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EFFECT OF PEG-INDUCED DROUGHT STRESS ON SEED GERMINATION AND INITIAL GROWTH OF THREE CUCUMBER CULTIVARS

ABSTRACT: As the most common abiotic stress, drought seriously affects crop production. Since the impact of drought stress varies among species, this study aims to evaluate drought tolerance of domestic cucumber cultivars in the initial stages of growth under PEG-induced drought conditions. The germination assay was performed using three different cucumber varieties (Tajfun, Sunčani Potok and NS Kir) at four different drought levels (0, -0.15, -0.49, and -1.03 MPa). The results demonstrated that all the tested cucumber cultivars are sensitive to drought during germination and initial plant growth, while the osmotic potential of -0.49 MPa can be considered the tolerance threshold for cucumber.

KEYWORDS: *Cucumis sativus* L., initial seedling growth, PEG-induced drought, seed germination, seedling vigour index

INTRODUCTION

Drought is one of the most common abiotic stresses that adversely affect agriculture and food security in the era of climate change. Due to global warming, water loss occurs both in the soil and at the plant level (Seleiman et al., 2021). According to current estimates, one-fifth of the world's population will be affected by a serious water deficit as the air temperature increases by an estimated 2 °C above the current level (Ray et al., 2019; Seleiman et al., 2021). The severity of drought is determined by many factors, including the amount of rainfall and its distribution, soil moisture-holding capacity and evaporative requirements (Khan et al., 2018). According to Radić et al. (2018), up to 70% of the total arable land is affected by drought.

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The impact of drought on crop production depends on the plant species and the growth stage. Many crops are highly vulnerable to water deficit if it occurs at germination or seedling growth. Since seed germination is related to vegetative and generative plant growth in later stages, low moisture availability during seed germination impedes plant distribution and diversity (Lu et al., 2022). Previous studies demonstrated that drought may have detrimental effects on the seed germination and vegetative growth of rice (Evamoni et al., 2023), maize (Radić et al., 2018), wheat (Bayoumi et al., 2008), carrot and watermelon (Steiner and Zuffo, 2018) and other species, with significant differences between the tested varieties. Different taxa may exhibit distinct adaptation methods of survival under drought stress (Bhatt et al., 2020; 2022). Assessment of plant drought tolerance during seed germination can give insights into the patterns of population persistence and community assembly, as well as clarifying the potential effects of climate change on these attributes (Bhatt et al., 2022).

However, establishing and preserving pure water potential in soil, in order to assess drought tolerance, is almost impossible (Hellal et al., 2018). One of the most effective ways to examine the effects of drought stress on germination is to induce osmotic potential using various osmotic materials, such as polyethylene glycol (PEG), mannitol or salt treatments. Polyethylene glycol (PEG-6000), a harmless, non-ionic polymer with a defined molecular weight, causes drought stress without affecting cells or causing physiological injuries (Qi et al., 2023). PEG 6000 is often used in this type of research because it has excellent water-absorbing properties and may simulate drought stress by dehydrating plant cells (Murillo-Amador et al., 2002).

Cucumber (*Cucumis sativus* L.) is one of the most significant vegetable crops in the world. According to Campobenedetto et al. (2020) and Das et al. (2024), cucumber is the most cultivated vegetable globally and the fourth vegetable most frequently consumed in salads. As reported by Liu et al. (2016) and Kim et al. (2019), cucumbers have a high transpiration rate and sensitivity to drought. Plant growth and fruit yield of cucumbers are adversely affected by exposure to soil water deficits, which may also result in decreased fruit quality (Farag et al., 2019). Different osmotic potentials of PEG solution, which represent the tolerance threshold of cucumber, vary depending on the genotypes and range from 0.5% to 10% PEG solution (De Suoza Neta et al., 2024; Li et al., 2022). In Serbia, information about drought sensitivity and drought tolerance threshold for domestic cucumber cultivars is currently lacking. The study aims to identify the tolerance thresholds for domestic cucumber cultivars and investigate their susceptibility to drought.

MATERIALS AND METHODS

Experimental (Plant) materials

The seeds of three different cucumber cultivars (Tajfun, Sunčani Potok, and NS Kir) were obtained from the Department of Vegetable and Alternative

Crops, Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad. Seeds of all the examined cucumber cultivars were produced at the Rimski Šančevi experimental field of the Institute of Field and Vegetable Crops, Novi Sad, in 2023.

PEG-induced drought

Three different polyethylene glycol (PEG 6000) (Sigma Aldrich, St. Louis, MO, USA) treatments, -0.15, -0.49, and -1.03 MPa, were used to assess the response of cucumber cultivars to artificial drought. The respective solutions were prepared according to the procedures set by Money et al., (1989). Distilled water was used as a control.

Germination assay

Prior to the germination test, cucumber seeds were disinfected with 5% (v/v) sodium hypochlorite for 5 minutes and then rinsed thoroughly with distilled water thrice. Working samples consisted of 3 x 100 seeds. The cucumber seeds were placed in plastic boxes (240 × 150 mm) with filter paper moistened with the respective solutions. The samples were incubated for eight days in a germination chamber at 25 °C (ISTA, 2024). Germination energy, i.e. germination first count, was determined four days after seed placement in the germination chamber by counting the normal seedlings with well-developed essential structures. Final germination, abnormal seedlings, shoot and root length, and fresh seedling weight of the 10 normal seedlings were determined eight days after seed placement in the germination chamber (Conviron CMP 4030, Winnipeg, Canada). Dry weight of the cucumber seedlings was determined after drying the seedlings in an oven at 80 °C for 24 hours (Tamindžić et al., 2023).

The seedling vigour index (SVI) was determined according to the procedure of Abdul-Baki and Anderson (1973) and calculated using the following formula:

$$\text{SVI} = \text{Final Germination (\%)} \times \text{Seedling Length (cm)}$$

Following the procedure of Channaoui et al. (2019), the shoot elongation rate (SER) and root elongation rate (RER) were determined using the following formulas:

$$\text{SER} = \frac{\text{Shoot length on the } 8^{\text{th}} \text{ day} - \text{Shoot length on the } 4^{\text{th}} \text{ day}}{\text{Time duration between two measurements (days)}}$$

$$\text{RER} = \frac{\text{Root length on the } 8^{\text{th}} \text{ day} - \text{Root length on the } 4^{\text{th}} \text{ day}}{\text{Time duration between two measurements (days)}}$$

Statistical analysis

The statistical software Statistica 10 (StatSoft, Inc., 2007) was used to perform an analysis of variance. Duncan's multiple range test was employed to separate the mean values at the probability level of $p<0.05$.

RESULTS AND DISCUSSION

The results showed that cucumber cultivars differed in their response to drought stress (Table 1). As confirmed by the analysis of variance, drought level significantly altered all the tested parameters of the different cucumber cultivars. Moreover, all the tested parameters were significantly affected by the cultivar and cultivar × drought interaction.

Table 1. Two-way analysis of variance for the examined parameters of three cucumber cultivars under different artificial drought conditions

Trait	Cultivar	Drought level	Cultivar × drought level
Germination Energy	0.0000***	0.0000***	0.0000***
Final Germination	0.0000***	0.0000***	0.0000***
Abnormal Seedlings	0.0000***	0.0000***	0.0003***
Shoot Length	0.0000***	0.0000***	0.0000***
Root Length	0.0000***	0.0000***	0.0000***
Fresh Seedlings Weight	0.0000***	0.0000***	0.0000***
Dry Seedlings Weight	0.0000***	0.0000***	0.0000***
Seedling Vigour Index	0.0000***	0.0000***	0.0000***
Shoot Elongation Rate	0.0000***	0.0000***	0.0000***
Root Elongation Rate	0.0000***	0.0000***	0.0000***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns – non-significant

In general, drought caused a decrease in all the examined parameters at a certain level (Table 2; Table 3). The control groups of the examined cucumber cultivars showed differences in germination energy, which ranged between 87.7% and 93.0%. The decrease in osmotic potential led to a decrease in germination energy compared to the control. A significant decrease was observed in cv. Tajfun and cv. Sunčani Potok at -0.49 MPa, while a significant decrease in germination energy occurred in cv. NS Kir at an osmotic potential of -1.03 MPa. Cucumber cultivars differed in their final germination as well; cv. Sunčani Potok had the lowest germination percentage of 88.0% in control, while the highest final germination was recorded in cv. NS Kir (96.0%). Final germination decreased in all the tested cultivars with the decrease in osmotic potential.

Compared to the control, a significant decrease in final germination was observed under drought at an osmotic potential of -0.49 MPa, while the greatest decrease was recorded at an osmotic potential of -1.03 MPa, which was significantly lower compared to control and at other tested drought levels. A similar pattern was also observed in the abnormal seedling occurrence. A significant increase in abnormal seedlings was noted under moderate drought (-0.49 MPa), while the highest was observed under severe drought (-1.03 MPa). Numerous studies confirmed the detrimental effects of drought on seed germination of different crops, such as carrot and eggplant (Steiner and Zuffo, 2018), pea (Petrović et al., 2021), maize (Radić et al., 2018), tomato (Esan et al., 2018) and spinach (Zargar et al., 2023), which is in agreement with the results obtained in the study. Germination is reduced under drought conditions due to reduced water infusibility via the seed coat and hampered water absorption (Channaoui et al., 2019; Hossain et al., 2024). Various factors, such as decreased water availability which impedes reserve mobilization, hormonal and enzymatic activities, respiration and protoplasm dilution required for effective embryonic growth, are considered responsible for the decrease in germination percentage (Li et al., 2021; Hossain et al., 2024).

Furthermore, initial growth was also affected by drought (Table 2). However, the response of cucumber cultivars to drought levels varied in terms of the shoot or root length. Shoot length of cv. Tajfun significantly decreased with the decrease of osmotic potential at -0.49 MPa and -1.03 MPa, while shoot length decreased gradually with the decrease of osmotic potential in cv. Sunčani Potok and cv. NS Kir. A similar pattern of root length decrease was observed in cv. Sunčani Potok and cv. NS Kir, where a significant reduction of root length was observed at -0.49 MPa and -1.03 MPa, compared to the control. In contrast, root length of cv. Tajfun decreased gradually with the decrease of osmotic potential. Shoot and root length of the tested cucumber cultivars, as crucial indicators of drought resistance, demonstrated that these cultivars are drought-sensitive, especially at a lower osmotic potential of PEG-induced drought. Cucumber cultivars also differed in their response to different levels of osmotic stress, indicating different levels of resistance. These findings corroborate the previous studies conducted on wheat (Peršić et al., 2022), maize (Mustamu et al., 2023) and tartary buckwheat (Hossain et al., 2024), indicating that seedling growth gradually declines as PEG concentration increases.

Table 2. Germination and initial plant growth parameters of cucumber cultivars under different artificial drought conditions

Cucumber cultivar	Drought Level	Germination Energy (%)	Final Germination (%)	Abnormal Seedlings (%)	Shoot Length (mm)	Root Length (mm)
Tajfun	Control	87.7 ± 1.5 a	93.3 ± 1.2 a	5.0 ± 1.5 c	47.7 ± 1.7 a	78.0 ± 2.0 a
	-0.15 MPa	86.7 ± 1.2 a	93.0 ± 1.0 a	6.3 ± 0.6 bc	46.8 ± 1.6 a	75.2 ± 1.0 b
	-0.49 MPa	73.7 ± 1.2 b	78.3 ± 0.6 b	8.0 ± 1.0 b	29.2 ± 0.3 b	50.3 ± 1.0 c
	-1.03 MPa	70.0 ± 1.0 c	72.3 ± 2.5 c	12.3 ± 0.6 a	27.8 ± 0.3 b	47.3 ± 0.8 d
	p	0.0000	0.0000	0.0000	0.0000	0.0000
Sunčani Potok	Control	82.3 ± 2.1 a	88.0 ± 1.0 a	5.7 ± 1.2 c	63.2 ± 1.7 a	57.7 ± 1.8 a
	-0.15 MPa	82.0 ± 1.5 a	87.3 ± 0.6 a	6.3 ± 0.6 c	58.7 ± 1.3 b	57.0 ± 1.7 a
	-0.49 MPa	70.7 ± 2.1 b	74.3 ± 0.6 b	11.0 ± 1.0 b	39.3 ± 0.6 c	52.0 ± 1.3 b
	-1.03 MPa	72.0 ± 1.0 b	72.7 ± 0.6 c	14.7 ± 1.2 a	25.5 ± 0.5 d	48.0 ± 1.3 c
	p	0.0000	0.0000	0.0000	0.0000	0.0000
NS Kir	Control	93.0 ± 2.0 a	96.0 ± 1.7 a	1.7 ± 0.6 b	74.2 ± 0.8 a	114.3 ± 0.8 a
	-0.15 MPa	93.0 ± 0.0 a	95.3 ± 1.2 a	2.0 ± 1.0 b	69.3 ± 0.8 b	113.0 ± 1.0 a
	-0.49 MPa	90.3 ± 1.2 a	91.7 ± 1.5 b	4.3 ± 0.6 a	46.3 ± 1.3 c	70.7 ± 2.6 b
	-1.03 MPa	86.3 ± 1.5 b	90.0 ± 1.0 c	5.3 ± 0.6 a	38.0 ± 0.5 d	58.3 ± 2.0 c
	p	0.0000	0.0000	0.0000	0.0000	0.0000

Data are presented as Means ± SD (n=3). Means within each trait followed by the same letters are not significantly different (Duncan's multiple range test, $p \leq 0.05$).

Regarding biomass accumulation, in cv. Tajfun and cv. NS Kir, an osmotic potential of -1.03 MPa, followed by -0.49 MPa significantly reduced fresh and dry seedling weight compared to the control (Table 3). In cv. Sunčani Potok, fresh seedling weight decreased gradually with the decreased osmotic potential, while dry seedling weight was reduced only at -1.03 MPa. Lower reduction in biomass accumulation during drought suggests that a genotype is more resistant to drought. Similar results were also obtained on tartary buckwheat (Hossain et al., 2024).

Seedling vigour index decreased gradually with the decrease in osmotic potential in all tested cultivars (Table 3). Hellal et al. (2018) also pointed out that seedling vigour index decreases with an increase in drought stress, which is in accordance with our results. Additionally, Magar et al. (2019) reported that PEG treatment up to -1.5 MPa reduced maize seedling vigour index, while Hellal et al. (2018) also observed the same reduction in barley.

The tested cultivars responded differently to osmotic stress in terms of the shoot elongation rate (SER) (Table 3). A significant decrease in SER was observed in cv. Sunčani Potok at -0.15 MPa and lower and in cv. Tajfun and cv. NS Kir at -0.49 MPa and -1.03 MPa, respectively. Contrary to this, all tested cultivars had a significantly decreased root elongation rate (RER) at an osmotic potential of -0.49 MPa and lower compared to the control, while no significant decrease was observed at mild stress levels. In this regard, PEG concentration proved to have a major impact on the histological size variations of root tissues, including the cortex, stele and epidermis (Mustamu et al., 2023). According to Zeid and Shedeed (2006), plant growth is a complex process of cell division and cell growth. It is considered one of the most drought-sensitive physiological processes due to the reduction of turgor pressure. The reduction in growth occurs due to impaired mitosis, cell elongation and expansion caused by drought (Wach and Skowron, 2022). Despite differences in cultivar response to certain drought stress levels, the results of shoot and root elongation rate confirmed drought sensitivity in all the tested cucumber cultivars.

Table 3. Biomass accumulation and growth-related parameters of cucumber cultivars under different artificial drought conditions

Cucumber Cultivar	Drought Level	Fresh Seedling Weight (g)	Dry Seedling Weight (g)	Seedling Vigour Index (SVI)	Shoot Elongation Rate (SER)	Root Elongation Rate (RER)
Tajfun	Control	2.84 ± 0.04 a	0.294 ± 0.001 a	1172.9 ± 22.2 a	8.58 ± 0.6 a	12.69 ± 0.6 a
	-0.15 MPa	2.82 ± 0.05 a	0.292 ± 0.003 a	1134.6 ± 23.2 b	8.51 ± 0.3 a	12.04 ± 0.4 a
	-0.49 MPa	1.87 ± 0.04 b	0.185 ± 0.001 b	622.8 ± 9.1 c	4.91 ± 0.1 b	7.48 ± 0.4 b
	-1.03 MPa	1.71 ± 0.03 c	0.176 ± 0.005 c	543.7 ± 20.7 d	4.58 ± 0.1 b	6.83 ± 0.2 b
	p	0.0000	0.0000	0.0000	0.0000	0.0000
Sunčani Potok	Control	2.66 ± 0.05 a	0.206 ± 0.003 a	1063.4 ± 24.3 a	12.5 ± 0.3 a	8.25 ± 0.5 a
	-0.15 MPa	2.54 ± 0.04 b	0.207 ± 0.006 a	1010.1 ± 24.0 b	11.4 ± 0.3 b	8.42 ± 0.3 a
	-0.49 MPa	2.28 ± 0.01 c	0.198 ± 0.006 a	678.9 ± 17.7 c	7.60 ± 0.1 c	5.92 ± 0.5 b
	-1.03 MPa	1.62 ± 0.03 d	0.174 ± 0.005 b	535.3 ± 11.2 d	4.56 ± 0.1 d	5.10 ± 0.5 b
	p	0.0000	0.0000	0.0000	0.0000	0.0000
NS Kir	Control	2.96 ± 0.03 a	0.286 ± 0.003 a	1809.5 ± 21.6 a	9.79 ± 0.4 b	11.68 ± 0.4 a
	-0.15 MPa	2.89 ± 0.02 a	0.284 ± 0.003 a	1738.3 ± 19.8 b	11.17 ± 0.6 a	11.42 ± 0.4 a
	-0.49 MPa	2.80 ± 0.07 b	0.264 ± 0.008 b	1072.7 ± 46.9 c	8.27 ± 0.2 c	4.83 ± 1.1 b
	-1.03 MPa	2.47 ± 0.01 c	0.224 ± 0.003 c	867.1 ± 22.7 d	7.07 ± 0.2 d	3.75 ± 0.3 b
	p	0.0000	0.0000	0.0000	0.0000	0.0000

Data are presented as Means ± SD (n=3). Means within each trait followed by the same letters are not significantly different (Duncan's multiple range test, $p \leq 0.05$).

CONCLUSION

Based on the obtained results, it can be concluded that domestic cucumber cultivars are susceptible to drought. All tested cultivars can withstand mild drought in the initial phase of plant growth, such as seed germination and initial seedling growth. However, all examined parameters of the tested cucumber cultivars declined at an osmotic potential of -0.49 MPa (moderate drought), which can be considered as the tolerance threshold for cucumber.

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УТИЦАЈ ПЕГ-ИНДУКОВАНЕ СУШЕ НА КЛИЈАВОСТ СЕМЕНА И ПОЧЕТНИ ПОРАСТ БИЉАКА ТРИ СОРТЕ КРАСТАВЦА

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САЖЕТАК: Као најчешћи абиотички стрес, суша озбиљно утиче на производњу усева. С обзиром да утицај стреса од суше варира међу врстама, ова студија је имала за циљ да процени толеранцију домаћих сорти краставца на сушу у почетним фазама раста у условима вештачке суше. Лабораторијско испитивање је изведено коришћењем три различите сорте краставца (Тајфун, Сунчани поток и НС Кир) и четири различита нивоа суше (0, -0,15, -0,49 и -1,03 MPa). Резултати су показали да су све испитиване сорте краставца осетљиве на сушу у фази клијања и почетног пораста биљака, као и да се осмотски потенцијал од -0,49 MPa може сматрати прагом толеранције за краставац.

КЉУЧНЕ РЕЧИ: *Cucumis sativus* L., индекс вигора семена, клијавост семена, почетни пораст изданака, PEG-индукована суша