Seed Priming with Proline as Promising Tool to Mitigate Water Stress on Squash Cultivars

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ABSTRACT

Proline is considered endogenous regulator that alleviates environmental stresses as water stress. This study investigated the effect of seed priming with proline on summer squash plant to mitigate water stress. Four squash cultivars were used SQ250, Behera, Sp 11/4 and Kaha5/7. The seed priming were hydro-priming (as a control) and priming seeds that primed with 20 mmol proline for 24 hours. The study was conducted in greenhouse where the squash plants were subjected to three irrigation levels (100% FC (Field capacity), 75% FC and 60% FC) in three replicates. The results revealed that water stress significantly decreased all measured physiological parameters (plant height, number of leaves, dry weights, fresh weights, fruit weights and relative chlophyll content index), while proline contents in plants increased with water stress Meanwhile seed priming with proline significantly ameliorate squash tolerance to water stress through improve these parameters. Sp11/4 and Kaha5/7 gave the highest fruit weights so they are the recommended squash cultivars that can be used under water stressed condition after seed priming with proline.

Keywords: Squash, water stress, proline, seed priming.

INTRODUCTION

Last decades many countries are subjected to severe environmental conditions as drought due to climate change (IPCC, 2013). Drought is a major abiotic stress and a limiting factor for plant production and threatens food security. Water stress decreases plant morphological and physiological parameters then reduces plant productivity (Badr Eldin et al., 2022). There is a need to find methods that alleviate plant response to water stress. One of the effective methods that alleviate water stress and improve plant tolerance to environmental stresses is seed priming. Seed priming is a pre-sowing treatment, an effective method for increase seed germination and plant growth under stress condition. Seed priming initiates tolerance mechanisms and accelerate later stages of plant growth (Johnson et al., 2021). There are many priming agents and methods as chemical priming, biological, hormonal priming, osmopriming and hydropriming (Ashraf et al., 2018). Seed priming has many advantages as accelerate germination through increase enzymes activities (Varier et al., 2010). Also is shorting emergency time by 28% even in stress conditions (Qamar et al., 2022). Primed seeds have higher antioxidant defense that increase plant tolerant to different environmental stresses (Farooq et al., 2017). There are many endogenous regulators that uses in seed priming (chemical priming) to increase plant tolerance as proline (Sako et al., 2020).

Proline is an amino acid that accumulates in plants subjected to stress (Sadeghipour,2020). It is considering endogenous regulator that increase plant tolerant to environmental stresses (drought, temperature and salinity). Where it plays as an osmolyte for osmotic adjustment. Also, it increases water uptake potential, increase activity of enzymes, protect members through binding to hydrogen bonds and improve protein stability (Burritt, 2012; Hossain et al., 2019). Several researches revealed that application of exogenous proline at low concentration increases plant tolerance. The main methods to applied exogenous proline are seed priming or foliar application. It increases plant tolerance to salt stress as showed by Singh et al. (2018). Also, it is adversely affected drought stress as revealed by Farooq et al. (2017).

Summer squash is used worldwide as a human food also it used in medical purposes where it is recognized with highly amount of zinc, vitamin α and β carotene, vitamin C, minerals and antioxidant (Adepoju and Adepanjo, 2011; Jacobo-Valenzuela et al., 2011). It has a shallow root and it is very sensitive to drought. The excess or reduction irrigation rate that applied to squash plants affects adversely on yield and yield component (El-Dewiny, et al., 2011). The objective of our research was studying the effect of proline seed priming on tolerance of summer squash cultivars to water stress.

MATERIAL AND METHODS

Greenhouse experiment was achieved in pots at Experimental Station of the Faculty of Agriculture, Alexandria University, in Alexandria, Egypt. The maximum and minimum temperatures through the experimental period were 23 ^{0}C and 16 ^{0}C and average humidity was 68%. Four squash cultivars were used SQ250, Behera, Sp 11/4 and Kaha5/7. The squash seeds

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were obtained from Giza Agricultural Research Center. The experimental design was split split with three replicates.

Seeds were priming using two treatments, hydro-priming treatment with using distilled water as a control and proline priming treatment (20 mmol). Seed were primed with priming solution for 1 day under dark condition at room temperature. After priming seeds, five seeds were planted in each 5 Kg sandy clay loam pots in October 2021. Two plants were chosen in each pot to complete growth to harvesting (after two months). Table (1) showed the soil analysis according to Black (1965). Field capacity (FC) and permanent wilting point (PWP) were measured according to Israelsen and Hansen (1962) method using pressure plate. Pots were fully irrigated in two weeks until appearing of the first leaves then irrigation treatments were applied until harvesting. There were three treatments 1000 ml for 100% of the Field capacity (FC) (No stress) as a control (I100%), 750 ml for 75% (I75%) and 600 ml for 60% (I60%) of the Field capacity. The irrigation was two times a week and the amount of water was added to replace water loss by measured pot weights and added water to the required level of water. All agricultural practices were carried out according to the recommendations of Ministry of agriculture Egypt in squash planting. The chlorophyll content was measured through the planting period by a Minolta SPAD chlorophyll meter (SPAD-502 plus, Konica Minolta Sensing, Japan) according to Yadava (1986). Number of leaves per plant, Number of masculine flowers per plant, and Number of feminine flowers per plant were measured during the planting period. Other physiological parameters were measured after harvesting through removed plants from soil then transfer to laboratory, washed the plant with water to remove soil particle then weigh fresh weights, measured plant height. Proline content was determined according to Bates et al. (1973). Weigh 0.1gm of dry leaf and were homogenized in 10 ml of sulphosalicylic acid, 2ml each of acid ninhydrin and glacial acetic acid were added to 2ml filtrate. The samples were heated at water bath. The mixture was extracted with toluene. The free toluene was measured using Spectrophotometerically at 52٠ nm.

Statistical analysis

The experiments were achieved in three replicates using split split design. A statistical analysis was according to Snedecor and Cochran (1989), using Co-state software program. Three irrigation levels were (I100, I60 and I75), hydropriming (control) and proline seed priming using four squash cultivars.

Table 1. Physical and chemical properties of the experimental soil

Soil Physical characteristic	Value	Soil chemical characteristic	Value
Sand	50.5%	pH	8.1
Silt	30.1%	EC (ds/m)	1.5
Clay	19.4%	Ca^{2+} (Meq/l)	4.5
Soil Texture	Sandy loam	Mg^{2+} (Meq/l)	2.5
Bulk density (gm/cm^3)	1.3	K^+ (Meq/l)	2
Field capacity (gm^3/gm^3)	0.3	Cl^- (Meq/l)	3
Wilting point (gm^3/gm^3)	0.12	OM (%)	0.20%

RESULTS AND DISCUSSION

Water stress reduced all physiological growth parameters for squash plant as showed below. All vegetative growth parameters as plant height, fresh weights, dry weights and number of leaves which were an average for four squash cultivars are decreased with water stress (Nilanthi et al., 2014), because water deficit decrease transpiration, intake, transport of nutrient and metabolism (Farooq et al., 2009).

Plant weight:

Figure (1) showed a significant decrease in plant height with increasing water stress. The plant height was 53.67 cm at I100% and reduced to 50.08 cm, 45.08 cm at I75% FC and I60%, respectively. Seed priming with proline enhanced the three irrigation levels. The highest value of plant height was 58.12 cm at I100+proline. Also seed priming with proline improve other treatments I75+proline (52.25 cm) and I60+proline (49.5 cm). Ambreen et al. (2021) found that seed priming with proline increase plant height and fresh and dry weights in wheat. Increase of plant height with proline priming could be described because it is a growth regulator which is the antioxidative potential of cells. Increase cell multiplication and expansion in comparison with control (Qamar et al., 2021).

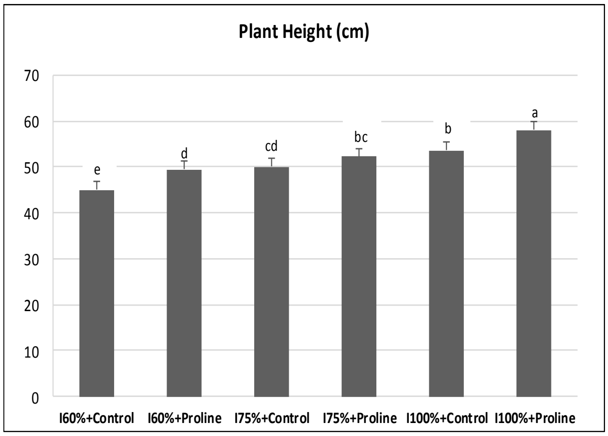

Fig.1. Effect of seed priming with proline and irrigation levels on plant height (cm) Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

Fresh and dry weights:

Water stress decreased fresh and dry weights (Figure 2, Figure 3). There was a significant decrease between irrigation levels. I100% was 298.4 gm, I75% (190.4 gm) and I60% (169.8 gm) for the control. All values improved by seed priming with proline, they became 243.5 gm, 273.7 gm and 260.5 gm for I100%+proline, I75%+proline and I60%+proline, respectively. The same trend was found for dry weights (Figure 3). The seed priming with proline increase fresh and dry weight through improving plant uptake of mineral nutrient and photosynthesis (Athar et al., 2009).

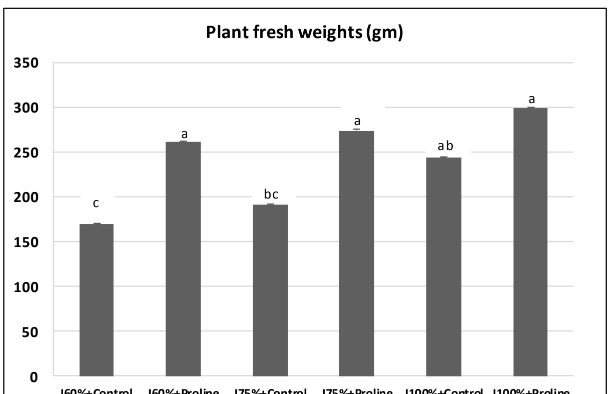

Fig. 2. Effect of seed priming with proline and irrigation levels on plant fresh weight (gm). Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

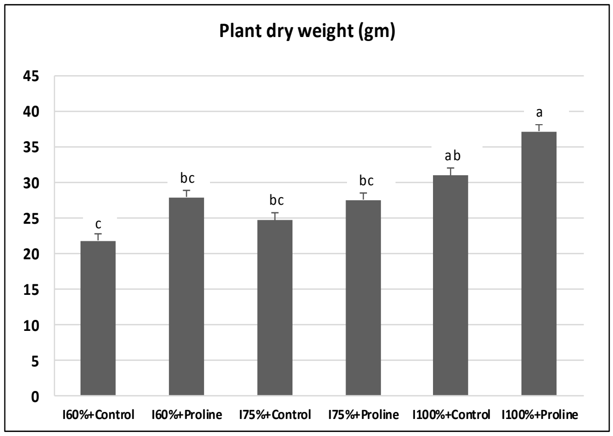

Fig. 3. Effect of seed priming with proline and irrigation levels on plant dry weight (gm). Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

Number of leaves:

There was a reduction in number of leaves per plant with increase water stress as shown in figure (4). Also, proline priming improved this character. The proline seed priming increased the nitrogen availability for cellar metabolism that increase plant growth as number of leaves (Zhang and Becker, 2015).

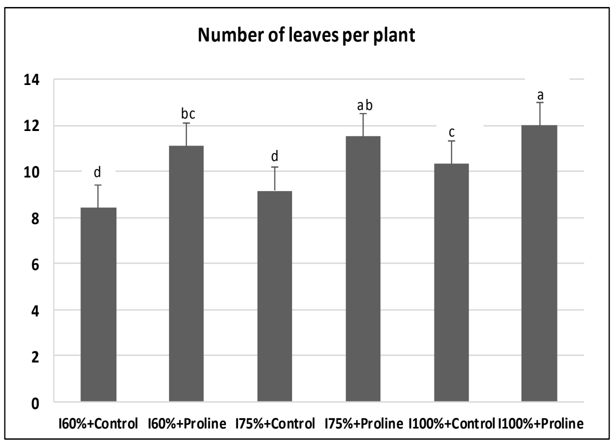

Fig. 4. Effect of seed priming with proline and irrigation levels on number of leaves per plant. Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

Relative Chlorophyll content index:

Relative chlorophyll was measured by SPAD apparatus. The more stressed plant the less chlorophyll content. There was a significant difference between treatments in chlorophyll content indexs (Figure 5). Water stress decreased chlorophyll content in leaves where it was 46.7 in I60+control. Seed priming with proline induced this effect. Because proline is considering an osmotic defender to water stress caused increase in photosynthesis pigments and chlorophyll content (Merwad et al., 2018).

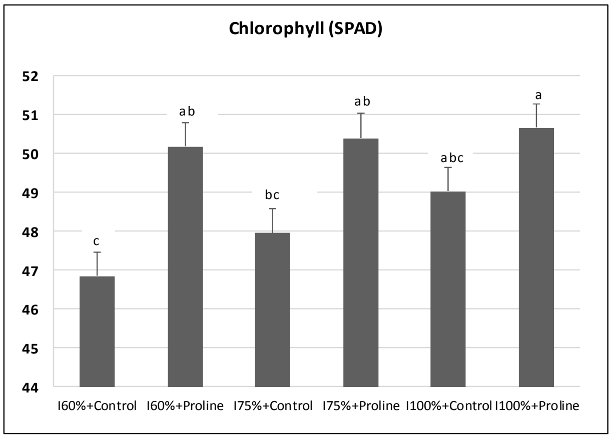

Fig. 5. Effect of seed priming with proline and irrigation levels on relative chlorophyll content. Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

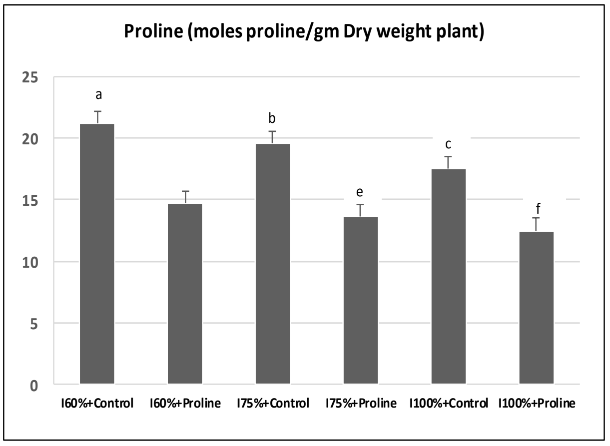

Fig. 6. Effect of seed priming with proline and irrigation levels on proline content. Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

Proline content:

Proline accumulates during water stress due to increase proline biosynthesis precursors (Ashraf et al., 2018). There was a positive correlation between proline accumulation in plants and water stress. Figure (6) showed the significant difference between proline content in plant and water stress. The highest proline concentration was 21.24 moles proline/gm Dry weight plant in I60%+control that decreased to 19.57 moles proline/gm Dry weight plant in I75%+control and 17.56 moles proline/gm Dry weight plant in I50%+control. Exogenous proline alleviated this effect by reduction in proline content in plant because priming with proline adversely affect plant response to water tolerance. It became 14.72 moles proline/gm Dry weight plant in I60+proline.

Fruit weight:

The fruit weight decreased with water stress (Figure 7). The highest fruit weight was treatment I100%+proline (1480 gm), the lowest value was 1045 gm in I60%+control. This lowest height plant was increased by 35% (1411 gm) by priming with proline. Analysis of variance showed significant increasing in fruit weight in all irrigation treatment using seed priming with proline compare with control.

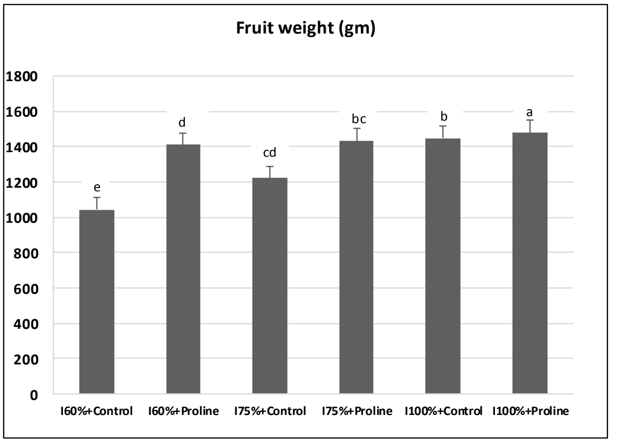

Fig. 7. Effect of seed priming with proline and irrigation levels on fruit weight (gm). Means followed by different lowercase letters indicate significant differences between treatments based on LSD test (p = 0.05)

The interactions between irrigation rates, seed priming with proline and squash cultivars:

Table (2, 3) showed that there were significant difference in the interaction between irrigation rates, proline priming and squash cultivars with squash growth parameters and yield attributes. In table (2) the highest plant height was 61.66 cm in Kaha5/7 in I100% + proline priming but the smallest plant height was 34 cm in Behera in I60%+control which increased to 42.66 cm with proline priming. Also, the highest number of leaves (13) and fresh weight (400.9 gm) was in Kaha5/7 in I100%+proline priming. While the lowest value in number of leaves in Behera in I60%+ control was 7, and the smallest fresh weight in SQ250 in I75%+control. The best squash cultivar in fruit weights was Sp11/4 (1546.6 gm) in 100%+proline priming then Kaha5/7 (1486.6 gm) in the same treatment. There was almost no-significant between squash cultivars in I60%+control (that has the smallest fruit weight) except Kaha5/7 that increased little bit.

When we compare fruit weight in I100%+ control for Sp11/4, the value was 1421.3gm. In contrast the fruit weight increased in I60%+proline priming to 1460 gm. So Sp11/4 can be used in water stress after seed priming with proline. In Kaha5/7 there was no-significant between I100%+ control (1451.6 gm) and I60%+proline (1433.3 gm), so Kaha5/7 can be used also with water stress condition after seed priming with proline.

Table (4) showed a highly significant difference in relative chlorophyll content between cultivars. Beside the priming with proline improved the chlorophyll content in comparison with control in all cultivars. As described before proline increase photosynthesis pigments.

Seed priming with proline reduced the adversely effect of water stress, through decreased the proline content in stressed conditions. The highest stress condition was in Behera I60%+control (23.12 moles proline/gm Dry weight plant) The proline content reduced it to 19.88 moles proline/gm Dry weight plant in I60%+seed priming. So, proline improve plant tolerance to water stress

In addition to seed priming with proline increased flower production due to their connection with growth hormones production and enhanced flowering (Xu et al., 2015), that revealed in number of both masculine and feminine flower in cultivars primed with proline.

Finally, Improvement in growth due to proline is cultivars specific. Also, the concentration of proline is varying between plants (Athar et al., 2009).

Table 2. Effect of irrigation rates and seed priming with proline and their interaction on squash cultivars plant growth parameters

Irrigation	Proline	cultivers	Plant height (cm)	Number of leaves /plant	Fresh weight (gm)	Dry weight (gm)	Fruit weight (gm)
I60%	Control	Behera	34o	7k	164.63ij	22.53efgh	1040.3g
		SQ250	53.66efghi	9.66ghi	127.36jk	22.6efgh	1031.6g
		Kaha5/7	44.33lmn	8.33j	223.3efgh	21.73fgh	1067fg
		Sp 11/4	48.33jkl	8.66ij	163.9ij	20.1gh	1041.6g
	Proline priming	Behera	42.66mn	11.6bcd	244cdef	32.17cde	1360d
		SQ250	50ijk	10.6defg	203.1fghi	22.8efgh	1390cd
		Kaha5/7	56.66bcdef	11.6bcd	362.1a	34.8bc	1433.3bc
		Sp 11/4	51hij	10.3efgh	232.96defg	21.67fgh	1460bc
I75%	Control	Behera	41.33n	8.3j	206.77fghi	22.83efgh	1129f
		SQ250	55.33cdefg	9.6ghi	115.97k	23.13defgh	1216.3e
		Kaha5/7	49.66ijk	9.3hij	242.07cdefg	22.63efgh	1273.3e
		Sp 11/4	51.66ghij	9.3hij	196.83ghi	30cdefg	1268.6e
	Proline priming	Behera	46.66klm	12abc	309.65b	33.4cd	1390cd
		SQ250	55defgh	10.3efgh	128.37jk	18.1h	1390cd
		Kaha5/7	57.66abcde	12.3ab	391.73a	35.47bc	1472.6b
		Sp 11/4	55.33cdefg	11.3bcde	265.1bcde	23.13defgh	1480ab
I100%	Control	Behera	42.66mn	10fgh	293.87b	32.1cde	1460.3bc
		SQ250	59.66ab	10.6defg	178.2hi	25.77cdefgh	1462b
		Kaha5/7	52ghij	10.6defg	271.57bcd	35bc	1451.6bc
		Sp 11/4	54.66defgh	10fgh	230.4defg	31.07cdef	1421.3bcd
	Proline priming	Behera	53fghi	11.6bcd	376.23a	53.7a	1446.6bc
		SQ250	58.66abcd	11cdef	128.47jk	17.17h	1440bc
		Kaha5/7	61.66a	13a	400.9a	44.27ab	1486.6ab
		Sp 11/4	59.3abc	12.3ab	287.87bc	33.23cd	1546.6a

Values followed by the same alphabetical letter(s) in common, within a particular group of means in each character, do not significantly differ, using revised LSD test at 0.05 level of probability.

Table 3. Effect of irrigation rates and seed priming with proline and their interaction with squash cultivars on relative chlorophyell content, proline content, number of masculine and number of feminine

Irrigation	Proline	cultivars	Chlorophyll (SPAD)	Proline (moles proline /gm Dry weight plant)	Number of masculine /plant	Number of feminine /plant
I60%	Control	Behera	50.53abcde	23.12a	4h	1f
		SQ250	47.63cdefg	21.77b	5.3def	2.3bcd
		Kaha5/7	46.7fgh	21.63b	5.3def	1.6def
		Sp 11/4	49.63efgh	20.63c	6cd	2cde
	Proline priming	Behera	49.6abcdef	19.88cd	5efg	2cde
		SQ250	52.13ab	19.59de	5efg	2.3bcd
		Kaha5/7	47.7cdefg	19.20de	6.3bc	3a
		Sp 11/4	52.1ab	18.66ef	7.3a	2.3bcd
I75%	Control	Behera	49.27abcdefg	17.98fg	5efg	1.3ef
		SQ250	48.9bcdefg	17.98fg	4.6fgh	2.6abc
		Kaha5/7	43.36h	17.12g	6cd	2.3bcd
		Sp 11/4	45.86gh	15.98h	7ab	2.3bcd
	Proline priming	Behera	49.58abcdef	15.36hi	4.6fgh	2cde
		SQ250	50.46abcde	14.65ij	5.6cde	2.6abc
		Kaha5/7	48.66bcdefg	14.47ij	6.3bc	2.3bcd
		Sp 11/4	52ab	14.40ij	7.3a	3ab
I100%	Control	Behera	49.66abcdef	13.99h	5.6cde	1.3ef
		SQ250	52.83a	13.85jk	4.6fgh	2.6abc
		Kaha5/7	46.16fgh	13.83jkl	7.6a	2cde
		Sp 11/4	47.43defg	12.89klm	7ab	1.6def
	Proline priming	Behera	49.76abcdef	12.86lm	4.3gh	3ab
		SQ250	51.16abc	12.75m	6.3bc	3ab
		Kaha5/7	50.73abcd	12.75m	6cd	2.3bcd
		Sp 11/4	51abcd	11.62n	7ab	3.3a

CONCLUSION

The study concluded that seed priming with proline alleviate adversely effect of water stress and increase morphological growth parameters and yield attributes in summer squash. Seed priming with proline is a benefit method that could be used in squash planting in semi-arid conditions. Sp11/4 and Kaha5/7 are the recommended squash cultivars used under water stressed condition after seed priming with proline.

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الملخص العربي

نقع البذور بالبرولين كأداة واعدة لتقليل الاجهاد المائي على أصناف الكوسة

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يعتبر البرولين منظمًا داخليًا يخفف من الضغوط البيئية مثل الإجهاد المائي. بحثث هذه الدراسة في تأثير نقع البذور بالبرولين على نبات الكوسة الصيفية للتخفيف من الإجهاد المائي.استخدمت أربعة أصناف من الكوسة وهي (SQ250 و Behera) وKaha5/7. Sp 11/4 . تم نقع البذور اما نقع مائي ككنترول أو نقع ببرولين بتركيز ٢٠ ملي مول. أجريت الدراسة في البيوت المحمية. حيث تعرضت نباتات الكوسة لثلاثة مستويات ري (١٠٠٪ سعة حقلية و٧٥٪ سعة حقلية و٦٠٪ سعة حقلية في ثلاث مكررات. أوضحت النتائج أن الإجهاد المائي أدى إلى انخفاض معنوي في جميع الخصائص الفسيولوجية المقاسة (طول النبات، عدد الأوراق، الأوزان الجافة، الأوزان الطازجة، أوزان الثمار ومحتوى الكلوفيل النسبي). وفي الوقت نفسه أدت الي انخفاض محتوي البرولين في النبات مع الاجهاد المائي، في أثناء ذلك نقع البذور بالبرولين زود بشكل كبير من تحمل الكوسة للإجهاد المائي وذلك من خلال تحسين هذه الخصائص. أعطت كل من Sp11 / 4 و Kaha5 / 7 أعلى أوزان للثمار. لذا فهي من أصناف الكوسة الموصى باستخدامها تحت ظروف الإجهاد المائي بعد نقع البذور بالبرولين.

الكلمات الافتتاحية: الكوسة– الاجهاد المائي– البرولين– نقع البذور.