars*, object of the study, was highly effective on producing a satisfactory number of explants on MS media.

The combination of GA_3 with BAP was effective in improving the explants development, organogenesis stage and possibilities for minituber production from potatoes plants during this experimental work. The technique used in our study might be a possible one, for cloning of other potatoes cultivar plants.

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