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Putrescine and boron treatments increase seed quality in Melon (*Cucumis melo* var. *inodorus*)

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Abstract

In agriculture, seed quality and high yield are directly related to each other. Hybrid seed production is difficult and expensive, and as it were not enough, the rate of empty seeds at the end of production is high, which reveals the importance of studies in seedling emergence. Empty seed formation is frequently encountered in melons and causes low seed productivity. Efforts to eliminate this situation and improve seed germination and emergence quality will have positive effects on both producers and consumers. In this study, it was aimed to determine the effects of boron and putrescine on seed number per fruit, seed germination, and seed emergence rates in Cucumis melo var. inodorus. The seeds used in the research were produced from plants of the SR-21 and Sİ-8 genotypes to which boron and putrescine were applied in the spring-summer growing period of 2023. In research, germination and emergence rates, germination and emergence times, germination and emergence index parameters in seeds were investigated. Seed quality parameters were positively affected by boron and putrescine applications, boron increased seed quality compared to the control. Putrescine was the application that had the best effect on seed quality. As a result, it was determined that putrescine (90.16%; 92.83% respectively), boron (83.83%; 94.67% respectively), and boron+putrescine combinations (78.16%; 84.17%) increased the germination and emergence rate compared to the control group (%55, 58.17 respectively or % increases can be given compared to the control group). In conclusion, to produce higher quality seeds, breeders and seed companies could apply putrescine and boron to the plant before hand pollination.

Keywords: Boron, Putrescine, Melon, Seed

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INTRODUCTION

Melon is an annual vegetable belonging to the *Cucurbitaceae* family (Pitrat, 2016; Wan Shafiin et al., 2021) and spread around the world from Africa. Although there is no clear information about its origin and area, it can be cultivated in subtropical and tropical regions (Abraham-Juarez et al., 2018; Kesh and Kaushik, 2021). In Türkiye, 1.6 million tonnes of melons are produced in an area of 62.7 thousand hecares. The most melon is produced in the provinces of Denizli (126 000 tons), Konya (117 000 tons), Adana (116 000 tons), and Antalya (115 thousand tons) (TUIK, 2023). However, considering the effects of global climate change on Türkiye and in the world, it is important to implement practices that will both protect plants and seeds from this change and increase production and quality.

Boron, a microelement, is effective in both generative and vegetative tissues in plants and also plays an important role in cell wall formation, plant physiology, and increasing seed yield by supporting fertilization (Kumar et al., 2012). If boron is low in the soil, it causes losses in plant production areas (Shorrocks, 1997) and also negatively affects pollen formation, seed quality, and germination (Sillanpaa, 1982; Dell et al., 2002). In a study, it was stated that the low germination rate in seeds was due to the insufficient amount of boron in pollen (Pinho et al., 2010). Boron was recently applied to lentils (Khattab et al., 2016), flax (Jankowski et al., 2016), canola (Khan et al., 2016), olives (Gündeşli and Nikpeyma, 2016), peas (Sharma and Sharma, 2016), corn (Andric et al., 2016). It has been found to increase seed yield and quality in sunflower (Silva et al., 2016), beans (Parry et al., 2016), and radish (Dev, 2010; Namlı et al., 2022). In addition, since it is known that boron increases the quality of pollen in watermelons (Adıgüzel et al., 2023d), it is thought that it may have a positive effect on the seeds what is the seed.

Putrescine is a polyamine which positively affects growth and development in plants and increases tolerance to stress conditions (Liu et al., 2015; Thomas et al., 2020; González-Hernández et al., 2022). Studies have shown that putrescine application in vegetables increases the marketing period in melon (Lester, 2000), yield and quality in onion and artichoke (Amin et al., 2011; El-Abagy et al., 2010), and prevents cold damage in cucumber (Zhang et al., 2009). In addition, in a limited number of studies on seeds, it has been reported that putrescine increases the germination and viability rates in corn (Hussain et al., 2013) and the germination rate of semi-viable seeds in melon (Adıgüzel et al., 2023c). As a result of the putrescine application in lemon, by increasing ovule longevity and ovule quality the effective pollination period was increased and at the end, seed quality and germination level were also increased (Karabıyık, 2024). When these positive effects of boron and putrescine on plants are evaluated, it is thought that seed quality would be increased if boron and putrescine are applied together. In this context, although there is a study conducted on lemon (Karabıyık, 2024) about the effects of putrescine on the seed, as our knowledge there is no study on *Cucurbitaceae* species. This study aimed to determine the seed yield, full seed rate, germination and emergence rates of seeds harvested from the fruits of plants treated with boron, putrescine, and boron+putrescine.

MATERIALS AND METHODS

This study was carried out in a greenhouse at Research Application Areas of Çukurova University Faculty of Agriculture Department of Horticulture in 2024 and the seed tests were carried out in the Seed Technologies Laboratory of the same department.

Materials

In this study, SR-21 and Sİ-8 genotypes from inodorus group melon genotypes (*Cucumis melo* var. *inodorus*) were used. The seeds used in the research were obtained as a result of self-pollination of putrescine and boron-treated SR-21 and Sİ-8 genotypes in the spring-summer growing season of 2023. The used plants were in optimum care conditions. And only one fruit was allowed for every plant.The boron and putrescine treatments were made as follows: (i) Boron application [150 g/100 L EtiDot 67 (21% boron) applied twice with the help of a foliar back sprayer when the plants are at the 6-leaf stage and the small fruit stage]; (ii) Putrescine application [at the first male flower signs begin to appear on the plants, a single application of 0.25 mM 1.4 diaminobutane dihydrochloride by spraying the plants with a back sprayer]; (iii) Boron + Putrescine [application of boron and putrescine together as stated above] (iv) Control [only water treatment].

Seed Analysis

Seeds were harvetsed from each mature fruit obtained by inbreeding and left to ferment for 3 days. After the fermentation process, the seeds were washed and left to dry. Total and full seed were counted, and seed number and fully developed seed ratio were recorded. The obtained seeds were planted separately for each genotype and treatment for germination and emergence tests in February 2024. Randomly selected full seeds obtained from 5 fruits derived from each plant were used for both germination and emergence tests. Seed germination and emergence tests were carried out according to ISTA rules (ISTA, 2007) in 4 replicates with 100 seeds in each replication. For seed germination tests, 90 mm plastic petri dishes were used, and the seeds were germinated in the petri dishes between filter paper in an oven (Memmert) at 25°C for 7 days. For seed emergence tests, river sand was used, and emergence tests were performed in the growth chamber under 16 hours of light and 8 hours of darkness conditions for 10 days. Rate, duration, and index were calculated by counting the germinating and emerging seeds every day (Ellis and Robert, 1980; Demir et al., 2008).

Statistical Analysis

The experiment was set up according to the randomized design with 3 replications and statistical analyses were carried out according to randomized plots factorial experimental design on the basis of varieties and applications, each period within itself. The data were subjected to variance analysis in the JMP 13.2.0 package program and the differences between the means were classified at the 5% significance level according to the LSD test. Arc-sin transformation was applied to percentage values.

RESULTS AND DISCUSSION

The number of seeds harvested from fruits is given in Table 1. The total number of seeds was singificanty affected by genotype, treatment, and genotype x treatment interaction. In general, Sİ-8 (688.5 seed/fruit) produced more seeds than the SR-21 (527.5 seed/fruit) genotype, and all treatments increased the total number of seeds by approximately 30% compared to the control. The highest increase was obtained from boron + putrescine treatment (826.3 seed/fruit) to the Sİ-8 genotype and the lowest number of seeds was obtained from the control treatment to the SR21 genotype (477.7 seed/fruit).

Although the number of seeds is important in breeding studies, the high rate of full seeds provides information about the accuracy of the pollination process. The full seed rates obtained from the combinations used in the study are given in Table 1. The highest fully developed seed rate was obtained by applying putrescine to SR-21 genotype as 92.86% and the lowest was found to be 64.82% when both boron and putrescine were applied to Sİ-8. The SR-21 genotype (83.95%) had more full seeds than Sİ-8 genotype (70.80%) and the putrescine treatment increased the rate of fully developed seeds.

Table 1. Effect of Boron and Putrescine Treatments on Total Seed Number and Full Seed Rate of Different Genotypes.

Application	Total Seed Number (seed/fruit)			Full Seed Rate (%)		
	Genotype		Application average	Genotype		Application average
	SR-21	Sİ-8		SR-21	Sİ-8	
Control	477.7 e	543.0 de	510.3 B	87.08 b	67.80 ef	77.44 B
Boron	593.7 cd	678.7 bc	636.2 A	73.93 d	78.69 c	76.31 BC
Putrescine	527.7 de	706.0 b	616.8 A	92.86 a	71.88 de	82.37 A
Boron+ Putrescine	511.0 de	826.3 a	688.7 A	81.94 c	64.82 f	73.38 C
Genotype average	527.5 B	688.5 A		83.95 A	70.80 B	
LSD(Genotype):43.07 ***; LSD(Application): 60.92*** *; LSD(Genotype x Application): 86.15**				LSD(Genotype): 1.53***; LSD(Application): 2.16***; LSD(Genotype x Application): 3.05***		

NS: Not Significant; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Different uppercase letters were used for application average and genotype average. Different lowercase letters were used for genotype x application. And arc/sin transformation was made to percentage values.

The data on seed germination and emergence rates were given in Table 2. The table showed that the differences between genotypes were not statistically important while it was significant in terms of treatment and genotype x treatment showing that the treatments can be effective for all melons. In this context, when the treatment averages were evaluated, the highest value was obtained from putrescine with 90.16%, followed by boron application with 83.33%. The lowest value was obtained from the control application as 55.00%.

In terms of seed emergence rates, it was determined that the differences between the data were non-significant on the basis of genotypes, but important in terms of treatment and genotype x treatment interaction as it was in germination tests. The data showed that the highest values were obtained from boron and putrescine applications with 94.67% and 92.83%, respectively. At the same time, the lowest value in the mantioned parameter was again in the control application with 58.17%.

In this study, the effects of putrescine and boron application were examined, putrescine (90.16%; 92.83%, respectively), boron (83.83%, 94.67%, respectively) and boron it was found to increase the rate of the results are consistent with other studies.

The results showed that the application of putrescine and boron to melon plants increased seed germination and emergence rates.

Table 2. Effects of Boron and Putrescine Treatments on Seed Germination and Emergence Rate in Seeds of Different Genotypes (%).

Application	Seed Germination Rate (%)			Seed Emergence Rate (%)		
	Genotype		Application average	Genotype		Application average
	SR-21	Sİ-8		SR-21	Sİ-8	
Control	70.00 c	40.00 d	55.00 C	61.66 d	54.67 d	58.17 C
Boron	85.00 ab	82.67 bc	83.83 AB	98.67 a	90.66 bc	94.67 A
Putrescine	85.00 bc	95.33 a	90.16 A	88.33 c	97.33 ab	92.83 A
Boron+ Putrescine	81.33 bc	75.00 bc	78.16 B	80.00 c	88.33 c	84.17 B
Genotype average	80.33	73.25		82.17	82.75	
LSD(Genotype): N.S.; LSD(Application): 8.34***; LSD(Genotype x Application): 12.07*				LSD (Genotype): N.S.; LSD(Application): 0.69***; LSD(Genotype x Application): 9.75**		

NS: Not Significant; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Different uppercase letters were used for application average and genotype average. Different lowercase letters were used for genotype x application. And arc/sin transformation was made to percentage values.

When the seed germination time was examined, it was determined that there was no significant change between the periods and seed germinated within 3-4 days in all applications (Table 3). When looking at the seed emergence times, the differences were not statistically significant, but the longest seed emergence time was in the control treatment (5.56 days) and the shortest in the putrescine (4.09 days). Considering the seed emergence times, the latest emergence was observed in the control group (7.00 days) in SR-21, while all other treatments were statistically in the same statistical group and emerged between 3.66-4.71 days.

Table 3. Effects of Boron and Putrescine Treatments on Seed Germination and Emergence Time in Seeds of Different Genotypes (day).

Application	Seed Germination Time (day)			Seed Emergence Time (day)		
	Genotype		Application average	Genotype		Application average
	SR-21	Sİ-8		SR-21	Sİ-8	
Control	3.26	3.00	3.13	7.00 a	4.12 b	5.56
Boron	3.00	3.03	3.02	4.71 b	4.79 b	4.75
Putrescine	3.00	3.00	3.00	3.66 b	4.52 b	4.09
Boron+ Putrescine	3.03	3.00	3.00	4.27 b	4.43 b	4.35
Genotype average	3.06	3.01		4.91	4.46	
$LSD_{(Genotype)}$: N.S.; $LSD_{(Application)}$: N.S.; $LSD_{(Genotype\ x\ Application)}$: N.S.				$LSD_{(Genotype)}$: N.S.; $LSD_{(Application)}$: N.S.; $LSD_{(Genotype\ x\ Application)}$: 1.75*.		

NS: Not Significant; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Different uppercase letters were used for application average and genotype average. Different lowercase letters were used for genotype x application.

The results of germination and emergence speed index are given in Table 4. When the seed germination and emergence speed index data were examined, it was determined that the differences were statistically significant in terms of genotype, application, and genotype x application interaction.

The germination speed index was higher in the Sİ-8 genotype (6.08) than in the SR-21 genotype (5.84). In terms of treatment averages, the highest value was recorded in the putrescine application with 7.85, and the lowest value was obtained from the control application with 2.77, meaning that the germination speed index was increased by approximately 35% with this application. In genotype x application interaction, the values range between 1.01 (SR-21 x control) and 8.22 (SR-21 x boron) in the SR-21 genotype, and between 4.53 (Si-8 x control) and 8.20 (Si-8 x putrescine) in the Si-8 genotype.

When the emergence speed index was examined, it was determined that the highest values were in the SR-21 genotype (4.93) and the lowest values were in the Sİ-8 (4.09). The treatment averages showed that the highest values were in the boron (6.12) and putrescine (6.23) treatments and the lowest seed speed index values were again in the control group (1.33). In application and genotype combinations, SR-21 x boron (7.73) and SR-21 x putrescine (7.26) had the highest values, and SI-8 x control (1.96) and SR-21 x control (0.30) had the lowest values (Table 4).

Table 4. Effect of Boron and Putrscine Treatments on Seed Germination and Emergence Speed Index of Different Genotypes.

Application	Germination Speed Index			Emergence Speed Index		
	Genotype		Application average	Genotype		Application average
	SR-21	Sİ-8		SR-21	Sİ-8	
Control	1.01 d	4.53 c	2.77 C	0.30 d	1.96 c	1.33 C
Boron	8.22 a	7.00 b	7.61 AB	7.73 a	4.50 b	6.12 A
Putrescine	7.50 ab	8.20 a	7.85 A	7.06 a	7.40 b	6.23 A
Boron+ Putrescine	6.63 b	7.50 ab	7.06 B	4.63 b	4.50 b	4.57 B
Genotype average	5.84 B	6.08 A		4.93 A	4.09 B	
$LSD_{(Genotype)}$: 0.46***; $LSD_{(Application)}$: 0.65***; $LSD_{(Genotype\ x\ Application)}$: 0.92***				$LSD_{(Genotype)}$: 0.50**; $LSD_{(Application)}$: 0.70***; $LSD_{(Genotype\ x\ Application)}$: 0.99***		

NS: Not Significant; ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Different uppercase letters were used for application average and genotype average. Different lowercase letters were used for genotype x application.

Studies have shown that boron increases the pollen germination rate and the velocity of pollen germination (Goldberg et al., 2003; Şensoy et al., 2003; Ansari and Chowdhary, 2018; Fang et al., 2019; Hidayat et al., 2019, Adıgüzel et al., 2022; Adıgüzel et al., 2023d) which in turn provides a more efficient fertilization process. In studies conducted on lentils (Khattab et al., 2016), flax (Jankowski et al., 2016), canola (Khan et al., 2016), olives (Gündeşli and Nikpeyma, 2016), and peas (Sharma and Sharma, 2016) boron treatment increased the seed germination rate. It has also been stated that there was a significant rise in the number of seeds in corn (Andric et al., 2016), sunflower (Silva et al., 2016), beans (Parry et al., 2016) and radish (Dev, 2010; Namlı et al., 2022) by boron applications.

In the presence of putrescine, the ovule longevity was increased (Akbaş ve Solmaz, 2019; Erol ve Sarı, 2019; Solmaz and Yıldız, 2020; Karabıyık, 2024), providing the pollen tube to reach the ovule in a longer period. At the same time, putrescine provides plants to overcome some stress conditions like salt (Ekinci et al., 2019; Yuan et al., 2019; Islam et al., 2020), temperature (Zhang vd., 2009; Palma et al., 2016; Lu et al., 2022; Sharma et al., 2023) and drought stresses (Farsaraei et al., 2021; Li et al., 2021; Ma et al., 2022). The effects of putrescine on plants, fruit, and seeds were reported in apples (Costa et al., 1985), lemons (Karabıyık, 2024), onions (Amin et al., 2011), etc. before. In the light of this knowledge for boron and putrescine, it could be said that by increasing fertilization efficiency and pollen-ovule interaction, there could be stronger seeds with higher germination capacity. In recent studies, the application of 20 and 50 ppm putrescine on pepper plants increased germination and root capacity (Khan et al., 2016). Koç et al. (2014) reported that in the presence of putrescine, the germination rate was increased in bean genotypes exposed to salt stress by repressing the negative effects of salt stress. Moreover, in a previous study in melon seeds, the germination was increased by approximately 40% with putrescine dipping treatment even in semi-viable seeds (Adıgüzel vd., 2023c). In another study conducted on lemon, it was determined that 0.05 and 0.1 m Mol putrescine treatment to plants increased the seed germination rate by approximately 40% (Karabıyık, 2024). In this study conducted on melon genotypes, seed germination was increased by approximately 63% with the application of putrescine to melon plants. At the same time, this increase was 50% in boron and 42% in boron+putrescine treatment compared to the control group. So, it could be easily said that putrescine only or boron and putrescine in a couple could have a constant effect on the germination and emergence performance of melons. Seed number and quality affects from environment (Alqudah et al., 2011; Pervez et al., 2009; Sehgal et al., 2018), genotype (Jat et al., 2016), grafting (Suárez-Hernández et al., 2022; Adıgüzel et al., 2023b; Alam et al., 2023), flower sex (Adıgüzel et al., 2023b) and different treatments (Kuzucu and Dumlupınar, 2017; Ozdemir et al., 2019; Acharya et al., 2020; Alam et al., 2022; Yousef, et al., 2023). In another study of our team, it was reported that the total number of seeds varied between 773 and 291 number the rate of fully developed seeds varied between 36% and 97% which was thought as this higher numbers was due to the rapid advancement of pollen tubes to the ovules, depending on the flower sex structure (Adıgüzel et al. 2023b). In addition, it has been concluded that healthy seed formation is not caused by pollen, but is related to the homogeneous development of seed ovules. In this study, the total number of seeds increased compared to the control, and putrescine treatment came to the fore in the proportion of fully developed seeds. Putrescine has effects on ovule longevity (Karabıyık, 2024), reducing stress factors (Thomas et al., 2020), ensuring the development of healthy male and female flowers (Singh et al., 2014) and accordingly increasing fruit quality (Sayyad-Amin et al., 2018). As a result of the study, the highest seed and fully developed seed ratio was obtained from putrescine treatment. This proves that putrescine can produce healthy seeds.

Seed germination and emergence time analysis is an important parameter as it provides information about how many days it takes for the seeds to germinate (Ranal and Santana, 2006; Sarma, 2024). In this way, information is obtained about whether the seeds germinate faster or slower depending on their genetic structure, age, or treatments. In this case, ensuring earliness, especially in obtaining seedlings, is proportional to the germination time of the seeds. The emergence time of a seed depends on the availability of reserves within the seed (Taiz and Zaiger, 2002). The emergence time was between 7 and 10 days; the germination time was between 2 and 7 days in recent studies (Adıgüzel et al., 2023a, Adıgüzel et al., 2023b). The parameter in question in this study varied between germination time 3.00 and 3.26; emergence time 3.66 and 7.00 days. In a study conducted on viable and semi-viable seeds, the dipping of melon seeds to putrescine solution did not provide any significant increase (Adıgüzel et al., 2023c). However, in this study, there was a difference of approximately 1.5 days due to the emergence time of root tip in the emergence tests. So, it could be concluded that the increase of emergence time could be originated from the inner putrescine level of the plant with the efficient pollination and fertilization process of the flower. It was also concluded as the reserves could be accumulated in the seeds with putrescine applications and strengthen the seed. However, this opinion needs more detailed study.

The germination and emergence speed index shows the initial strength of the seed (Copeland and McDonald, 2012). In addition, seeds lose their strength vigor at the stage before losing their ability to germinate (Sivritepe, 2012). In this sense, germination and emergence speed index are important in determining seed quality. In this study, compared to control, the germination speed index increased 3 times with putrescine, boron, and boron x putrescine treatments and the emergence rate increased approximately 4-6 times. From this, it can be concluded that the germination index of putrescine acting on the mother plant will increase significantly, thus stronger seeds that provide emergence in a shorter time can be obtained. In general, the seeds that were initially strongest in terms of germination and viability were obtained by applying putrescine and boron treatments which had higher values than the control. In a study conducted on apple (Naija et al., 2009) and olive (Rugini and Mencuccini, 1985) it was showed that the increase in inner putrescine content increases the root formation in the plant and in the scions indicating that the putrescine has an effect on root initiation process.

In this study, the seed germination capacity in terms of germination and emergence rates, germination and emergence times, and germination and emergence speed index were increased by putrescine and/or boron treatments. The results of this study and recent studies together showed that this result originated from better plant fertilization success with superior and rapid root formation and in turn this shows a better germination capacity with putrescine and boron treatments.

CONCLUSION

In this study, where the effects of boron and putrescine applications on melon plants were examined, it has been determined that the seed germination capacity is affected by putrescine and boron treatments. In this context, although boron had a greater effect on seed germination, it still had an increase in germination rate than control treatments. Putrescine was the most effective treatment that increased the total number of seeds and the rate of filled seeds. Considering that the treatments strengthen the ovules and support the development of the pollen tube, it is thought that the embryos formed inside the seeds should also be quite developed. Thus, germination and emergence rates in this study were significantly higher than the control treatment, which supports this idea. In conclusion, putrescine treatment for the plant is an effective treatment for seedling growth by increasing the seed quality.

For a more profitable production, growers and seedling producers can be advised to spray their plants with 0.25 m Mol putresin and the specified amount of boron.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that there are no actual, potential or perceived conflicts of interest for this research article.

Author contribution

P. A and Ş. K: Study data analysis, conceptualization and design of the research; P. A: Wrote the original draft; and İ. S: Editing and preparation of the manuscript. All authors have read and approved the manuscript.

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