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Effect of Smoke Solution of Sage (*Salvia officinalis* L.) on Root and Shoot Growth of Grass Pea (*Lathyrus sativus* L.)

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A R T I C L E I N F O

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A B S T R A C T

In this study, the effect of different concentrations of smoke solution derived from sage (*Salvia officinalis* L.) on root and shoot growth of grass pea (*Lathyrus sativus* L.) was investigated in pots, in petri dishes (*in vivo*) and *in vitro* conditions. Smoke solution was obtained from hookah method and different concentrations (25%, 50%, 75%, 100%) were prepared by diluting the stock solution with distilled water and, distilled water was used as control. Solutions were used starting water of petri and perlite media and to prepare MS0 for *in vitro* condition. Nodal segments of grass pea seedlings as explants were cultured on MS0 medium *in vitro*. Plant nutrients, antioxidants, organic or inorganic chemicals, and plant growth regulators are commonly used for plant development both *in vivo* and *in vitro*. However, their use has risks in terms of economic costs as well as nature, environment and human health. Therefore, use of naturally derived chemicals in these applications has great advantages. Observations for *in vivo* conditions were determinate after 7 days from sowing and 15 days after *in vitro* culture. The longest root length (6.089 cm) was determined in 75% smoke solution of sage and, while the longest shoot length (3.026 cm) was obtained from 100% smoke solution of sage on petri media. In perlite media, the highest root and shoot length were observed in pure water (control). *İn vitro* conditions, although shoot formation was above 85% in all applications, root formation was under 33%. The shortest shoot length was obtained from smoke solutions of 100% (5.02 cm), the longest shoot length was obtained from 25% and 75% concentrations of smoke solution, respectively 8.35 and 8.94 cm.

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Introduction

Grass pea (*Lathyrus sativus* L.) is annual and herbaceous legume rich in protein belonging to Fabaceae family. It is tolerant to drought, moderate to salinity, many diseases and pests (Campbell 1997). Moreover, It can successfully grow in low fertility soils and drought conditions with very low input requirements (Croft et al., 1999, Abd-El Moneim et al., 2000). Because of these features, grass pea has begun to gain importance again in recent years. Especially, it is seen as one of the important plants from the point of sustainable agriculture and effects of global climate change (Kumar et al., 2011). Although grass pea can germinate in a short time in laboratory, its germination in field does not occur before 14-15 days even under in suitable conditions. If grass pea is sown in the spring, harvest period varies between 85-110 days depending on genotype (Basaran et al., 2016). Therefore, it is of great importance to rapid germination and seedling growth in terms of yield for this plant that has a short vegetation period.

In recent years, effects of smoke solutions on germination and seedling growth of different plants were investigated and, it showed positive effect on about 1200 species (Dixon et al., 2009). Effect of components in smoke on germination was first introduced by De Lange and Boucher (1990), and then intensive studies have been performed in this regard. Smoke contains hundreds of compounds. It has been reported that the effect on germination results especially from butenolide (3-methyl-2Hfuro [2,3-c] pyran-2-one) (Van Staden et al., 2004). Temperature rise, rapid mineralization and effects of components in ash were also investigated in order to explain the effects of smoke solutions on germination. Smoke solutions are effective on seedling growth and breaking dormancy. It also encourages root development and flowering (Taylor and Van Staden, 1996). Ghebrehiwot et al. (2012) found that seeds treated with smoke solution had longer roots and shoots, but this effect altered with among species and also differed with temperature application. Smoke solutions which contained active substances were obtained by burning proteins, plants etc., and its effects on germination of many plant seeds were examined (Flematti et al., 2004, Light et al., 2005, Jain et al., 2006, Flematti et al., 2007, Kepczynski et al., 2010). It was reported that smoke, smoky water, active substance (butenolide etc.) had different effects on germination, shoot and root development for each species and variety (Downes et al., 2010).

In this study, effect of the smoke solution derived from sage (*Salvia officinalis* L.) on shoot and root development of grass pea (*Lathyrus sativus* L.) was investigated in pots and petri. In addition, shoot and root formation frequency, number of node, number of branch and plant weight of grass pea were monitored *in vitro* conditions.

Materials and Methods

This study was carried out at Laboratory of Field Crops Department, Faculty of Agriculture, Bozok University, Yozgat.

Seeds of variety 'Gurbuz-2001' of grass pea (*Lathyrus sativus*. L.) were obtained from Faculty of Agriculture, Bozok University. In this study, mature seeds were used for germination in pots and Petri dishes. Nodal segments were also used as explant's source for direct shoot organogenesis.

Preparation of smoke solution (stock): Smoke solutions obtained by burning aerial parts of sage (*Salvia officinalis* L.) according to Van Staden et. al. (2004) and Ghebrehiwot et al. (2012) with some modifications. The water pipe system (nargile) was used for this process and negative pressure was provided with compressor. The aerial parts dried of 10 g sage were burned. And, smoke was completely collected through 1 liter of water. The stock solution was filtered through a rough filter paper. The stock solution was diluted with distilled water, to prepare four different concentrations of solution (100%, 75%, 50%, 25%) and, distilled water was used as a control.

Pot experiment: Fifteen seeds of grass pea were sown at a dept of 3cm in pots (10×10 cm) filled with perlite. After sowing, first irrigation was made with solutions, and for next irrigations, distilled water was used. The pots were conducted in laboratory conditions (20-25°C), daylight. Each treatment was repeated 3 times. Measurements of seedling, root and shoot development were made about on 7th day after sowing.

Petri experiment: The seed surfaces were sterilized in 20% sodium hypochlorite (ACE, Turkey commercial NaOCl was used as a stock in the sterilization process, 5% NaOCl) prior to the germination test (Subaşı and Güvensen 2010). Twenty five seeds were placed on filter paper in 100×10 mm Petri dishes and, each treatment was repeated 4 times. Each petri was irrigated with 6 ml of stock solutions. Then, the seeds were incubated at 24±2°C for 16 hours light and 8 h dark conditions at the climate chamber for germination. At the end of 7 th days, average lengths of shoot and root were determined.

In vitro experiment: The seed surfaces were sterilized 20 min in 20% sodium hypochlorite. After sterilization, the seeds were rinsed 3-4 times with sterile distilled water. The sterilized seeds were cultured in MS mineral salts and vitamins (Murashige and Skoog 1962) containing 0.64% agar (Duchefa) without any growth regulators (MS0) in sterile magenta incubated at 24±2ºC in 16 h photoperiod for seedling growth. Seven days after sowing, single nodal segment of seedlings was dissected under aseptic conditions and used as explant. In the experiments, MS medium containing 3% sucrose and 0.64% agar were used. The media were prepared using distilled water (control) and 4 different concentrations (25%, 50%, 75%, 100%) of smoke solutions. The pH of the media were adjusted to 5.8 with 1 M HCl or 1 M NaOH. All the culture media were sterilized by autoclaving at 121°C, 118 kPa pressure for 20 min. Five explant was put in magenta and, incubated at 24±2°C under 16/8h light/dark (3000 lux) for shoot initiation. Each treatment was repeated four times. At the end of 15th day, rate of shoot formation (%), rate of root formation (%), shoot length/plant (cm), fresh plant weight/plant (mg), number of nod/plant and explant rate of lateral branch formation (%), number of lateral branch/plant were determined.

All data were subjected to analysis of variance (ANOVA) and the means were compared with Duncan's multiple range tests. The statistical analysis was performed using SPSS 13.0 package program. Data given as percentages were subjected to arcsine transformation (Snedecor and Cochran 1967) before statistical analysis.

Results and Discussion

In perlite application, effects of smoke solutions on root and shoot lengths of grass pea were found to be statistically significant ($P<0.01$). The highest (6.476 cm) and the lowest (4.476 cm) root length were obtained from control and 50% smoke solution, respectively. The highest shoot length was observed 100% smoke solution (4.669 cm,). The lowest shoot length was noted as 1.807 cm in 50% smoke solution and 1.923 cm in 75% smoke solution. As a result, it was found similar results about effects on root and shoot lengths of smoke concentrations. In perlite application, root and shoot lenght were the highest in control and 100% smoke solution, while they were the lowest in 50% smoke solution (Table 1).

After germination of grass pea seeds in petri, effects of smoke solutions on root and shoot lengths were examined, the differences among root and shoot lengths were found to be statistically significant. It was observed that the highest root length was in 25%, 75% and 100% smoke solutions (5.810, 6.089 and 5.840 cm, respectively), whereas the lowest was in control solution as 3.742 cm. Similarly, the highest shoot lengths were determined as 3.026 cm in 100% smoke solution (Table 2).

Table 1 Effects of different concentrations of sage smoke solutions on root and shoot lengths of grass pea in perlite environment

Concentration	Control	Smoke solutions (%)			
		25	50	75	100
Root length (cm)	6.476^{a}	4.707^{ab}	4.476^{b}	4.830^{ab}	5.561^{ab}
Shoot length (cm)	4.215^{ab}	2.623^{bc}	1.807^{c}	1.923^{c}	4.669^{a}

Values shown in a line followed by different small letters are statistically different using Duncan's test at 1% level of significant

Table 2 Effects of different concentrations of sage smoke solutions on root and shoot lenghts of grass pea in petri environment

Concentration	Control	Smoke solutions (%)			
		25	50	75	100
Root lenght (cm)	3.742^{c}	5.810^{a}	4.962^{b}	6.089^{a}	5.840^{a}
Shoot lenght (cm)	1.349^{c}	2.283^{bc}	1.743^{bc}	2.610^{ab}	3.026^{a}

Values shown in a line followed by different small letters are statistically different using Duncan's test at 1% level of significant

Table 3 Effects of different concentrations of sage smoke solutions on seedling growth of grass pea in vitro condition

P	SF	RF	SL/P	FPW	NN/P	ERLBF	NLB/P
MS	93.3	33.3	5.43^{ab}	94.63^{b}	4.12^{ab}	55.0^{c}	1.33^{c}
25 %	85.0	0.0	8.35^{a}	128.48^{a}	4.41^{a}	60.0^{bc}	1.91^{bc}
50 %	95.0	15.0	7.23^{ab}	109.25^{ab}	2.25^{bc}	95.0^{a}	3.59^{a}
75 %	90.0	10.0	6.58^{ab}	86.91^{b}	1.94^{c}	90.0^{abc}	3.15^{ab}
100 %	90.0	10.0	5.00^{b}	87.65^{b}	3.90^{ab}	92.5^{ab}	2.96^{ab}

P: Parameter, SF: Shoot formation (%), RF: Root formation (%), SL/P: Seedling length/Plant (cm), FPW: Fresh plant weight (mg), NN/P: Number of nod/Plant, ERLBF: Explant rate of lateral branch formation, NLB/P: Number of lateral branch/Plant (%), Values shown in a column followed by different small letters are statistically different using Duncan's test at 1 % level of significant

Figure 1 Root and shoot lengths of germinated grasspea seeds in perlite (a) and petri (b) applications

This study, effects of sage smoke solutions on root and shoot lengths of grass pea which have an important place in plant growth were examined. According to statistical analysis, 100% smoke solution was found to be more effective for root and shoot development for petri and perlite conditions (Figure 1).

There are many studies on smoke solution or smoke-derived compounds that can possibly be used in improving and promoting the growth of agricultural and horticultural crops such as red rice (Doherty and Cohn 2000), maize (Modi 2004), rice (Kulkarni et al., 2006), bean (Van Staden et al., 2006), okra (Kulkarni et al., 2007), tomato (Kulkarni et al., 2008), and onion (Kulkarni et al., 2010). Moreover, Chumpookam et al. (2012) reported that 10 different concentrations (from 0% to 10%, v/v) of smoke solution derived dry rice straw (*Oryza sativa* cv. Japonica) promoted root and shoot lengths of papaya seedlings. Also, Ghebrehiwot et al. (2008) informed that **s**moke solution treated seeds produced significantly longer shoots or roots.

In vitro application, the effect of smoke solution on shoot and root formation from explant obtained from single nodal segments of grass pea was not significant. But the differences among shoot length, fresh plant weight, number of nod, explant rate of lateral branch formation and number of lateral branch under different smoke solutions were statistically significant (Table 3).

Shoot formation from nodal segments was observed in each treatment (Table 3). The highest percentage of shoot formation (95%) was noted in 50% concentration of smoke solution, but the lowest (85%) was in 25% smoke solution. Kendir et al. (2009) were reported that shoot formation from MS medium containing BA (Benzyladenine) and NAA (Naphtalene avetic acid) was 93.33%, but it was 100% under TDZ (Thidiazuron) concentrations. In the present study, root formation ranged from 33% to 0%, in MS0 and 25% smoke solution, respectively. However, it was revealed that there is statistically insignificant differences between applications for root and shoot formations. Root formation of grass pea *in vitro* was recorded as 78.7% on half-strength MS medium containing 2.85 μM IAA (Indole acetic acid) application (Barik et al., 2005), as 76.56% on MS basal medium supplemented with 2 mg / l IBA (Barpete et al., 2014) and as 40% on half strength of MS basal medium containing 1.0 mg/l NAA (Saha et al., 2015). Whereas, Kendir et al. (2009) notified that successful root formation was determined in MS medium containing NAA (91.67%). But, Taylor and Staden (1998) notified the weak root growth in smoke solution applications. Likewise, in this study, root formations observed in 15 days'culture *in vitro* were found to be weak.

Sparg et al. (2005) reported that seedling lengths obtained from 1:1000 smoke solution in *Albuca pachychlamys* and *Tulbaghia violacea* were higher than 1: 500 and 1:2000 applications, but *Merwilla natalensis* exhibited similar seedling lengths in all consentrations. Also, Kulkarni et al. (2008) tried concentrations of smoke-water or butenolide solutions and observed higher plant height in tomato with 1:500 concentration of smoke solution compare to butenolide (1 nM) and 1:2000 smoke solution. In our study, it was determined that the highest seedling length was 8.35 cm in 25% smoke application, also increasing smoke concentrations decreased seedling lengths. It was obtained 5.00 cm from 100% smoke concentration. Barik et al. (2005) reported that the highest shoot length in grass pea was 4.1 cm in MS medium containing BA and NAA. Kendir et al. (2009) were recorded that maximum shoot length (6.23 cm) on MS medium supplemented with BAP and NAA and increase in the concentration of BAP (6-Benzylaminopurine) to 4 mg/l had a negative effect on shoot growth of grass pea. Similarly, *in vitro* application in our study, increasing concentration of smoke solution had a negative effect on shoot growth. But smoke solutions were observed more effective than application of plant growth regulator *in vitro*. And, it was showed that explants didn't induced phenolic compounds *in vitro*. However, it was observed that increase in the concentration of smoke solution (over 50%) had a positive effect on root and shoot growth in perlite and petri applications. This indicated that different concentrations of smoke solutions will have different effects in different environment on different species.

In addition, whereas the highest fresh plant weight (128.48 mg) and number of nod per plant (4.41 plant/number) were obtained from 25% smoke solution, maximum explant rate of lateral branch formation (95%) and number of lateral branch per plant (3.59 plant/number) were obtained from 50% smoke solution. Also, it was determined that there were no significant differences regarding explant rate of lateral branch formation and number of lateral branch at 50%, 75% and 100% smoke solution (Table 3). In addition high concentrations of smoke solutions exhibited negative effect on fresh plant weight. Similarly, Sparg et al. (2005) determined that 1:2000 smoke solution resulted in significantly greater seedling mass for both *A. Pachychlamys* and *T. violacea* than 1:1000 and 1:00 smoke solutions.

Conclusion

Speed germination and uniform seedling are highly crutial for effective production in agriculture. High yield and quality can only be guaranteed by the strong seedlings. Strong seedling is more resistant to biotic and abiotic stress factors and weed competition which are main problems of agriculture. Also, when there is lack of suitable conditions for germination in field applications, seeds is likely to be infected by some fungal pathogens into seedbed because of late germination. Roche et al. (1997) suggested that high concentrations of smoke solution may confer protection against predation and microbial attack to seeds and perhaps also to seedlings. It is commercially important that seeds in the field of agriculture have a high rate of healthy plant growth in addition to rapid and uniform growth. Therefore, smoke applications have the potential use so as to obtain healthy and vigorous seedling in cultivation of horticultural and field crops (Light and Van Staden 2004).For this reason, when it is made some priming applications to optimize seed germination by considering environmental conditions and changing features belonging to plant species (or varieties), it can be affected positively both directly seed germination and indirectly plant growth. Smoke solutions, one of the priming applications, have been studied by many researchers (Flematti et al., 2004, Van Staden et al., 2004, Light et al., 2005, Jain et al., 2006, Flematti et al., 2007, Kepczynski et al., 2010). According to previous studies, it was reported that concentrations of smoke, smoky water, active substance (butenolide etc.) had different effects on germination, shoot and root development for each species and variety, also shown effect of inhibitory or encouragement (Downes et al., 2010). However, many studies were reported that smoke or butenolide acts as gibberellic acid on germination (Nelson et al., 2009).

In our study, the effect of sage smoke solutions on germination of grass pea seeds was investigated. Statistically, in perlite application, although the highest root and shoot formations was determined in control and 100% smoke solutions, the lowest in 50% smoke solution. But, in petri dish application, the highest was observed in 75% and 100% smoke solution, the lowest in control.

In vitro application, smoke concentrations exhibited almost the same stimulatory and inhibitory effects on shoot and root formation. The highest shoot and root formations were observed in 50% smoke solution (excluding control). Also, the highest explant rate of lateral branch formation, and number of lateral branch were obtained from 50% smoke solution. But, the highest shoot length, fresh plant weight, number of nod were observed in 25% smoke solution. When considered ex vitro, in vitro and other studies, it may be thought that different concentrations of smoke solutions have different effects in different environment on different species.

As a result, it was found that sage smoke solution had effective on shoot and root developments of grass pea seeds. Also this effect was determined to changed depending on the concentrations.

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