

TISSUE CULTURE IN MEDICINAL PLANT OF SUMAC (*Rhus coriaria*)

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**ABSTRACT**

Sumac (*Rhus coriaria*) from Anacardiaceae is an important medicinal plant that contains tannin, dextrose, and miristin. Sumac has many medicinal properties such as antiseptic, antispasm, antioxidant and styptic. There is port about propagation of *Rhus coriaria* through tissue culture. In this research callus induction and regeneration of sumac were studied. Axillary buds were used as explants. Buds were sterilized by 0.02% (w/v) mercuric chloride for 3 min., 70% ethanol for 2 min. and 30% NaOCl for 15 min. The explants were cultured on MS medium supplemented with different hormones such as IAA, BA, IBA, and TDZ. An experiment was carried out using a completely randomized design. There was no significant difference among media for callus initiation. The best shoot induction medium was MS with 1 mg/l BA + 0.5 mg/l IAA. Rooting of shoots occurred on MS medium supplemented with 1 mg/l IBA. The plantlets were successfully transferred to soil.

**KEYWORDS:** Medicinal plant, *Rhus coriaria*, tissue culture, regeneration, propagation.

**INTRODUCTION**

Sumac (*Rhus coriaria*) a member of the Anacardiaceae family is a shrub used in pharmaceutical and cosmetic preparation, food coloring or preservations, veterinary practices and leather processing technologies (Zargari, 1996). It contains many compounds that are useful economically in medicine (Eftekhari *et al.*, 2001). The main component of Sumac extract is tannic compound (Amin *et al.*, 2001). Sumac is a shrub with a long history application in traditional medicine and Iranian cuisine. It is grown wild in the region from the Canary Island over the Mediterranean area to Iran and Afghanistan (Rayne *et al.*, 2007). In Iran it is grown in Mazandaran, Khorasan, Shiraz, Ghazvin, Azarbayegan, Ghom and Hamedan (Rechinger, 1969). The word of "Sumac" is derived from Aramaic word "Sumaqa" which means red. Aside from some studies on sumac as an antioxidant, an anti hyperglycemic, and an anti hyperuricemic agent, many articles have been recently published on antimicrobial activities of sumac because of the advent of new microbial resistances and the need to find new antimicrobial agents (Ahmadian *et al.*, 2008). Fazeli *et al.*, (2008) reported that sumac has a considerable antimicrobial effect on skin bacteria. The results of a research showed that sumac controls xanthine oxidase and decreases uric acid in a gouty person (Candan, 2003).

Ozcan and Haciseferogullari (2004) analyzed sumac (*Rhus coriaria* L.) fruits and showed the following composition: moisture (9.6%), oil (7.4%), protein (2.6%), fiber (14.6%), ash (1.8%) and water-soluble extract (63.8%).

Reports showed that increasing of sumac is not possible via cutting and study on soil media showed that soil medium has a significant effect on cuttings germination but had no significant effect on survival (Doroudi *et al.*, 2008).

Plant tissue culture offers a potential of delivering large quantities of disease-free, true-to-type healthy stock within a short span of time (Hussain *et al.*, 2007).

The present study attempted to do callus induction and micropropagation of Sumac (*Rhus coriaria*).

**MATERIALS AND METHODS**

The plant material was obtained from the Arboretum of Khorasan Razavi Agriculture and Natural Research Center, Mashhad, Iran. Axillary buds were used as explants. Buds were washed with tap water for 30 minutes. The surface disinfection of explants was accomplished by dipping buds in 2% mercuric chloride for 3 min., 70% ethanol for 2 minutes and 30% NaOCl for 15 min. Afterwards, buds were rinsed several times with autoclaved distilled water under a laminar airflow cabinet. MS basal medium (Murashige and Skoog, 1962) at its full strength was used for callus and shoot induction and proliferation. MS medium was also supplemented with different hormones such as IBA, BA, BAP, TDZ and IAA (Table 1). There is no report about tissue culture of sumac so we used some hormone combinations from tissue culture of anacardiaceae plants.

**Table 1.** Combinations of plant hormones for callus induction and shooting.

Number	Combinations of hormone
1	0.01 mg/l IBA+ 2 mg/l BA
2	0.1 mg/l IBA+ 1.5 mg/l BAP
3	4 mg/l TDZ
4	5 mg/l IAA/0+ 1 mg/l BA
5	0.01 mg/l IBA+ 1.5 mg/l BAP

For the rooting of *in vitro* shoots, half and full strength MS medium was either supplemented with IBA and NAA. For rooting, 2 cm *in vitro* grown shoots were transferred to rooting medium.

In addition, all media were supplemented with 30g/l sucrose and adjusted to pH 5.8. Agar-agar was used to solidify the media. For removing phenol compound explants were sub cultured every week. Shoot induction was observed after two months. In order to increase the number of shoots, the explants were sub cultured on the same medium after a regular interval of 28-30 days.

For the purpose of hardening and acclimatization to the field condition, plantlets were taken out of culture medium. The roots of plantlets were washed with tap water in order to remove even the traces of the nutrient medium. The plantlets were then transferred to the pots containing sterilized sand. The pots were kept in the culture room. Adequate water was given to the plantlets at an interval of two-to-three days. After further hardening the plantlets were shifted to the soil in pots and then were shifted to the normal growth condition of the field.

The cultures were kept in the growth room at 25 ±2°C in 16 h photoperiod. The experiment was laid out following complete randomized design and was analyzed using SAS software.

**RESULT**

In order to identify the best medium for callus induction ANOVA with 3 replications was carried out, but there wasn't significant difference between medium for callus initiation.

Shoot induction response for buds explants was satisfactory on MS basal media. The results indicated that MS medium was supplemented with 0/5 mg/l IAA +1mg/L BA was the best medium for shoot regeneration (Table 2, 3 and Fig. 1, 2, 3).

Root induction was also observed in this study using MS basal media supplemented with different concentrations (Table 3, 4 and Fig. 3, 4, 5). But the best medium for rooting was MS+1 mg/l IBA because of the most rooting (Table, 3 and Fig. 4, 5).

**TABLE 2-** ANOVA of completely random design for shooting

Source	Df	Sum of Squares	Means square	F value
model	4	23093/33	141/3333	27/94*
error	10	2066/666	235	
Total	14	25160		

\*: Significant different at 5%

**TABLE3.** Comparison of mean values of shooting

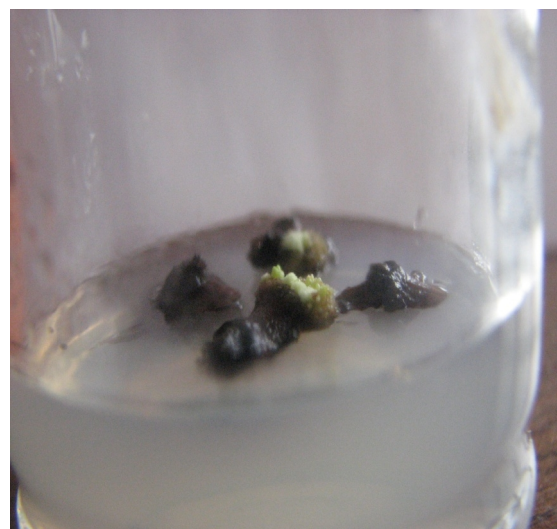
Medium	Duncan groups	Means
1	a	93/33
2	c	13/33
3	c	6/67
4	a	100
5	b	66/67

**TABLE 3.** ANOVA of completely random design for rooting percentage.

Source	DF	Sum of Squares	Mean Square	F Value
Model	4	6173.33	1543.33	19.29
Error	10	800	80	
Total	14	6973.33		

**TABLE 4.** Comparison of mean values of rooting percentage.

Rooting Medium	Duncan groups	Means
1/4 MS	b	16.66
½ MS+ 0/5 µM IBA	b	20
½ MS + 8 mg/l IAA	b	6.66
½ MS + 0/54 µM NAA	b	3.33
MS + 1 mg/l IBA	a	60



**Figure 1.** Shoot initiation on the explants of sumac in MS medium supplemented with 1 mg/l BA + 0.5 mg/l IAA.



**Figure 2.** Shooting of sumac after 2 month



**Figure 3.** Propagation and root induction of Sumac



**Figure 4-** Plantlet of Sumac in MS medium supplemented with 1 mg/l IBA.



**Figure 5-** Plantlet of sumac

## DISCUSSION

There was not any significant difference between treatments of callus induction. But significant difference between treatments of shooting showed that combination of several hormones affected shooting. Comparison of mean value indicated that MS medium supplemented with 0.5 mg /l IAA +1mg/L BA was the best medium for shoot propagation. This indicated that high concentration of BA is suitable for shoot induction.

Our findings were not in harmony with Cetiner *et al.* (2002) who showed that MS medium containing 4 mg/l

BAP is suitable for shoot development of *Pistacia vera* from anacardiaceae. Behboodi (2002) reported that IBA and BAP resulted in maximum growth and shoot formation in *Pistacia vera*.

The best medium for rooting was MS+1 mg/l IBA. This result showed that IBA have more efficient than NAA for root initiation. Also half strength MS was not suitable for root induction. Benmahioul *et.al.* (2009) reported that shoots were rooted *in vitro* in half-strength MS medium containing 12.3 mM IBA in *Pistacia vera* L. High bud proliferation (six shoots per explants) was achieved when using 17.8 mM BA combined with 0.5 mM IBA (Benmahioul *et al.* 2009).

These methods yielded a large number of shoot (about more than 100 shoots from one bud) within short period of time (40-50 days). The total period for seedlings was 60-70 days, considering root induction. A high frequency of plantlet regeneration increases the chance of obtaining transformed plant on genetic transformation procedures and short tissue culture time is helpful in reducing the time and cost. This method could also be used to produce essence and raw materials for pharmaceutical industry.

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