Study of Callus Induction in Common Sage
(Salvia Officinalis L.)

Mehrdad Ghasemi Lemraski¹, Mostafa Eftekhari², Miad Faraj³, Saeid Samadi Zarrini⁴*

1. Department of Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Agronomy and Plant Breeding Sciences, College of Aburaihan, University of Tehran, Tehran, Iran.
3. Young Researchers and Elite Club, Jouybar Branch, Islamic Azad University, Jouybar, Iran.

*Corresponding author email: s_samadi101@yahoo.com

ABSTRACT: The harmful effects of chemicals and the side effects of chemical drugs on human health have widely focused global attention on herbal drugs and medicinal plants. The increasing use of medicinal plants in the world is more than enough to show the significance of cultivating and producing such plants. Salvia L. is the largest genus of plants in the mint family, Lamiaceae, and comprises more than 900 species all over the world. Common sage, a well known species, generally refers to Salvia officinalis L. and is used widely in herbal medicine. Despite the increasing need that exists for mass reproduction of this plant, there is little information about its propagation methods. This experiment was conducted to investigate possibility of callus induction in Sage and the effect of different levels of BA-6-benzylaminopurine and NAA-naphthaleneacetic acid hormones and their interaction on callus volume. Data analyses were done by SAS software. The ANOVA procedure showed that the effect of BA-6-benzylaminopurine hormone on callus volume was significant at 1% probability level. The effect of NAA-naphthaleneacetic hormone on callus volume was also significant at 5% probability level. Two hormones interaction on callus volume was also significant at 1% probability level. According to the results, it was determined that 4mg/l² level of BA-6-benzylaminopurine hormone and 3mg/l³ level of NAA-naphthaleneacetic hormone are the best levels for callus induction in Sage.

Key words: Sage, Callus, BA-6-benzylaminopurine, NAA-naphthaleneacetic, hormone.

INTRODUCTION

Harmful effects of chemicals on human health and the side effects of chemical drugs, due to the wide range of international attention on a herbal and medicinal plants. Growing Approach of the world wide use of medicinal plants, makes clear the importance of these plants produced more. Genus Salvia L. is the largest plant genus in the family of Lamiaceae (Lamiaceae) (Labiate) that In different parts of the world has more than 900 species of herbaceous and woody species (Mozaffarian, 1998). This genus belongs to the subfamily Nepetoideae and tribe Mentheae (Anonymous, 2009). This genus is one of the few genus that called, Salvia (sage) in general.

If no moderators (modifiers) are used, then the term sage is generally called Salvia officinalis (common sage), but if it used with modulators (modifiers), it refers to any member of this genus. Ornamental species is commonly known by their genus named Salvia. The genus is widespread throughout the Old World and America continents, with three distinct variations: Central and South America (approx. 500 species); Central Asia and the Mediterranean Sea (250 species); East Asia (90 species) (Walker et al., 2004). Many species of this genus are used as ornamental plants (usually because of attractive flowers) and sometimes for ornamental and aromatic leaves. One of the known species, Salvia officinalis L. Or common sage is widely used in cooking, as an ornamental plant and pasture, and is used in herbal medicine. Sage is native to the Mediterranean region, but is cultivated in many parts of the world. The term officinalis refers to medicinal use of plants and officina, was a traditional warehouse of monastery to store herbs and medicines (Steam, 2004). Sage is grown in parts of Europe for oil extraction The extract of this herb contains chemical Compounds such as Cineole, Borneol and Thujone (Anonymous, 2008). Many properties have mentioned For this herb, such as anti-seizure and anti-cough (Zargari, 1991). Sage leaf extract can be safe and effective in the treatment of hyperlipidemia (high cholesterol levels) (Lee et al., 1998). Nowadays, the introduction of synthetic antioxidants limited due to its toxicity and the attention of the medical community focus on use and finding natural antioxidants (Asgari et
al., 2002). Today, medicinal herbs are the member of economically important plants that used in the form of raw and processed in traditional and modern industrial medicine. The fact is that active pharmaceutical ingredients can have different quality and quantity, depending on species, habitats and harvest periods (Currier et al., 2000). Tissue culture is a collection of lab methods that rely on TotiPotency property of an organ, tissue, cell, or even part of a cell, produce a complete and consistent plant with the intended purpose (Piri et al., 2006). Tissue culture techniques in medicinal plant can lead toward the production of Special metabolites, gene transfer, germplasm conservation or development of plant micropropagation (Lima et al. 2001; Rout et al., 2000). Today’s vegetable cultivation techniques has become so a powerful tool for plant reproduction (Sebastiana, 2004). In order to promote callus production level, optimization of medium components such as hormonal compounds are the prerequisites for the commercial production of secondary metabolites (Sada, 1989; Yamakawa et al, 1983).

One of the ways to reproduction and diversify in Salvia officinalis, is to produced callus in in vitro conditions. Despite of the increasing need for mass reproduction of this plant, there is a little information about the methods of its reproduction. Therefore, this study was aimed to determine the proper environment for callus production and micropropagation of this invaluable medicinal plant.

MATERIALS AND METHODS

In this study, seeds of Salvia (Salvia officinalis L.) were collected in the June 2011 at height of lar hillsides (fars province) with latitude 27 degrees and 68 minutes north, 54 degrees 34 minutes east longitude and 828 meters from the surface sea. At the beginning, seeds were washed in running water for 15 minutes and then were immersed in soapy water for 15 min too. Then were sterilized in a laminar air flow hood by 70% alcohol (3-2 min) and 1% sodium hypochlorite containing 3-2 drops of Tween 20 (20-15 min). After each step, the seeds were washed several times with sterile distilled water. Sterilized seeds were sown on medium MS (Murashige, 1962). The PH of medium was set with using NaOH and HCl one normal between 5/6 to 5/8.

Seeds were stored at room with temperature about 25±2 ° C and 70% relative humidity for a period of 16 h light and 8 h dark. After two months, roots were placed on simple MS medium containing various concentrations of BA-6-benzylaminopurine hormone (0mg l⁻¹, 3mg l⁻¹, 4mg l⁻¹ and 5mg l⁻¹) and various concentrations of NAA-naphthaleneacetic hormone (0mg l⁻¹, 1mg l⁻¹, 2mg l⁻¹ and 3mg l⁻¹). Separation of the mini samples done under air flew laminar and completely sterile conditions. Mini samples were initially stored in dark medium with temperature about 25-27 ° C for one month and then was moved to the dark environment with 16 hours of light and 8 h of darkness and after a month the volume of callus was measured. This experiment was set up as a factorial experiment based on completely randomized design with three replications. The data were analyzed using with SAS (version 6.12) and the procedures were described by SAS. The measurements of treatments were compared and grouped using LSD tests at the 0.05 significance level.

RESULTS

Effect of BA Hormone

An effect of BA-6-benzylaminopurine hormone on the volume of sage callus was significant at the 1% level (Table 1).

The mean comparison of different levels of BA-6-benzylaminopurine hormone showed a significant difference on the level of 4 mg l⁻¹ per liter, and had the most of the callus mass. The minimum amount of callus related to the hormone levels of 3 mg l⁻¹ per liter (Table 2).

Effect of NAA Hormone

Effect of NAA-naphthaleneacetic hormone on the sage callus size was significant at the 5% level probability (Table 1). The mean comparisson of the different levels of NAA-naphthaleneacetic hormone showed that the 2 mg l⁻¹ per liter level, had the significant difference with other hormone levels and had the most of the callus mass. The lowest mass of callus was related to the 1 mg l⁻¹ per liter (Table 3).

| Table 1. Analysis of variance of Study of callus induction in common sage |
|-----------------------------|------|-----|
| S.O.V.                      | MS   | DF  |
| BA-6-benzylaminopurine hormone | 5.61 | 3   |
| NAA-naphthaleneacetic hormone | 3.75 | 3   |
| Interaction effect          | 4.33 | 9   |
| Error                       | 0.94 | 32  |

*, ** and ns: Significant at the 5 and 1 % probability and non significant, respectively

Interaction of two Hormones
The interaction of two hormones on sage callus mass was significant at 1% level probability (Table 1). The significance of the interaction effects indicates that the effects of various concentrations of each hormone on callus induction was varied on various concentration of other hormones. The means of the comparison of the hormones interaction showed that the interaction effect of 4 mg/l per liter of BA-6-benzylaminopurine hormone with 3 mg/l per liter NAA-naphthaleneacetic has the greatest callus mass.

<table>
<thead>
<tr>
<th>Hormone density (mg/l)</th>
<th>0</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callus mass (mm³)</td>
<td>361.4</td>
<td>254.7</td>
<td>572.1</td>
<td>465.5</td>
</tr>
</tbody>
</table>

Table 2, Mean comparison of BA-6-benzylaminopurine hormone

<table>
<thead>
<tr>
<th>Hormone density (mg/l)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callus mass (mm³)</td>
<td>379.5</td>
<td>281.4</td>
<td>571.7</td>
<td>477.3</td>
</tr>
</tbody>
</table>

In each column, means with the same letters have no significant difference. (α = 5%)

RESULTS AND DISCUSSION

The results indicating that using different concentrations of plant growth regulators, has the different results. According to the results, the most callus mass obtained in 4 mg/l per liter BA-6-benzylaminopurine hormone level and 2 mg/l per liter NAA-naphthaleneacetic. Also the interaction between the two hormones in 4 mg/l per liter BA-6-benzylaminopurine hormone levels and 3 mg/l per liter NAA-naphthaleneacetic has the most callus mass. In research conducted by Bvita et al., (2000) it was reported that, the best callus induction medium in the sage, was the MS medium with 10.47 m.mol NAA-naphthaleneacetic and 4.5 m.mol BA-6-benzylaminopurine. In lavender (Lavandula officinalis) also, MS medium containing 4.5 m.mol NAA-naphthaleneacetic plus 9 m.mol BA-6-benzylaminopurine is defined as the best callusing medium (Dronne et al., 1999). Regeneration of the Seeding through tissue culture techniques, is a basic requirement for the use of molecular genetic technologies in a particular species. Researches has shown that the ability of tissue culture and plant regeneration from callus is related to the genetics and many genes in the nucleus and cytoplasm could control it (Wan et al., 1988).

Totally, by the survey was conducted, the 4 mg/l per liter hormone level of BA-6-benzylaminopurine and 3 mg/l per liter of NAA, was suggested as the best level of callus induction of Salvia. We can improve the micropropagation efficiency by further study and investigation of other factors on callus induction and plant regeneration. Passing through callus phase is associated with changes in plant Svmakvn in the plant which these features are used to produce plants with desirable agronomic characteristics. Whereas from the fast and medium standard (callus phase) of organs and seedlings on explants used for rapid propagation of plants to (Lee et al., 1988; Tapia, 1996). In the most of researches that are done in conjunction with tissue culture of ornamental plants, researchers concluded that BA-6-benzylaminopurine and NAA-naphthaleneacetic hormones are the most effective growth regulators used for shoot proliferation and rooting, respectively (Mikkelsen et al., 1978).

In general, researches of scientists intimates that appropriate concentration of hormone for callus induction in explants and reproduction of seedling from these calluses will be different based on plant species, hormone type used in culture environment, growth stage of maternal plant and type of explant. So, according to intended purpose in tissue culture processes and type of used explant, appropriate hormonal treatment should be used (Stiekema et al., 1988; Tapia, 1996).

REFERENCES


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