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Full Paper

Effects of melatonin on flowering, seed yield and seed quality of onion under salt stress

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Abstract: The effects of melatonin on flowering, seed yield and seed quality of onion (*Allium cepa* L.) under salt stress conditions were investigated. Onion bulbs were incubated for 24 hr before planting in 1, 2, 4 and 8 µM melatonin solutions. Salt stress was imposed on the plants four times after planting by an irrigation programme using 100 mM, 200 mM and 400 mM NaCl. The data recorded were: number of leaves, leaf length, number of flowers, flower stalk length, main flower stalk length (from bulb neck to flower structure), expected number of seeds, 1000-seed weight, seed creation percentage, number of seeds per bulb, germination rate, initial count value, germination peak day, germination index, germination peak value, average daily germination, germination value and average germination day. At 400 mM salinity stress, no flowering was observed even though green leaves formed from the bulbs. At 200 mM salinity, only 8 µM melatonin solution provided flowering of the plants. As a result of testing to determine seed quality, seeds obtained from plants grown under 100 mM salt stress with supplementation of 2, 4 and 8 µM melatonin gave similar results, althought at 200 mM salinity, a dose of 8 µM melatonin gave significantly highest values. When all the results are evaluated together, it can be suggested that an application of 8 µM melatonin may preserve the seed yield and seed quality of onion under salt stress condition.

Keywords: *Allium cepa*, onion, melatonin, abiotic stress, salt stress

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INTRODUCTION

Onion (*Allium cepa* L.) is a two-year vegetable type and its commercial cultivation is made with seeds. However, seed production requires various cultural processes, unlike bulb cultivation. It is known that seed yield and quality of onions are affected by genotype, growing season, location and production methods. Abundant and high-quality onion seed production is directly related to environmental conditions. To have a good stock of quality seeds and associated production technology is very important for a superior productivity of onion [1].

Onion seed production is one of the most labour-intensive processes for vegetable crops. The most important reason for this is that onion seed production takes two or even three years. This period depends on the biological characteristics of the variety and growing area [2]. Salinity is an important stress factor limiting crop productivity. More than 800 million hectares of land worldwide are affected by salinity. A significant part of these areas is excluded from agricultural production programme [3]. This situation causes a loss of hundreds of millions of dollars each year. Although onion is classified as a salt-sensitive crop, with a salinity threshold level of 1.2 dSm^{-1} for bulb yield [4], there are scarce studies examining the effects of salinity on onion seed yield and quality. However, it is known that salinity negatively affects seed yield and quality in other plant species such as mungbean, safflower and maize [5-7].

It has been proven in various studies that plant defense system can be strengthened by exogenous application of substrates such as melatonin, thereby reducing the negative effects of salinity. Melatonin ($C_{13}H_{16}N_2O_2$) was first described in the bovine adrenal gland (epiphysis) in 1958 [8]. This compound was initially considered to be a neural hormone (neurohormone) since the epiphyseal tissue is only found in the nervous system of vertebrates and the functions of melatonin are hormonal. Therefore, it was thought to be synthesised only in the pineal tissue [9]. Melatonin in plants was first found as a result of research conducted by two different research groups in 1995 [10, 11].

Many studies have reported that melatonin applications reduce the level of stress deformation. In these studies increases in electrolyte permeability and malondialdehyde level in cucumber [12], primary root and hypocotyl elongation in sunflower [13], chlorophyll degradation in rice [14], and decrease in abscisic acid level in cucumber [15] have been reported. Melatonin application in beans attenuates the effect of alkaline stress damage and leads to significant improvements in seedling length, fresh weight and chlorophyll content [16]. In addition, less damage occurs in pepper plants treated externally with 5 μM melatonin to provide tolerance to chilling stress in the seedling stage [17]. Hanci and Bingöl [18] investigated the effects of melatonin on garlic under salinity stress in the early plant growth phase and reported that 2 μM melatonin application had the highest effect on leaf length under 400 mM salinity. Hanci and Tuncer [19] reported a positive effect of exogenous melatonin and L-tryptophan on the size of lettuce plants grown under salt stress.

There are limited studies investigating the effects of melotin on onion. Hanci et al. [20] evaluated the effects of different concentrations of L-tryptophan and melatonin on the germination of onion, leek, black carrot and radish seeds exposed to salt stress. In general, all melatonin doses slightly increased the maximum germination rate and germination index values under 300 mM NaCl stress condition. The germination index of seeds obtained under 200 mM salt stress and 8 μM melatonin application was found to be higher than other seed lots.

The effects of 1-μM, 2-μM, 4-μM, and 8-μM doses of melatonin on yield and quality of onion plants under salt stress were investigated in another study conducted by the same team of this study. At the end of the study, a wide variation was found among genotypes, salinity levels and melatonin doses. By evaluating the results statistically, it was concluded that appropriate melatonin doses seemed to vary according to genotype [21]. In the present study the effects of melatonin application on yield and quality of onion seeds grown under salt stress are investigated.

MATERIALS AND METHODS

'Corum Moru' onion variety, widely cultivated in Turkey, was used as plant material in the study. Equal-sized onion bulbs were incubated for 24 hr before planting in the dark at 21°C in 1, 2, 4 and 8 µM melatonin solutions [20-21]. The solution volume was adjusted to cover the entire root disc. Bulbs of the control group were kept in pure water without melatonin under the same conditions. The bulbs were planted in February 2019 in 23-litre pots which were filled with garden soil, peat and perlite at 1: 1: 1 ratio, under rain-sheltered conditions. All irrigation programmes were done according to the conditions and humidity level of the growing environments.

A total of 60 onion bulbs were planted in 3 replications, with 5 hormone doses and 4 levels of irrigation water salinity. The first and second irrigations were done with normal tap water. Then one out of every 3 irrigations was done with a saline solution (100 mM, 200 mM and 400 mM NaCl). In total, 12 irrigations were made, 4 of which were with salt water. The following measurements and observations were made regarding plant growth (at the beginning of flowering), flowering, seed yield and seed quality: number of leaves (NL) and leaf length (LL) at the beginning of flowering; number of flowers (NF); flower stalk length (FSL), main flower stalk length (MFSL) (from bulb neck to flower structure) and expected number of seeds (ENS) (six seeds for each flower) as the membrane on the flower structure fully opens; seed 1000-grain weight (SGW), seed creation percentage (SCP) and number of seeds per bulb (NSB) after the seed harvest; germination rate (GR), initial count value (ICV), germination peak day (GPD), germination index (GI), germination peak value (GPV), average daily germination (ADG), germination value (GV) and average germination day (AGD). The data were subjected to analysis of variance (ANOVA). The LSD (minimum significant difference) test was used to determine the differences between applications.

RESULTS AND DISCUSSION

Morphological Parameters and Seed Yield

The number of leaves on each bulb recorded at the beginning of flowering, is shown in Table 1. As a result of the statistical analysis, no significant difference is observed between different melatonin applications in terms of the number of leaves. The longest leaf length was measured after the plants completed forming new leaves. Also, there is no statistically significant effect of melatonin dose on leaf length. The source of variation for this parameter is irrigation water salinity. As expected, increasing salt concentration causes a decrease in leaf length (Table 1).

The flowering was observed only in plants treated with 4 and 8 µM melatonin at 200 mM salt dose; no flower was observed in 400 mM salt stress applications. When the membrane on the flower structure was fully opened, each flower was counted without damaging the female and male organs. The effects of melatonin and irrigation water salinity on this parameter are found to be significant ($P \leqslant 0.05$). No flowering was observed at 400 mM salinity level. At 200 mM salt dose, flowering was observed only in plants treated with 4 and 8 µM melatonin (Table 1). The highest flower numbers were detected at 2, 4 and 8 µM melatonin doses on non-stressed plants (511.33, 498.00, and 519.67 respectively). However, these values are statistically included in the same group.

Table 1. Effects of salt stress and melatonin application on different morphological properties of onion at the different stages of seed production period

Salinity (mM)	Melatonin (µM)	NL	LL (cm)	NF	FSL (cm)	MFSL (cm)	ENS	SGW (g)	SCP (%)	NSB
0	0	31.00	36.33	482.50 b	2.55 cde	69.50 ab	2895 a	4.16 bc	23.29 a	674
	1	34.67	37.83	354.00 b	2.85 bc	70.50 ab	2124 b	3.61 de	20.14 ab	376.5
	2	33.67	39.83	511.33 a**	3.10 ab	65.00 bc	3068 a	4.88 a	15.65 bc	481.67
	4	34.00	39.67	498.00 a	2.25 def	79.00 a	2988 a	4.09 bcd	19.56 ab	586
	8	21.67	41.07	519.67 a	2.90 abc	69.00 abc	3118 a	3.79 cd	18.21 ab	609.67
	Mean	*31.00*	*38.95a*	*473.10*	*2.73*	*70.60*	*2838.60*	*4.11*	*19.37*	*545.57*
100	0	32.67	39.10	235.00 bc	1.95 f	57.50 cd	1410 bc	3.87 bcd	12.33 cd	187
	1	31.67	34.50	0	0	0	0	0	0	0
	2	32.67	36.83	244.00 bc	2.00 f	45.50 e	1464 bc	3.09 ef	10.24 cd	172.5
	4	24.00	38.33	132.67 cd	2.23 def	49.00 de	796 cd	4.40 ab	7.92 d	71.33
	8	37.00	36.33	183.67 cd	2.08 ef	63.33 bc	1102 cd	2.38 g	12.02 cd	132.33
	Mean	*31.60*	*37.02a*	*159.07*	*1.65*	*43.07*	*954.40*	*2.75*	*8.50*	*112.63*
200	0	26.33	30.93	0	0	0	0	0	0	0
	1	19.00	28.40	0	0	0	0	0	0	0
	2	28.67	36.00	0	0	0	0	0	0	0
	4	28.67	31.73	93.00 de	2.60 cd	49.00 de	558 de	2.64 fg	8.76 d	46
	8	26.00	35.00	200.33 cd	3.36 a	78.33 a	1202 cd	2.95 f	15.42 bc	185.33
	Mean	*25.73*	*32.41b*	*58.67*	*1.19*	*25.47*	*352.00*	*1.12*	*4.84*	*46.27*
400	0	19.00	26.37	0	0	0	0	0	0	0
	1	25.67	24.23	0	0	0	0	0	0	0
	2	22.00	25.83	0	0	0	0	0	0	0
	4	29.67	29.67	0	0	0	0	0	0	0
	8	21.67	32.00	0	0	0	0	0	0	0
	Mean	*23.60*	*27.62c*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*
LSD value		*15,37*	*3.95*	*124.57*	*0.50*	*11.93*	*747.41*	*0.55*	*5.56*	*193.29*

Note: Differences between values with different letters in the same column are statistically significant *(*$p \leqslant 0.05$*)*. NL=number of leaves, LL=leaf length, NF=number of flowers, FSL= flower stalk length, MFSL=main flower stalk length, ENS=expected number of seeds, SGW=seed 1000-grain weight, SCP=seed creation percentage, NSB=number of seeds per bulb

The effects of melatonin and irrigation water salinity on petiole of each flower were found to be significant ($p \leqslant 0.05$). Application of 8 µM melatonin at 200 mM salt stress gives the longest flower stalk length of 3.36 cm, followed by 2 µM melatonin applied to the control group (Table 1). The lenght between the onion neck and the flower structure was measured after the flower formed the seed. The effect of melatonin and irrigation water salinity on this parameter, which varies between 45.50-79.00 cm, is found to be statistically significant ($p \leqslant 0.05$). The longest flower stalk (79.00 cm), is obtained in the control set (0 mM salt stress) with 4 µM melatonin, while 100 mM salt stress and 2 µM melatonin application give the shortest flower stem length (Table 1).

In botanical terms the fruit of the onion has three carpels and each carpel has two ovaries. Based on this information, the expected seed amount (six seeds for each flower) is calculated from the average number of flowers. The effects of melatonin and irrigation water salinity on this parameter are found to be significant ($p \leqslant 0.05$). The expected number of seeds varies between

558-3,118; 200 mM salt stress and 4 µM melatonin application give the lowest expected seed number (558) (Table 1). Also statistically significant ($p \leqslant 0.05$) is the 1000-grain weight, which varies between 2.64-4.88 g. The highest 1000-grain weight was obtained from 2 µM melatonin application under 0 mM salt stress (4.88 g), while the lowest was obtained from 4 µM melatonin application under 200 mM salt stress (2.64 g). As for the number of seeds per bulb, as the salt level increases, significant losses in the number of seeds occur. The application of 8 µM melatonin gives the highest number of seeds (besides control) under 200 mM salt stress.

The effects of melatonin and irrigation water salinity on seed creation percentage were found to be significant ($p \leqslant 0.05$). This value varies between 7.92-23.29% in the seed-forming plants (Table 1). In general, increased stress condition leads to a serious decrease in the seed creation percentage. At 200 mM salt stress, 4 µM melatonin application leads to 8.76% seed creation while this value increases to 15.42% in 8 µM melatonin application. It is remarkable that this obtained value is higher than all the values observed under 100 mM salt stress.

Seed Quality

Germination tests were carried out following International Seed Testing Association rules [22]. The seeds were placed in a petri dish with two layers of filter paper. Seeds that have 2-mm radicles were considered as germinated.

The germination rate (%) was calculated at the end of the trial period. It is seen in the Table 2 that the application of 8 µM melatonin under 200 mM salt stress has a 100% germination rate. It was observed that melatonin application against 200 mM salt stress has an improved effect on the germination rate (Table 2). It was also observed that 2 µM and 4 µM melatonin doses increase the initial count value in non-salt stressed seeds. In addition, the application of 8 µM melatonin under 200 mM salt stress causes a significant increase in the initial count value compared to 4 µM melatonin application (Table 2).

The initial count value (percentage of normal seedlings germinated on the first count day, as specified in the germination test for each species) was obtained by determining the normal germination rate on the sixth day after sowing [22]. Does of 2 µM and 4 µM melatonin increase the first count value in the control group. In addition, the application of 8 µM melatonin under 200 mM salt stress leads to a significant increase in the initial count value compared to 4 µM melatonin application (Table 2).

Germination peak day (GPD) is the day when the maximum daily germination occurs during the entire germination trial [22]. This period is expected to prolong under stress factors. In 100 mM salt stress condition, this value is 11.33 days in the group without melatonin, while it is 10.17 days in the non-salt stress condition (Table 2). The 2 µM melatonin application under 100 mM salt stress positively affects the germination peak day (9.83 days).

The application of 8 µM melatonin at 200 mM salt stress significantly increases the germination index (calculated using the following formula [23]: $GI = \Sigma\ n / d$, where n=no. of normal seedlings obtained on day d, d=days counted from the beginning of the test). The germination index of seeds obtained under 200 mM salt stress and 8 µM melatonin application was found to be higher than those of other seed lots. In addition, melatonin doses of 2 µM, 4 µM and 8 µM have a positive effect on the germination index in the non-stressed group (Table 2).

Table 2. Effects of salt stress and melatonin application on different seed quality parameters of onion during seed production

Salinity (mM)	Melatonin (μM)	GR (%)	ICV (%)	GPD (day)	GI	GPV	ADG	GV	AGD
0	0	100 a*	80.00 bc	10.17 b	14.45 cd	9.98 cd	0.48 a	4.76 bc	6.02 a
	1	81.67 b	63.33 de	9.50 bc	12.59 f	8.87 def	0.39 b	3.71 def	5.93 ab
	2	100 a	86.67 b	9.22 c	18.05 ab	11.53 b	0.48 a	5.49 b	4.63 ef
	4	100 a	98.33 a	6.67 d	18.96 ab	15.18 a	0.48 a	7.23 a	4.03 f
	8	94.44 a	71.11 cd	9.56 bc	15.69 c	9.95 cd	0.45 a	4.51 cd	5.27 cd
	Mean	*95.22*	*79.89*	*9.02*	*15.95*	*11.10*	*0.46*	*5.14*	*5,18*
100	0	98.33 a	68.33 d	11.33 a	15.66 bc	9.00 def	0.47 a	4.23 cd	5.88 abc
	1	0	0	0	0	0	0	0	0
	2	85.00 b	63.33 de	9.83 bc	14.69 cd	9.03 de	0.40 b	3.84 def	5.33 bcd
	4	85.00 b	53.33 ef	10.17 b	12.92 def	8.39 ef	0.40 b	3.48 ef	6.06 a
	8	86.67 b	63.33 de	11.67 a	12.80 ef	7.57 fg	0.41 b	3.11 f	6.31 a
	Mean	*71.00*	*49.66*	*8.60*	*11.21*	*6.80*	*0.34*	*2.93*	*4,72*
200	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	4	60.00 c	43.33 f	9.67 bc	10.80 g	6.18 g	0.28 c	1.79 g	5.10 de
	8	100 a	90.00 ab	9.00 c	19.86 a	11.20 bc	0.48 a	5.33 b	4.00 f
	Mean	*32.00*	*26.67*	*3.73*	*6.13*	*3.48*	*0.15*	*1.42*	*1,82*
400	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0
	Mean	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0,00*
LSD value		*6,57*	*10.07*	*0.91*	*1.78*	*1.43*	*0.03*	*0.80*	*0.64*

Note: Differences between values with different letters in the same column are statistically significant ($p \leqslant 0.05$). GR=germination rate, ICV=initial count value, GPD=germination peak day, GI=germination index, GPV=germination peak value, ADG=average daily germination, GV= germination value, AGD=average germination day

Germination peak value (GPV) is the number of days the maximum germination occurs divided by numerical expression of the day [24]. A dose of 4 μM melatonin gives the highest value in the control group (0 mM salt). In addition, while 8 μM melatonin application under 200 mM salt stress has a positive effect, 4 μM melatonin application under 200 mM salt stress has a negative effect on the value (Table 2).

The average daily germination (ADG) is the total number of germinated seeds divided by total count days [19]. At 200 mM salt stress, 8 μM melatonin application shows the most prominent effect on ADG (Table 2).

Germination value is calculated according to the following formula [23]: GV = germination peak value x daily germination. While 4 μM melatonin application gives the highest germination value in the control group (0 mM salt), the opposite is observed when salt stress is 100 and 200 mM. The application of 8 μM melatonin at 200 mM salt stress causes an increase in this value (Table 2).

Average germination day is calculated according to the following formula [23]: AGD = Σ (n × d) / N, where n = no. of seeds germinated per day, d = no. of days from start of the test, and N = total no. of seeds germinated at end of the trial. In the salt-free control group the mean germination day value decreases in parallel with increase in melatonin dose. However, this reduction is reversed with a dose of 8 μM melatonin. At 100 mM salinity, 4 and 8 μM melatonin doses prolong this period (Table 2).

CONCLUSIONS

The exogenous application of melatonin significantly increases several parameters of onion flowers and seeds under both normal and salinity conditions. When all results are considered together, it can be said that although it varies depending on salt doses, 8 μM melatonin application seems to have positive effects on flowering, seed yield and seed quality under salt stress conditions. These results, therefore, provide evidence that melatonin plays a role during the flowering physiology of onion in both salinity and normal conditions.

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