Journal of Medicinal Plants and By-products (2013) 1: 53-56

Original Article

Influence of Plant Growth Regulators and Media on *In vitro* Propagation of *Sorbus aucuparia* L.

Mitra Emam* **and Abas Ghamarizare**

Department of Biotechnology, Research Institute of Forests and Rangelands, P.O. Box 13185-116, Tehran, Iran

Article History: Received: 09 January 2013/Accepted in revised form: 29 June 2013

Abstract

Aucuparia is an important slow growth forest tree with medicinal, industrial and ornamental uses which is applied for reforestation in high altitude of mountain lands. This species has been endangered at northern forests of Iran; therefore micropropagation of adult trees by bud culture may help to reforestation. The best sterilizing treatment was the buds washing with $HgCl_2$% 0.1 solution for 7 minutes in Autumn. The highest of shoot regeneration were taken in DKW medium with BA (0.5 mg/l), IBA (0.1 mg/l) and TDZ (0.05 mg/l). Rooting of shoots was done in modified MCM medium supplemented with 1.5 mg/l of IBA in dark condition. The plantlets were acclimated in green house.

Key words : *Sorbus aucuparia*, Micropropagation, Bud culture

Introduction

Sorbus aucuparia is a hardy forest tree species that grows from in the upper tree line in mountains of northern Iran. It is from Rosaceae family that applied for reforestation in mountainous regions. It is used for pulping and furniture. It's fruit is important food for birds and game in mountainous regions. It is propagated mainly by seeds, that must be stratified for long period [1]. Conventional vegetative propagation is not successful for *S. aucuparia* and using of seed propagation is yielding highly variable progeny for continuous pulpwood supplies. Foresters also have recently appreciated the value of vegetative propagation as a source of uniform trees of known genotype and it can be a proper tool to manipulate the environmental factors and plant material homogeneity [2]. Only few reports have so far been published on *in vitro* culture of Sorbus. Most investigations have focused on clonal propagation, embryo and callus culture and protoplast isolation [3]. Regeneration of plants from callus and protoplast has not yet made great progress [4-7], then in this study, micropropagation of *S. aucuparia* by bud culture has been carried out.

Materials and Methods

Shoot tips and nodal segments were separated as explants from mature trees of *S. aucuparia.* L at Sangdeh- Sari Province in northern forests of Iran. For explant collection, mature trees were selected (30-45 years old) from different seasons. For surface sterilization, the explants were immersed in mercuric chloride solution (0.1%) for different applying times and cultured under aseptic conditions.

Explants were cultured on nutrient media that consisted of: MS, modified MS (1/2 Nitrate) and DKW supplemented with a gradient range of hormones (Table 1). Media were solidified with 0.68% w/v agar.

Sucrose in 3% and 200 mg/l PVP (poly vinyl pyrolidone) were added in media and these media were autoclaved at 120 °C under 1.06 kg/cm^2 pressure for 20 min. Incubation culture conditions were 16h light, Light intensity in 5250 Lux and 22-25 °C. Explants were embedded into the media (5 explants/plate × 6 plates). After 30 days, the growth parameters including shoot and bud numbers as proliferation rate and shoot length (cm) were recorded. Data sets were analyzed at randomized

*Corresponding author: Department of Biotechnology, Research Institute of Forests and Rangelands, P.O. Box 13185-116, Tehran, Iran
E-mail Address: mitraemam@yahoo.com

complete block design (RCB) by using of SPSS software (version 13) ($p \leq 0.05$). The means of data compared by Duncan's multiple rang test at $\alpha \leq 5\%$ level when a significant difference was found between treatments.

In rooting phase, the six growth regulator treatments were employed, that was MCM medium with $1/_2$ Macroelements in liquid and solidified state, supplemented with IBA in (1- 1.5 mg/l), (Table 2). Shoots were selected at 15-20 mm pieces. Explants at these treatments were embedded in the medium (5 explants/plate × 5 plate). After 2 months, rooting and necrosis plant percents were recorded.

In vitro derived plantlets were transferred to 4:1:4 peat: perlite: vermiculite pots with polyethylene caps in greenhouse condition.

Results

In sterilization phase, the best treatment was washing explants with $HgCl_2$ (0.1% solution) in 7 minutes in autumn (Table 3, Fig. 1-a). In comparison of other media, nodal segments placed on DKW medium produced many shoots (8-10 No) for 6-7 weeks. The highest proliferation rate was obtained on DKW medium with 0.5 BA plus 0.1 IBA and 0.05 mg/l TDZ (Table 4, Fig. 1-b). Growth indices such as shoot and bud proliferation, had significant difference in according to hormone treatments but Media was affected on shoot length growth significantly. The addition of 2iP instead of BAP ameliorated shoot elongation. The using of two different multiplication medium improved shoot condition. Microshoots with 15 mm height were transferred in rooting media. These shoots formed adventitious roots in liquid MCM medium with IBA 1.5 mg/l for 3-4 weeks (Table 5, Fig. 1-c).After 4 weeks, rooted plantlets were cultured in potting mixture (peat: perlite: vermiculite 4:1:4 V/V) and established in green house condition, successfully (Fig. 1-d).

Table 1 Shooting different treatments(Media and hormones)

hormone (mg/l) Medium	BA	TDZ	IBA	2iP
DKW	0.5	0.05	0.1	-
DKW	-	0.05	0.1	0.5
DKW	0.25	0.05	0.1	0.25
MS	0.5	0.05	0.1	-
MS	-	0.05	0.1	0.5
MS	0.25	0.05	0.1	0.25
1/2MS	0.5	0.05	0.1	-
1/2MS	-	0.05	0.1	0.5
1/2MS	0.25	0.05	0.1	0.25

+: Presence of Growth Regulator, - : No- Growth Regulator

Table 2 Rooting treatments

IBA (mg/l) / Medium	Solid MCM	Liquid MCM
T1	1	-
T2	1.5	-
T3	-	1
T4	-	1.5

+: Presence of Growth Regulator, - : No- Growth Regulator

Table 3 Results of sterilization treatments

Season	Treatment ($HgCl_2$ 0.1%)	Viability%	Infection%	Necrosis%
Spring	3 min	32	10	58
Summer	5 min	48	42	10
Automn	7 min	70	12	18
Winter	9 min	45	48	7

Table 4 Analysis of variance in different media on growth indices (shoot and bud proliferation and shoot length).

Source	df	Mean Square		
		Bud proliferation	Shoot proliferation	Shoot length
Medium	2	0.422 ^{ns}	1.43 ^{ns}	0.698**
Hormone	2	26.6**	26.43**	0.368 ^{ns}
Medium*Hormone	20	3.751 ^{ns}	6.23**	0.317 ^{ns}
Error	108	2.8	3.46	0.195

(**: significant different (p≤0.01), *: significant different (p≤0.01), ns: non- significant)

Table 5 Rooting percentage of tissue cultured shoots

Medium Hormone	Rooting %	Shoot necrosis %
Solid MCM ‹ IBA1	10	15
Solid MCM‹ IBA1.5	17	35
Liquid MCM ‹ IBA1	31	30
Liquid MCM‹ IBA1.5	35	45

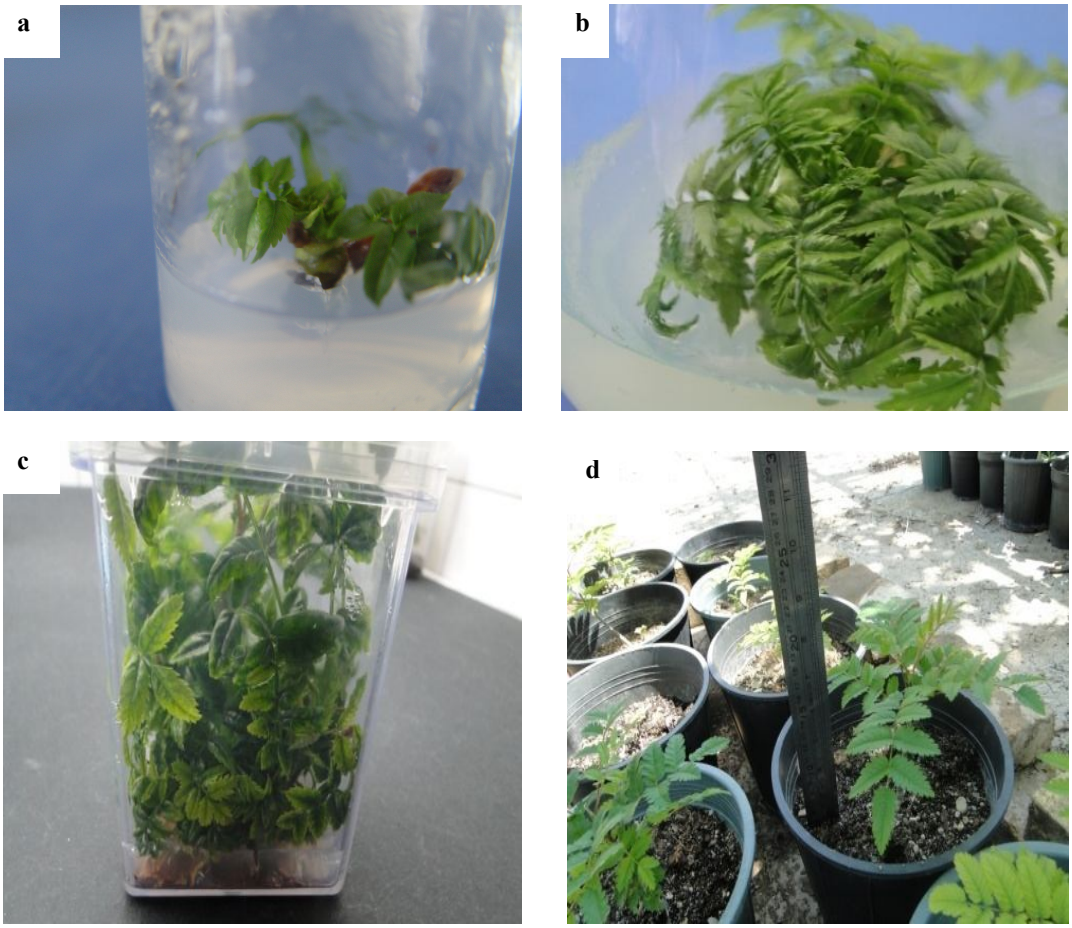

Fig. 1 The different stages of *S. aucuparia* micropropagation. a: Established bud, b: Proliferation of shoots, c: Rooting of shoots, d: Acclimated plantlets

Discussion

In the sterilization phase, the best treatment was immersion of buds in $HgCl_2$ solution (0.1%), formerly used by Chalupa [1] on *S. aucuparia*. Increasing of PVP in media had good effect on clamping the reaction of tannins. In fact, the tannin constitutes a physiological inhibitor [8,9]. Chalupa [1] also, mentioned to these growth inhibitors in culture of mature plants.

In our research, good multiplication and shoot growth, obtained in the DKW (10) medium with BAP and TDZ in comparison of the other media. One of the effective factors in applyment of culture medium is the kind of salts and ionic strength of its [11]. The maximum of data in ionic strength of media was about DKW medium. In contrary of our research, the findings of Chalupa [3-7] on tissue culture studies of Sorbus species showed that shoot multiplication on MS medium was better than other media. TDZ is the active cytokinin that induces shoot proliferation in plus of BA. Chalupa [5-7] also mentioned this subject. The addition of IBA in low concentration stimulated proliferation and shoot elongation that already found by Bonga and Aderkas [12].

Half strength of liquid MCM medium plus 1.5 mg/l of IBA was also found to be best in rooting. Auxins are involved in the process of adventitious root formation [13]. In some other woody plants, IBA is commonly used to promote root initiation [14]. In our study, IBA in the rooting medium had good effect on root formation of *S. aucuparia*shoots. These cloning plants after acclimation were employed for reforestation in high altitude of mountain lands.

Novel advances in micropropagation of forest trees have opened great opportunities for mass propagation of selected valuable genotypes [15]. Selection of explants, composition of nutrient media, concentration of phytohormones and methods used for micropropagation had significant effects on shoot multiplication and rooting rates of *S. aucuparia* explants. The clonal propagated plants will be used for tree improvement and reforestation programes. Then we recommend that by transferring of this protocol to promoters and producing of these trees in large numbers by tissue culture methods, we will able to revive of ruined mountain lands in northern forests of Iran.

Acknowledgments

This research was supported by Research Institute of Forests and Rangelands, Iran.

References

1. Chalupa V. *In vitro* propagation of mature trees of *Sorbus aucuparia* L and field performance of micropropagated trees. J Forest sci. 2002;48:529-535.
2. Morabito D, Mills D, Prat D and Dizengremel P. Response of clones of *E. microtheca* to NaCl *in vitro*. Tree Physiol. 1994;14:201-210.
3. Chalupa V. Micropropagation of mountain ash (*Sorbus aucuparia* L) and wild service tree (*Sorbus torminalis* L.). In: Bajaj YPS (eds.) Biotechnology in Agriculture and Forestry, Vol 18, Springer-Verlag publication, 1992, pp. 211-225.
4. Chalupa V. Clonal propagation of broud- leaved forest trees *in vitro*. Communication Institute for Cech. 1981;12: 255-271.
5. Chalupa V. *In vitro* propagation of willows, European mountain ash (*Sorbus aucuparia L*) and black locust. Biol Plantarum. 1983;25:305-315.
6. Chalupa V. Effect of benzyl amino purine and thydiazuron on *in vitro* shoot proliferation of *Tilia cordata Mill*., *Sorbus aucuparia* L. and *Robinia pseudo acaciae* L. Biol Plantarum. 1987;29:425-429.
7. Chalupa V. *In vitro* propagation of small – leaved linden, black locust and mountain ash (*Sorbus aucuparia L.*) and growth of tree cultivated *in vitro.* Lesnictvi. 1988; 34:705-720.
8. Custodio L, Carneiro M F, Romano A. Microsporogenesis and anther culture in carob tree (*Ceratonia siliqua* L.). Scientica Hort. 2005;104:65-77.
9. Souayah N, Khouja ML, Khaldi A, Rejeb MN, Bouzid S. Breeding improvement of *Laurus nobilis* L. by conventional and *in vitro* propagation techniques. J Herbs, Spices & Med Plants. 2002;9:101-105.
10. Driver JA, Kuniyuki H. *In vitro* propagation of Paradox walnut root stocks (*J. hindsii* J. regia*). Hortscience. 1984; 19:507-509.
11. McCown B H and Sellmer J C. Media and physical environment. In: Bonga J M, Durzan D J (eds.) Cell and Tissue Culture in Forestry, VoL 1, Martinus Nijhoff Publishers, 1982, pp. 1-17.
12. Bonga J M and Aderkas P V. *In vitro* Culture of Trees. Kluwer Academic Publishers. 1992, p. 236.
13. Glock P, Delaporte K, Collins G and Sedgley M. Micropropagation of juvenile tissue of *E. erythonema* X *Eucalyptus stricklandii* cv. Urrbrae Gem. *In vitro* Cell Develop Biol Plant. 2006;42:139-143.
14. Onay A. *In vivo* and *in vitro* micrografting of pistachio (*Pistacia vera* L.). Turkish J Biol. 2003;27:95-100.
15. Assareh M H and Henney M J. Effects of CO_2 - enriched and non- enriched environments on the growth rate of *Eucalyptus microtheca*. Research reports 1996/1997,UCD. 1998; pp.140-141.