PLANT STRESS INDUCED BY EXCESSIVE SUCROSE AND AGAR CONCENTRATION ON *IN VITRO* GERMINATION AND PLANTLET GROWTH OF *LAURUS NOBILIS* L. (LAURACEAE)

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Abstract. *Laurus nobilis* L. a member of Lauraceae family, is a beautiful medicinal and aromatic, evergreen plant. In this study shortening of *in vitro* germination time and the appropriate sucrose and agar concentration for this approach were investigated. Excessive sucrose concentration negatively affected germination percentage, root length, shooting percentage and shoot length in Murashige and Skoog Medium (MS). Medium without sucrose (0 g/L sucrose) having the highest germination rate (96.3%), the highest root length per plant (3.84 cm), the highest shooting rate (96.3%) and the highest shoot length per plant (1.68 cm) in all concentrations of sucrose used (0, 10, 20 and 30 g/L) after 4 weeks of culturing. Although sucrose and agar interactions exerted effects on the germination percentage, additionally all concentrations of agar used (3, 6 and 9 g/L) showed that adding excessive amounts to the growth medium was not necessary to induce germination and shooting in general. 0 mg/L sucrose and 3 g/L agar found optimum composition of the media for both rooting and shooting in all used media. This protocol will be useful for rapid and economic large-scale *in vitro* cultivation of *Laurus nobilis* to obtain aseptic seedlings.
Keywords: *recalcitrant seed culture, tissue culture, organic additives, medium specification, rooting, shooting*

Introduction

Developing an efficient and rapid system for *in vitro* tissue and organ culture is highly dependent on plant genus, species, physical and chemical culture environment. The content of culture media for germination, shooting, rooting and multiplication has an enourmous efficacy on costs in large-scale commercial manufacturers producing ornamental plants, vegetable seedling, fruit sapling and secondary metabolites as well as governmental or private scientific research and development centers.

During the past decades different media have been developed for *in vitro* plant culture. One of the known important components are carbon sources such as sucrose. Sucrose is mostly used 2-4% to bring in the young explant ready to use carbon (Gamborg et al., 1976). Especially 3% concentration is choosen in lots of studies as known recommendation of (Murashige and Skoog, 1962). In some cases high level of sucrose can be caused decreasing *in vitro* germination or the other growth parameters (Jo et al., 2009; Huh et al., 2016). The second known components are gelling agents such as agar. Generally, if solid media is aimed to use, agar is added 0.6-0.8% ratio to increase media viscosity (Debergh, 1983). In some cases increasing ratio of the gelling agent led to a progressive decrease in

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adventitious shoot per explant (Owens and Wozniak, 1991; Casanova et al., 2008). On the contrary, decreasing ratio of the agent cause turning media solid to liquid is not suitable in some conditions such as germination, rooting or shoot multiplication in some plants. Because of inability of the explant to hold on the surface and subside to the bottom cause undesired physical condition as non-stability of the explant and oxygen deficiency for the plant parts. The model plants for the study, bay laurel (*Laurus nobilis* L.) belongs to the family Lauraceae family which include valuable genus (Werff and Richter, 1996; Judd et al., 1999; Marques, 2001). Bay laurel are dioecious, evergreen tree or shrub known as laurel, bay laurel or sweet bay (Marzouki et al., 2009). Nearly all plant parts have been used as medicinal-aromatic, ornamental, plant-animal health and environmental purpose for a long time (Patrakar et al., 2012; Chalal et al., 2017). Active chemicals of most of these usage obtained from traditionally grown or naturally existing plants. The high economic value of the species in Lauraceae family has caused these to be destroyed in the natural habitats over years as our long term observations. Optimized tissue culture techniques can provide protection of natural habitats. There are a few studies for different purposes given some information the *in vitro* propagation of *Laurus nobilis* L. (Rady et al., 1999; Souayah et al., 2002; Al-Gabbiesh et al., 2014; Nadarajan and Pritchard, 2014; Royandazagh, 2019). The objective of the present study was to evaluate the effect of sucrose and agar concentrations on *in vitro* germination, primary root and primary shoot growth of bay laurel (*L. nobilis*) in *in vitro* seedlings to achieve scientific and economic gain.

Materials and Methods

The research was carried out at Kocaeli University, Plant Tissue Culture and Biotechnology Laboratory during 2018-2019. The fruits of laurel were picked up from only one tree that have been observing for many years, at natural habitats of Kocaeli City in Turkey. As soon as the bay laurel fruits were collected at 2018 November, 24; they were brought to the laboratory and the fruit flesh was peeled with the help of paper towel. One day after this procedure, the seed coats were removed with fingernail to obtain naked seed. The naked seed (the average weight of 100 naked seeds was 0.574 g/seed) kept in refrigerator (8 ºC) until the next day to use. Seeds were washed with running tap water for 1 hour and soaked for 40 min. in 20% commercial bleach (Na-hypochloride 5≤) solution and rinsed in two changes of sterile distilled water. Seeds were cultured on MS medium (Murashige and Skoog, 1962) with full micro, macro elements and vitamins on four trade mark sucrose ($C_{12}H_{22}O_{11}$-MW:342.29) (0, 10, 20 and 30 g/L) and three trade mark agar-agar concentration (3, 6 and 9 g/L). The pH of the medium was adjusted to 5.6 with 1 M KOH or 1 M HCl prior to autoclaving for 15 min. at 121 ºC. The media were filled in sterile glass petri plates in 6 cm diameter subsequently sterile-naked seeds were placed on the surface of media and kept in culture room at 23 ºC under dark condition for four weeks. Each experimental treatment combination consisted of two factors; sucroseXagar (4X3). The experiment laid out in completely randomised design with 3 replications. In only one repeat which was carried out with 3 glass petri dishes each of which contained only one seed. Results were evaluated weekly after cultures along four weeks. The recorded parameters were germination percentages (%), root length (cm), shooting percentages (%) and shoot length (cm) of the newly germinating seeds. The data were subjected to analysis of variance and significant differences among the treatments were tested using two-way ANOVA and means were separated by Duncan Multiple Range Test at $P \leq 0.05$.

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Results and Discussion

Germination started as of the first week and negative effect of high sucrose concentration observed and statistically detected by the time. There was no positive or negative effect of agar concentration on germination percentage along 4 weeks and at the end of study (*Tables 1, 2, 3, 4; Fig. 1*). The combination of sucrose and agar in used MS medium significantly affected *in vitro* seed germination percentage by the second week. At the same time root length was also statistically and negatively affected by high sucrose concentration at the end of the experiment.

Table 1. *Laurus nobilis L. germination percentage and root length at the end of the 1st week in the modified MS medium*

Germination Percentage (%) at the end of the 1st week					**Root Length (cm) at the end of the 1st week**				
	3 g/L Agar	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose*** **		**3 g/L Agar**	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose*** **
0 g/L Sucrose	33.3*	22.2	33.3	**29.6 A**	**0 g/L Sucrose**	0.17a****	0.10ab	0.10ab	**0.12 A**
10 g/L Sucrose	22.2	11.1	33.3	**22.2 AB**	**10 g/L Sucrose**	0.13ab	0.03b	0.13ab	**0.10 AB**
20 g/L Sucrose	22.2	11.1	0.0	**11.1 AB**	**20 g/L Sucrose**	0.06b	0.03b	0.00b	**0.03 AB**
30 g/L Sucrose	0.0	0.0	0.0	**0.0 B**	**30 g/L Sucrose**	0.00b	0.00b	0.00b	**0.00 B**
Mean of Agar**	**19.5**	**11.1**	**16.7**		**Mean of Agar****	**0.09**	**0.04**	**0.06**	

*N.S.; No significant difference in sucroseXagar concentration interaction in germination percentage, **N.S.; No significant difference in agar concentration in germination percentage and in root length, ***Capital letters denote significantly differences in sucrose concentration in germination percentage and in root length, ****Lower-case letters denote significantly differences in sucroseXagar concentration interaction in root length

Table 2. *Laurus nobilis L. germination percentage and root length at the end of the 2nd week in the modified MS medium*

Germination Percentage (%) at the end of the 2nd week					**Root Length (cm) at the end of the 2nd week**				
	3 g/L Agar	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose****		**3 g/L Agar**	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose****
0 g/L Sucrose	88.9a***	100.0a	66.6abc	**85.2 A**	**0 g/L Sucrose**	1.23a***	0.80bc	1.25a	**1.09 A**
10 g/L Sucrose	88.9a	66.6abc	88.9a	**81.5 A**	**10 g/L Sucrose**	0.88ab	0.46bc	1.03ab	**0.79 AB**
20 g/L Sucrose	66.6abc	66.6abc	77.7ab	**70.3 A**	**20 g/L Sucrose**	0.37bc	0.56bc	0.79bc	**0.57 BC**
30 g/L Sucrose	22.2c	55.5bc	33.3bc	**37.0 B**	**30 g/L Sucrose**	0.13c	0.85bc	0.67bc	**0.55 C**
Mean of Agar*	**66.7**	**72.2**	**66.6**		**Mean of Agar******	**0.65 B**	**0.67 B**	**0.94A**	

*N.S.; No significant difference in agar concentration in germination percentage, **Capital letters denote significantly differences in sucrose concentration in germination percentage and in root length, ***Lower-case letters denote significantly differences in sucroseXagar concentration interaction in germination percentage and in root length, ****Capital letters denote significantly differences in agar concentration in root length

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Table 3. *Laurus nobilis L. germination percentage and root length at the end of the 3rd week in the modified MS medium*

	Germination Percentage (%) at the end of the 3rd week					**Root Length (cm) at the end of the 3rd week**			
	3 g/L Agar	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose****		**3g/L Agar**	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose******
0 g/L Sucrose	100.0a***	100.0a	88.9a	**96.3 A**	**0 g/L Sucrose**	2.96	2.24	1.9	**2.37**
10 g/L Sucrose	88.9a	66.6ab	100.0a	**85.2 AB**	**10 g/L Sucrose**	2.08	1.49	2.45	**2.01**
20 g/L Sucrose	66.6ab	66.6ab	77.7ab	**70.3 BC**	**20 g/L Sucrose**	1.52	2.44	2.28	**2.08**
30 g/L Sucrose	33.3b	66.6ab	66.6ab	**55.5 C**	**30 g/L Sucrose**	1.80	1.50	1.49	**1.59**
Mean of Agar*	**72.2**	**74.9**	**83.3**		**Mean of Agar***	**2.09**	**1.92**	**2.03**	

*N.S.; No significant difference in agar concentration in germination percentage and in root length or sucroseXagar concentration interaction in root length, **Capital letters denote significantly differences in sucrose concentration in germination percentage, ***Lower-case letters denote significantly differences in sucroseXagar concentration interaction in germination percentage, **** N.S.; No significant difference in sucrose concentration in root length

Table 4. *Laurus nobilis L. germination percentage and root length at the end of the 4th week in the modified MS medium*

	Germination Percentage (%) at the end of the 4th week					**Root Length (cm) at the end of the 4th week**			
	3 g/L Agar	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose******		**3 g/L Agar**	**6g/L Agar**	**9g/L Agar**	**Mean of Sucrose******
0 g/L Sucrose	100.0a***	100.0a	88.9 ab	**96.3 A**	**0 g/L Sucrose**	4.22**	3.93	3.37	**3.84 A**
10 g/L Sucrose	88.9ab	66.6ab	100.0 a	**85.2 AB**	**10 g/L Sucrose**	3.70	2.91	3.76	**3.46 AB**
20 g/L Sucrose	66.6ab	88.9ab	77.7 ab	**77.7 AB**	**20 g/L Sucrose**	2.72	3.02	3.76	**3.17 AB**
30 g/L Sucrose	44.4b	77.7ab	66.6 ab	**62.9 B**	**30 g/L Sucrose**	2.63	2.14	2.93	**2.57 B**
Mean of Agar*	**72.2**	**83.3**	**83.3**		**Mean of Agar***	**3.32**	**3.00**	**3.46**	

*N.S.; No significant difference in agar concentration in germination percentage and in root length, **N.S.; No significant difference in sucroseXagar concentration interaction in root length, *** Lower-case letters denote significantly differences in sucroseXagar concentration interaction in germination percentage, **** Capital letters denote significantly differences in sucrose concentration in germination percentage and in root length

Shooting started after two weeks of culture and similarly shooting percentage and shoot length statistically affected from high sucrose concentration negatively and sucroseXagar interaction after 4 weeks (*Tables 5, 6 7, 8; Fig. 2*). Similarly, there were no positive effect of agar concentrations on shooting percentage along 4 weeks of study. Agar doses showed effectiveness on shoot length in 2nd and 3rd week but at the end of the study the effect has disappeared and agar concentrations showed similarity in statistics.

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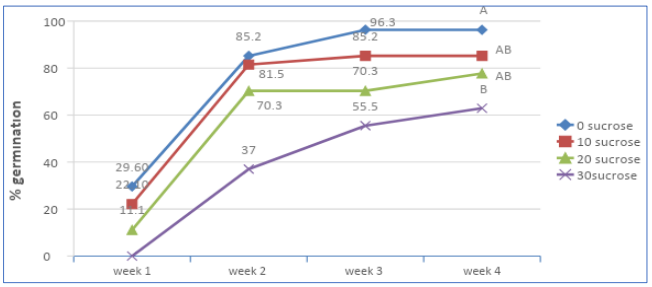

Figure 1. *Germination percentage in all sucrose concentrations along 4 weeks*

Table 5. *Laurus nobilis L. shooting percentage and shoot length at the end of the 1st week in the modified MS medium*

	Shooting Percentage (%) at the end of the 1st week					Shoot Length (cm) at the end of the 1st week			
	3 g/L Agar	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose*** **		**3 g/L Agar**	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose*** **
0 g/L Sucrose	0*	0	0	**0**	**0 g/L Sucrose**	0*	0	0	**0**
10 g/L Sucrose	0	0	0	**0**	**10 g/L Sucrose**	0	0	0	**0**
20 g/L Sucrose	0	0	0	**0**	**20 g/L Sucrose**	0	0	0	**0**
30 g/L Sucrose	0	0	0	**0**	**30 g/L Sucrose**	0	0	0	**0**
Mean of Agar**	**0**	**0**	**0**		**Mean of Agar****	**0**	**0**	**0**	

*N.S.; No significant difference in sucroseXagar concentration interaction in shooting percentage and shoot length, **N.S.; No significant difference in agar concentration in shooting percentage and shoot length, ***N.S.; No significant difference in sucrose concentration in shooting percentage and shoot length

Table 6. *Laurus nobilis L. shooting percentage and shoot length at the end of the 2nd week in the modified MS medium*

	Shooting Percentage (%) at the end of the 2nd week					Shoot Length (cm) at the end of the 2nd week			
	3g/L Agar	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose ****		**3 g/L Agar**	**6 g/L Agar**	**9g/L Agar**	**Mean of Sucrose ** **
0 g/L Sucrose	88.9a***	100a	66.6abc	**85.2 A**	**0 g/L Sucrose**	0.28ab***	0.26ab	0.37a	**0.30**
10 g/L Sucrose	77.8ab	33.3bc	88.9a	**66.7 A**	**10 g/L Sucrose**	0.27ab	0.25ab	0.32a	**0.28**
20 g/L Sucrose	66.6abc	55.5abc	66.6abc	**62.9 AB**	**20 g/L Sucrose**	0.18ab	0.21ab	0.24 ab	**0.21**
30 g/L Sucrose	22.2c	55.5abc	33.3bc	**37.0 B**	**30 g/L Sucrose**	0.10b	0.18ab	0.30 ab	**0.19**
Mean of Agar*	**63.9**	**61.1**	**63.9**		**Mean of Agar *****	**0.21C**	**0.23B**	**0.31 A**	

*N.S.; No significant difference in agar concentration in shooting percentage, **N.S.; No significant difference in sucrose concentration in shoot length, *** Lower-case letters denote significantly differences in sucroseXagar concentration interaction in shooting percentage and shoot length, **** Capital letters denote significantly differences in sucrose concentration in shooting percentage, *****Capital letters denote significantly differences in agar concentration in shoot length

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Table 7. *Laurus nobilis L. shooting percentage and shoot length at the end of the 3rd week in the modified MS medium*

Shooting Percentage (%) at the end of the 3rd week					Shoot Length (cm) at the end of the 3rd week				
	3 g/L Agar	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose *****		**3 g/L Agar**	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose *****
0 g/L Sucrose	100a*	100a	66.6abc	**88.9 A**	**0 g/L Sucrose**	0.92abc*	0.67bc	1.18a	**0.92 A**
10 g/L Sucrose	88.9ab	55.5bc	88.9ab	**77.8 AB**	**10 g/L Sucrose**	0.58c	0.67bc	1.12ab	**0.79 AB**
20 g/L Sucrose	66.6abc	55.5bc	77.7ab	**66.6 BC**	**20 g/L Sucrose**	0.43c	0.59c	0.81abc	**0.61 B**
30 g/L Sucrose	33.3c	66.6abc	55.5bc	**51.8 C**	**30 g/L Sucrose**	0.50c	0.53c	0.60c	**0.54 B**
Mean of Agar**	**72.2**	**69.4**	**72.2**		**Mean of Agar******	**0.61 B**	**0.62 B**	**0.93 A**	

* Lower-case letters denote significantly differences in sucroseXagar concentration interaction in shooting percentage and shoot length, ** N.S.; No significant difference in agar concentration in shooting percentage, *** Capital letters denote significantly differences in sucrose concentration in shooting percentage and shoot length, **** Capital letters denote significantly differences in agar concentration in shoot length

Table 8. *Laurus nobilis L. shooting percentage and shoot length at the end of the 4th week in the modified MS medium*

Shooting Percentage (%) at the end of the 4th week					Shoot Length (cm) at the end of the 4th week				
	3 g/L Agar	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose *****		**3 g/L Agar**	**6 g/L Agar**	**9 g/L Agar**	**Mean of Sucrose *****
0 g/L Sucrose	100a*	100a	88.9ab	**96.3 A**	**0 g/L Sucrose**	1.87a*	1.40abcd	1.77ab	**1.68 A**
10 g/L Sucrose	88.9ab	55.5bc	88.9ab	**77.8 AB**	**10 g/L Sucrose**	1.28abcd	1.60abc	1.65ab	**1.51 A**
20 g/L Sucrose	66.6abc	77.7ab	77.7ab	**74.0 BC**	**20 g/L Sucrose**	0.77c	0.72c	1.37abcd	**0.95 B**
30 g/L Sucrose	33.3c	66.6abc	66.6abc	**55.5 C**	**30 g/L Sucrose**	0.83c	0.89cd	1.05bcd	**0.93 B**
Mean of Agar**	**72.2**	**75.0**	**80.5**		**Mean of Agar****	**1.19**	**1.15**	**1.46**	

* Lower-case letters denote significantly differences in sucroseXagar concentration interaction in shooting percentage and shoot length, ** N.S.; No significant difference in agar concentration in shooting percentage and shoot length, *** Capital letters denote significantly differences in sucrose concentration in shooting percentage and shoot length

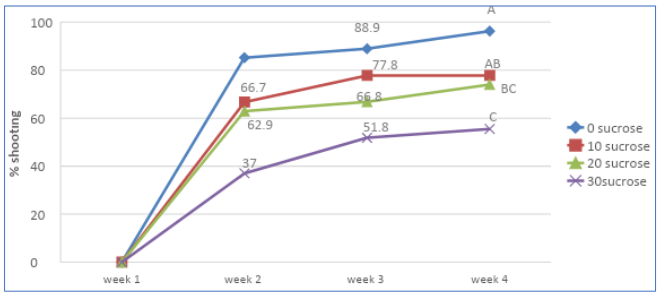

Figure 2. *Shooting percentage in all sucrose concentrations along 4 weeks*

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Studies are available outlining the ineffectiveness of excessive agar or sucrose doses in *in vitro* cultures. For example; Rabaiolli et al. (2017) demonstrated better *in vitro* rhizogenesis was achieved when ½ WPM medium supplemented with 30 cm^3 vermiculite without agar than 3.5 g/L or 7 g/L used agar for primary and secondary root percentages in *in vitro* and survival rate and leaf number in *ex vitro* in *Handroanthus chrysotrichus*. Suthar et al. (2011), in their work on *Boswellia serrata* in *in vitro*, studied agar at the rate of 0.0; 0.2; 0.4; 0.6; 0.8% w/v in MS+0.5 mg/L BAP+0.05 mg/L NAA for shoot multiplication from shoot clusters and 0.0 to 1% (w/v) agar in rooting medium containing 0.5 mg/L IBA+ 0.25 mg/L NAA+ antioxidants solution from rooting from shoots. According to their results 0.2% agar concentration was the best in number of shoots and 0.0% agar gave the highest rate of shoot length, number of leaves, fresh and dry weight, chlorophyll a, b and total. Similarly, they found that 0.0 % agar gave the highest rooting percentage, number of roots, root length and shoot length. Casanova et al. (2008) studied on agar concentration (0, 2, 4, 6, 8, 10 and 12 g dm^3) and vessel closure for *Dianthus caryophyllus in vitro* culture. They emphasized that the highest organogenic response was obtained in MS medium solidified with 2 g dm^3 agar (17.7 shoots per petal) and the least response was obtained in MS medium with 12 g dm^3 (4.3 shoots per petal) after 30 days of culture. Cortés-Olmos et al. (2018) studied on different sucrose (20 and 30 gL^{-1}) and agar (8 and 10 gL^{-1}) in full and half strength MS medium for *Lophophora williamsii*. They found that neither agar and sugar concentrations nor MS strength changed seed germination percentage after 49 days. But seedling size and areoles per seedling found higher in lower sucrose (20 gL^{-1}) and lower agar (8 gL^{-1}) than higher ones (30 gL^{-1} sucrose and 10 gL^{-1} agar). In another study, Gürel and Gülşen (1998) studied on two cultivars of *Prunus amygdalus* shoot tip *in vitro* culture in MS media. Sucrose concentrations were 2, 3, 4, 5 and 6% at 0.7% agar and agar were examined at the rate of 0.5; 0.6; 0.7; 0.8 and 0.9% at 3% sucrose. They emphasized that not in initiation stage but both multiplication and transplantation stage highest and lowest sucrose levels negatively affected shoot production and growth rate of developing shoots. 3 and 4% sucrose level found better in this stages. In addition, they found that the increasing concentration of agar caused a decrease in the growth of shoot tip explants in initiation stage 0.5; 0.6 and 0.7% agar were found significantly better than 0.8 and 0.9 agar on shoot development. Schulze et al. (2017) studied on *in vitro* germination of *Prunus lusitanica*. They used MS media with or without GA_3 and with different BA with two sucrose level (30 and 60 gL^{-1}). Their findings showed that radical and shoot emergence were greater on media with 30 gL^{-1} sucrose than with 60 gL^{-1}. In *Hancornia speciosa* cultured *in vitro*, dos Santos et al. (2017) observed that increasing sucrose concentration up to 60 g L^{-1} reduced germination speed and seedling height when they use (15, 30, 45 and 60 g L^{-1} sucrose) after 60 days of *in vitro* culturing of the naked embryos.

Conclusion

In conclusion, the results of this study revealed that increasing sucrose concentration as carbon source negatively influence germination efficiency of *Laurus nobilis* from naked seeds derived from mature female tree (*Fig. 3*). Among all the treatments, all parameters recorded to evaluate seedling growth of *Laurus nobilis* showed better performance in 0 g/L of sucrose than 10, 20, and 30 g/L in MS medium. Higher concentration of sucrose caused lateness in germination and shooting with reduced germination and shooting rate, root and shoot length (*Figs. 4, 5, 6, 7*). When sucrose doses

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are ignored and only agar doses are considered, there were no differencies in all parameters at the end of the experiment excepting middle stage of experiment. When sucrose and agar interaction were examined, 0 g/L sucrose with 3 g/L agar and 0 g/L sucrose with 6 g/L agar were found 100% in both germination and shooting percentage than 9 g/L agar. This results supports the hypothesis that agar as viscosity source and sucrose as carbon source reduces the availability of nutrients in media to the plants. Moreover, root and shoot length found the highest in 0 g/L sucrose with 3 g/L agar among all the treatments (0, 10, 20, and 30 g/L sucrose with 3, 6 and 9 g/L agar). The decreased germination and seedling capacity in seed explants may result from a decrease in the amount of water available in the medium that have high rate of sucrose and agar. In this perspective, it must be underlined sucrose and agar compositions should be experienced in all plant genus and explant types for commercially or academically valuable application to lower the cost and to achieve the right results from other tested treatments.

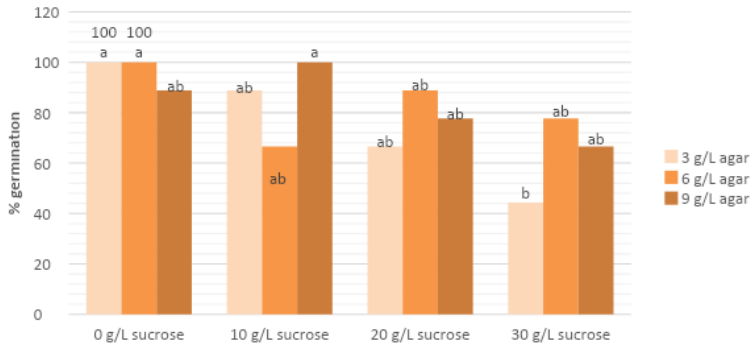

Figure 3. *Germination percentage in all sucrose and agar concentrations at the end of the 4th week*

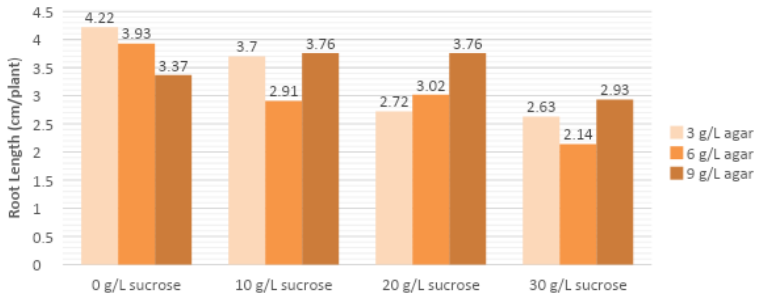

Figure 4. *Root length per plant in all sucrose and agar concentrations at the end of the 4th week*

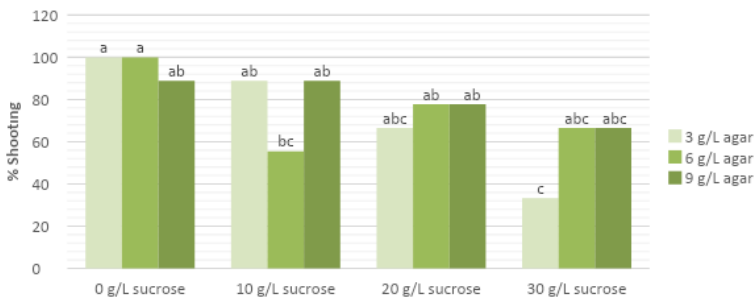

Figure 5. *Shooting percentage in all sucrose and agar concentrations at the end of the 4th week*

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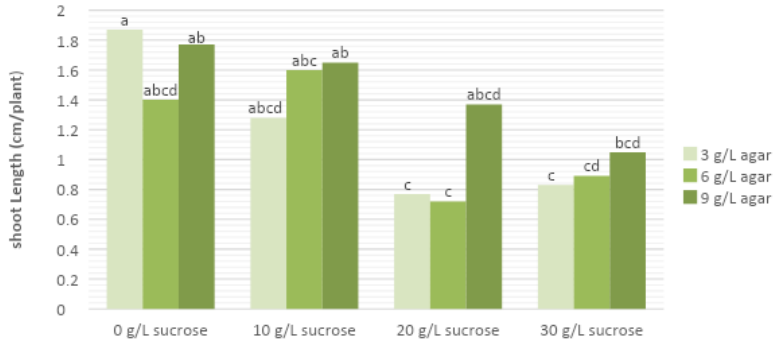

Figure 6. *Shoot length per plant in all sucrose and agar concentrations at the end of the 4th week*

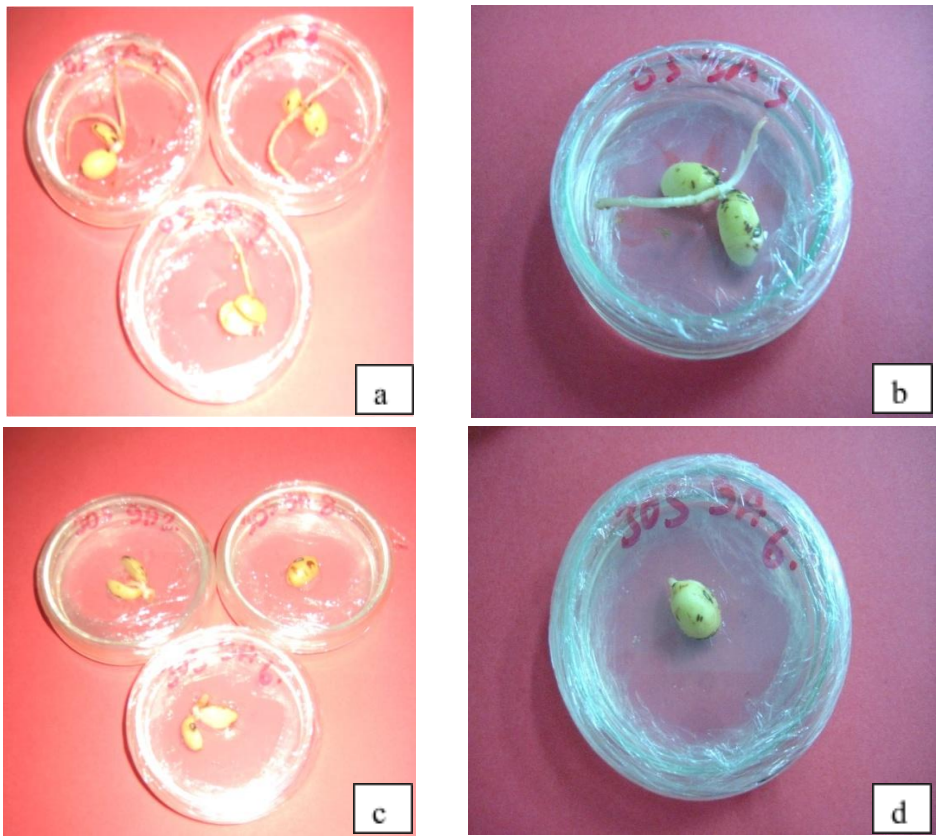

Figure 7. *Gemination and shooting of Laurus nobilis L. in different sucrose and agar concentration after 4 weeks; (a, b) successful germination and shooting at 0 g/L sucrose and 3 g/L agar, (c, d); poor germination and shooting at 30 g/L sucrose and 3 g/L agar*

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