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Dose-dependent of melatonin and gibberellin priming improve seed germination and growth indices of *Salvia officinalis* L.

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Abstract

The importance of medicinal plants is increasing day by day and their consumption is increasing. One of the obstacles in cultivating these plants is the low germination of their seeds due to dormancy. Melatonin and gibberellin are important growth regulators that have decisive roles in the growth and development of plants. To identify an effective priming dose of these regulators on seed germination, seedling growth and dry matter of *Salvia officinalis* L. in the lab experiment, effect of ten levels of hormone priming (50, 100, 150, and 200 ppm of gibberellin and 50, 100, 150, 200, 250, and 300 mM of melatonin) along with control in the complete random design with three replicates were tested. Results showed that although all levels of melatonin and gibberellin priming caused increase in seedling growth indices (percentage and germination rate, seed vigour index, root and plumule length, and seedling dry weight), compared to control, their effects strongly was dose-dependent. The least and the most germination percentage belonged to control (30 %) and 300 mM melatonin (90 %) levels, respectively. Albeit, the application of melatonin priming at 100 mM had the highest seed vigour index (18.4 %) and root length (2.04 cm) due, seemingly, to low plumule length, highest increase in seedling dry weight compared to control level was obtained at 200 ppm gibberellin (3-fold) and 300 mM melatonin (2.5-fold), respectively. Findings of the present study reveal that the priming of *S. officinalis* seeds by 300 ppm of melatonin and 200 mM of gibberellin may be played a crucial role in the germination and growth indices of this aromatic plant even under stress conditions.

Keywords: Growth indices, hormone priming, Seed germination, Seedling dry weight, Vigour index.

Introduction

Today, due to special role of medicinal plants in the pharmaceutical industry, it is necessary that these plants be cultivated more and more (Ekor, 2014). Salvia (*Salvia officinalis* L.) is an important perennial medicinal plant which is cultivated in the Mediterranean, Southeast Africa, Central Asia and South America. It has been used for ornamental, medicine and food (Kintzios et al., 1999).

Germination as the first stage of plant development is one of the critical and sensitive stages in the plant life cycle. It is a key process in plant emergence that is controlled by genetic, hormonal and environmental factors (Meyer and Pendleton, 2000).

Seed priming is one of the applied techniques effective on germination and increases the speed of seed germination and seedling establishment, which can strengthen the seedling's ability to absorb water and nutrients and provide more use of sunlight (Finch-Savage et al., 2004). There are several different methods for seed priming, including osmopriming, hydropriming, matrix priming, hormonal priming and biopriming. Melatonin (N-acetyl-5-methoxy-tryptamine) and gibberellin are plant growth regulators whose application as seed priming has a significant effects on plant growth and development stages such as seed germination, stem elongation, leaf development and yield (Khan et al., 2020). Seed priming with plant growth regulator (such as gibberellin and melatonin) can improve germination and growth under stress and control conditions by affecting germination, growth and development processes (Khan et al., 2020).

However, the positive effects of seed priming with gibberellin and melatonin on germination, growth and yield of crops such as rapeseed (Khan et al., 2020) and soybean (Wei et al., 2015) has been reported; but these effects may vary depending on the concentration of the hormones and the plant species.

Accordingly, the current study was conducted to determine the most appropriate concentration of gibberellin and melatonin (that can be used in priming) affecting germination and growth characteristics of *S. officinalis* L.

Materials and method

The experiment was carried out as a completely randomized design with three replications in 2021 at the Lorestan University. *Salvia officinalis* L. seeds were prepared from Pakan Bazr Company of Isfahan, Iran. Experimental treatments including 11 levels of seed priming: control (without pretreatment) and seed priming with gibberellin (50, 100, 150, 200, 250 and 300 ppm) and melatonin (50, 100, 150, 200, 250 and 300 µM). For priming, the seeds were placed in the hormones solutions for 6 hours under dark and then air dried (Khan et al., 2020). Thirty seeds were planted in each petri dish (9 cm in diameter) on two layers of Whatman filter paper. Then 8 ml of distilled water containing fungicide (Carboxin Thiram 1 per thousand) was added to each petri dish. The petri dishes were placed inside a growth chamber at a temperature of 25± 2 °C.
Two-millimeter root emergence was considered as a criterion for germinated seeds (ISTA, 2007). Germinated seeds were counted consecutively every 24 hours and continued for up to a week. Final germination percentage (FGP), average germination time (MGT), germination rate (GR) and seedling vigor index (VI) were estimated using Equations 1, 2, 3 and 4, respectively:

$$FGP = (\frac{Ni}{N}) \times 100 \quad (1)$$

Where Ni: total number of germinated seeds on the last day of counting; N: total number of seeds

$$MGT = \sum (n * d)/N \quad (2)$$

Where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total

number of seeds germinated at the termination of the experiment (Ellis and Roberts, 1981).

$$GR = 1/M\ GT \quad (3)$$ where GR= germination rate

$$VI = \sum (FGP \times SL)/100 \quad (4)$$ Where, FGP: final percentage of germination, and SL: seedling length.

Two weeks after planting, five seedlings were randomly selected and the length of root and shoot were determined with a ruler. Seedling dry weight of 10 seedlings from each experimental unit was measured after oven drying at 75 °C for 48 h. Data analysis was performed with SAS.9.4 software and graphs were drawn with Excel 2010 software.

Results and Discussion

The results of analysis of variance showed that the effects of priming treatments on all studied traits (germination percentage and rate, vigor index, root and shoot lengths and seedling dry weight) were significant (P≤0.01, Table 1). All levels of gibberellin and melatonin primings increased the percentage and germination rate and growth characteristics such as vigor index, root and shoot lengths and dry weight of *S. officinalis* seedling compared to the control (Figure 1-A, B, C, D, E and F).

The highest germination percentage (90%) was obtained by melatonin 300 mM and the lowest (30%) in the control, respectively (Figure 1-A). The highest germination rate was obtained by gibberellin 50 and 100 ppm primings (0.418 and 0.403, respectively) and the lowest (0.170) in the control (Figure 1-B). The highest vigor index and radicle length were obtained by pretreatment of melatonin 100 mM (18.4 and 2.04, respectively) and the lowest in the control (1.55 and 0.382, respectively) (Figure 1-D and C). The highest seedlings dry weight was obtained by the gibberellin 200 ppm pretreatment (0.008 g/seedling) and melatonin 300 mM (0.007 g/seedling) and the lowest in the control (0.002 g/seedling) (Figure 1-F).

Our findings show that it is possible to improve the establishment, germination and growth indicators of *S. officinalis* seeds with the application of gibberellin and melatonin growth regulators priming, but this improvement is highly dose-dependent.

In similar results, the positive effect of seed priming by plant growth regulators gibberellin and melatonin has been reported depending on the type and concentration of the hormone and the type of plant in the conditions on the germination, growth and yield of some agricultural plants (Khan et al., 2020; Wei et al., 2015).

Table 1. ANOVA analysis of gibberellin and melatonin priming effect on percentage, germination rate and some growth characteristics of *Salvia officinalis* L.

S.O.V	Df	Mean square					
		Percentage germination	Germination rate	Seed vigor index	Root length	Plumule length	Seedling dry weight
Treatment	4	764**	0.017**	86.5**	0.979**	0.007**	0.00001**
Error	10	77.2	0.001	9.03	0.142	0.002	0.000002
Total	14	-	-	-	-	-	
CV (%)	-	11.9	13.2	30.6	35.1	25.4	26.5

**and ^{ns} indicate significant at 0.01 probability and non-significant, respectively.

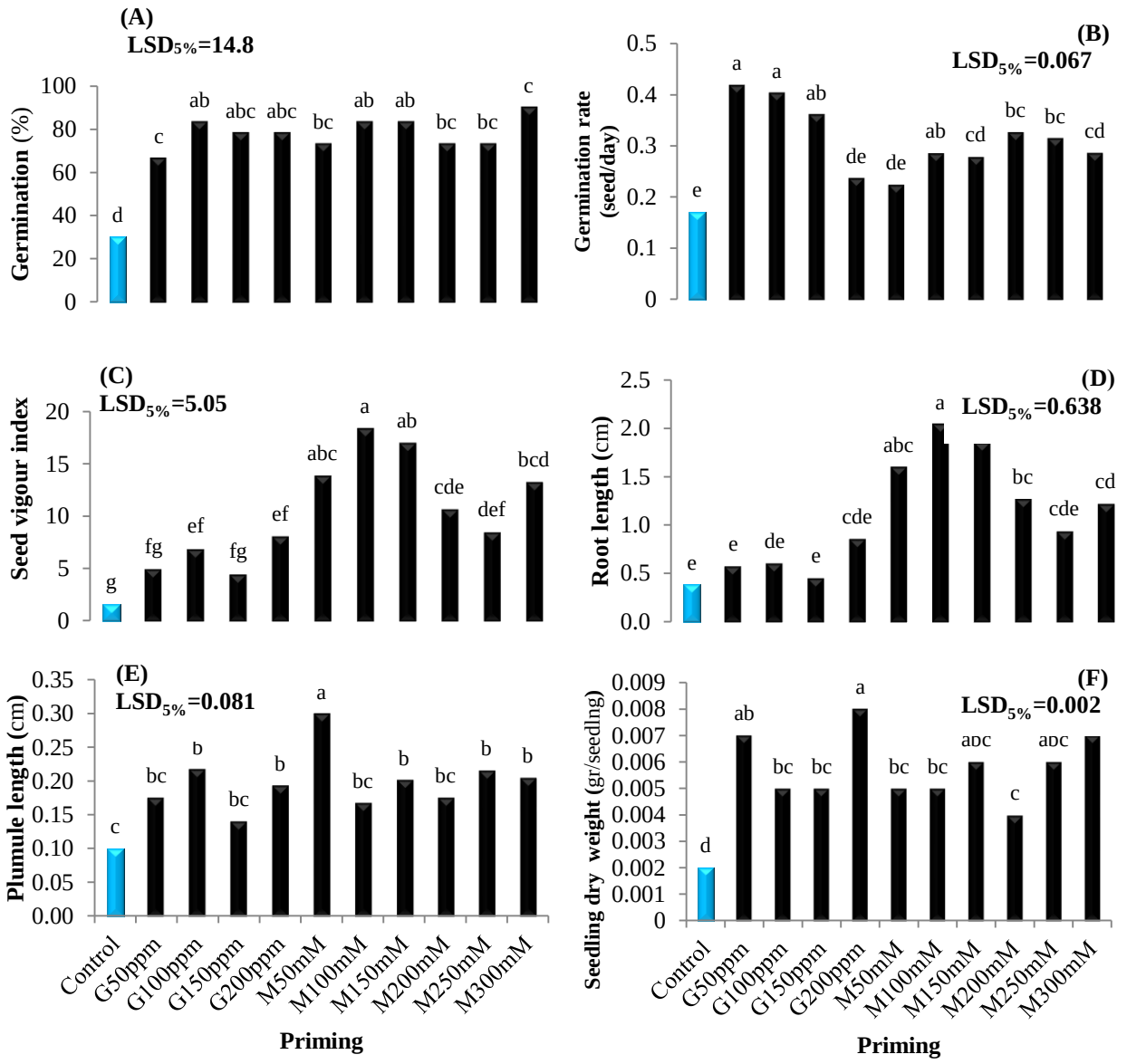

Figure 1. Effects of gibberellin and melatonin primings on germination percentage (A), germination rate (B), seed vigour index (C), root length (D), plumule length (E), and seedling dry weight (F) of *Salvia officinalis* L. G and M indicate gibberellin and melatonin, respectively. For each trait, means with the same letters do not have statistically significant differences at 5% level of probability according to LSD.

Conclusions and Outlook

Findings of the present study reveal that the improvement of germination and growth characteristics of *Salvia officinalis* L. is strongly dose-dependent of melatonin and gibberellin priming. So that, our findings showed the priming of 300 ppm of melatonin and 200 mM of gibberellin may be played a crucial role in the germination and growth indices of this aromatic plant even under stress conditions.

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