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RESEARCH ARTICLE

DROUGHT ALLEVIATION USING GLUTATHIONE IN CANOLA PLANTS

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Abstract

Drought is one of most important environmental factors inhibiting photosynthesis and decreasing growth and productivity of plants. A Pot experiment was conducted in the greenhouse of the National Research Centre to evaluate the effect of glutathione GHS100 and 200 ppm, on Canola plants grown under different irrigation levels .Growth, yield, some chemical constituents and osmotic pressure (OP) were tested in plants. Plant height, number of green leaves, leaf area, and total dry weight positively responded to GHS treatments when canola plants received water every 7 days. Number of seeds and yield of seeds/plant responded to the interaction between irrigation intervals and glutathione spraying with 100 ppm glutathione was more effective than 200 ppm under 7 and 14 days irrigation intervals. The highest value of carbohydrate was obtained by spraying 100 ppm GHS and irrigation with 7 days intervals. Oil percentage only increased with application 100 and 200 ppm GHS under 7 days intervals. Application of glutathione showed slight increase in osmotic pressure of canola plants and 100 ppm GHS increased proline under 14 days irrigation intervals.

Introduction

Brassica nupas (Canola) is a member of Brasicaceae which is covered with more bloom than other species in this family. Canola has been especially developed for oil by the Canadian scientists, where they reduced the amount of erucic acid in the newly bred variety. Canola constitutes low saturated fatty acids (6%) and high non-saturated fat. Canola has 50% less saturated fat than corn oil (Wiess, 1983). Canola cultivated area in Egypt is relatively very small due to the strong competition in cropping system with the strategic crops during winter season. It could be successfully cultivated in the newly reclaimed soils outside of Nile valley and delta areas for narrowing the gap of edible oil (Gallab and Sharaan, 2002 and Mekki, 2013). Drought strikes plants when transpiration rate is greater than rate of water absorption (Bray, 1997). Damages to plant caused by drought stress are variable depending on the level and duration of the stress and environmental factors (Glantz, 1994). Deficit water strategy is one of the management practices for coping with drought and shortage of water in arid and semiarid regions (Shabani, et al. 2013).To cope with water scarcity different approaches are proposed to decrease water consumption and increase water use efficiency, These methods included use of high yielding varieties (Mekki, 2013), fertilization (Bybordi and Ebrahimian (2013), growth regulators (Ullah, et al. 2012) or antioxidant application (Sakr and Arafa, 2009). Under both natural and agricultural conditions plants are often exposed to various environmental stresses. Drought is one of most important environmental factors inhibiting photosynthesis and decreasing growth and productivity of plants. It is one of the major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang, et al. 2003).

Glutathione (GHS) is a tripeptide with a gamapeptide linkage between the amino group of cestine and the carboxylic group of glutamate side chain (Pompella, et al. 2003). As prototype antioxidant, has been involved in cell protection in noxious effect of excess oxidant stress both directly or cofactor of glutathione peroxidases. Glutathione is one of the major endogenous antioxidants in plants, known to play an important role in plant defense mechanisms. Glutathione functions as a substrate in antioxidative defense mechanisms by conjugating to toxic electrophilic

compounds, scavenging free radicals, and reducing peroxides. Several important catalytic enzymes that utilize glutathione in defense mechanisms, such as glutathione S-transferase (GST) and glutathione reductase (GR) show differential patterns of activity in plant tissues exposed to environmental stress conditions such as; cold, drought and wounding treatments (James and Davis, 2004 and Yılmaz, 2006).
Therefore, the objective of this work is to investigate the effect of glutathione on growth, yield, some chemical constituents and osmotic pressure in leaves of canola plants grown under different irrigation intervals.

MATERIALS AND METHODS

Pot experiment was conducted in the green net of the National Research Centre during the winter season of 2012-2013 to evaluate the effect of spraying glutathione on growth, yield and some chemical constituents and osmotic pressure in leaves of canola plants grown under different irrigation intervals. The treatments were as follows:
Irrigation intervals: Plants irrigated every week (7 days), 2 weeks (14 days) and three weeks (21 days). **Glutathione**: Spraying canola plants with 100 and 200 ppm and control plants sprayed with distilled water. The experiment included 9 treatments 3 irrigation intervals treatments and three glutathione treatments, with 8 replicates.
Seeds of canola (Prassica nupus L) variety Serw 4 were sown on the 1st of Dec., 2012 and in earthenware pots (72 pots) filled with 12 kg of sandy loam soil. The physical and chemical properties of this soil were illustrated in Table (1). Plants were thinned twice the first after 10 days and the second after 21 days from sowing. Calcium super phosphate (15.5% P_2O_5) and potassium sulfate (48.5% K_2O) in the rate of 3.0 and 1.5 g/pot were added by broadcasting before sowing. Ammonium sulfate was added in two equal portions in the rate of 1.5 g/pot twice at 21 days from sowing and two weeks after the first one. Irrigation interval treatments were applied at 21 days till two weeks before harvesting.

Osmotic pressure determination

Osmotic pressure in fresh samples was determined according to the method described by **Gusev (1960**).

Chemical determinations

Two plants from each pot were collected, cleaned, dried at 70 °C in an electric oven for three days and ground in a stainless steel mill. The following determinations were investigated.

Nitrogen and protein determination

Digestion and N determination was done according to the methods described by micro Kejeldahel methods as described by **Pregel (1945)**.

Carbohydrate percentage determination

Total carbohydrate was determined according to the method of Smith, et al. (1956).

Oil percentage determination

Oil percentage was determined according to the methods described in the A.O.A.C. (1970).

Proline determination

Proline determination was done using the method described by: Wren and Wiggall (1965).
Collected Data was subjected to the proper statistical analyses as described by Snedecor and Cochran (1990).

RESULTS AND DISCUSSION

Table (1): Physical and chemical properties of soil under experimentation.

Physical properties			
Clay %		18.00	
Sand %		57.25	
Silt %		29.75	
Soil Texture		Sandy loam	
Chemical analysis			
PH (1:3)		7.25	
EC. (1.53)		1.1 dS/m-1	
Available macronutrients (ppm)		**Available micronutrients**	
N	189.10	B	3.42
P	3.14	Fe	15.14
K	259.75	Mn	21.81
Ca	65.15	Zn	1.18
Mg	73.18	Cu	1.31
		Al	0.78

Growth and yield

Irrigation intervals: The plant height of irrigated plants every two weeks (67.8cm) exceeded those irrigated every one week or irrigated every three weeks. Number of green leaves/plant followed the same trend of plant height (Table, 2). Area of leaves was not affected by extension of irrigation interval, except for extension to three weeks. Total dry weight positively responded to GHS treatments when canola plants received water every 7 days. Prolonging irrigation to 21 days decreased dry mass with the first concentration (100 ppm glutathione) and showed slight increase with 200 ppm glutathione exceeding the control (Table 2).

Illustrated data in Table (3) showed the effect of irrigation interval on canola yield and its attributes, where the highest values were obtained when irrigating plants every two weeks. Seeds weight per plant increased by 36.0% and 387.10%, and number of seeds per pod increased by 4.95% and 82.03%, when irrigating plants every two weeks compared with one week and three weeks intervals, respectively. The drought stress occurs in the plant when the received water is less than water loss. This may results from either water over loss or reduction in the absorption or both cases (Kuchaki and Alizade, 1995). The drought stress affects different aspects of plant growth and causes reduction and delay of germination, reduction of shoots growth, and reduction of dry matter production. The reduction of osmotic potential and water potential, turgescence removing, and stoma closure as well as growth failure are symptoms of water stress (Singh and Patel, 1998). Gul and Ahmed (2004) noticed that vegetative growth recorded in term of plant height, number of leaves , number of branches and fresh biomass/plant was much reduced under 6 days intervals. They added that there was no much differences in leaf area between 2 and 4 days irrigation intervals but under 6 days intervals, it caused considerable reduction. Growth of plants depends on cell expansion and enlargement which is probably most sensitive physiological aspect of a plant to water deficit leading to reduce plant productivity (Larson, 1992).

Table (2): Effect of glutathione and irrigation intervals on growth of canola.

Irrigation intervals	GHS ppm	Plant height, cm	leaves No.	Leaf area, mm^2	Fresh weight (g): Stem	Fresh weight (g): Leaves	Fresh weight (g): Total	Dry weight (g): Stem	Dry weight (g): Leaves	Dry weight (g): Total
7 days	D.W.	47.3	9.67	31.50	10.12	28.02	38.14	0.70	1.81	2.51
	100	58.3	11.67	25.94	7.21	31.78	38.99	1.00	1.87	2.87
	200	53.3	11.00	42.80	7.46	12.60	20.08	1.30	3.32	4.62
14 days	D.W.	63.7	10.17	30.85	6.19	22.13	28.32	1.33	2.36	3.69
	100	78.7	15.67	41.00	6.71	48.00	54.71	2.28	3.08	5.36
	200	61.0	14.33	28.33	8.02	9.79	17.81	0.56	1.30	1.86
21 days	D.W.	42.7	9.33	42.80	7.63	20.48	27.81	1.40	1.52	2.92
	100	51.3	8.67	28.33	6.06	37.11	43.17	0.39	1.31	1.70
	200	45.0	11.33	16.78	5.15	19.47	24.62	0.75	1.52	2.27
Mean values under the effect of Irrigation intervals	7	46.3	10.78	33.41	8.26	24.13	32.39	1.00	2.33	3.33
	14	67.8	13.39	33.39	6.97	26.64	33.61	1.39	2.25	3.64
	21	46.2	9.78	29.30	6.28	25.69	31.97	0.85	1.45	2.30
Mean values under the effect of glutathione rates	D.W.	51.2	9.71	35.05	9.51	23.54	33.05	1.14	1.90	3.04
	100	62.8	12.00	31.76	10.59	38.96	49.55	1.22	2.09	3.31
	200	53.1	12.22	29.30	9.77	17.13	26.90	0.87	2.05	2.92
LSD at 5% level	Ir.In.	9.72	2.58	N.S	N.S	N.S	8.69	N.S	N.S	1.37
	GHS.	7.05	1.72	N.S	N.S	1.96	8.01	N.S	1.19	N.S
	Ir.In x GHS	N.S	2.98	14.76	N.S	3.40	13.86	N.S	N.S	3.34

Table (3): Effect of glutathione and irrigation intervals on yield of canola.

Irrigation intervals	GHS ppm	No of pods/plant	Seeds weight/plant, g	No of seeds/pod	Weight of 100 seed, g
7 days	D.W.	43.3	2.19	23.3	0.28
	100	56.7	4.37	21.7	0.38
	200	50.0	3.44	21.7	0.29
14 days	D.W.	52.7	3.77	23.2	0.31
	100	74.0	6.66	23.3	0.37
	200	85.0	3.15	23.0	0.22
21 days	D.W.	53.3	0.73	11.7	0.15
	100	56.7	0.57	16.7	0.17
	200	38.3	0.44	10.0	0.18
Mean values under the effect of Irrigation intervals	7	49.8	3.33	22.2	0.32
	14	70.6	4.53	23.3	0.27
	21	49.4	0.93	12.8	0.17
Mean values under the effect of glutathione rates	D.W.	49.8	2.23	19.9	0.25
	100	62.2	4.20	20.6	0.31
	200	57.8	2.34	8.3	0.23
LSD at 5% level	Ir.In.	N.S	0.81	3.90	0.013
	GHS.	2.14	0.63	4.68	0.04
	Ir.In x GHS	8.96	1.08	N.S	N.S

D.W. = Distilled water GHS.=Glutathione II.=Irrigation intervals

Growth and photosynthesis are two of the most important processes suppressed partially or completely by water stress (Kramer and Boyer, 1995). Several investigations concluded that this may be attributed to the effect on enzymes and oxidative defense (Niu, et al. 2013). Moisture stress affecting photosynthesis and carbohydrate building (Pinheiro and Chaves, 2011 and Tarighaleslami, et al. 2012).

Glutathione

Data in Table (2 & 3) for growth and yield showed that the enhancing effect of 100 ppm glutathione exceeded those induced by application of 200 ppm concentration, except for leaves number and area. Reduced glutathione, and reduced ascorbate, the two major water soluble antioxidants in photosynthetic and non-photosynthetic tissues, reacting directly or indirectly with reactive oxygen species, contribute to maintain the integrity of cell structures and the proper functions of various metabolic pathways (Konkline, et al. 1997). Eid, et al. (2011) noticed that glutathione increased plant height, number of branches, fresh and dry weight of herb and flowers and number of flowers in marigold (Tagetes erecta L.) plants.

Glutathione x irrigation intervals

Plant height and number of green leaves increased by GHS application (100ppm) under tested irrigation intervals. Area of leaves was reduced by application of 100 or 200 ppm GHS (Table 2). Total dry weight positively responded to GHS treatments when canola plants received water every 7 days and negatively responded to irrigation every 14 days but prolongation to 21 days dry mass decreased with 100ppm GHS and tended to increase with 200 ppm compared to the control. Moreover the highest total dry weight was attained by spraying plants with 100 ppm glutathione and irrigation every 14 days.

The interactive effects of irrigation intervals and glutathione spray amount on yield of canola plants. Data presented in (Table 3) showed that plant response to 100 ppm glutathione exceeded that of 200 ppm under 7 and 14 days irrigation intervals. However, under 21 days intervals, 100 ppm glutathione slightly affected number of pods but 200 ppm antioxidant concentration negatively affected this character (Table 3). Among the non-enzymatic compounds, glutathione and ascorbate are essential plant metabolites that regulate major cell functions and play a pivotal role in antioxidant defense (Noctor and Foyer, 1998). Exposure of plants to unfavorable conditions such as drought, high temperature or salinity can increase the production of reactive oxygen species (ROS) single oxygen, hydrogen peroxide, superoxide radicals, and hydroxyl radicals to protect themselves against these toxic oxygen intermediates. Plants employ defense that included the enzymes such as suproxide dismutase, catalases, ascorbate peroxidases, glutathione-s-transferases and glutathione reductase that catalyze the scavenging of ROS (Rexas, et al. 2000).

Carbohydrate, oil and protein

Irrigation intervals

Protein and carbohydrate percentages were reduced by extending the irrigation interval , meanwhile, oil percentage increased with 14 days intervals and decreased with 21 days intervals to be less than the control (Table 4). This means that extension of irrigation interval adverse markedly the carbohydrate and protein building. Such result was confirmed by Pinheiro and Chaves (2011) and Tarighaleslami, et al. (2012). The plant reacts to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration. As a consequence, the diffusion of CO_2 into the leaf is restricted, less water in tissues and this in turn affected the carbohydrates building (Kramer and Boyer, 1995).

Glutathione: It was clear that carbohydrate content increased as sprayed with GHS but protein and oil percentages were not affected (Table 4). Eid, et al. (2011) found that application of glutathione (100 or 200 ppm) was effective in increasing carbohydrate percentage and amino acid concentrations.

Glutathione x irrigation intervals

Spraying plants with 100 ppm GHS under 7 days irrigation intervals showed the highest value of carbohydrate .Oil percentage only increased with application of 100 and 200 ppm GHS under 7 days intervals .

Table (4): Effect of glutathione and irrigation intervals on chemical constituents of canola plants

Irrigation intervals	GHS ppm	Carboh -ydrate	Protein %	Oil %	Proline ppm	Osmotic pressure
7 days		52.7	22.85	23	2.48	5.12
	D.W.	86.9	24.81	25	4.69	6.60
	100	64.9	24.69	27	2.40	5.67
14 days	200	56.0	23.38	31	1.94	5.36
	D.W.	59.8	24.31	31	3.01	4.77
	100	55.5	23.33	27	7.72	6.61
21 days	200	59.7	20.87	22	1.09	6.07
	D.W.	60.0	23.88	21	2.64	6.20
	100	65.0	23.44	21	3.21	5.30
Mean values under the effect of Irrigation intervals	200	68.2	24.12	25.0	3.19	6.46
	7	57.1	23.69	29.7	4.22	5.58
	14	61.6	22.78	21.3	2.31	5.86
Mean values under the effect of glutathione rates	21	56.1	23.45	25.3	1.84	5.52
	D.W.	68.9	24.33	25.7	3.45	5.86
	100	61.8	23.84	25.0	4.44	6.53

D.W. =Distilled water GHS.=Glutathione II.=Irrigation intervals

Osmotic pressure and proline

Irrigation intervals

Data in Table (4) showed the decrease in osmotic pressure with the increase in periods between irrigations. Huang and Redman, 1995) mentioned that osmotic adjustment play a crucial role in plant adaptation to drought.

Glutathione

Application of glutathione showed slight increase in osmotic pressure of canola plants and 100 ppm GHS increased proline under 14 days irrigation interval Table (4). Water uptake is enhanced by the accumulation of solutes to lower the tissue water potential and by improving root growth, and water loss through evaporation is limited by closing stomata, restricting shoot growth and accelerating leaf senescence (Nakashima, et al. 2009). Exogenous amino acids application caused significant increase in proline contents (Burbulis, et al. 2013).

Glutathione x irrigation intervals

The response of OP to application of GHS was similar under 4 and 21 days between irrigations but under 7 days intervals was different which decreased with the 100 ppm treatment (Table 4).. Glutathione is one of the major endogenous antioxidants in plants known to play an important role in plant defense mechanisms. Glutathione S-transferase is a GSH dependent detoxifying enzyme in plants, which catalyzes the conjugation of GSH (Yilmaz, 2006). The response of OP to application of GhS was similar under 4 and 21 days between irrigations but under 7 days intervals was different which decreased with the 100 ppm treatment and tended to increase to be more than that was shown with control plants. The foliar spray of GB could markedly alleviate the water stress (Cao, et al. 2013).

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