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The effect of media and plant growth regulators on embryo culture of *Salvia leriifolia*:

Abstract*:*

Salvia leriifolia (*Lamiaceae*) is an endemic endangered plant of Khorasan and Semnan province from Iran. Poor germination of seeds for this plant is a serious problem tomass production. The first step to improve this precious plant is to produce sterile plantlets in order to prepare explants of appropriate vigor. In this study, in vitro culture of *Salvia leriifolia* embryo was performed through a factorial experiment in the form of completely random design including culture medium in 3 types (MS.1/2MS,B5), BAP of 4 levels(0,1,2,3 mgl^{-1}), NAA at 3 levels(0,0.5,1 mgl^{-1}) and 3 replications. Statistical analysis was performed according to the JMP software. The results showed that, MS and 1/2MS appeared to be more efficient than B5 medium and significant differences were observed. Efficient concentrations of BAP and NAA were 1 mg/lit and had a significant effect in regeneration and growth of embryos. So that the entire seedling were obtained ten days after planting .Direct regeneration with minimal Somaclonal variation through embryo culture in MS and 1/2MS media supplemented with 1mg/lit BAP and NAA asthe bestoptionforfast accessto resistant seedling totheusabilitygenetic engineering and biotechnology and propagation of *Salvia leriifolia* is recommended.

Key words: embryo culture, growth regulators, *Salvia leriifolia*.

1. Introduction:

Salvia leriifolia from family *Lamiaceae* is endemic of Khorasanrazavi and Semnan provinces in IRAN. There are different reports in medical properties of this plant. Ant pain activity of leaf extract of *S.leriifolia*in concentration of 500 mg/kg in comparison of 5 mg/kg diazepam (Hosseinzadeh and Larry, 2000).Essence of this plant could be useful in cure of Alzheimer'sdisease because of inhibitory effect at butyl colin esterase (Loizze et al, 2009). Tumult properties of leaf extract could becomparing with diclofenac medicine (Hosseinzadeh and Yavary, 2009). Water and alcoholic of leaf extract of *S.leriifolia* blocking the creation and developing of ulcer in mouse and this advantage resemble of sucralfatedrug (Hosseinzadehet al, 2000). Different experiments showed many anti-microbial effects of various sections of plant (Modarres et al, 2009;Modarres and Abrishamchi, 2011).Ant proliferation properties of *S.leriifo*lia on oncogenic cells of human demonstrated (Loizze et al, 2009). The leaf and root of *S.leriifolia* have strong anti-oxidant properties and prevent as oxidation of sunshade oil which compare with anti-oxidant in food industry such as botilited hydroxyl toloen (BHT) and α-tocoferol (Hosseinzadeh and Yavary, 1999;Khodaparast et al, 2006).

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There are many reports which show remove of coat and cotyledon from embryo play important role in shortening of dormancy of seed and propagation and also MS, half-strength MS and B5 mediums are useful for embryo culture in different plants (Craverol and Cointry, 2007;Toosi et al, 2010). Thereto, add of suitable treatment of auxin and cytokinin growth regulators to embryo mediums cause improvement of growth quality in plantlets (Keiichi et al, 2005). There is not any investigate on culture of *S.leriifolia* in vitro until now. According to medicinal, industrial and tore important of this endemic and endangered plant, use of plant tissue culture and biotechnology methods for reformation , retinue of germplasm, and increase of secondary metabolites is necessary. This paper describes the preliminary results for the establishment of*Salvia leriifolia* plants from embryo culture through organogenesis.

2. Material and Methods:

2.1 Plant material

The seeds of *S.leriifolia* Benth were collected at Bajestan in south of Khorasanrazavi province, Mashhad, Iran.

2.2 Media

For the propagation by direct organogenesis MS (Murashige & Skoog 1962), half strength MS and B5 (Gamborg et al, 1968) media were used.

2.3 Embryo culture

The seeds sterilized for 30 seconds in ethanol 70%,sodium hypochlorite 3% for 5 min and then rinsed three times with sterile water. After removing the coat of seeds, embryos emitted carefully and were transplanted into vials supplement with 30 gram/l sucrose, 7 gram/l agars. pHwas adjusted to 5.8 before autoclaving. Media differed in concentrations of growth regulators and light conditions of culture.

2.4 Plant growth regulators

For effect of growth regulators on embryo growth, different concentration of BAP (0,1,2,3 mg/l) and NAA (0,0.5,1 mg/l), separately and combinational add to three mediums. For each medium it was used 12 treatments and for each treatment 10 vials totally 360 embryos were cultured. After 4 weeks, percentage of growth embryo, leaf numbers, fresh and dry weight of plantlets were evaluated.The effect of NAA and BAP were tested. Six combination of growth regulators were used in experiment.The experiment was carried out in factorial plan with completely random design with 3 replications. In each vial 2 embryo explants were inoculated. For each variant the number of leaf/plantlet, fresh and dry weight, length of shoot and root were considered]

2.5 Statistical analysis

The results are presentated as mean values± standard errors. The data on leaf regeneration, fresh and dry weight were subjected to analysis of JMP with the means separation ($p<0.05$) by HSD test. The charts were draw by Excell 2007.

3. Results

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The result of embryo culture show in almost all of the treatment, coleorhiza after 24 hours as culture time start to growth. This is notable that embryo no produce callus in all of treatments.

The percentage of regeneration in MS, 1/2MS and B5 was 98.4%, 97.5% and 66% respectively. The result of data analysis after 4 weeks showed plantlet regeneration, fresh dry weight in MS and 1/2MS is more than B5 medium significantly but there was no significant different in MS and 1/2MS mediums.Data analysis also showed 1mg/l BAP cause significant increase in length of shoot and root than instancebut with increase BAP concentrations the fresh and dry weight and length of shoot decreased. The least length of shoot was in 3mg/l BAP and 0.5mg/l NAA+3mg/l BAP but the length of root was not significant different with instance. The result showed NAA cause significant increase of length of shoot in0.5mg/l. however in 0.5 and 1mg/l NAA the length of root decrease significantly but the other qualities were not affecting of NAA.Average comparison of different treatment showed the maximum rate of dry weight and length of shoot was in 0.5mg/l NAA and 1mg/l NAA+1mg/l BAP which has significant difference with the other treatments. Also length of root is more than other treatment in 1mg/l BAP and 1mg/l BAP+1mg/l NAA (figure1). Addition to characterizewith increase BAP until 3mg/l with NAA the average of dry weight and length of shoot and leaf number decrease significantly.The result of experiments showed the treatment of 1mg/l NAA+1mg/l BAP in MS and 1/2MS mediums was the best treatment for growth of embryo. At this treatment fresh and dry weight, length of root and shoot was significant difference with the others.

4. Discussion:

Low viability and little percentage of seeds in *Salvia leriifolia* is a main problem for propagation of this plant. The results showed embryo culture in vitro is a suitable method for propagation because of rate germination of embryo. Khodaparast and Hoseini (2004) reported that remove of coat-seed cause increase germination into 40%. There are many reports which showed separate of embryo as seed coat and cotyledons cause rapid germination (Craverol and Cointry,2007; Sanchez-Zamora et al,2006). The survey of mediums displayed MS and 1/2MS mediums is more suitable than B5, which referred to effectiveness of MS medium to rate of minerals. In agreement with this idea, the embryos of *Salvia brachyodon* have better growth in 1/2 MS (Misic et al, 2006). They are concluded 1mg/l BAP has positive effect on embryo growth, whereas in higher concentrations has decrease effect on length of embryo and its qualities. They also showed with increase of auxin concentration the length of root decrease. It is maybe in embryo of *S.leriifolia*, the amount of auxin is enough and enhance of outer auxin without add cytokinin prevent of root growth.The inhibitory effect of auxin on ethylene biosynthesis induced by auxin (Taiz and Zeiger, 2006). On the other hand, maybe the interior cytokinin is not enough, therefore exist of cytokinin could be positive effect on embryo growth and produce of plantlets. At this paper the best treatment for rapid growth of embryo was MS and 1/2MS + 1 mg/l NAA and BAP. This is conformingtoembryo culture of *Synsepolumdalcificum* in MS medium (Ogunsola and Ilorico, 2008). Moreover Kelichi et al (2005) concluded that regeneration of plantlets of Japanese morning gloryin medium containing different treatment of auxin and cytokinin, only attain in MS medium that have NAA and BAP. Also this research uphold of report of Chin et al (1998) for embryo culture of 11 plant speckles. Try to this report the embryo of these plants which have problem in seed germination have better growth in MS medium with 1 mg/l NAA and 1 mg/l BAP. Also use of 2gr/l active charcoal in these medium have positive effect on growth embryo significantly. Active charcoal could be main role in absorb of additional hormone and oxidant phenol, chemical material which inhibitor of growth and organogenesis (Thomas, 2008).

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Number of leaf(M.S)	*Fresh weight (M.S)*	*Dry weight (M.S)*	*Root length (M.S)*	*Shoot length(M.S)*	*df*	*S.O.V*
33.879*	0.193*	0.002*	29648.324*	2867.969*	3	BAP
17.388ns	0.163ns	0.001ns	30817.463*	1072.115*	2	NAA
82.666*	0.835*	0.008*	14569.019*	702.143*	2	Media
16.981ns	1.443*	0.011*	23955.870*	649.939*	6	NAA*BAP
36.370*	0.557ns	0.002ns	36600.315*	1289.689*	6	BAP*Media
86.685*	2.455*	0.015*	75060.574*	2274.213*	4	NAA*Media
21.611*	0.990*	0.004*	38112.981*	644.453*	12	BAP*NAA*Media
22.120	0.134	0.004	66539.33	1096.167	72	error

Table1: The analysis of variance for factorial design for three treatments. Number are means of square and * is significance in α=5%. ns is not significant.

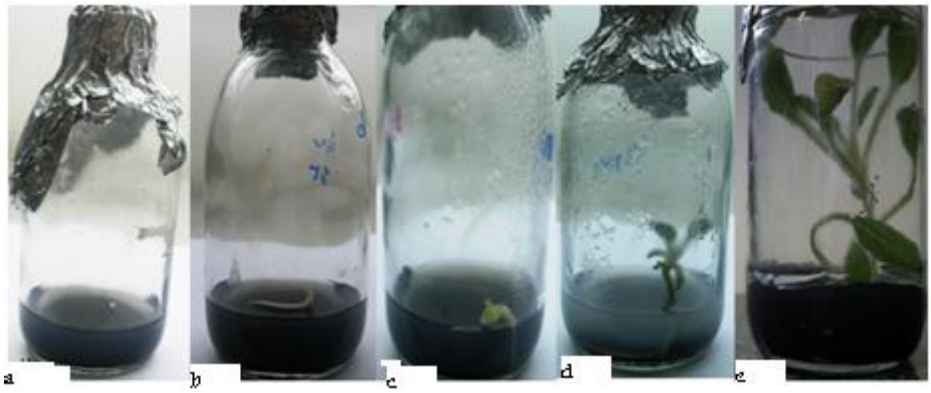

Figure1- embryo growth of *S.leriifolia* in MS medium containing 1mg/l NAA+1mg/l BAP.a) embryo cultured in MS medium b) embryo after 4days c) embryo after 1week d) 2weeks after culture e) 1months after culture.

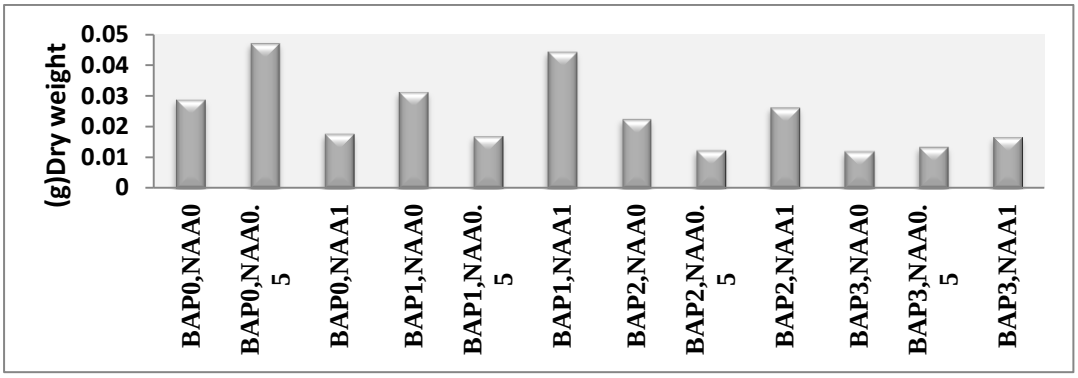

Figure 2- comparison of dry weight for regeneration plantlets in three culture media containing different BAP and NAA hormones and their interactions

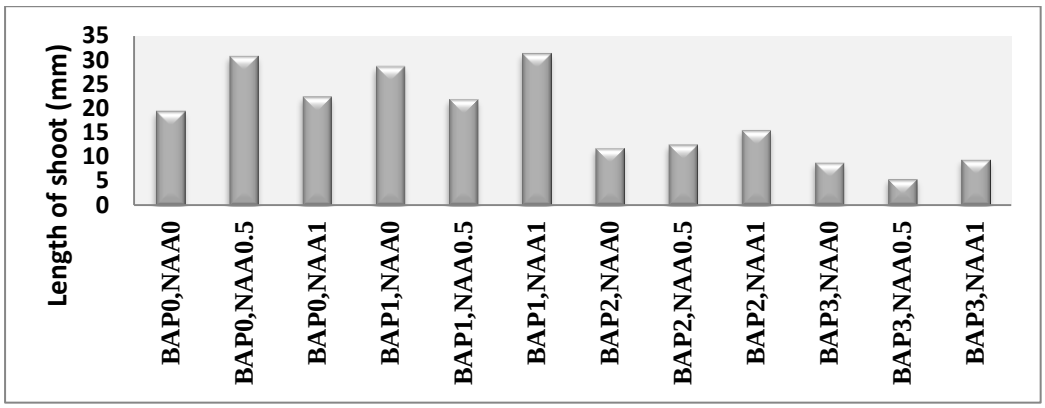

Figure 3- comparison of length of shoot for regeneration plantlets in three culture media containing different BAP and NAA hormones and their interactions

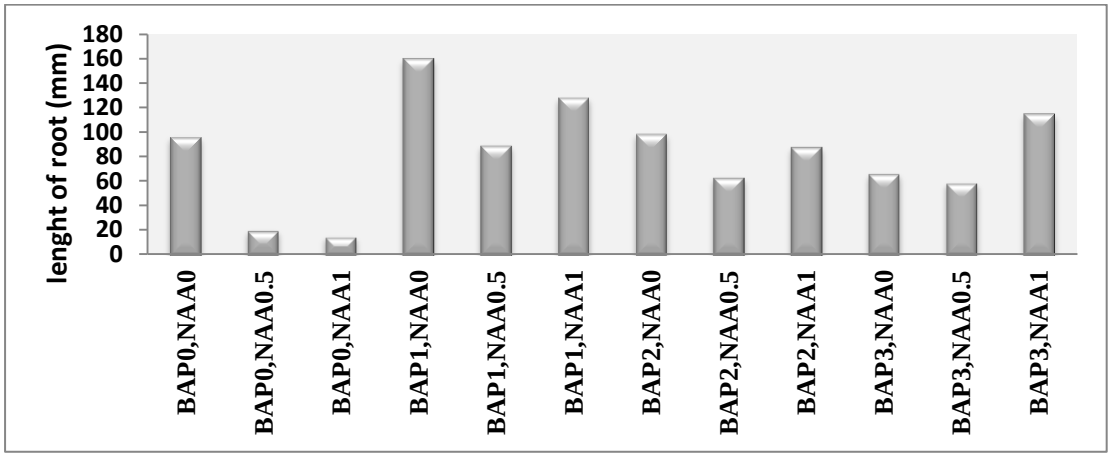

Figure 4- comparison of length of root for regeneration plantlets in three culture media containing different BAP and NAA hormones and their interactions

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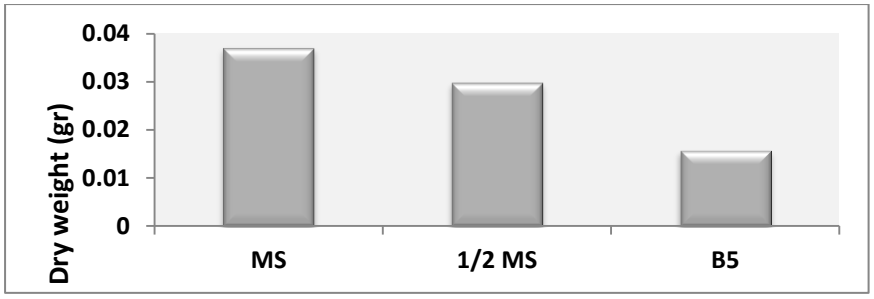

Figure 5- comparison of dry weight for regeneration plantlets in three culture media containing different BAP and NAA hormones and their interactions

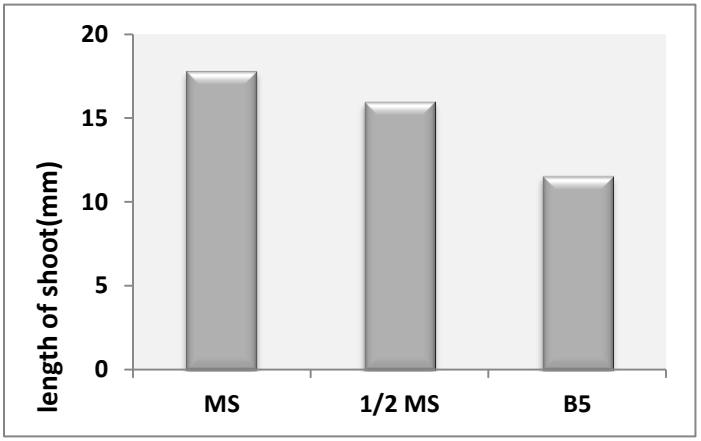

Figure 5- comparison of length of shoot for regeneration plantlets in three culture media

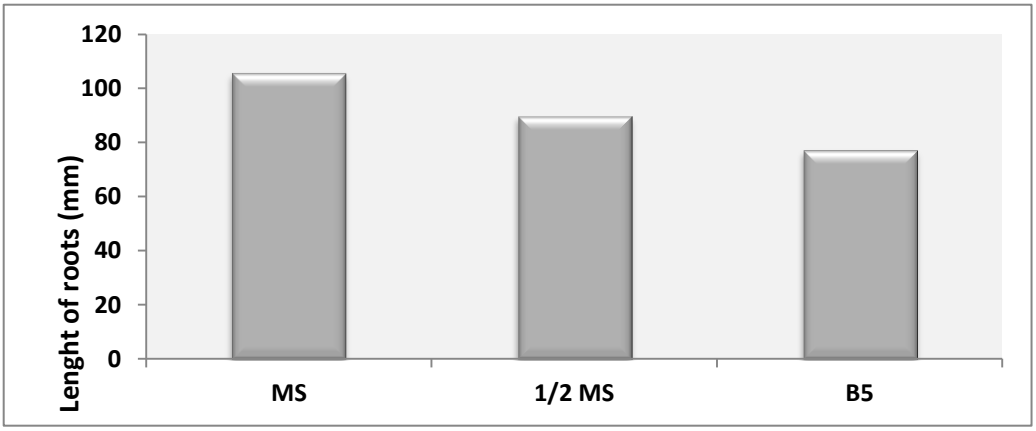

Figure 6- comparison of length of roots for regeneration plantlets in three culture media

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