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In Vitro Propagation of Blue Honeysuckle /*Loniceraedulis* /

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Abstract: *The micropropagation method of blue honeysuckles varieties/Zolushca, Sindrella,9-15/ introduced in Mongolia was developed .The sterilization explants based on application of 70 % ethanol followed by calcium hypochlorite and highest disinfection percentage was obtained for varieties Zolushca in the sterilization solution with 6%calciumhypochlorite added 1-2 drops of Tween20.*

Proliferating medium according to Gamborg's B5 media supplemented with TDZ at concentration 0.2mg/l gave the best shoot multiplication rate of all three varieties.

The best rooting of varieties was achieved on Gamborg's B5media supplemented with 0.5mg/l NAA. Acclimatization of rooted plantlets increased their survival rate in the greenhouse.

Keyword: *In vitro condition, explant, culture, disinfection, plantlets, micro-propagation, medium, variety.*

1. INTRODUCTION

At present, it is an immediately developing urban agriculture, mining, large buildings, and population growth and density increases, there is an abrupt change of ecology.

Thus, frost hardy species is suitable and potential as a cultivated small fruit crop such as honeysuckle in the climatic conditions of Mongolia.

In many countries honey suckle is considered to be a new species of plants or chardist attention.

Last years, Russian and Canadian varieties of honey suckle has been introduced in Mongolia. But traditional propagation method of honeysuckle species by hardwood cuttings has disadvantage- low percentage of root establishment. Therefore, in vitro culture method is promising for production of more plant material in a shorter period. However, several micro propagation protocols have been reported, unfortunately these are not broadly applicable because of effectiveness of the culture medium seems to the highly genotype-species.

In the presented paper, in vitro propagation and acclimatization ex vitro were studied to provide an efficient plant production system of edible honey suckle.

2. MATERIALS AND METHODS

The initial plant material was selected vegetative axillary buds of honeysuckle varieties *Sendrella*/Canadian /, *Breeding line 9-15*/ Canadian/and *Zolushca* /Russian/.

2.1. Disinfection of Explant

The axillary buds were surface disinfected with70% ethanol for 30 seconds followed by 6% solution of calcium hypochlorite for 20 min and subsequently washed 3 times with distilled water.

For disinfection compare studied two solutions such as calcium hypochlorite along and with 1-2 drops Tween 20.

2.2. Culture of Explants in In-Vitro Condition

The shoots /buds/ was cultured in flasks each with 25ml of Gamborg'sB5 medium solidified with 0.7% agar. Initial medium contained TDZ (Thidiazuron) at concentration of 0.2mg 1^{-1}. The experiments on micropropagation were done on stabilized shoot culture (after six months culturing in in-vitro conditions).

Media for rooting enriched with NAA (Naphthalene Acid) 0.5mg 1^{-1}.The pH of medium was adjusted to 5.7 before autoclaving.

The plantlets of honeysuckle were cultured in flasks transferred to growing chamber room. This room condition was a 16 h photoperiod provided by cool –white fluorescent lamps and 24-26^0 C.

The experiment was conducted in 2 replications for3 varieties, each of them consisted of 10 flasks with 5 shoots. The experiment was repeated 3 times.

About one month later plantlets were transferred to the soil in vivo condition.

3. RESULTS

The results of explant sterilization procedures are recorded in table1.The sterilization procedures were successful and enabled obtaining different percentage of disinfected explants of three varieties. Of the 50 explants of varieties 70-80 % were disinfected in sterilized solution with 6%calciumhypochlorite added Tween20. It was better thanTween20 free solution (Table1).

Table1.*The result of sterilization of explant*

Varieties	Number of explant	% of disinfected explants ,	
		Type of a sterilized solution	
		6%calciumhypochlorite	6%calciumhypochlorite+ Tween 20
		20 minutes	
Zolushca	50	60	80
Sindrella	50	40	70
9-15	50	60	75

The highest disinfection percentage was obtained for varieties *Zolushca* in the sterilization solution with Tween20. The contaminated explants were discarded and other uncontaminated explants produced shoots.

The high of new shoots formed on media with TDZ at concentration 0.2mg/l for three varieties. Especially *Zolushca* variety has produced more biomass and 8.5 new shoots per explant (Table2).

Table2. *Number and length of newly – formed shoots of three blue honeysuckle varieties*

Media	**Zolushca**		**Sindrella**		**9-15**	
	average per explant					
	Number of shoot	Length of shoots. mm	Number of shoot	Length of shoots. mm	Number of shoot	Length of shoots. mm
Gamborg's B5	3.1	40	2.0	30	3.3	40
Gamborg's B5 + TDZ 0.2mg/l	8.5	41	4.4	32	6.5	42

However, the media Gamborg's B5 was effective for shoot induction, but non observed root initiation for all varieties (Pic 1).

The media Gamborg's B5 supplemented with 0.5mg/l NAA was effective for root induction or first roots were observed 14 days after inoculation shoots/ Pic 2/.

Rooted plants was acclimated in peat substrate for 10 days and after that transplanted in to greenhouse. Survival rate of plantlets was high or 90-93.5% (Pic 3.4).

Pic1. *Plantlets of Honeysuckle*

Pic2. *Root induced on the media containing NAA0.5mg/l*

Pic3. *Plant lets after acclimatization*

Pic4.*Transplanted plantlets in to the greenhouse*

4. DISCUSSION

The sterilization procedure was satisfactory, resulted in 60-80% of disinfected explants, depending on varieties and disinfection solutions. The highest disinfection percentage was obtained for varieties *Zolushca* in the sterilization solution with 6%calciumhypochlorite added 1-2 drops of Tween20.It was previously reported that plant explant could be successfully surface sterilized using solution of 10 %calcium hypochlorite for 10 min and result was similar or percentage of disinfected explants obtained 65,9% (EwaDziedzic, 2008). Sodium hypochlorite solution (Karhu, 1997) or mercuric chloride (Sedlak, 2007) are used for sterilization solution.

In our studies for shoot proliferation was performed on Gamborg's B5 media supplemented with TDZ at concentration of 0.2mg/l. Other researchers were reported, that proliferation media could be used MS with salt reduced by 1/4withdifferent concentrations of cytokine in such as 1- 2 mg/l BAP, IBA 0, 1mg/l.

Result of our study has been shown that the Gamborg's B5media supplemented with 0.5mg/l NAA was effective for root induction. In Ewa Dziedzic's experiment, the media with low nutrient concentration WPM was more effective in root induction than media rich in mineral salts. Sedlak and Paprstein rooted honeysuckle shoots on MS medium supplemented with 2.5 mg/l IBA achieving 100 % rooting.

5. CONCLUSION

Gamborg's B5 media supplemented with TDZ at concentration 0.2mg/l gave the best shoot multiplication rate of all three varieties. The best rooting of varieties was achieved on Gamborg's B5media supplemented with 0.5mg/l NAA. Acclimatization of rooted plantlets increased their survival rate in the greenhouse.

REFERENCES

[1] Atarsaikhan, T. (2007). Result of study varieties of blue honey suckle. Master thesis, Darkhan-uul,

[2] Bors, B. (2004). Propagation and Nursery management Class Notes. University of Saskachewan, Canada

[3] E.F.George. (1996). Plant propagation by tissue culture. Part 2 in practice.

[4] KarhuS.T. (1997). Axillary shoot proliferation of blue honeysuckle. Plant cell, tissue culture, organ culture 48: 195-201,

[5] Sedlak, F. Paprstein (2007). In virto propagation of blue honeysuckle. Journal of Fruit and Ornamental Plant Research.

[6] EwaDziedzic. (2008). Propagation of blue honeysuckle in vitro culture. Journal of Fruit and Ornamental Plant Research .vol 16. 93-100

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Institute of Plant and Agricultural Sciences / IPAS/ is one of the research branches of Mongolian University of Life Science.

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