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Determination of Selenium dose assessment and optimization for heat- tolerance in cucumber (*Cucumis sativus* L.)

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Abstract

The current study was conducted to identify the best level of selenium (Se) for inducing thermo-tolerance capacity in genotypes of cucumber. The study was performed in a growth chamber. Seeds of one heat-sensitive (Desi Cucumber) and one heat-tolerant cucumber genotype (Safoora F1-Hybrid) were sown in plastic pots. The heat-stress (40/30 °C) was applied after leaf emergence. Different levels of selenium as a foliar spray (0, 0.2, 0.7 and 0.8 ppm) were applied 15-day after stress induction. The treatment of selenium (0.7 ppm) significantly more effect on the shoot length and the highest shoot length (95.87 cm) was observed in Safoora FI- Hybrid followed by Dasi Cucumber (36.41 cm). Similarly, maximum root length (58.57 cm) was recorded in Safoora F1 Hybrid while the lowest root length (40.17 cm) was noted in control. Similarly, findings were observed in Desi Cucumber at the same level of selenium at 0.7 ppm. The best leaf area was also observed in Safoora F1-Hybrid (57.82 cm^2), while the minimum leaf area was recorded in the control 36.56 cm^2. Overall results showed that selenium @0.7 ppm was the best appropriate level of selenium to increase the thermo-tolerance perspective in the best genotypes of cucumber.

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Introduction

Cucumber is an important vegetable crop that is grown all over Pakistan. It belongs to the family cucurbitaceous. It is commonly cultivated in tropical and subtropical areas of the world (Wang *et al.*, 2012; Renner *et al.*, 2019). It is native to Asia and has been cultivated since 3000 years ago (Wehner and Guner, 2004; Siddique *et al.*, 2017. In Pakistan, the production of cucumber is 65597 tones under 3549 hectares of land during the year 2018 (FAO, 2019). The optimum temperature required for the growth of cucumber is 20 ˚C - 32 ˚C. Cucumber can grow on a variety of soils. The cucumber is very sensitive to frost by causing serious injuries in the fruit. Its cultivated area reduced approximately 49.000 hectares. Its cultivated area reduced approximately 49.000 hectares. The decrease in optimal yield is due to an environmental issue like drought stress, temperature stress, the severe influence of salt stress, waterlogging and nutrient application (Ramakrishna and Gokare, 2011).

Environmental factors such as heat stress and drought significantly affect crop production all over the world (Khan *et al.*, 2015). These stresses regularly disturb the morphological, biological and physiological systems of the plant's development which reduced the yield of crop Christensen and Christensen, 2007). However, heat stress is a major factor affecting crop production due to increasing temperature that damage the biochemical process which reduced the heat tolerance capacity of plants.

It has been previously reported that two-thirds production of cucumber in the world attained from China (Shetty and Wehner, 2002). Foliar application and seed treatment of micro-nutrients (Sulpher, Boron, Iron, Zinc and Selenium) have been used to overcome the adverse effect of temperature, drought and salt as well as to maintain the growth of plants. Among these nutrients, selenium is used to create tolerance of heat stress in the crops. Due to tolerance capability against abiotic stress Se is also vital such as infrared (UV) radiation, salt stress and drought stress in plants. The effect of Se has been previously reported in lettuce, cucumber, ryegrass, okra and tomato (Djanaguiraman *et al.*, 2005; Hawrylak-Nowak *et al.*, 2010). Under UV-induced oxidative pressure, it has the capability of interruption in senescence. It maintenance the growth of mature plantlets and leaf area of plants (hartikainen *et al.*, 2000). It is also reported that seed treatment or side dressing of Se showed less reclamation in crops under high-temperature regimes than foliar spray (Curtin *et al.*, 2006). Hence, the present study was planned to explore the effect of foliar application selenium on the growth of thermo-sensitive and thermo-tolerant genotypes of cucumber under heat stress.

Materials and methods

The current study was planned for the optimization of the best dose of selenium under heat-stressed for genotypes of cucumber. Seeds of selected genotypes of cucumber were collected from Vegetable Research Institute, Ayyub Agriculture Research Institute, Faisalabad. This study was conducted in the growth chamber Department of Horticulture, University College of Agriculture, University of Sargodha, Punjab, Pakistan. The seeds were collected from Vegetable Research Institute, Ayyub Agriculture Research Institute, Faisalabad and sown in plastic pots filled with peat moss as growth media.

The treatments were replicated 5-times and 6-seed per pot were sown. 4-plant per pot was adjusted by thinning of weak and less vigorous seedlings. Hoagland nutrient solution @ half strength (0.5) was given to plants as a nutrient source (Aron, 2015) at a 10-day interval. The growth chamber was adjusted to 23/13˚C day /night temperature with 75% relative humidity, light intensity 550 µmol m^{-1} s^{-1} from fluorescent tubes and photoperiod (11.5 hours). After true leaf emergence; heat-stress (40/30 ˚C) was applied and other conditions were maintained the same as for 23/13˚C. Selenium @ 0 ppm (control), 0.2 ppm, 0.7 ppm and 0.8 ppm were foliar-applied 15-day after heat stress induction on the seedlings to recognize the best level of selenium for the alleviation of adverse effects of heat stress and induction of thermo-tolerance in tested cucumber genotypes. The experiment was laid out under CRD two factor factorial arrangements. The data regarding the following parameters were taken as:

Shoot and root length (cm)

5-plant per replication were randomly selected from both genotypes to measure the shoot and root length in centimeter (cm) by using a meter rod.

Fresh and dry weight of shoot (g)

After the measurement of shoot length, the fresh weight (g) of shoots was measured by using a digital balance. However, the plant samples were oven-dried (Memmert-110, Schawabach, Germany) at 72 °C for 48 h to measure the dry weight of shoot for both genotypes of cucumber.

Fresh and dry weight of roots (g)

Similarly, fresh and dry weight of roots was also measured the same as fresh and dry weight of shoot was measured for both genotypes of cucumber.

Leaf area (cm^2)

The leaf area of seedlings of both genotypes of cucumber was calculated by using leaf area meter (Ll-3100; LI-COR Inc., Lincoln, NE, USA).

Statistical analysis

After the collection of the data, the data was statistically analyzed. The experiment was two factorial (Treatments × genotypes), set up in a completely randomized design (CRD) with 5-replications per treatment.

Data were statistically analyzed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using LSD at a 5% probability level (Steel *et al.*, 1997).

Results and discussion

Effect of selenium on shoot length of selected genotypes of cucumber under heat stress

The data showed that selenium significant ($p< 0.05$) improved shoot length of both cucumber genotypes when heat stress was applied at (40/30 °C) (Table. 1).

The treatment of selenium (0.7 ppm) significantly affect the shoot length and the highest shoot length (95.87 cm) was observed in Safoora FI- Hybrid followed by Dasi Cucumber (36.41 cm). However, the highest shoot length was observed in Safoora FI hybrid (78.44 cm) as compared to Dasi Cucumber (29.94 cm).

Table 1. Effect of selenium on shoot length of selected genotypes of cucumber under heat stress.

Shoot length (cm)			
Treatments	Safoora F1-Hybrid	Desi Cucumber	Means (Treatment)
Control	58.67^c	22.47^e	40.57^c
Se (0.2 ppm)	68.72^b	26.48^e	47.60^b
Se (0.7 ppm)	95.87^a	36.41^d	66.12^a
Se (0.8 ppm)	90.52^a	34.43^d	62.48^a
Means (Genotypes)	78.44^a	29.94^b	
LSD ($p < 0.05$)			
Treatments (T)	4.01		
Genotypes (G)	2.88		
T×G	4.60		

n = 5 replications (4 seedlings per replication), any two mean with different letters are significantly different; Ns = not-significant.

The results showed that shoot length significantly increased with the increase of selenium levels. The findings of the current study were also supported by the finding of (Xue and Piironen, 2001), they stated that the foliar application of selenium stimulates shoot length, fresh weight, dry weight, leaf area and yield in lettuce. Similarly, (Turakainen *et al.*, 2004) also found the same results in green tea and potato.

They found that in young potato plants Selenium promoted starch accumulation in the upper leaves and later in mature roots. Germ, (2007) observed that Se spray enhanced the height of plants and fresh weight of potato tuber.

Table 2. Effect of selenium on root length (cm) of selected genotypes of cucumber under heat stress.

Root length (cm)			
Treatments	Safoora F1-Hybrid	Desi Cucumber	Means (Treatment)
Control	40.17ab	22.45c	21.98c
Se (0.2 ppm)	46.98b	24.99c	26.09c
Se (0.7 ppm)	58.57a	33.32a	45.87a
Se (0.8 ppm)	54.89a	30.17b	42.53b
Means (Genotypes)	50.15a	27.73b	
LSD ($p < 0.05$)			
Treatments (T)	4.30		
Genotypes (G)	2.05		
T×G	4.02		

n = 5 replications (4 seedlings per replication), any two mean with different letters are significantly different; Ns = not-significant.

Effect of selenium on root length of selected genotypes of cucumber under heat stress

The root length of selected genotypes significantly increased by the application of selenium when the heat was applied @ (40/30 °C). The results showed that the maximum highest root length (58.57 cm) was recorded in Safoora F1-Hybrid while lowest root length (40.17 cm) was noted in control when selenium was applied @ 0.7 ppm. Similarly, findings were also observed in Desi Cucumber at the same level of selenium @ 0.7 ppm (Table 2). (Germ *et al.*, 2007) has been reported that selenium significantly increased root length of *Daucus carota* and *Raphanus sativus*.

Table 3. Effect of selenium on fresh weigh of shoot for selected genotypes of cucumber under heat stress.

Fresh weight of Shoot (g)			
Treatments	Safoora F1-Hybrid	Desi Cucumber	Means (Treatment)
Control	3.26c	1.38e	2.32d
Se (0.2 ppm)	4.06b	1.57de	2.81c
Se (0.7 ppm)	5.84a	2.13d	3.99a
Se (0.8 ppm)	5.28a	1.84de	3.56b
Means (Genotypes)	4.61a	1.73b	
LSD ($p < 0.05$)			
Treatments (T)	4.05		
Genotypes (G)	2.10		
T×G	4.90		

n = 5 replications (4 seedlings per replication), any two mean with different letters are significantly different; Ns = not-significant.

It may have argued that selenium significantly improved the root growth of plants and enhanced the absorptions capacity of nutrients from the soil which ultimately increased the growth performance of plants (Turkainen *et al.*, 2004. Kong *et al.*, 2005) observed that selenium is a fundamental part of selenoproteins that play a significant role in improved shoot and root length through energy metabolism, protection of antioxidant, gene expression and transcription in plants under stress.

Effect of selenium on fresh weight of shoot for selected cucumber genotypes under heat stress

The treatment of selenium @ (0.7 ppm) significantly ($p< 0.05$) effect on the fresh weight of shoot for selected genotypes of cucumber under heat stress at

(40/30 ˚C) day and night temperature. However, the maximum fresh weight of shoot (5.84 g) was observed at 0.7 ppm followed by (5.28 g) at selenium (0.8 ppm) and (4.06 g) at 0.2 ppm while (3.26 g) at control in Safoora F1-Hybrid. Similar findings were noted Dasi Cucumber at the same levels of selenium application. Generally, it was observed that selenium applications significantly improved the fresh weight of shoot for both genotypes of cucumber at heat stress (40/30 ˚C) as shown in (Table 3).

Table 4. Effect of selenium on dry weight (g) of shoots for selected cucumber genotypes**.**

Dry weight of Shoot (g)			
Treatments	Safoora F1-Hybrid	Desi Cucumber	Means (Treatment)
Control	0.04^{d}	0.02^{f}	0.06^{d}
Se (0.2 ppm)	0.08^{c}	0.03^{ef}	0.07^{c}
Se (0.7 ppm)	0.12^{a}	0.05^{e}	0.09^{a}
Se (0.8 ppm)	0.10^{b}	0.04^{ef}	0.07^{b}
Means (Genotypes)	0.09^{a}	0.04^{b}	
LSD ($p < 0.05$)			
Treatments (T)	4.06		
Genotypes (G)	2.92		
T×G	4.63		

n = 5 replications (4 seedlings per replication), any two mean with different letters are significantly different; Ns = not-significant.

The results revealed that heat stress reduced the leaf area, shoot fresh biomass, shoot dry biomass, root length and shoot length of both genotypes of cucumber. It was observed that foliar spray (0.7 ppm) of selenium is an efficient, viable, and effective approach for the application of fertilizers and improving fertilizer use efficiency. Selenium foliar treatment (0.7 ppm) plays a positive role in improving heat tolerance of selected cucumber genotypes as compared to other selenium applications. The highest biomass accumulation with selenium foliar spray may be due to the diffusion of selenium ions that takes place from the surface of leaves to epidermal cells. The similar result observed by Germ *et al.* (2008), investigated that foliar selenium application increased growth, leaf area and fresh biomass of carrot at reproductive stages under heat stress condition. Our results are in line with the results of Germ *et al.* (2005), they found that selenium foliar application improved water holding capacity and filtered solar UV-B in pumpkins. Germ *et al.* (2007 stated that selenium improved the yield, fresh weight, dry weight of potato and pumpkin under heat stress.

Table 5. Effect of selenium on fresh weight of root for selected genotypes of cucumber under heat stress.

Dry weight of Shoot (g)			
Treatments	Safoora F1-Hybrid	Desi Cucumber	Means (Treatment)
Control	0.41^{b}	0.20^{e}	0.30^{d}
Se (0.2 ppm)	0.41^{b}	0.22^{d}	0.31^{c}
Se (0.7 ppm)	0.46^{a}	0.25^{c}	0.35^{a}
Se (0.8 ppm)	0.43^{a}	0.23^{d}	0.33^{b}
Means (Genotypes)	0.42^{a}	0.24^{b}	
LSD ($p < 0.05$)			
Treatments (T)	4.11		
Genotypes (G)	2.62		
T×G	4.52		

n = 5 replications (4 seedlings per replication), any two mean with different letters are significantly different; Ns = not-significant.

Effect of selenium on the dry weight of shoots for selected cucumber genotypes under heat stress

A significant improvement in the dry weight of shoot was recorded in both genotypes of cucumber as a result of selenium application under heat stress. The treatment of selenium @ 0.7 ppm significantly increased dry weight of shoot and the highest dry weight (0.12 g) was recorded in Safoora F1-Hybrid.

Similarly, the maximum dry weight of shoot (0.05 g) was observed at 0.7 ppm while dry weight of shoot (0.02 g) at control in Dasi Cucumber.

Table 6. Effect of selenium on dry weight (g) of root for selected cucumber genotypes.

Dry weight of Roots (g)			
Treatments	Safoora F1-Hybrid	Desi Cucumber	Means (Treatment)
Control	0.01[c]	0.003[d]	0.007[c]
Se (0.2 ppm)	0.02[b]	0.004[d]	0.012[b]
Se (0.7 ppm)	0.06[a]	0.006[d]	0.033[a]
Se (0.8 ppm)	0.04[a]	0.005[d]	0.023[a]
Means (Genotypes)	0.03[a]	0.003[b]	
LSD ($p < 0.05$)			
Treatments (T)	4.21		
Genotypes (G)	2.42		
T×G	4.35		

n = 5 replications (4 seedlings per replication), any two mean with different letters are significantly different; Ns = not-significant.

In general, it was found that as well as Se concentrations increased from control to 0.7 ppm), it improved the dry weight of shoot in both genotypes of cucumber under heat stress (Table 4). It may have argued that selenium significantly improved the root growth of plants which ultimately improved the performance nutrients uptakes in plants which play an important role in the growth and development. The foliar application of Se effects significantly, it stimulates development in many crops like carrot, radish, garlic and onion under heat stress. It was concluded from the present research that Se foliar spray (0.7 ppm) was more effective than other treatments for increasing dry and fresh biomass of cucumber seedlings of both genotypes. It was also concluded that its high concentration can cause damage to the leaf surface. Therefore, the concentration of solution at fertigation and foliar application of Se should be selected with care, based on recommendations. However, Lyons *et al.* (2004) described that foliar application is less efficient than seed treatment at vegetative growth. It was found from recent research that the plants showed maximum root and shoot length and biomass (fresh and dry) in both genotypes of cucumber by Se foliar application, while a non-significant difference was recorded between other Se treatments. Similar results found by Turakainen *et al.*, (2004), they described that Se foliar application effect on fresh and dry biomass of plants, it improved the vegetative growth in several species. Yao *et al.* (2009) informed that Se foliar application increased the root activity (growth and uptake) under water-stressed and enhanced the dry matter of plants. Our results confirmed by the finding of Djanaguiraman *et al.* (2005), concluded that selenium enhanced the shoot and root dry weight by regulating the water status under drought stress.

The treatment of increasing selenium caused a significant variation in terms of plant fresh weight under heat stress (40/30 °C). The maximum fresh weight of root (0.46 g) was achieved at 0.7 ppm in Safoora F1-Hybrid, whereas, lowest fresh weight (0.41 g) was revealed at control. Desi Cucumber revealed the highest root fresh weight at 0.7 ppm (0.25 g), whereas, lowest fresh weight (0.20 g) was observed at control. Overall it was observed that as the dose of Se increased, shoot length of both genotypes was improved as shown in Table (5). Selenium can increase the tolerance of plants to UV-induced oxidative stress, delay senescence, root fresh and dry biomass, and promote the growth of aging seedlings Xue *et al.*, 2001; Pennanen *et al.*, 2002). Recently it has been shown that selenium can regulate the water status of plants under drought conditions and heat stress which increased fresh weight shoots as well as roots (Kuznetsov *et al.*, 2003). Hartikainen *et al.* (2000) reported about the growth-promoting effect of selenium in ryegrass by increased the root and shoot biomass of plants. Senescence stress is partly counteracted with enhanced antioxidation which is associated with an increased glutathione peroxidase (GSH-Px) activity. Although some studies have evaluated the effect of hardness, temperature, pH and other parameters on selenium toxicity, sulfate has perhaps been most widely studied with selenium uptake and toxicity in aquatic and terrestrial organisms that improved the biomass of plants (Sappington, 2002).

Table 7. Effect of selenium on the leaf area of selected genotypes of cucumber under heat stress.

Dry weight of Roots (g)			
Treatments	Safoora F1-Hybrid	Desi Cucumber	Means (Treatment)
Control	36.56^{c}	12.53^{e}	24.54^{c}
Se (0.2 ppm)	45.94^{b}	16.37^{de}	31.15^{b}
Se (0.7 ppm)	57.82^{a}	21.69^{d}	39.75^{a}
Se (0.8 ppm)	53.58^{a}	18.49^{de}	36.04^{a}
Means (Genotypes)	48.47^{a}	17.27^{b}	
LSD ($p < 0.05$)			
Treatments (T)	4.11		
Genotypes (G)	2.12		
T×G	4.15		

n = 5 replications (4 seedlings per replication), any two mean with different letters are significantly different; Ns = not-significant.

Effect of selenium on dry weight (g) of roots for selected genotypes of cucumber under heat stress

The results showed that the dry weight of roots significant ($p < 0.05$) effected by the application selenium of both genotypes under heat stress (40/30˚C) (Table 6). When averaged over treatments, the maximum dry weight of root (0.033 g) observed at control followed by the treatment of selenium @ 0.7 ppm. Safoora F1-Hybrid showed maximum (0.06 g) dry weight of root at 0.7 ppm, while a minimum dry weight of root (0.01 g) was revealed at control. On the other hand, Desi Cucumber revealed (0.006 g) values of root dry weight at 0.7 ppm as compared to control (0.007 g). It was recorded that (0.7 ppm) dose of Se improved the dry biomass of Safoora F1 Hybrid and Desi Cucumber as compared to control and other concentrations under heat stress @ 40/30 ˚C. It was explored from a recent study, selenium foliar application (0.7 ppm) showed maximum root dry in Safoora F1 Hybrid and Desi Cucumber as compared to other treatments. Kuznetsov *et al.* (2003) described that selenium could regulate the water status of plants and keep them fresh and dry biomass of plants in summer vegetables under drought and high temperatures. Wang *et al.* (2012) described from their research that high levels of selenium inhibited photosynthesis through reducing the light energy absorbed by the aerial part of plants and damage photosynthetic process of potato which results in the lower production of starch that decreases biomass of plants. This increment may also be attributed to the facts that Se enhances the activities of soil microbes which mobilizes the unavailable nutrients into a readily available form, which results in increased shoot fresh and dry biomass, yield and average fruit weight.

Effect of selenium on leaf area of selected cucumber genotypes under heat stress

The leaf area of both genotypes of cucumber was significantly affected by different concentrations of selenium when heat stress was applied at 40/30°C. It was observed that the best leaf area was observed in Safoora F1-Hybrid (57.82 cm^2) at Se level (0.7 ppm), while the minimum leaf area (36.56 cm^2) was recorded in the control. Similarly, in Desi Cucumber highest leaf area (21.69 cm^2) was observed at (0.7 ppm) compared to control (12.53 cm^2). In general, the highest leaf area (48.47 cm^2) followed by Desi Cucumber (17.27 cm^2). It was reported by Tian *et al.* (2012) that foliar spray effectively improved the antioxidant and leaf area, root fresh, dry weight and leaf area, therefore, enhanced the tolerance of the plants to high-temperature stress. Similarly, selenium application enhanced the distribution of photosynthates from leaves to fruits resulting in enhanced growth, leave area and yield of a crop Terry *et al.* (2002) reported that selenium application increase in acidity, iron oxides and organic matter root, leaf area, shoot fresh and dry weight high clay content of soil decrease the bioavailability of selenium to plants. Yao *et al* (2009) stated that Se foliar application maintained the growth of plants and increased the leaf area under heat stress. It may regulate water status and increase biomass production by the activation of antioxidant activity and leaf area of heat-stressed plants. Optimal Se concentration and Se often put forth a dual effect on plant growth. At a low dosage, it can stimulate the growth of plants and leaf area and respond to many types of environmental stresses, while at high quantities, it can act as pro-oxidant and reduced the leaf area (Feng *et al.*, 2013).

Conclusion

It is concluded from the current study that selenium significantly effects both genotypes of cucumber under heat stress. However, the treatment of selenium 0.7 ppm more effective treatment of selenium application which improved the fresh weight of shoots, roots and dry weight of shoot s, roots as well as leaf area of both genotypes under heat stress.

Conflict of Interest

The authors have no conflict of interest in this research study.

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