

EFFECT OF GLUTATHIONE AND HYDROGEN PEROXIDE AND THEIR INTERACTION IN THE YIELD AND ITS COMPONENTS OF *VIGNA RADIATA* L. PLANT

Eman H. Al-Hayany*

Depart. of Biology, College of Education for Pure Science (Ibn Al-Haitham), Univ. of Baghdad, Baghdad, Iraq.

Abstract

Two field experiments during the growth season of spring and autumn planting periods at 2014 for *Vigna radiata* L. plant in the botanical garden of the Department of Biology/College of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad. The experiments aimed to study the effect of glutathione and hydrogen peroxide and their interaction in the yield and its components. Glutathione concentrations were (0, 25, 50, 75, 100) mg.L⁻¹ and the hydrogen peroxide concentrations were (0, 5, 10, 15) mmol.L⁻¹, both experiments were carried out using Randomized Complete Block Design (R.C.B.D). The results show that glutathione, in particular at the concentration of 100 mg. L⁻¹ resulted in a significant increase in the number of pods per plant by an increase of 45.60% and 52.13%, the number of seeds per pod 17.43% and 16.23%, the weight of 100 seeds was 22.15% and 22.43% as well as the yield of seeds 52.77% and 43.70% for two planting periods at respectively. The number of pods were increased at the concentrations 15 mmol.L⁻¹ of hydrogen peroxide were 42.77% and 52.13%, the number of seeds per pod 22.93% and 22.39%, the weight of 100 seeds 24.14% and 24.86% for both planting periods at respectively. While the yield of seeds increased at rate 16.60% for spring planting period only, as the results show significant effect of interaction between glutathione and hydrogen peroxide on the yield and its components.

Key words: Glutathione, hydrogen peroxide, Vigna radiata L.

Introduction

Mung bean (*Vigna radiata* L.) plant belongs to the family Fabaceae (Al-Kateb, 1988), which is a summer herbal crop branched into a semi erect, a short crops in their growth period and is cultivated with two spring and autumn planting periods (Ali *et al.*, 1990a). *V. radiata* was implant for many purposes, including the production of seeds, which are consumed as human food because they contain a high percentage of protein 29%, which is rich in amino acid lysine, whose quantity in grains is low and the carboxylic substances.

It also uses as green fodder for animals, and green fertilizer to improve soil properties (Ali *et al.*, 1990b). As well as it is an important source of protein, therefore *V. radiata* is important in maintaining soil fertility by supplying nitrogen in the symbiosis process. It was also found that *V. radiata* leaves provided an amount of 37-

40 kg of nitrogen per hectare of soil (Anwar and Rashad, 2010). Tri-peptide glutathione is the most abundant in plant tissue and represents multiple roles in cellular metabolism and is a powerful shorthand for Reactive Oxygen Species (ROS) (Tauz and Grill, 2000). It is an antioxidant which is defined as a protein compounds that possess a delicate position consisting of a number of active amino acids that have a role in preventing free radicals from interacting with cellular constituents or minimizing their effect (Locato et al., 2007; Rouhier et al., 2008).

Hydrogen peroxide is an effective oxygen Reactive Oxygen Species (ROS) that is more stable at the cell level, it plays a biological role in the plant by sending chemical signals that lead to the resistance of the plant to stress and that the signal acts on the gene expression (Hung *et al.*, 2005), with low concentrations as a partial signal causing the plant tolerance against the biotic and non-biotic stresses (Mitter *et al.*, 2004). The high concentrations result in the release of the factors that

^{*}Author for correspondence: E-mail: emanhh1973@gmail.com

are responsible for programmed cell death (Dat *et al.*, 2000). This study aims to study the effect of glutathione and hydrogen peroxide and their interaction in plant yield and its components.

Materials and Methods

Experiment site: Two field experiments was conducted at first the spring planting period and the second at autumn planting period during the growth season 2014, in the botanical garden of the department of biology/College of Education for Pure Sciences (Ibn Al-Haitham)/University of Baghdad for the purpose of studying the effect of glutathione and hydrogen peroxide and their interaction in the yield and its components of mung bean (*V. radiata* L.) plant.

Seed source

The seed seeds have been obtained from the local market

Design of experiment

The experiment was designed in accordance with the Randomized Complete Block design (R.C.B.D.). As a working experience (4×5) and with three repeaters, the following factors included:

- 1. Five concentrations of glutathione 0, 25, 50, 75, 100 mg. L⁻¹.
- 2. Four concentrations of hydrogen peroxide 0, 5, 10, 15 mmol. L-1. The number of experimental units for each experiment was 60 experimental units. The land was prepared and processed for cultivation and well settled where it was divided into experimental units measuring (1×1) m² planted on four lines and a distance of 30 cm between one line and another. The soil of the field was analyzed before planting in the laboratories of the department of soil and water resources/College of Agriculture/ University of Baghdad by taking samples at a depth of 0-30 cm. Phosphate fertilizer super triple calcium phosphate was added before planting 20% P (Shukla and Chandel, 2006) and 100 kg. Ha⁻¹ (Saad *et al.*, 2000).

The seeds of *V. radiata* L. plant (local species) were planted with spring planting period in 20/3/2014 and autumn planting period 5/6/2014 and at a rate of 24 kg.H⁻¹ (Ali *et al.*, 1990a). The land of the experiment was handcrafted and watered when needed and the spring planting period was harvested in 1/6/2014, while the autumn planting period was harvested in 24/8/2014.

Preparation of glutathione

Glutathione solution was prepared at concentrations (0, 25, 50, 75, 100) mg.L⁻¹, and the concentrations were

sprayed immediately after preparation early in the morning with the pressing sprayer on the plants at the stage of four leaves and sprayed the control of distilled water for spring and autumn planting periods.

Preparation of hydrogen peroxide

Hydrogen peroxide solution was prepared at concentration (0, 5, 10, 15) mmol.L⁻¹, and the seeds of the mung bean plant have been soaked for 12 hours (Gondim *et al.*, 2010) and the control treatment was soaked in distilled water and for both the first and second planting periods.

Plant yield and its components

Number of pods. Plant⁻¹. The number of plants per plant has been calculated and the rate of three plant randomly per experimental unit at harvest.

Number of seeds per pod

The mean number of seeds in the pod for the three plants was calculated at harvest.

Weight 100 seed

A random sample of 100 seeds was taken after mixing the seed for each experimental unit and weighed in a sensitive balance.

Seed yield gm.m⁻²

It was calculated by the seed yield per m² of each experimental unit at harvest.

Statistical analysis

The results were analyzed by statistical program (SAS, 2010).) and means were compared using the lowest least significant difference (L.S.D) at the 0.05 probability level.

Results and Discussion

The tables 1 and 2 showed significant increase in the mean number of pods per plant by increasing the concentration of glutathione, when glutathione concentration is raised from zero mg.L⁻¹ to 100 mg.L⁻¹, the mean number of pods increased from 12.81 pods to 6 18.6 pods for spring planting period and from 18.28 pods to 22.03 pods for autumn planting period, with an increase of 45.66% and 52.13% for both planting periods at respectively.

The increase in the number of pods it is may be due to the role of glutathione in the increase the number of branches and the growth of the flowering group, or the increase may be due to the fact that glutathione small molecules oxidizing and reductive have a role in the formation of flowers and acid salicylic acid as well as the plant's defense (Rouhier *et al.*, 2008), in addition the

salicylic acid it has a role in increasing the concentration of cytokine and the gibberellin as well as the transmission of water and nutrients from the source to the downstream as it works in inhibition of abscisic acid and enzyme pectin methylase aseterase and metabolism of ethylene (Gharib and Hegazi, 2010; Gupta, 2011) and this result is consistent with the findings (Abd Elwahed and Abouziena, 2014; El-Awadi *et al.*, 2014; Sadak *et al.*, 2014) on the wheat plant.

Tables 1 and 2 also showed a significant increase in the rate of pod number per plant by increasing the concentration of hydrogen peroxide when the concentration of hydrogen peroxide is raised from zero mmol.L⁻¹ to 15 mmol.L⁻¹.

The mean number of pods per plant increased from 12.18 pods to 17.39 pods for spring planting period and from 15.50 pods to 23.58 pods for the second planting period, with an increase rate of 42.77% and 52.13% for two planting periods at respectively. This increased may be due to the fact that hydrogen peroxide stimulates the division and elongation of cells and the formation of secondary walls and works to improve the dynamics of the roots, length and number, leading to a high absorption of nitrogen that is reflected in the growth and plant yield (Hameed et al., 2004; Liao et al., 2004). As well as hydrogen peroxide works in the induction the genes that responsible for nutrients necessary for plant growth such as calcium and potassium (Desikan et al., 2004; Liu et al., 2004; Wendehenne et al., 2004) or possibly due to the fact that hydrogen peroxide works in the stimulation of molecular signals responsible for plant hormones (Quen et al., 2008).

Table 1 and 2 confirm that there is a significant effect of the interaction between the concentration of glutathione and the concentration of hydrogen peroxide and the highest value of the number of plants per plant, 25.56

Table 1 : The effect of glutathione and hydrogen peroxide in the number of pods.plant⁻¹ (Spring planting period).

Concentration	Conce	Concentration of glutathione mg.L ⁻¹					
of H ₂ O ₂ mmol.L ⁻¹	0	25	50	75	100	Mean	
0	9.61	9.50	14.89	14.33	12.56	12.18	
5	16.33	15.69	15.55	16.78	14.10	15.69	
10	10.39	14.66	12.00	11.55	25.56	14.83	
15	14.89	13.42	15.89	20.33	22.44	17.39	
LSD0.05	Glu	Glutathione $\times H_2O_2 = 7.360$					
Mean	12.81	13.32	14.58	15.75	18.66		
LSD 0.05		Glutath	ione =	3.680			

Table 2 : The effect of glutathione and hydrogen peroxide in the number of pods.plant⁻¹ (Autumn planting period).

Concentration	Conce						
of H ₂ O ₂ mmol.L ⁻¹	0	25	50	75	100	Mean	
0	11.33	19.67	15.00	15.50	16.00	15.50	
5	17.11	13.67	22.00	18.67	24.00	19.09	
10	20.67	18.78	15.89	22.00	27.11	20.89	
15	24.00	24.22	25.33	23.33	21.00	23.58	
LSD 0.05	Glu	Glutathione $\times H_2O_2 = 4.166$					
Mean	18.28	19.08	19.55	19.87	22.03		
LSD 0.05		Glutath	ione =	2.083			

pods and 27.11 pods, when the concentration treatment is 100 mg.L⁻¹ and 10 mmol.L⁻¹ and for both planting periods at respectively, while reaching the lowest value of pods number for one plant were 9.61 pods and 11.33 pods when the concentration was zero for both the experiment and the two planting periods at respectively.

Tables 3 and 4 showed a significant effect of increasing the mean number of seeds in the pod by increasing the concentration of glutathione, when the concentration was raised from zero mg.L-1 to 100 mg.L-¹ the mean number of seeds in the pods has increased from 8.43 to 9.90 for the root of the seed from 8.62 to 10.08 seeds of autumn planting periods by an increase rate 17.43% and 16.93% and for two planting periods at respectively. This increased in the mean number of seeds in pod may be due to the that multifunction of the glutathione during the development of seeds, it represents a role in the stages of the seeds growth and the division of cells involved in the formation and protection of this cell from oxidation. The humidity content differs in the seeds as well as the metabolic activity, the length of the seed growth and as a result the sources of ROS vary greatly in the stages of growth of seeds (Bailly, 2004). The seeds evolve by the process of photosynthesis and respiration, which makes the photosynthesis and the electronic transport chain in mitochondria as source for ROS (Mittler, 2002). Besides that glutathione has a role in defending cells against oxidation and participates in plant growth and control of the cycle of cell (Potters et al., 2004) and this result is consistent with (Sadak et al., 2014) on wheat plant.

Table 3 also showed a significant effect of increasing the mean number of seeds in the pod by increasing the concentration of hydrogen peroxide from zero mmol.L⁻¹ to 15 mmol.L⁻¹. The mean number of seeds in the pod increased from 8.37 to 10.29 seed at spring planting period and from 8.44 to 10.33 seed at autumn planting period,

with an increase of 22.93% and 22.39% for two planting periods respectively.

The increase in the number of seeds in the pod may be due to the fact that Hydrogen peroxide has many basic roles in the metabolism of the plant and is involved in a wide variety of interactions and the sequencing of signals necessary for all aspects of the growth of root hairs, differentiation of wood, the lignification, organization the opening and closure of stomata and participates in the metabolism and natural growth of the plant (Checseman, 2007), or may be due to the fact that hydrogen peroxide acts in stimulating the division and elongation of cells and formation of the secondary walls and is working to improve the number, length, coefficient and viability of the roots and this is reflected positively in the growth and yield of the plant (Hameed *et al.*, 2004; Liao *et al.*, 2004).

Table 3 has a significant effect of the interaction between the concentration of glutathione and the effect of hydrogen peroxide concentration on the number of seeds in the pod, the highest value of seeds number was 10.93 seed at concentration 100 mg.L⁻¹ of glutathione and 15 ml mol.L⁻¹ of hydrogen peroxide during spring

Table 3 : Effect of glutathione and hydrogen peroxide in the number of seeds per pod (Spring planting period).

Concentration	Conce	Concentration of glutathione mg.L ⁻¹					
of H ₂ O ₂						Mean	
mmol.L ⁻¹	0	25	50	75	100		
0	5.93	8.80	8.00	8.93	10.20	8.37	
5	8.73	9.73	8.47	9.87	8.40	9.04	
10	8.80	8.93	9.13	9.13	10.07	9.21	
15	10.27	9.93	10.20	10.13	10.93	10.29	
LSD 0.05	Glu	Glutathione $\times H_2O_2 = 1.322$					
		0.591					
Mean	8.43	9.35	8.95	9.52	9.90		
LSD 0.05		Glutath	ione =	0.661			

Table 4: Effect of glutathione and hydrogen peroxide in the number of seeds per pod (Autumn planting period).

Concentration	Conc						
of H ₂ O ₂ mmol.L ⁻¹	0	25	50	75	100	Mean	
0	5.00	7.93	9.27	9.77	10.22	8.44	
5	9.72	9.44	9.00	9.89	8.78	9.37	
10	10.33	9.33	9.67	9.00	9.33	9.53	
15	9.44	9.00	10.55	10.66	12.00	10.33	
LSD 0.05	Glu	tathion	$e \times H_2$	$O_2 = N.5$	S.	H ₂ O ₂ =	
Mean	8.62	8.93	9.62	9.83	10.08		
LSD 0.05		Glutath	ione =	0.713			

planting period. Table 4 showed no significant effect of interaction between the two experimental works during autumn planting period.

The results in tables 5 and 6 showed a significant effect in increasing the mean of 100 seeds, when the concentration of glutathione increases from zero mg.L⁻¹ to 100 mg.L-1, the mean weight of 100 seeds increased from 3.05 to 3.755 gm during spring planting period, and from 3.61 gm to 4.42 seeds during autumn planting period, with an increase of 22.95% and 22.43% for two planting period at respectively. This increase in the mean weight of 100 seeds resulted from increasing glutathione concentration may be due to the multifunction of glutathione during the development of seeds. It plays a role in the stages of seed growth and common cell division in its composition and protection from oxidation (Bailly, 2004). The seeds evolve in photosynthesis and respiration, which makes the photosynthesis and the electron transport chain in mitochondria source of (ROS) (Mittler, 2002).

It may be due to the glutathione are small oxidized and reductive molecules that have a role in the formation of flowers, salicylic acid and plant defense signals (Rouhier *et al.*, 2008). It is believed that salicylic acid increases the metabolism of CO₂ and the production of dry matter and regulate distribution from source to downstream, which is the seeds (Ouda *et al.*, 2007). This result in agreement with (El-Awadi *et al.*, 2014) who are stated that the increase 100 seeds weight when treating two varieties of wheat with glutathione.

Table 5 and 6 also confirmed that there was a significant effect in increasing the mean of 100 seeds, when hydrogen peroxide increased its concentration from zero mmol.L-1 to 15 mmol.L-1 the mean of 100 seeds was increased from 3.23 gm to 4.01 gm during spring planting period and from 3.58 gm to 4.47 gm during spring planting period with increase rates of 24.14 % and 24.86% for two planting period at respectively. This increase may be due to the fact that hydrogen peroxide acts to induce molecular signals that are responsible for plant hormones such as abscisic acid (ABA), Ethylene, Jasmouate (JA), salicylic acid (SA) (Quen et al., 2008). As well as hydrogen peroxide has a role in the induction of the genes responsible for elements necessary for plant growth such as calcium and potassium (Desikan et al., 2004; Liu et al., 2004).

The results of tables 5 and 6 also showed a significant effect of the interction between the effect of glutathione concentration and the effect of hydrogen peroxide concentration, with the highest value of 4.99 gm and 4.98 gm at the concentration of treating glutathione 100 mg. L⁻¹ and 15 mmol.L⁻¹ of hydrogen peroxide for two planting

Table 5 : Effect of glutathione and hydrogen peroxide in the weight of 100 seeds (gm) (Spring planting period).

Concentration	Concentration of glutathione mg.L ⁻¹						
of H ₂ O ₂ mmol.L ⁻¹	0	25	50	75	100	Mean	
0	2.11	3.73	3.71	3.29	3.33	3.23	
5	3.32	3.56	3.42	3.52	3.43	3.45	
10	3.32	3.53	3.36	3.55	3.26	3.40	
15	3.45	3.51	4.16	3.95	4.99	4.01	
LSD 0.05	Glu	Glutathione $\times H_2O_2 = 0.6869$					
Mean	3.05	3.58	3.66	3.58	3.75		
LSD 0.05		Glutath	ione =	0.3435			

Table 6 : Effect of glutathione and hydrogen peroxide in the weight of 100 seeds (gm) (Autumn planting period).

Concentration	Conc	Concentration of glutathione mg.L-1				
of H ₂ O ₂ mmol.L ⁻¹	0	25	50	75	100	Mean
0	2.10	4.23	3.90	4.09	3.57	3.58
5	2.06	3.49	3.96	4.16	4.24	3.98
10	4.01	3.84	4.57	3.71	4.91	4.21
15	4.29	4.52	3.99	4.61	4.98	4.47
LSD 0.05	Glu	H ₂ O ₂ =				
		0.3451				
Mean	3.61	4.02	4.11	4.14	4.42	
LSD 0.05		Glutath	ione =	0.3858		

periods at respectively. The lowest value was 2.11 gm when the concentration of treating glutathione zero mg. L⁻¹ and zero mmol.L⁻¹ of hydrogen peroxide during spring planting period. The lowest value for 100 seeds was 2.06 gm when the concentration of treating glutathione zero mg.L⁻¹ and 5 mmol.L⁻¹ hydrogen peroxide during autumn planting period.

The results of tables 7 and 8 were demonstrated significant effect in increasing the mean yield of seeds gm.m², when the glutathione concentration was raised from zero mg.L¹¹ to 100 mg.L¹¹. The mean seed yield increased from 22.71 gm.m² to 34.56 gm.m² during spring planting period and from 21.92 gm.m² to 31.50 gm.m² during autumn planting period, with an increase rate of 52.17% and 43.70% for two planting periods at respectively. The reason for the increase in the yield of seeds to the role of glutathione in increasing the components of the yield and glutathione represents an important role in control and maintain the oxidation system within the cells, as it is important in the photosynthesis (Hell and Bergmann, 1990).

The increase may be due to the fact that glutathione antioxidant acts to protect cells from crashing by free

Table 7: The effect of glutathione and hydrogen peroxide on the yield of the seeds gm.m² (Spring planting period).

Concentration	Conce	Concentration of glutathione $mg.L^{-1}$					
of H ₂ O ₂ mmol.L ⁻¹	0	25	50	75	100	Mean	
0	20.27	23.42	23.09	30.42	35.59	26.56	
5	20.27	25.14	28.91	28.00	30.59	26.58	
10	24.15	25.94	32.06	29.16	33.27	28.92	
15	26.17	29.79	31.73	28.36	38.79	30.97	
LSD 0.05	Glu	Glutathione \times H ₂ O ₂ = N.S.					
		2 2					
Mean	22.71	26.07	28.95	28.98	34.56		
LSD 0.05		Glutath	ione =	3.489			

Table 8 : The effect of glutathione and hydrogen peroxide on the yield of the seeds gm.m² (Autumn planting period).

Concentration	Conce						
of H ₂ O ₂ mmol.L ⁻¹	0	25	50	75	100	Mean	
0	15.19	18.60	21.74	25.26	28.47	21.85	
5	23.03	23.81	22.89	24.71	27.97	24.48	
10	25.93	15.64	27.50	28.49	38.80	27.27	
15	23.52	30.96	23.63	32.50	30.75	28.27	
LSD 0.05	Glu	Glutathione $\times H_2O_2 = 5.169$					
		$H_2O_2 = N.S.$					
Mean	21.92	22.25	23.94	27.74	31.50		

radicals. Additionally, it helps to keep cells in active form as well as to increase enzymatic activity (Mamdouh, 1995). The glutathione is a small oxidizing and reductive molecules have important role in the formation of the flowers and salicylic acid as well as the plant's defense signals (Rouhier *et al.*, 2008). Salicylic acid has a role in improving the growth of plants, increasing the efficiency of photosynthesis, CO₂ metabolism and increase the accumulation of dry matter (Yazdanpanah *et al.*, 2011). This result is consistent with (El-Awadi *et al.*, 2014; Sadak *et al.*, 2014) who indicated that the increased yield of grains when spraying wheat plant with different concentration of glutathione.

Table 7 also illustrated the significant effect of hydrogen peroxide in increasing the mean yield of the seed when increasing the concentration of hydrogen peroxide from zero mmol.L⁻¹ to 15 mmol.L⁻¹ the mean of seeds yield increased from 26.56 gm.m² to 30.97 gm.m² and with an increase rate of 16.60% during spring planting period and the concentration 15 mmol.L⁻¹ did not different significantly from the concentration 10 mmol.L⁻¹. The hydrogen peroxide caused an increase in the mean vegetative, flowering and root growth, and the exposure

of the seeds to stress hydrogen peroxide led to an increase in H_2O_2 content within the cell (Lin and Kao, 1998) as well as the hydrogen peroxide is working to kill the pathological causes or acts as stimulating genes that reduce pathological injuries (Al-Ghazi, 2013).

The reason for the increase in the rate of the seed yield may be cause by stimulating hydrogen peroxide to cell division and its elongation as it acts in the formation of secondary walls. As well as it improves the number and length of the roots and increase its vitality (Hameed et al., 2004; Liao et al., 2004) or possibly due to the fact that hydrogen peroxide induces genes responsible for the nutritional elements necessary for plant growth and specifically the potassium and calcium elements (Desikan et al., 2004; Liu et al., 2004). This result is consistent with the result (Al-Ghazi, 2013) on the maize plant.

As the results of table 8 showed a significant effect of interaction between the effect of glutathione concentration and the effect of hydrogen peroxide, the highest value of the seeds yield was 38.80 gm.m² when the concentration of treating glutathione 100 mg.L¹ and 10 mmol.L¹ of hydrogen peroxide, the lowest value of 15.19 was recorded when the concentration of treating glutathione zero mg.L¹ and zero mmol.L¹ of hydrogen peroxide during autumn planting period.

References

- Abd Elwahed, M.S.A. and H.F. Abouziena (2014). Efficacy comparison of Stearic Acide, Glutathione and Salicylic Acid on wheat (*Trticum aestivum* L.) cultivars productivity in Sandy soil. *Int. J. Plant Soil Sci.*, **3(6):** 554-574.
- Al-Ghazi, A. K.A.M. (2013). The role of potassium in the tolerance of yellow maize plants (*Zea mays* L.) for drought and hydrogen peroxide stresses. PhD. thesis, College of Agriculture, University of Baghdad, Iraq.
- Al-Kateb, Y. M. (1988). Classification of Seed Plants, University of Baghdad, Ministry of Higher Education and Scientific Research.
- Ali, H.G., T.A. Issa and H.M. Jadan (1990a). Legumes crops. Higher Education presses in Mosul, Iraq.
- Ali, H. Ch., E. Talib and H.M. Jadaan (1990b). Legume crops Al-Hikma house for printing and publishing, Baghdad, Iraq. Pp 58-68.
- Anwar, H. and F.M. Rashad (2010). Supply response of Potato in Bang Ladesh; vector correction approach. *J. Appl. Sci.*, **10 (11):** 895-2010.ISSN 1812-5654.
- Bailly, C. (2004). Active oxygen species and antioxidants is seed biology. Seed Sci. Res., 14: 93-107.
- Bozso, Z., P.G. Ott, A. Szamari, A.C. Zelleng, G. Varga, E. Besenyei, E. Sardi, E. Banyai and Z. Klement (2005). Early detection of Bacterium-induced bas al resistance in

- Tobacco leaves with diaminobenzidine and dichloro flouorescein diacetate. *J. Phyt. Pathol.*, **153:** 596-607.
- Checseman, J.M. (2007). Hydrogen Peroxide and Plant Stress: A challenging Relationships. Global Science books. *Plant Stress*, **1(1)**: 4-15.
- Dat, J. V., S. Enabeele, E. Vranova, M. Van Montagu, D. Inze and F. Vanbreusegem (2000). *Dual action of the active oxyen species during plant stress responses cell. Mol. Life Sci.*, **57:** 779-795.
- Desikan, R., M.K. Cheng, A. Clarke, S. Golding, M. Sagi and R. Fluhr (2004). Hydrogen peroxide is a common signal for darkness and, ABA-induced stomatal closure in *pisum sativum*. Funct. Plant Biol., **31:** 913-920.
- El-Awadi, M. El., S.R. El-Lethyand K.G El-Rokiek (2014). Effect of the Two Antioxidants, Glutathione and Ascorbic acid on Vegetative Growth, yield and some biochemical changes in two wheat cultivars. *J. Plant Sci.*, **2(5)**: 215-221.
- Gharib, F. A. and A.Z. Hegazi (2010). Salicylic acid ameliorates germination, seedling growth, phytohormones and activity in bean (*Phaseolus vulgaris* L.) under cold stress. *J. Amer. Sci.*, **6(10):** 675 683.
- Gondim, F. A., E.G. Filho, C.F. Lacerda, J.T. Prisco, A.D. Neto and E.C. Marques (2010). Pretreatment with H_2O_2 in maize seeds: effect on germination and seedling acclimation to salt stress. *Baraz. J. Plant Physiol.*, **22(2)**: 103-112.
- Gupta, S. D. (2011). Reactive oxygen species and antioxidant in higher plants.CRC Press, En field, New Hampshire, USA.
- Hameed, A., S. Farooq, N. Iqbal and R. Arshad (2004). Influence of Exogenous application of hydrogen peroxide on root and seedling growth on wheat (*Triticum aestivum L.*). *Int. J. Agri. Biol.*, **6(2)**:-366-369.
- Hell, R. and L. Bergmann (1990). Y- Glutamylcysteine synthetase in higher plants: catalytic properties and subcellular localization. *Plant*, **180**: 603-612.
- Hung, S., C. Yu and C.H. Lin (2005). Hydrogen peroxide functions as a stress signal in plants. *Botanical Bulletin of Acadamia Sinica*, **46:** 1 10.
- Liao, M., I.R.P. Fillery and J.A. Patta (2004). Early vigorous growth is a major factor influencing nitrogen up take in wheat. *Funct. Plant Biol.*, **31:** 121 -129.
- Liu, Q., Z. Yu and G. Kuang (2004). Ethylene signal transduction in arabidopsis. *J. Plant Physiol. Mol. Boil.*, **30(3):** 241-250.
- Lin, J. and C.H. Kao (1998). Effect of Oxidative stress caused by hydrogen peroxide on senescence of rice leaves. *Bot. Bull. Acad. Sci.*, **39:**161-165.
- Locato, V., M.C. Depint and L. De Gara (2009). Different involvement of Mitochondrial plastidial and cytosolic ascorbate glutathione redox enzymesin heat shock responses Physiol. *Plant*, **135**: 296-306.
- Mamdouh, M.A. (1995). Glutathione regulation of glutathione S-transferase and peroxidase activity in herbicide treated

- Zea mays. Plant Physiol. Biochem., 33: 185-192.
- Mitter, R., S. Vanderauwera, M. Gollery and F. van Bareusegem (2004). Reactive oxygen gene network of plant. *Trends plant. Sci.*, 9110: 490-498.
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance, *trends Plant Sci.*, 405 -401.
- Ouda, S.A., T. El-Mesiry and M.S. Gaballah (2007). Effect of using stabilizing agents on increasing yield and water use efficiency in barly grown under water stress. *Austral. J. Apll. Sci.*, **1(4):** 571 -577.
- Potters, G., N. Horemans, S. Bellone, J. Caubergs, P. Trost, Y. Guisez and H. Asard (2004). Dehydroascorbate influences the plant cell cycle through a glutathione –Independent Reduction mechanism. *Plant Physiol.*, **134(4):** 1479-1487.
- Quen, L. J., B. Zhang, W.W. Shi and H.Y. Li (2008). Hydrogen peroxide in plants; A Versatile molecule of reactive oxygen species network. J. Intergr. Plant Biol., 50(1): 2-8.
- Rouhier, N., S.D. Lemaire and J.P. Jacquot (2008). The Role of Glutathione Photosynthetic organisms: emerging function for Glutaredoxins and Glutathionylation." *Annual Review of plant Biology*, **59:** 143-166.
- Saad, T. M., S.F. Hasan, B. Al-Rawi (2000). Response of the

- yield and its components and other characteristics to mung bean seedling rates. *Iraq J. Agric. Sci.*, **32(3):** 107-112.
- Sadak, M. SH., S.R. El-Lethy, M.A. Ahmed and K.G El-Rokiek (2014). Response of two cultivars of wheat plant foliar treatment of Glutathione. *Middle East j. Agric. Res.*, **3(4):** 732-737.
- SAS. (2010). Statistical Analysis system, User¹/₄s Guide.Statistical.Version 9.1th ed.SAS. *Inst. Inc. Cary.* N.C.USA.
- Shukla, R.S. and P.S. Chandel (2006). A text book of plant ecology Schand and Company Ltd. Ramnagar, New Delhi.
- Tauz, M. and G. Grill (2000). The role of Glutathione in stress Adaptation of plants. *Phyton (Horn, Austria)*, **40(3)**: 111-118
- Wendehenne, D., J. Dummer and D.F. Klessing (2004). Nitric oxide: a new player in plant signaling and defense responses. *Curr. Opin. Plant Boil.*, 7: 449-455.
- Yazdanpanah, S., A. Baghizadeh and F. Abbasi (2011). The interaction between drought stress and salicylic acid and ascorbic acid on some biochemical characteristics of *Satureja hortensis. Afric. J. Agric. Res.*, **6(4)**: 798-807.