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COMPARISON OF AGAR AND BROTH MEDIA AND VITRIFICATION IN *IN VITRO* CULTURING OF SUMAC

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ABSTRACT
Sumac (*Rhus coriaria L.*) is referred to any of the flowering plants in the genus "Rhus" and related genera in Anacardiaceae family grown in Iran. This plant consists of various chemical compounds, including tannic acid, tannin, dextrose, and myristein. This herb is economically important given its use in cosmetics, pharmaceuticals, foods, and green spaces. The present study aimed to compare the rooting ability of sumac seedling in solid and liquid media. The evaluation of the effect of solid and liquid media was accomplished using agar (8 g/l) and broth media, one of which had a filter sheet as a support. The tests were performed in form of a randomized factorial design with two factors (*i.e.*, the first factor consisted of three levels and the second factor encompassed two levels) in three replications. The result showed that agar medium resulted in the highest rate of rooting growth (44.83%), whereas the broth medium had the lowest rate (4.5%) in this regard. On the other hand, the rate of rooting growth in the broth medium with a support was obtained as 8.5%. It seems that the agar medium more increased the rooting ability of the seedling, compared to the broth media with and without support.

KEYWORDS: Sumac, Medium, Rooting, Agar.

INTRODUCTION

Sumac (*Rhus coriaria L.)* is in Anacardiaceae family and contains several chemical compounds, such as tannic acid, tannin, dextrose, and myristein (Amin, 1991). In the past, sumac was used as an astringent for the management of diarrhea and mouth bleeding; furthermore, it was applied as an antiperspirant (Zargari, 2014). Recent studies have shown that sumac extract affects a number of intestinal bacteria (Fazeli, 2005). Sumac is economically important in cosmetics, pharmaceuticals, foods, and green spaces (Bloshenko, 1996). This plant has a broad vegetative propagation and produces dense clones in form of a basal shoot. One study shows that a pre-heating of 120-140°C for 15 min would increase the germination of the Sumac seeds under different conditions, such as heat, ash, pH, water potential, and ethylene (Neeman *et al.*, 1999). Sumac seeds lack germination ability in their natural habitat (*i.e.*, Binalood mountain ranges in Khorasan, Iran) and are unable to germinate with various hormone compounds (Daroodi, 2010). There is limited evidence on the proliferation of sumac using tissue culture methods. One of the problems of broth culture is the incidence of vitrification, which has been observed in liquid medium for apple and peach, clove, and artichoke (Bagheri, 2008). According to the results obtained by Deberg (1983) and Zio and Hallway (1983), vitrification occurs as a result of high humidity in the growth tube, very low agar concentration in the solid medium, or culturing on a broth medium (Bagheri, 2008). With this background in mind, the present study was conducted to compare the rooting ability of sumac seedling in liquid and solid media.

MATERIALS AND METHODS

This study was conducted in the Cell Culture and Tissue Culture Laboratory of the Agricultural Biotechnology Research Institute of Iran (Branch of the East and North East) to compare the effect of solid, liquid, and supported liquid media on the rooting ability of sumac seedling. This end was accomplished by using one agar medium and two broth media, one of which contained a filter sheet as a support to be used as a control. In order to evaluate the effect of agar on the rooting ability of the samples, the regenerated stem and leaf calluses were transferred from N and H media (Table 1) into the Murashige and Skoog medium (MS) medium with 1 mg/l indole-3-butyric acid hormone and 8 g agar and broth (containing a filter sheet as a support) (Fig. 1 and Table- 2).

This experiment was performed using a randomized factorial design with two factors in three replications. The first factor included three levels (*i.e.*, agar medium, broth medium, and broth medium with a filter sheet as a support), whereas the second factor had two levels (*i.e.*, N and H hormonal compositions), both of which were carried out in three replications. This test involved the investigation of the independent and interactive effects of agar and regeneration hormonal composition on root creation.

TABLE 1. Media used in the present study and their hormonal composition: MS: Murashige and Skoog, BA: 14C-benzyladenine, IAA: Indole-3-acetic acid, IBA: indole-3-butyric acid

Media name	Basic media	Sucrose (r/l)	Agar	Hormonal content
N	MS	30	8 g	1 BA+0.5 IAA
H	MS	30	8 g	2 BA+0.01 IBA

FIGURE 1. Effect of 1 mg/l Hormonal IBA (Indole Butyric Acid) in a medium with Agar on rooting growth

TABLE 2. Impact of agar on sumac rooting: MS: Murashige and Skoog, IBA: indole-3-butyric acid

Basic media	Sucrose (g/l)	Hormonal content	Agar
MS	30	1 mg/l IBA	8 g
MS	30	1 mg/l IBA	No agar
MS	30	1 mg/l IBA	Filter sheet as support and no agar

RESULTS

1. Impact of Agar on Rooting

Analysis of variance for the rooting characteristics of regenerated samples for the first factor, which included the agar effect on rooting, revealed a significant difference between various types of media (P≤0.5; Table 3). Comparison of mean values resulted in the classification of the agar medium, broth medium with filter sheet, and broth medium without a support in classes a, b, and c, respectively.

In this regard, agar medium had the highest percentage of rooting generation (44.83%). On the other hand, the lowest percentage of rooting generation (4.5%) was related to the broth medium without a support. In addition, the broth medium with a filter sheet for support had 8.5% rooting generation (Table 4).

TABLE 3. Analysis of variance regarding agar impact on rooting growth (*A significant difference based on Duncan's test at a probability level of 5%)

Source of changes	Degree of freedom	Sum of squares	Mean squares	F value	Coefficient of variation
Model	5	10696.94	*2139.38	663.95	9.31
Error	12	38.66	3.22		
Corrected Total	17	10735.61			

TABLE 4. Comparison of the mean effect of agar on rooting growth using Duncan's test, P-value was significant at 0.05.

Type of MS media	Duncan's grouping	Mean
Agar media	a	44.83
Broth with a filter sheet as support	b	8.5
Broth	c	4.5

2. Effect of the Best Regeneration Hormonal Composition on Rooting Growth

The analysis of variance for the rooting characteristics of the regenerated samples for the second factor, which included the effect of regeneration hormonal compositions on rooting, demonstrated a significant difference among various hormonal compositions (P≤0.5). Based on the comparison of mean values, N hormonal composition was classified as a, whereas the H hormonal composition was categorized as b. In this respect, the N hormonal composition had the highest percentage of rooting

(30.22%), while the H hormonal composition showed a rooting generation rate of 8.33% (Table 5).

3. Interactive Effect of Regeneration Hormonal Composition and Agar on Rooting Growth

The analysis of variance for the rooting characteristics of the regenerated samples for the third factor, which included the effect of regeneration hormonal compositions and agar on rooting, revealed the highest percentage of rooting (72.66%) in the N hormonal composition in the agar medium. In addition, the lowest rooting percentage was found in the H hormonal composition in the broth medium with a rooting generation of 0%.

According to the results, the H hormonal composition had 17% rooting generation in the agar medium, whereas the N hormonal composition had a rooting generation rate of 9% in the broth medium. In addition, the N and H hormonal compositions showed the rooting generation rates of 9% and 8% in the broth medium with a support, respectively (Table 7).

TABLE 5. Comparison of the mean effect of the best hormonal compositions on rooting growth using the Duncan's test: P-value was significant at 0.05.

Type of medium of hormonal compositions	Duncan's grouping	Mean
N	Na	30.22
H	Hb	8.33

TABLE 6. Analysis of variance regarding the interactive effect of regeneration hormonal compositions and agar on rooting growth (*A significant difference based on Duncan's test at a probability level of 5%)

Source of changes	Degree of freedom	Analysis of variance	Mean squares	F value
Type of medium	2	5925.77	*2962.88	919.52
Callus inducing hormonal composition	1	2159.05	*2156.05	669.12
Interactive effect of hormonal compound and type of medium	2	2615.11	*1307.55	405.79

TABLE 7. Comparison of the mean interactive effect of the type of medium and callus-inducing hormonal compositions on the rooting of cultured sumac explants

Type of medium	Callus inducing hormonal composition	Mean rooting
Agar	H	17±0.48
	N	72±0.35
Broth with a filter sheet as a support	H	8±0.7
	N	9±0.33
Broth	H	0
	N	9±0.33

DISCUSSION

In the present research, the rooting ability of sumac regenerated explants obtained from N and H hormonal compositions was evaluated in agar, broth without a support, and broth with a filter sheet (as a support) media. According to the results, the highest rooting percentage was observed in the agar medium (44.8%), whereas the lowest rate was related to the broth medium (4.5%). In addition, the broth medium with a support had a rooting generation rate of 8.5%. Accordingly, there was a significant difference among the three evaluated media in terms of rooting growth rate (P=0.05). Results also demonstrated a significant difference between the N and H hormonal compositions, where the N hormonal composition yielded a more efficient rooting generation (P≤0.5).

Reduced percentage of rooting in the broth medium was due to the drowning of shoots in the medium and lack of proper aeration. In addition, the reduction of explants quality in the broth medium might be due to the reduced transpiration of the plant and calcium absorption where there is a high in vitro humidity level, which is associated with the browning and destruction of tissues due to the decomposition and destruction of the tissues (Kaar Amad and Moghaddam, 2014, Ruzic *et al*., 2000). According to our findings, there was a higher rate of rooting growth in the broth medium with a support, compared to that in the broth medium without a support. The broth medium can cause a form of physiological impairment known as vitrification, which happens when there is excess water in the culture medium. However, the enhancement of agar concentration properly prevents vitrification (Bagheri, 2008).

Agar forms a gel that is able to absorb compounds (Kamal al-Dini, 2006). This substance acts similar to the active coal method in removing the cellular waste products from the medium. This property can prevent the absorption of some chemical compounds by the cultured tissue. One of the substances that can be absorbed by agar is cytokinin. Generally, agar gel has a negative electrical load and causes positive polarity in the cell membrane (Kamal al-Dini, 2006). In addition, the success persistence of this gel may be related to its complex absorption and electrical capability (Kamal al-Dini, 2006). Vitrification has been observed in the broth medium for apple and peach, clove, and artichoke (Bagheri, 2008).

The results obtained by Deberg (1983) and Zio and Hallway (1983) indicated that vitrification was the result

of a high humidity level in the growth tube, significantly low agar concentration in the agar medium, or culturing on a broth medium (Bagheri, 2008). In 1983, Deberg concluded that the enhancement of agar concentration properly prevented vitrification (Bagheri, 2008). Zaraveshan *et al.* (2005) observed that benzyl adenine hormone independently led to the incidence of vitrification (Zaroushan and Bernard, 2013). They also reported that in a medium free of benzyl adenine, the presence of salicylic acid improved the return to the normal state (Zaroushan and Bernard, 2013). Accordingly, it was concluded that, the use of broth culture medium is not recommended for the in vitro culturing of clove, and agar culture medium is more efficient in this regard (Zaroushan and Bernard, 2013).

On the other hand, Karamad *et al*. reported that the rooting percentage was higher in the broth medium with Gisela 6 as the base, compared to the agar medium (Kaar Amad and Moghaddam, 2014). However, in the mentioned study, the broth culture medium reduced the quality and survival of the seedlings and was more efficient for the seedling survival, compared to the agar culture (Kaar Amad and Moghaddam, 2014). This was due to the accumulation of moisture on the walls of the containers in form of drops (Kaar Amad and Moghaddam, 2014). In a study performed by Kharazi *et al*. the broth medium resulted in a higher speed of clove bud proliferation, compared to the agar medium (Kharazi and Nemati, 2012). However, vitrification was higher in the agar medium (Kharazi and Nemati, 2012). Accordingly, they did not recommend using broth medium for the in vitro cultivation of cloves (Kharazi and Nemati, 2012).

CONCLUSION

Our findings are in congruence with those of the mentioned studies since the highest rate of rooting growth was observed in the agar medium. In addition, no favorable rooting was obtained in the broth without support and broth medium with support due to the lack of suitable aeriation, incidence of vitrification, and possibility of the release of BA (cytokinin) residues from the hormonal compositions in the broth medium. The higher rooting growth in the N hormonal composition, compared to that in the H hormonal composition, in the agar medium was due to the fact that the former contained cytokinin hormone half time less than the latter; therefore, it exerted lower preventive effect on rooting. As the findings of the present study indicated, agar medium was the best medium for the rooting of sumac explants.

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