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Effect of Glycine Betaine and Chitosan on Water Stress Tolerance of Summer Squash Plants.

Doklega, S. M. A.[1]; S. T. M. El-Afify[1]; E. A. Ibrahim[2] and S. A. A. Salem[1*]

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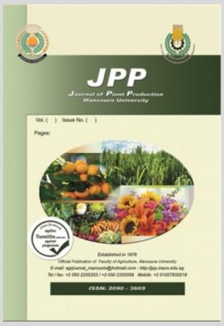
[1]Vegetable and Floriculture Dept., Faculty of Agriculture, Mansoura University, Egypt
[2]Vegetable Research Dept., Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.



ABSTRACT

Two field experiments were conducted at private farm in Belqas, Dakahlia Governorate, Egypt during the two summer seasons of 2020 and 2021 to study the effect of irrigation intervals (7 and 14 days), control (priming and foliar with tap water), glycine betaine seed priming (150 ppm), glycine betaine seed priming (150 ppm) plus glycine betaine foliar application (700 ppm), chitosan seed priming (150 ppm), and chitosan seed priming (150 ppm) plus chitosan foliar application (150 ppm), and their interactions on yield, chemical constituents and quality of summer squash cv Eskandrani. Irrigation intervals every 7days increased significantly yield, total chlorophyll, N, P, K and protein percentages of fruits meanwhile irrigation intervals every 14 days increased significantly phenols, CAT in leaves, VC and carbohydrates in fruits. Glycine betaine or chitosan seed priming with foliar applications had similar effect on most studied characters, which significantly increased in all studied parameters compared with control. The interaction between irrigation every 7 days and chitosan seed priming (at 150 ppm) with foliar application of chitosan (at 150 ppm) recorded the highest values in fruit length, diameter, fruit dry matter, total yield (ton/fed), total chlorophyll, N, P and K and protein percentages in fruits, while phenols, CAT of leaves, VC and carbohydrates in fruits recorded the highest values with the interaction between irrigation every 14 days and glycine betaine seed treatment at 150 ppm with foliar application of glycine betaine (at 700 ppm) compared with other treatments. Therefore, these treatments enhanced the yield under water stress conditions.

Keywords: squash, irrigation, chitosan, glycine betaine.

INTRODUCTION

Water stress is an abiotic stress which has a negative effect on growth and productivity of plant Parkash and Singh, 2020. The relationship between crop yield and water stress can be shown from irrigation experiments in which a major range of irrigation implementation Raza *et al.*, 2019.

Agriculture needs a large amount of irrigation water and this quantity will be increased in the future because the increase of population and the available amount of water to agriculture is declining worldwide because the greater incidence of drought caused by climate change and different human activities in recent years (World Bank, 2006).

Summer squash (*Cucurbita pepo*,L.) cv. Eskandrani is one of the major vegetables which, cultivated in most Mediterranean countries, and it is one of the most popular cucurbits vegetable crops grown in the world. It is eaten as boiled, fried or stuffed as immature fruits. It has various health and medicinal benefits of human. It is belong to family Cucurbitaceae which is rich in useful fibers, amino acids content and a lot of beneficial minerals. Squash fruits contain considerable amounts of carbohydrates, proteins, and vitamins. The area that was cultivated of summer squash in Egypt was nearly 25 thousand hectares, and produced about 456 thousand metric tons (EMARS, 2018). Root depth of summer squash plant is shallow so plant is sensitive to soil water content. Drought stress may damage summer squash plant. There are many strategies in plants to defend against drought stress by adapting themselves according to extremal conditions. Plants change their physiological metabolic processes for their survival (Saini *et al.*, 2020). Additionally, there are many strategies for alleviation of harmful effects of drought stress sush as using seed priming of anti-stress sub-stances such as glycine betaine and chitosan.

The technique of seed priming is soaking seeds in a particular solution with many of substances for a period of time, then it is stored in a dark room for priming, and washed thoroughly with deionized water, and dried to minimize the moisture content to <10% and used to germination after that. The purpose of seed priming is increase seed resistance to stressful conditions, and improve germination (Paparella *et al.*, 2015).

Seed priming stimulates the pre germination metabolic processes and increases the antioxidant system activity and the repair of membranes which promote seed vigor during germination (Ibrahim, 2016).

Nowadays, the use of glycine betaine and chitosan as natural anti-stress sub-stances has increased. They are environmentally friendly and easily degradable. Glycine betaine is amino acid which a vital tonic that is rapidly absorbed and transported through plant parts. It has a direct effect on the activity of plant enzymatic and the photosynthesis process and enhances its efficiency. Glycine betaine increased leaf area and shoot fresh biomass of plant (Cha-um *et al.*, 2013) on rice. Glycine betaine can be used to increase the extent of plant chlorophyll (a and b), height, yield and yield components in drought stress condition and normal condition (Miri and Armin, 2013). Glycine betaine has a vital role in resisting drought (Dawood and Sadak, 2014) in either plant accumulating or non-accumulating plants. GB priming enhances antioxidant enzyme activity, (Cheng *et al.*, 2018). It improves seed germination of plants, especially under stress conditions (Rakshit and Singh, 2018). Using glycine betaine

*** Corresponding author.**
E-mail address:samarnew97@gmail.com
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increased quality and yield of soybean (Malekzadeh, 2015) as seed priming, squash (Abdel-Mawgoud, 2017), cucumber (Youssef *et al*., 2018), as foliar application.

Chitosan is a natural and cheap bio polymer, which named chitin; it can be extracted from the exoskeletons of insects. Becker *et al.* (2000) pointed that chitosan include nitrogen in its formula basic unit and by extracting the acetyl group. It can be transformed into chitosan and turn it into amino (Sugiyama *et al.,* 2001). In addition, chitosan improved extracellular peroxidase activity (Ortmann and Moerschbacher, 2006). It enhanced a transportation of nitrogen in the functional leaves which improved growth and development of plant (Gornik *et al.,* 2008). Chitosan stimulates plant growth (Mondal *et al.,* 2012). Using chitosan increased quality and yield of vegetable plant such as broad beans (Abdel-Aziz, 2019) as seed priming, cucumber (Abd El-Hady and Abd-Elhamied, 2018), as foliar application.

This study was designed to know the effect of glycine betaine and chitosan seed priming with their foliar spray under normal and water stress conditions on quality, yield as well as enzymatic antioxidants activity of summer squash plants.

MATERIALS AND METHODS

1- Plant material and growth conditions:

Analysis of soil:

Before planting a soil sample was randomly collected at a depth of (0- 30) cm from the experimental field area from the soil surface before soil preparation to estimate some mechanical and chemical analysis of the used soil, as shown in Table 1. These characteristics were analyzed according to Buurman *et al.* (1996).

Table 1. Mechanical and chemical analysis of the experimental soil.

Properties	2020	2021	Properties	2020	2021
Soil texture	Clay-loam	Clay-loam	HCO3-	0.63	0.63
Sand (%)	14.20	13.72	SO4 --	0.03	0.04
Silt (%)	30.40	31.11	Ca++	0.60	0.62
Clay (%)	55.40	55.17	Mg++	0.31	0.32
Organic matter %	1.33	1.44	Na+	0.20	0.21
pH value	7.40	7.80	N	47.00	47.50
E C (mmohs/cm)	0.64	0.55	P	12.50	12.80
			K	201.00	208.00

2- The experimental design and treatments:

Two field experiments were performed in a clay loam soil at a private farm located in Belqas, Dakahlia Governorate, Egypt during the two summer seasons of 2020 and 2021, using summer squash plants. (*Cucurbita pepo* L.) cv. Eskandrani to achieve the study objectives.

Split-plot design was the experimental layout with three replicates. These experiments included ten treatments which were the combination between two irrigation intervals and five seed priming of glycine betaine and chitosan with their foliar spray and control (seed soaking and spraying with tap water). Two irrigation intervals were assigned in the main plots (every 7 and 14 days intervals starting after 1st irrigation). The irrigation numbers were totally 8 and 4 times, respectively, while seed priming and foliar spray were randomly distributed in the sub-plots. The plot area was 15 m^2, 3 ridges (each 5 m length and 1 m width). Seeds were planted on 1 st April of 2020 and 2021 seasons on one side of ridges with 50 cm spacing between plants at a rate of 2-3 seeds at hills at the depth of 1-2 cm of soil by hand. After germination plants were thinned to one plant per hill.

The treatments were arranged as follow:

A. Irrigation intervals:

1- Normal irrigation (at 7 days intervals).

2- Water stress (at 14 days intervals).

B. Antioxidant treatment:

1- Control (seed soaking and spraying with tap water).

2- Glycine betaine seed priming at 150 ppm.

3- Glycine betaine seed priming at 150 ppm with foliar application of glycine betaine at 700 ppm.

4- Chitosan seed priming at 150 ppm.

5- Chitosan seed priming at 150 ppm with foliar application of chitosan at 150 ppm.

For seed priming seeds were soaked for 12 hours with glycine betaine, chitosan at 150 ppm and tap water solution at the dark room at 25Cº. Then to remove priming agent from the seeds surface, the primed seeds were washed by deionized water and dried (48 h) to minimize the moisture content to <10% by blotting the paper and placed to an air- drying oven at 25Cº.

The normal agricultural practices of squash production were followed according to the recommendations of Egyptian Ministry of Agriculture. Fruits harvesting was done according to the standard characteristics of exportation.

3- Data recorded were as follows

Fruit yield and its components:

At harvest time, samples of squash fruits were harvested three times weekly. Twenty time harvests were done. Fruits were harvested, counted and weighted of each plot at the proper maturing stage and the following parameters were collected: fruit length, fruit diameter, dry matter of fruit and total fruit yield (ton $feddan^{-1}$) of squash plant.

Chemical composition of leaves:

Total chlorophyll was determined before the beginning of flowering in leaves according to Lichtenthaler and Wellburn (1983). Free phenolics, was measured according to Kahkonen *et al.* (1999) and Catalase (CAT) was measured according to Beers and Sizer (1951).

Chemical composition of fruits:

Representative samples of 10 squash fruits were randomly taken which harvest in the tenth time of harvest and the following parameters were determined nitrogen, phosphorus, potassium (%):fruits were oven dried at70 C^0 and N, P and K were determined according to Plummer (1978), Jackson (1958) and Piper (1950) respectively and protein (%) was calculated by multiplying the total nitrogen by the factor 6.25, Vitamin C (mg/100g) in squash fruits were determined according to the method reported in AOAC (2000), Total carbohydrates % was determined in squash fruits by the method described by Hedge and Hofreiter (1962).

Statistical analysis:

All statistical analyses were performed using analysis of variance technique by means of Costat computer software. Using the differences between individual pairs of treatment means were compared using Duncan Multiple Range Test at 5% according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Effect of irrigation intervals:

Regarding the effect of irrigation intervals, results in Tables 2, 4, 6, and 8 show significant increase in fruit length, fruit diameter, dry matter of fruit and total yield ton fed^{-1}, total chlorophyll, in the leaves, N, P and K and crude protein in squash fruits, when plants irrigated every 7 days intervals compared to irrigation every 14 days intervals except, phenols, CAT of leaves, VC and carbohydrates content of fruits recorded the highest values when plants irrigated every 14 days intervals as water stress in both seasons of study. These results may be due to that irrigation every 7 days were appropriate intervals to save the water around roots which caused good conditions to plant roots to absorb the required sufficient water and the available mineral elements, nitrogen and other macro- and micro-elements absorption, plant metabolism and in addition to the ability of soil to retain the water reasonable which reflected on photosynthetic process where the atmosphere in this period is sunny and hot and consequently on vegetative growth, chemical constituent, and this lead to significant increase on production of squash yield and fruit quality of squash plants. This finding is in agreement with (Kurunç and Unlukara, 2009) on okra and Ezzat *et al.* (2015) on Jerusalem artichoke plants.

On other side, irrigation every 14 days during the hot and dry condition caused counteracting the plant to water deficit stress which lead to significantly decreased characteristics of yield of squash plants because the increment the production of reactive oxygen species, superoxide, and hydroxyl radical in chloroplasts, and mitochondria those were negatively impacting various processes e.g. stomatal conductance, photosynthesis and growth thus the previous parameters significantly decreased. These results supported by the report of those obtained by Abd El-Mageed and Semida (2015) on summer squash and Ragab *et al.* (2015) on tomato

The decline in plant growth in response to water stress might be attributed to reductions in cell elongation due to growth-promoting hormones inhibition, leading to decrease in cell volume and limite of photosynthesis, growth and yield (Tezara *et al.*, 2005). Boutraa (2010) reported that water stress conditions cause a multitude of molecular, biochemical and physiological changes, adversely impacting in plant growth and development.

Water stress increased vitamin C and carbohydrates of fruit because both of free and total water in the leaf tissues were higher under the highest water quantity level (irrigation every 7 days) compared to water stress. Similar results are obtained by (Ezzat *et al.*, 2009) on potato and Hussein *et al.* (2011) on okra.

The increasing of water quantity applied to plants decreased the antioxidant because both of free and total water in the leaf tissues were higher (Ezzat *et al.*, 2009). Similar results were obtained by Unyayar *et al.* (2005) on tomato, Rai *et al.* (2021) on tomato.

Table 2. Fruit length, fruit diameter, dry matter of fruit and total yield (ton fed^{-1}.) of squash plants as affected by irrigation intervals and glycine betaine and chitosan seed priming and foliar application in 2020 and 2021 seasons.

Treatments		Fruit length (cm)		Fruits diameter (cm)		Dry matter %		Total yield feddan $^{-1}$ (ton)	
		2020	2021	2020	2021	2020	2021	2020	2021
Irrigation intervals	Normal irrigation at 7 days	12.3 a	12.6 a	3.2 a	3.3 a	12.2 a	12.2 a	13.7 a	14.0
	Drought stress at 14 days	11.3 b	11.0 b	2.7 b	2.6 b	11.6 b	11.6 b	8.8 b	8.7 b
Seed priming and foliar application	Control	11.2 c	10.7 c	2.7 c	2.7 c	11.6 e	11.5 e	8.9 c	8.6 c
	Seed priming glycine betaine 150 ppm	11.9 ab	12.0 b	2.9 b	2.9 b	11.8 d	11.8 d	10.7 b	10.8 b
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine 700 ppm	12.2 a	12.2 a	3.0 a	3.1 a	12.1 b	12.1 b	12.8 a	12.9 a
	Seed priming chitosan 150 ppm	11.8 b	11.9 b	2.9 b	2.9 b	11.9 c	11.9 c	11.0 b	11.2 b
	Seed priming chitosan150 ppm + foliar application chitosan 150 ppm	12.1 ab	12.2 a	3.0 a	3.1 a	12.2 a	12.1 a	13.0 a	13.3 a

Means of each column for every interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

Table 3. Fruit length, fruit diameter, dry matter of fruit and total yield (ton fed^{-1}.) of squash plants as affected by the interaction between irrigation intervals and glycine betaine and chitosan seed priming and foliar application in 2020 and 2021 seasons.

Treatments		Fruit length (cm)		Fruits diameter (cm)		Dry matter %		Total yield feddan^{-1} (ton)	
		2020	2021	2020	2021	2020	2021	2020	2021
Normal irrigation at 7 days	Control Tap water	12.0 bcd	11.8 d	2.9 c	2.9 d	11.8 e	11.8 c	10.8 c	10.5 c
	Seed priming glycine betaine 150 ppm	12.4 abc	12.8 bc	3.2 b	3.2 c	12.2 d	12.2 b	13.0 b	13.4 b
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine 700 ppm	12.7 a	12.9 a	3.3 a	3.5 a	12.4 b	12.3 b	15.8 a	16.3 a
	Seed priming chitosan 150 ppm	12.2 abc	12.6 c	3.2 b	3.3 b	12.2 c	12.3 b	13.4 b	13.4 b
	Seed priming chitosan 150 ppm+ foliar application chitosan 150 ppm	12.5 ab	12.9 ab	3.3 a	3.5 a	12.5 a	12.5 a	15.8 a	16.6 a
Drought stress at 14 days	Control Tap water	10.4 f	9.6 g	2.6 f	2.5 h	11.3 j	11.3 e	7.1 g	6.7 h
	Seed priming glycine betaine 150 ppm	11.4 de	11.2 f	2.6 e	2.5 g	11.5 i	11.5 d	8.4 f	8.1 g
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine 700 ppm	11.8 cde	11.6 e	2.7 d	2.6 f	11.8 g	11.8 c	9.7 e	9.5 e
	Seed priming chitosan 150 ppm	11.3 e	11.2 f	2.6 ef	2.5 g	11.5 h	11.6 d	8.7 f	9.0 f
	Seed priming chitosan 150 ppm+ foliar application chitosan 150 ppm	11.8 cde	11.4 e	2.7 d	2.7 e	11.8 f	11.8 c	10.3 d	10.0 d

Means of each column for every interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

Effect of antioxidant treatment:

Impacts of seed priming with antioxidants materials as seed priming and foliar spray of glycine betaine and chitosan are shown in 2, 4, 6, and 8. The previous parameters were significantly improved compared to control treatment in both seasons. These results may be attributed to the positive effects of antioxidants materials on fruit length, fruit diameter, dry matter of fruit, total yield (ton fed^{-1}), total chlorophyll, phenols and CAT in the leaves, N, P and K, crude protein vitamin C and carbohydrate percentages in squash fruits during normal and water stress conditions. Chitosan seed priming at 150 ppm with chitosan foliar application at 150 ppm was the best treatment in total dry matter of fruit, total chlorophyll, N, P, K, and protein percentages in fruits. Glycine betaine seed priming at 150 ppm with foliar application of glycine betaine at 700 ppm was the best treatment in CAT in squash leaves and vitamin C in fruits in both seasons. While chitosan seed priming at 150 ppm with foliar application of chitosan at 150 ppm and glycine betaine seed priming at 150 ppm with foliar application of glycine betaine at 700 ppm were had similar effect and recorded the best treatment in carbohydrate in fruits, phenols in leaves, fruit length, fruit diameter and total yield (ton fed^{-1}) compared with other treatments and control in both seasons.

Glycine betaine regulates the endogenous accumulation of soluble sugars and proline, and antioxidant system in seedlings, it soothes the enzymes and activities of protein complexes under stress conditions (Sakamoto and Murata 2002) which may also be a remarkable demonstration of increased seed germination characteristics under stressful condition. It has effect on metabolism and growth and its role as osmoprotectant (Ragab *et al,* 2015) and this enhances the chemical composition of fruits. These results are supported by the report of Abdel-Mawgoud (2017) on squash; Sadeghipour (2020) on cowpea as for glycine betaine seed priming; El-Shoura (2020) on summer squash and Nada (2020) on strawberry as for foliar application of glycine betaine.

Stimulating effect of chitosan on studied characters may be attributed to it enhances photosynthetic rate and improves chemical composition of squash fruits (Khan *et al.,* 2002). Chitosan reduces the accumulation of harmful free radicals and increases antioxidants and enzyme activities and improves essential nutrients and uptake and the availability of water and by adjusting cell osmotic pressure (Guan *et al.,* 2009). Chitosan is considering a new plant growth promoter such as GA3 which has reflected on the growth and yield of plant (El-Bassiony *et al.,* 2014). These results are agreed with Ibraheim and Mohsen (2015) on summer squash and Geries *et al.* (2020) on onion.

These results are supported by the report of Geries *et al.* (2020) on onion; Menendez and Rodriguez (2020) on soybean as for chitosan seed priming; Ibraheim and Mohsen (2015) on summer squash, Abd El-Hady and Abd-Elhamied (2018) on cucumber and Tantawy *et al.* (2021) on garlic as for chitosan foliar application.

Effect of interactions:

Concerning the interactions between irrigation intervals and seed priming with foliar application of antioxidants, the obtained results in Tables 3, 5, 7, and 9 demonstrated that the interaction with irrigation every 7 days and chitosan seed priming at 150 ppm with chitosan foliar application at 150 ppm recorded the highest values on total chlorophyll, dry matter of fruit, N, P, K and protein percentages of squash fruits compared to other treatments. Irrigation every 14 days and glycine betaine seed priming at 150 ppm with foliar application of glycine betaine at 700 ppm recorded the highest values of CAT compared to other treatments.

As well as, irrigation every 7 days and chitosan seed priming at 150 ppm with chitosan foliar application at 150 ppm and irrigation every 7 days and glycine betaine seed priming at 150 ppm with foliar application of glycine betaine at 700 ppm had similar effect and recorded the highest values of fruit length, fruit diameter and total yield (ton fed^{-1}) compared to other treatments.

Concentrating treatment of irrigation every 14 days and chitosan seed priming at 150 ppm with chitosan foliar application at 150 ppm and irrigation every 14 days and glycine betaine seed priming at 150 ppm with foliar application of glycine betaine at 700 ppm had similar effect and recorded the highest values of phenols in leaves, vitamin C and carbohydrate in fruit.

These results are supported by the report of Ragab *et al.* (2015) on tomatoes and El Afifi *et al.* (2018) on okra. These results may be due to the positive effect of the appropriate irrigation times and the role positive impact of glycine betaine and chitosan on plant as mention previously.

Table 4. Total chlorophyll content, phenol and CAT of squash leaves as affected by irrigation intervals and glycine betaine and chitosan seed priming and foliar application in 2020 and 2021 seasons.

Treatments		Total chlorophyll (mg/g FW)		Free phenolics (mg GAE g^{-1} DW)		CAT absorbance/ min/g Fw	
		2020	2021	2020	2021	2020	2021
Irrigation intervals	Normal irrigation at 7 days	1.0 a	1.0 a	35.9 b	35.9 b	0.4 b	0.4 b
	Drought stress at 14 days	0.9 b	0.9 b	41.0 a	41.4 a	0.7 a	0.8 a
Seed priming and foliar application	Control	0.9 e	0.9 d	36.5 c	36.8 c	0.5 e	0.5 e
	Seed priming glycine betaine 150 ppm	0.9 d	0.9 c	38.5 b	38.4 b	0.6 c	0.6 c
	Seed priming glycine betaine 150 ppm+ foliar application glycine betaine 700 ppm	0.9 b	0.9 b	40.2 a	40.2 a	0.7a	0.7 a
	Seed priming chitosan 150 ppm	0.9 c	0.9 b	37.3 bc	37.8 b	0.5 d	0.6 d
	Seed priming chitosan150 ppm+foliar application chitosan 150 ppm	1.0 a	1.0 a	39.9 a	40.0 a	0.6 b	0.6 b

Means of each column for every interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

Table 5. Total chlorophyll content, phenol and CAT of squash leaves as affected by the interaction between irrigation intervals, glycine betaine and chitosan seed priming and foliar application in 2020 and 2021 seasons.

Treatments		Total chlorophyll (mg/g FW)		Free phenolics (mg GAE g^{-1} DW)		CAT absorbance/ min/g Fw	
		2020	2021	2020	2021	2020	2021
Normal Irrigation at 7 days	Control Tap water	0.9 e	0.9 e	33.9 h	34.2 e	0.3 j	0.3 i
	Seed priming glycine betaine 150 ppm	1.0 d	1.0 d	35.8 f	35.3 e	0.4 h	0.4 h
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine700 ppm	1.0 b	1.0 b	37.9 e	37.6 d	0.5 f	0.5 f
	Seed priming chitosan 150 ppm	1.0 c	1.0 c	34.6 g	35.2 e	0.4 i	0.4 h
	Seed priming chitosan 150 ppm+ foliar application chitosan 150 ppm	1.0 a	1.0 a	37.5 e	37.4 d	0.5 g	0.5 g
Drought Stress at 14 days	Control Tap water	0.8 j	0.8 i	39.2 d	39.5 c	0.6 e	0.6 e
	Seed priming glycine betaine 150 ppm	0.9 i	0.9 g	41.2 b	41.6 ab	0.7 c	0.8 c
	Seed priming glycine betaine150 ppm+ foliar application glycine betaine 700 ppm	0.9 g	0.9 h	42.5 a	42.9 a	0.8 a	0.9 a
	Seed priming chitosan 150 ppm	0.9 h	0.9 g	40.1 c	40.5 bc	0.7 d	0.7d
	Seed priming chitosan 150 ppm+ foliar application chitosan 150 ppm	0.9 f	0.9 f	42.2 a	42.6 a	0.8 b	0.8 b

Means of each column for every interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

Table 6. Nitrogen (N), phosphorus (P), potassium (K) and protein percentages in squash fruits as affected by irrigation intervals and glycine betaine and chitosan seed priming and foliar application in 2020 and 2021 seasons.

Treatments		N %		P %		K %		protein%	
		2020	2021	2020	2021	2020	2021	2020	2021
Irrigation intervals	Normal irrigation at 7 days	2.1 a	2.2 a	0.2 a	0.2 a	1.8 a	1.8a	13.1a	13.8 a
	Drought stress at 14 days	1.7 b	1.8 b	0.2 b	0.2 b	1.4 b	1.4 b	11.1 b	11.6 b
Seed priming and foliar application	Control	1.8 c	1.8 d	0.2 e	0.2 e	1.5 e	1.5 e	11.3 d	11.6 d
	Seed priming glycine betaine 150 ppm	1.9 b	1.9 c	0.2 d	0.2 d	1.5 d	1.5 d	11.8 c	12.3 c
	Seed priming glycine betaine 150 ppm+ foliar application glycine betaine 700 ppm	1.9 b	2.1 b	0.2 b	0.2 b	1.7 b	1.7 b	12.3 b	13.1 b
	Seed priming chitosan 150 ppm	1.9 b	2.0 bc	0.2 c	0.2 c	1.6 c	1.6 c	12.1 bc	12.8 bc
	Seed priming chitosan150 ppm + foliar application chitosan 150 ppm	2.0 a	2.1 a	0.2 a	0.2 a	1.7 a	1.8 a	12.9 a	13.6 a

Means of each column for every interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

Table 7. Nitrogen (N), phosphorus (P), potassium (K) and protein percentages in squash fruits as affected by the interaction between irrigation intervals and glycine betaine and chitosan seed priming and foliar application in 2020 and 2021 seasons.

Treatments		N %		P %		K%		protein%	
		2020	2021	2020	2021	2020	2021	2020	2021
Normal irrigation at 7 days	Control Tap water	1.9 c	2.1bcd	0.2 e	0.2 e	1.7 e	1.7 e	12.1 c	13.3 bcd
	Seed priming glycine betaine 150 ppm	2.0 b	2.1abc	0.2 d	0.2 d	1.7 d	1.7 d	13.0 b	13.6 abc
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine 700 ppm	2.1b	2.2 ab	0.2 b	0.2 b	1.9 b	1.9 b	13.2 b	14.0 ab
	Seed priming chitosan 150 ppm	2.1 b	2.2 abc	0.2 c	0.2 c	1.8 c	1.8 c	13.1 b	13.7 abc
	Seed priming chitosan 150 ppm+ foliar application chitosan 150 ppm	2.2 a	2.3 a	0.2 a	0.2 a	1.9 a	2.0 a	14.0 a	14.5 a
Drought stress at 14 days	Control Tap water	1.6 f	1.6 g	0.2 j	0.2 i	1.3 j	1.2 j	10.5 g	10.0 g
	Seed priming glycine betaine 150 ppm	1.7 f	1.7 f	0.2 i	0.2 h	1.3 i	1.3 i	10.6 g	11.1 f
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine 700 ppm	1.7 e	1.9 de	0.2 g	0.2 f	1.5 g	1.5 g	11.4 e	12.3 de
	Seed priming chitosan 150 ppm	1.8 e	1.9 ef	0.2 h	0.2 g	1.4 h	1.5 h	11.2 f	11.8 ef
	Seed priming chitosan 150 ppm+ foliar application chitosan 150 ppm	1.8 d	2.0 cde	0.2 f	0.2 e	1.6 f	1.6 f	11.8 d	12.7 cde

Means of each column for every interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

Table 8. Vitamin C and carbohydrate contents in squash fruits as affected by irrigation intervals and glycine betaine and chitosan seed priming and foliar application in 2020 and 2021 seasons.

Treatments		vitamin C (mg/100g)		Total carbohydrate %	
		2020	2021	2020	2021
Irrigation intervals	Normal irrigation at 7 days	18.4 b	18.5 b	13.7 b	13.7 b
	Drought stress at 14 days	20.1 a	20.2a	16.1 a	16.1 a
Seed priming and foliar application	Control	18.6 d	18.6 d	13.9 c	13.9 c
	Seed priming glycine betaine 150 ppm	19.1 c	19.1 c	14.8 b	14.8 b
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine 700 ppm	20.0 a	20.1 a	15.7 a	15.7 a
	Seed priming chitosan 150 ppm	19.0 c	19.0 c	14.5 bc	14.4 bc
	Seed priming chitosan150 ppm + foliar application chitosan 150 ppm	19.7 b	19.8 b	15.7 a	15.6 a

Means of each column for every interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

Table 9. Vitamin C and carbohydrate contents in squash fruits as affected by the interaction between irrigation intervals and glycine betaine and chitosan seed priming and foliar application in 2020 and 2021 seasons.

Treatments		vitamin C (mg/100g)		Total carbohydrate %	
		2020	2021	2020	2021
Normal Irrigation at 7 days	Control Tap water	17.8 h	17.7 f	12.6 f	12.5 f
	Seed priming glycine betaine 150 ppm	18.3 g	18.3 e	13.5 e	13.5 e
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine 700 ppm	19.1 e	19.3 cd	14.7 cd	14.7 d
	Seed priming chitosan 150 ppm	18.2 g	18.2 e	13.1 e	13.1 e
	Seed priming chitosan 150 ppm+ foliar application chitosan 150 ppm	18.8 f	19.1 d	14.7 d	14.7 d
Drought Stress at 14 days	Control Tap water	19.5 d	19.5 c	15.2 c	15.2 c
	Seed priming glycine betaine 150 ppm	19.9 c	19.9 b	16.1 b	16.2 ab
	Seed priming glycine betaine 150 ppm + foliar application glycine betaine 700 ppm	20.9 a	20.9 a	16.8 a	16.7 a
	Seed priming chitosan 150 ppm	19.8 c	19.8 b	15.8 b	15.7 bc
	Seed priming chitosan 150 ppm+ foliar application chitosan 150 ppm	20.6 b	20.6 a	16.7 a	16.6 a

Means of each column for every interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

CONCLUSION

Irrigated squash plants every 7 days and seed priming with glycine betaine or chitosan 150 ppm with foliar spraying with glycine betaine 700 ppm or chitosan 150 ppm gave the highest values of quality, yield as well as enzymatic antioxidants activity. But we recommends the use of interaction treatment between irrigation every 14 days plus priming seed with chitosan 150 ppm and foliar spray with chitosan (150 ppm), it provides half number of irrigation water and it gave yield close to irrigation every 7 days without seed priming and without foliar spray.

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تاثير الجليسين بيتين والشيتوزان على تحمل نباتات الكوسة للاجهاد المائى

سمر محمد عبد الحميد دقليجة[1] و سمير طه محمود العفيفى[1] و ايهاب عوض الله ابراهيم[2] و سمر عابد احمد سالم[1]

[1]قسم الخضر والزينة ـ كلية الزراعة ـ جامعة المنصورة ـ مصر

[2]قسم بحوث الخضر ـ معهد بحوث البساتين ـ مركز البحوث الزراعية – مصر

الملخص

أجريت تجربتان حقليتان بمزرعة خاصة فى بلقاس – محافظة الدقهلية – مصر خلال الموسمين الصيفيين لعامى 2020- 2021 لدراسة تاثير فترات الرى كل (7 - 14 يوم) و(نقع البذور والرش بماء الصنبور كنترول – نقع البذور بالجليسين بيتين150 جزء فى المليون- نقع البذور بالجليسين بيتين150 جزء فى المليون والرش الورقى بالجليسين بيتين 700 جزء فى المليون – نقع البذور بالشيتوزان 150 جزء فى المليون - نقع البذور بالشيتوزان 150 جزء فى المليون والرش الورقى بالشيتوزان 150 جزء فى المليون والتفاعلات بينهم على المحصول والمكونات الكيماوية وصفات الجودة لنبات الكوسة صنف الاسكندرانى . الرى كل 7 ايام اعطى زيادة معنوية للمحصول, الكلوروفيل الكلى, النسب المئوية لكلا النيتروجين , الفوسفور, البوتاسيوم والبروتين فى الثمار, بينما اعطى الرى كل 14 يوم زيادة معنوية للفينولات, انزيم الكاتاليز فى الاوراق , فيتامين ج والكربوهيدرات فى الثمار. نقع البذور والرش الورقى بالجليسين بيتين او الشيتوزان كان لهما نفس التاثير على معظم الصفات المدروسة حيث ادى الى زيادة معنوية فى كل الصفات المدروسة مقارنة مع الكنترول. التفاعل بين الرى كل 7 ايام ونقع البذور بالشيتوزان 150 جزء فى المليون مع الرش الورقى بالشيتوزان 150 جزء فى المليون سجل اعلى القيم فى طول و قطرالثمار, المادة الجافة للثمرة, المحصول الكلى, الكلوروفيل الكلى, النسب المئوية لكلا النيتروجين والفوسفور والبوتاسيوم والبروتين فى الثمار بينما سجل الفينولات وانزيم الكاتاليز فى الاوراق وفيتامين ج والكربوهيدرات فى الثمار اعلى القيم بالتفاعل بين الرى كل 14 يوم ونقع البذور بالجليسين بيتين 150 جزء فى المليون والرش الورقى بالجليسين بيتين 700 جزء فى المليون بالمقارنة مع باقى المعاملات., لذلك تلك المعاملات تحسن الانتاج تحت ظروف الاجهاد المائى

الكلمات الدالة: الكوسة ، الرى، الشيتوزان ، الجليسين بيتين