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Influence of L-Tryptophan and Melatonin on Germination of Onion and Leek Seeds at Different Temperatures

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Abstract: The aim of the study was to evaluate the influence of different L-tryptophan and melatonin concentrations on several germination parameters of onion and leek seeds under low and high-temperature conditions. The experiments were carried out in laboratories of the Erciyes University. Seeds were treated with 125, 250, 375 ppm L-Tryptophan and 5, 10, 25 uM Melatonin for 24 hours, then subjected to germination tests at optimum (21 ºC) chilling stress (7 ºC) and hot temperature (35 ºC) conditions. Seeds not treated were considered as controls. Treatment of onion seeds with 125 ppm L-tryptophan had a significantly positive effect on final germination ratio at 7 ºC while in leek seeds, the 5 µM concentration of melatonin had a significant effect on the germination index at the same temperature. In both Allium species, hormone treatments had no effect on mean germination time. These values decreased depending on the increase in temperature. The extreme level of high temperature burned certain percent of seedlings, while at low temperature, seedlings were not harmed by cold domination.

Keywords: L-Tryptophan, melatonin, onion, leek, germination

1. Introduction

Especially after sowing in the early spring or autumn, slow and non-homogeneous germination, as well as a high number of abnormal seedlings, have been observed as a result of stress conditions (Borowski and Michalek, 2006). Also, compared to many vegetable crops, onion has quite short-lived seeds (Amjad and Anjum, 2002). This causes the onion seeds to be generally expensive (Black et al., 2006). For this reason, there has been a great interest to research practical and affordable techniques to improve seed germination, growth, and yield of onion plants (De Souza et al., 2014).

Pre-applications to increase seed germination are called as "seed priming". These applications also help to grow normal and vigorous seedlings. This makes the plants more resistant to biotic and abiotic stress conditions (Khan et al., 2009a). The use of plant growth regulators in the course of priming and other pre-sowing treatments can improve germination performance of many crops (Khan et al., 2009b). Small polycationic molecules, called polyamines, can be found in varying amounts in almost all organisms. Functions of the polyamines have been reported in several biological processes such as transcription, RNA alteration, protein synthesis and the modulation of enzyme activities (Takahashi and Kakehi, 2010). Polyamines are important for growth, development and cell growth in plants, can be accumulated in tissues and can undertake various functions under stress conditions. Polyamine type, concentration, and status of embryo dormancy can affect the germination of the seed (Farooq et al., 2011). Melatonin (N-acetyl-5-methoxytryptamine, MEL) is one of the best-studied biological molecules found in the cattle pineal gland in 1958 (Lerner et al., 1958). Since the detection of MEL in a single-cell photosynthetic organism *Gonyaulax polyedra* in the early 1990s (Hattori et al., 1995), research in plants has been continuing. In contrast to animals, the role of MEL in plants is less understood. Many studies have proposed that MEL can play a role as a plant growth regulator and bio-stimulator under stress conditions (Arnao and Hernandez-Ruiz, 2006). Application of MEL has increased germination performance of cucumber, *Phacelia tanacetifolia*, and pepper, (Posmyk et al., 2009; Tiryaki and Keleş, 2012; Korkmaz et al., 2017; Zhang et al., 2017).

L-tryptophan (3-indolylalanine, L-T) was first discovered by English chemist Frederick Gowland Hopkins in 1901. It is an essential amino acid not only for plants but also for animals, humans, and some bacteria (Frankenberger and Arshad, 1991). L-T which bearing an indole ring is a unique amino acid (Palego et al., 2016). It is a biologically active precursor of auxin, which, when applied exogenously, increases the levels of auxin in plant tissues. A positive response of L-T application in germination and growth performance of several crops has been reported by different researchers (Khodary, 1992; Parvez et al., 2000; Abbas et al., 2013; Antony et al., 2017).

Although numerous reports have been published to the investigation of the effects of different plant growth regulators in onion and leek seeds, any research has not been conducted to detect the role of L-T or MEL treatment on germination characteristics under stress conditions. Thus, the aim of this study was to evaluate the effect of different concentration of L-T and MEL on the several germination parameters of onion and leek seeds under low and high-temperature conditions.

2. Materials and Methods

This study was carried out in laboratories of the Erciyes University, in 2019. The onion seeds (*Allium cepa* L.cv. Valenciana) and leek seeds (*Allium porrum* L.cv. İnegöl-92) were provided by a local seller. The seeds were surface sterilized under aseptic conditions with 70% ethanol for 1 min, followed by 20% commercial Clorox (5.25% sodium hypochlorite) for 10 min. and then washed 2 to 3 times with distilled water (Hanci et al., 2012). The germination tests are designed according to Anonymous (1999) rules. Following disinfestation, seeds were soaked 20 mL of 0 (distilled water), 125, 250, 375 ppm L-T; and 5, 10, and 25 µM MEL solutions at 20 °C in darkness for 24 hours (Karaca, 2013). After treatment, seeds were rinsed under running water for 2 minutes and were kept to dry on paper towels for 4 hours. Then fifty seeds were placed on two layers of filter paper moistened with 5 mL of distilled water in covered 10 cm Petri dishes. A completely randomized design was used with four replications. Although germination experiments in ISTA (International Seed Testing Association) rules have been limited to 12 days for onion and leek, this period was considered as 21 days, as it was assumed that low temperature would prolong germination time. The Petri dishes were placed in a germination chamber in the dark at various temperatures (7 ºC, 21 ºC, and 35 ºC) for 21 days. Germinated seeds (with a protruded radicle of 2 mm long) were counted daily and conserved until end of the experiment. At the end of the study, Equations 1, 2, 3, and 4 were used to determine the effects of L-T and MEL on the germination of seeds (Al-Maskri et al., 2004; Li et al., 2007; Mercedes et al., 2007).

G-max= Germination rate (%)= (*G*/*T*) x 100 (1)

G-index= Germination Index= (1. day *G-max* / *Dt*1) + ……..……+ (*n*. day *G-max* / *Dtn*) (2)

G50 (day) = Time for germination of 50% seeds (3)

MGT= Mean germination time (Day)= [(1.day *G* x 1) +….….+ (*n*. day *G x n*)] / Total *G* (4)

Where *T* is the total seed number; *G* number of seeds which were germinated on the day; *Gt* number of days counted from the beginning of germination. Statistical analysis was conducted using the PAST3 software (Hammer et al., 2001). The data from the experiment were subjected to a general analysis of variance (ANOVA).

3. Results

3.1. Experiment I, Onion

The germination percentage (G-max, %), germination index (G-index) and shoot length (SL, cm) were significant for three factors (treatment, temperature, and their interaction). Fresh weight (FW, mg) was significant for both treatment and temperature; mean germination time (MGT, day) was for only temperature. The effects of pre-treatments on onion seeds are shown in Table 1 and 2. Germination was very low at 7 ºC and 35 °C, with 20.95% and 21.19%, respectively.

In addition, at 21 ºC, it has been found interesting that some treatments reduce the germination rates. The seeds incubated at 21 ºC degrees and not treated with hormones germinated at a 93.33% rate. All seeds were germinated at 21 ºC after 125 ppm L-T treatment (100%). However, increasing doses of L-T decreased the germination rate sharply at this temperature. This has also been observed for MEL treatments. However, the sharp decrease in MEL applied seeds was observed in the first dose. Under extreme temperature conditions (7 ºC and 35 ºC) the lowest doses used

Table 1. Some germination features of onion seeds at different temperatures[1]

Temperature (ºC)	Treatment	G-max (%)	G-50 (day)	G-Index	SL (cm)
7	Control	30.00 c-f	19.33 ab	1.56 f	0.43 e
	125 ppm L-T	35.00 cd	18.66 ab	1.86 f	0.43 e
	250 ppm L-T	23.33 c-g	20.00 a	1.18 f	0.37 e
	375 ppm L-T	15.00 e-g	19.00 ab	0.76 f	0.30 e
	5 µM MEL	13.33 f-g	17.00 b	0.82 f	0.30 e
	10 µM MEL	11.67 g	20.00 a	0.61 f	0.20 e
	25 µM MEL	18.33 d-g	18.33 ab	0.97 f	0.27 e
21	Control	93.33 a	3.67 e	22.79 a	7.67 a
	125 ppm L-T	100 a	3.67 e	20.93 a	9.00 a
	250 ppm L-T	63.33 b	4.33 de	12.99 b	7.33 ab
	375 ppm L-T	23.33 c-g	4.00 e	4.67 d-f	3.67 d
	5 µM MEL	36.66 c	6.67 d	8.96 b-e	4.67 cd
	10 µm MEL	21.66 c-g	4.00 e	4.56 d-f	5.67 bc
	25 µM MEL	21.66 c-g	11.00 c	4.25 ef	4.00 cd
35	Control	25.00 c-g	2.33 e	10.06 bc	0.33 e
	125 ppm L-T	33.33 cd	2.00 e	11.68 bc	0.67 e
	250 ppm L-T	18.33 d-g	2.00 e	7.51 c-e	0.30 e
	375 ppm L-T	25.00 c-g	2.67 e	7.51 c-e	0.30 e
	5 µM MEL	20.00 c-g	3.33 e	5.04 d-f	0.20 e
	10 µm MEL	15.00 e-g	4.00 e	4.59 d-f	0.20 e
	25 µM MEL	11.67 g	2.00 e	5.00 d-f	0.23 e

Ratios (Hormone, Temperature, Hormone x Temperature): G-Max: 19.8*, 63.6*, 6.5*; G50: 667.01*, 2.21^{ns}, 4.56*; G-Index: 13.4^{ns}, 66.9*, 5.8*; SL: 3.9*, 181.8*, 3.1*. *significant ($p < 0.01$), ns: not significant. F: Freedom, L-T: L-tryptophan, MEL: Melatonin, G-max: Final germination percentage, G-index: Germination index, G-50: Time for germination of 50% seeds, SL: Shoot length. Means within a group that have a different small letter are significantly different from each other.

Table 2. MGT and seedlings FW of onion at different temperatures

MGT (day)								
Temperature (ºC)	Control	125 ppm L-T	250 ppm L-T	375 ppm L-T	5 µM MEL	10 µM MEL	25 µM MEL	Average
7	19.26	19.05	19.59	19.63	17.33	12.83	19.08	18.11 a
21	5.87	7.55	8.16	7.28	6.76	6.61	10.90	7.59 b
35	2.81	4.21	3.22	2.93	4.75	6.00	2.44	3.76 c
Average	9.31	10.27	10.32	9.95	9.61	8.48	10.81	
FW (mg)								
Temperature (ºC)	Control	125 ppm L-T	250 ppm L-T	375 ppm L-T	5 µM MEL	10 µM MEL	25 µM MEL	Average
7	21.96	25.18	26.41	21.09	22.42	22.55	25.59	23.60 a
21	21.57	24.14	25.39	20.33	20.69	20.56	24.67	22.48 b
Average	21.76 C	24.66 B	25.90 A	20.71 D	21.55 C	21.55 C	25.13	

F Ratios (Hormone, Temperature, Hormone x Temperature): MGT: 0.6^{ns}, 135.9*, 1.5^{ns}; FW: 59.8*, 29.8^{*}, 1.0^{ns}. *significant ($p < 0.01$), ns: not significant. F: Freedom, MGT: Mean Germination Time, FW: Fresh weight of seedlings, L-T: L-tryptophan, MEL: Melatonin. Means within a group that have a different capital or small letter are significantly different from each other.

in L-T applications increased germination rates compared to the control. No effect was observed in MEL applications at these temperatures. The lowest germination index value was observed at 7 ºC. There was no statistical difference between the results obtained from all treatments at this temperature. The smallest shoot length values were also observed at this temperature. In terms of shoot lengths, at 21 ºC, 125 ppm L-T pre-treatment gave the highest value but there was no statistically significant difference with the control group.

Only the "temperature" had an effect on the MGT of the seeds. The MGT was reduced by increased temperature (Table 2). When evaluating the fresh weight of the shoots, the values obtained at 35 ºC were not taken into account because the shoots formed at this temperature were dried before the end of the experiment. The highest shoot fresh weights were obtained from 250 ppm L-T and 25 µM MEL pre-treatments.

3.2. Experiment II, Leek

According to the results, the differences were statistically significant depending on the temperature. Only the differences between the results of G-max and results of G-index were found to be significant depending on hormone applications. Among seeds not pre-treated with L-T or MEL, the highest germination (G-max 100%) was observed at 21 ºC (Table 3).

Considering the tested L-T and MEL pre-treatments, all concentrations had the favorable effect on germination (100%) at 21 ºC, although, this effect was observed only at 125 ppm L-T and 5 µM MEL concentration during the 7 ºC incubation (Table 4). At this temperature, increasing doses of hormones had a negative effect on the germination rate. The lowest germination rates in all applications were observed in seeds incubated at 35 ºC degrees. The percentage of seed germination decreased in accordance with further increments in the L-T and MEL concentration at this temperature compared to control.

Germination index was very low at 7 ºC and 35 ºC with 2.45% and 3.73%, respectively. Increasing doses of L-T had no effect on germination index at 7 ºC and 21 ºC, however, a small increase was observed at 35 ºC depending on increasing concentrations of L-T. The lowest concentration of MEL had a positive effect on germination index.

The highest mean germination time was observed at 7 ºC (17.26 day). There was no statistical difference between the results obtained from other temperatures (Table 4). A sharp reduction was observed at extreme temperature, which is evidenced significantly. No hormonal effect was observed on this feature.

4. Discussion

The results of the study showed that pre-treatment with L-T and MEL at low temperature may affect the seed germination of onions and leeks. Germination is slowing at low temperature and the germination time is prolonged (Grime et al., 1981). This could be related to the oxygen permeability via seed coat (Come and Tissaoui, 1973) or the effect of temperature on water uptake (Gulliver and Heydecker, 1973). In a study of onion seeds, it has been reported that increasing the temperature from 5 ºC to 30 ºC causes a rapid increase in the percentage of germination in both light and dark conditions (Abu-Rayyan, 2012). In our study, incubation at the 21 ºC increased the germination index compared to 7 ºC and 35 ºC conditions for both onion and leek seeds. Raising the temperature above 7 ºC (21, and 35 ºC) caused a rapid decrease in days to 50% germination in both L-T and MEL pre-treatments. At 7 ºC, 50% germination of seeds were achieved at 17th day in onion seeds. This could be related to the oxygen permeability via

Table 3. Some germination features of leek seeds at different temperatures

Temperature (ºC)	Hormones	G-max (%)	G-50 (days)	MGT (days)	SL (cm)
7	Control	38.33	16.67	16.68	0.83
	125 ppm L-T	48.33	16.33	16.65	0.83
	250 ppm L-T	35.00	17.33	17.69	0.70
	375 ppm L-T	36.67	16.67	17.02	0.63
	5 µM MEL	55.00	18.67	17.27	0.70
	10 µM MEL	33.33	18.00	17.76	0.70
	25 µM MEL	36.67	17.67	17.77	0.67
	Average	40.48 b	17.33 a	17.26 a	0.72 b
21	Control	100.00	3.00	3.57	9.67
	125 ppm L-T	100.00	3.00	3.85	10.33
	250 ppm L-T	100.00	3.33	4.02	11.67
	375 ppm L-T	100.00	3.33	3.78	7.33
	5 µM MEL	100.00	3.33	3.93	8.00
	10 µM MEL	96.67	3.67	4.27	9.00
	25 µM MEL	100.00	3.33	4.72	9.00
	Average	99.52 a	3.29 b	4.02 b	9.29 a
35	Control	21.67	4.33	4.44	0.20
	125 ppm L-T	8.33	3.67	4.83	0.20
	250 ppm L-T	11.67	3.33	10.89	0.30
	375 ppm L-T	13.33	3.00	3.58	0.20
	5 µM MEL	18.33	3.67	3.50	0.20
	10 µM MEL	10.00	4.00	6.83	0.20
	25 µM MEL	16.67	5.67	6.97	0.33
	Average	14.29 c	3.95 b	5.87 b	0.23 b

F Ratios (Hormone, Temperature, Hormone x Temperature): G-Max: 0.7^{ns}, 271.2*, 0.5^{ns}; G50: 474.81*, 0.74^{ns}, 0.45^{ns}; MGT: 0.8^{ns}, 92.4*, 0.5^{ns}; SL: 1.8^{ns}, 431.5*, 1.5*. *significant ($p < 0.01$), ns: not significant. F: Freedom, L-T: L-tryptophan, MEL: Melatonin, G-max: Final germination percentage, G-50: Time for germination of 50% seeds, MGT: Mean Germination Time, SL: Shoot Lenght. Means within a group that have a different small letter are significantly different from each other.

Table 4. Some germination features of leek seeds at different temperatures

Hormones	Temperatures (ºC)	G-index	FW (mg)
Control	7	2.43	2.26
	21	30.29	2.13
	35	5.61	
	Average	12.78 a	2.20 c
125 ppm L-T	7	3.04	2.61
	21	29.83	2.44
	35	2.22	
	Average	11.70 abc	2.52 b
250 ppm L-T	7	2.11	2.67
	21	27.98	2.62
	35	3.37	
	Average	11.15 bc	2.64 a
375 ppm L-T	7	2.24	2.26
	21	29.94	2.06
	35	3.87	
	Average	12.02 ab	2.16 c
5 µM MEL	7	3.26	2.15
	21	30.35	2.20
	35	5.35	
	Average	12.99 a	2.18 c
10 µM MEL	7	1.93	2.30
	21	26.89	2.16
	35	2.42	
	Average	10.41 c	2.23 c
25 µM MEL	7	2.12	2.65
	21	26.44	2.59
	35	3.26	
	Average	10.60 bc	2.62 ab

F Ratios (Hormone, Temperature, Hormone x Temperature): G-Index: 3.2^{*}, 1635.3^{*}, 0.9^{ns}; FW: 37.5^{*}, 13.5^{*}, 1.4^{ns}. *: Significant ($p < 0.01$), ns: Not significant, F: Freedom, L-T: L-tryptophan, MEL: Melatonin, G-index: Germination index, FW: Fresh weight of shoot, Means within a group that have a different small letter are significantly different from each other

seed coat (Come and Tissaoui, 1973) or the effect of temperature on water uptake (Gulliver and Heydecker, 1973). Pimpini et al. (1993) reported that very low germination rate was induced by temperature levels below 7.5 ºC. In addition, a result of slow water imbibition, while at optimum temperature, a relation was observed between water uptake and oxygen availability. A highly statistical superiority was induced by the interaction of temperature and light showed this superiority just at 5 ºC, 35 ºC, and 40 ºC which are unfavorable conditions to start germination (Fenner, 2000). Both onion and leek seeds had different responses to concentrations of L-T and MEL at different germination temperatures. L-T (especially 125 ppm) had a positive effect on the maximum germination rate of onion seeds at low temperature. At 21 ºC temperature, the hormone applications did not increase the maximum germination rate and germination index.

The results of hormones of different concentrations applied to increase germination ratio and index of onion seeds at low and high temperatures showed that L-T was more effective than MEL. In terms of concentration, 125 ppm was determined to be the most effective dose for L-T. However, in leek seeds, 5 µM MEL application was found to be more effective on germination rate at low temperature. Similar results were obtained from the study of Simlat et al. (2018). The mechanisms of the effect of MEL are not clearly understood, but all changes in plant metabolism occur because of its influence as an antioxidant, and its role in membrane stabilization and organizing of gene expression (Nawaz et al., 2016). However, some previous reports point out that the influence of MEL on seed germination bound up with on its concentration. Higher concentrations of MEL inhibited or had no influence on seed germination, whereas lower concentrations improved seed germination (Hernandez Ruiz et al., 2005; Chen et al., 2009; Wei et al., 2015). Zhang et al. (2013) reported that MEL had no effect on germination of cucumber seeds, regardless of the germination conditions. On the other hand, another report pointed to concentrations of 1- and 10-mM MEL appeared to have a beneficial effect on germination of red cabbage seeds under optimal conditions (Zhang et al., 2016). Also, Bajwa et al. (2014) reported that the exogenous MEL applying confers chilling tolerance in Arabidopsis. The effect of hormone applications on germination properties may vary depending on plant species. Similar results were obtained in Gibberellic acid (GA_3) which is one of the most investigated hormones on germination. For example, any germination were observed in *Origanum husnucan-baseri* seeds exposed to GA_3 application in the study of Caniş (2006). Similarly, in a study on germination of *Eranthis hyemalis* L. seeds by Tıpırdamaz and Gömürgen (2000), it was reported that application of GA_3 was not effective on germination at 23 °C. Nematollah et al. (2011) reported that GA_3 applications did not affect the germination percentages of *Allium hirtifolium* seeds. In a study conducted with *Datura stramonium* L. seeds, it was reported that GA_3 application significantly reduced germination (Kevseroğlu, 1993).

5. Conclusions

Although the positive effect of seed priming in MEL solutions on seed germination and plant quality, especially under stressful conditions, is well documented, reports that prove the positive effects of L-T is extremely limited. This is the first study investigating the effects of L-T and MEL on onion and leek under stress conditions.

The findings of this study show that the effects of the L-T and MEL are different for onion and leek seeds. The seeds of leek and onion incubated at 35 ^oC were germinated faster compared to other temperatures. The effect of the L-T and MEL on the germination rate at low and high temperature has been limited. The application of 125 ppm LT increased the germination rate slightly in onion seeds incubated at low temperature. However, this effect was not observed in germination index values. In leek seeds, in addition to 125 ppm LT, 5 μL MEL application increased the germination rate at low temperature. In both species, fresh weights of shoots obtained at low temperature were found to be higher. Our results suggest that pre-sowing with LT or MEL can be applied in agriculture to improve germination in onion and leek seeds. However, more extensive studies are needed to determine the appropriate doses.

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