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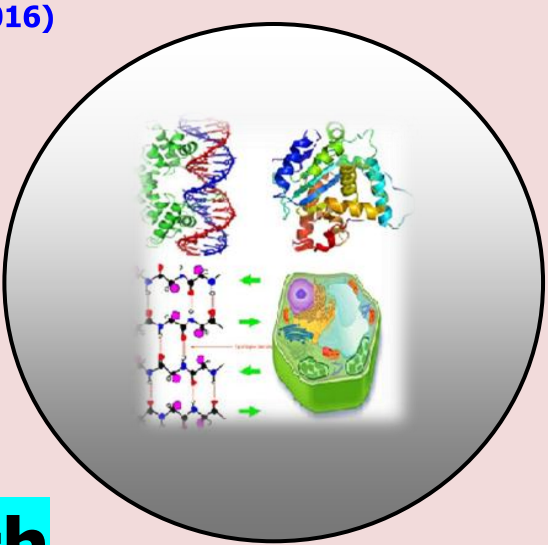

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RESEARCH PAPER

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Stimulating Properties of Components Glycyrrhizic Acid in Growth and Development of Wheat (*Triticum aestivum*)

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ABSTRACT

The effect of seed treatment before planting in different concentrations of zincglycyrrhizin acid (ZnGA) ($1x10^{-3}$ - $1x10^{-7}$ M) was studied on seed germination, seedling photosynthetic capacity, chlorophyll (Chl) contents, root activity and antioxidant enzymes activities of wheat. The result has indicated that seed germination rate was increased. The root length was elongated and root activity was increased. The seedling length was improved with increase in chlorophyll content, photosynthesis rate (Pn) and stomatal conductance (Gs). Antioxidant enzymes activities (SOD, CAT and POD) in leaves were increased. Variation in response of wheat to given zinc glycyrrhizin acid concentration was found. Effect of low concentration zinc glycyrrhizin acid was better than high concentration in the growth-regulatory activity among different zinc glycyrrhizin acid concentrations. The most suitable concentration found was $1x10^{-6}$M followed by $1x10^{-5}$M zinc glycyrrhizin acid. In conclusion, treatmenting with zinc glycyrrhizin acid can be used to improve wheat growth and development via modification in physiological and biochemical process.
Keywords: Zinc Glycyrrhizin Acid; Wheat; Seed Germination and Antioxidant Enzymes.

INTRODUCTION

Various exogenous compounds are applied to promote plants growth and induce abiotic resistance (Gokare, 2011, Rehman et all., 2014, Yasmeen et all., 2013). For example, foliar application of ascorbic acid, kinetin and glutamic acid promoted growth of Codiaeum variegatum L. plants (Azza et al., 2011)). Exogenous application of 20 mMproline as seed treatment increased growth, photosynthesis and antioxidant enzymes activities on Brassica juncea L. under natural conditions (Arif et al., 2013). Root application of 0.75 ppm selenium enhanced growth in mungbean (Phaseolusaureus Roxb.) through up-regulation of enzymes of carbohydrate metabolism (Malik et all., 2011). Biologically active compounds of steroids as signal molecules are one of exogenous compounds, which can exert plant growth and regulate gene expression to enhance defense reactions (Cécile et all., 2013). For example monoammonium salt of Glycyrrhizin acid could promote cotton seed germination (Akhunovet all., 2004) and (ZnGA) reported to regulate growth and development of wheat (Kushiev, et all.,2013). It is reported that the compounds of glycyrrhizin acid possesses multifunctional properties. Some components of glycyrrhizin acid inhibiting the growth and development of wheat rust disease, exhibits fungicidal activity (Khashimova et all., 2015). It is also reported to regulate plant growth and development (Lin et all.,2005) therefore, ZnGA has a strong potential application value in agriculture.

Reactive oxygen species are related to light-dependent events produced in plants even under optimal conditions. Therefore, photosynthetic cells are easily damaged by oxidative stress due to producing and consuming oxygen in metabolic processes. The superoxide radical (O. 2 -), singlet oxygen ($1O_2$) and H_2O_2 are major source of activated oxygen which injures the cellular components of proteins, nucleic acids and membrane lipids (Foyer, et all.,1994) It is reported that compounds of glycyrrizin acid induced accumulation of H_2O_2 in rice cells(Lin et all.,2005) and plants. It is involved in the oxidative burst and the induction of the reactive oxygen species (ROS) scavenging system (Agrawal et all., 2002)
Nonetheless, very little information is known about effects of compounds glycyrrizin acid on wheat seedlings growth and development. The present study evaluated the effect of different concentrations of some compounds of glycyrrhizin acid on wheat seed germination and seedling physiological responses.

MATERIALS AND METHODS

Plant Culture and Treatments

Wheat (*Triticum aestivum* L.) seeds were surface sterilized in 2% sodium hypochlorite solution for 10 min and rinsed three times with distilled water. Subsequently, rinsed seeds were soaked in 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125% glycyrrhizin acid compounds solution (supplied by Bioorganic chemistry institute of Academy Sciences of Uzbekistan) for 5 h. Seeds soaked in distilled water were taken as control. Germinated seeds were cultivated in a 500 ml beaker containing full Hoagland's nutrient solution and seedlings were grown in a controlled chamber. The average day/night temperature was kept at $25^{o}C/20^{o}C$, respectively, with a mean photoperiod of 12 h, relative humidity 80% and light intensity of 800 µmol m^{-2} s^{-1}. The Hoagland's nutrient solution was renewed every third day.

Growth Parameters

Daily counts of germinated seed were made. At 3-leaf stage, seedling height, root length and seedling fresh weight was recorded. Plant dry weight was obtained by oven-dried at $80^{0}C$ until a constant weight.

Root Activity

Root activity was measured by triphenyltetrazolium chloride (TTC) method (Steponkus, et all., 1967). Root samples of 0.5 g were fully immersed in solution containing 5 mL of 4% 2, 3, 5-triphenyl tetrazolium chloride (TTC) and 5 mL phosphate buffer. The mixtures were kept at 37℃ under dark conditions. After 2 h, 2 mL of 1 M H_2SO_4 was immediately added to mixtures to terminate the reaction. Roots were taken out and blotted with the filter paper, then fully grinded with 3-4 mL ethyl acetate. Red extract was exhaustively collected in test tube. The residue was washed 2 to 3 times with a small amount of ethyl acetate and graduated into test tube. Finally, a total of 10 mL was made with ethyl acetate. Color intensity measured at 485 nm. The reduction of TTC content was calculated according to standard curve.

Chlorophyll Content and Photosynthetic

Characters Fresh leaves 0.1 g were collected and extracted with 80% acetone and ethanol (v/v =1:1) for 24 h in the dark. Chlorophyll content followed spectrophotometrically as described by (Lichtenthaler et all., 1987). The photosynthetic rate (P_n) and stomatal conductance (Gs) were measured with a portable photosynthesis system (LI-6400, Lincoln, NE, USA).

Antioxidant Enzyme Activities

About 0.5 g samples was homogenized with 5 mL of extraction buffer (0.1 M phosphate buffer at pH 7.8), 0.1 mM EDTA, 1 g PVP). The homogenate were centrifuged at 10,000 × g for 15 min, and the supernatants were as crude to determine SOD, POD and CAT activity. Superoxide dismutase, CAT and POD activity were measured as described by Costa et al. (Costa et all., 2002), Cakmak and Horst (Cakmak et all.,1991) and Kochba et all.,1977) respectively. Protein concentration was estimated according to (Lowry, et all., 1951) using bovine albumin as standard.

Statistical Analysis

Each treatment was conducted with three replicates. All data were analyzed according to Duncan's multiple range test using the SPSS 14.0 software package.

RESULTS

Plant growth and biomass accumulation of compounds glycyrrhizin acid promoted wheat growth in terms of increased germination capacity, seedling fresh and dry weight and enhanced root length and seedling height (Table 1). Except for seeds treated by 1% ZnGA germination capacity reached above 95% than control with germination of 84%. There was apparent increase in seedling height when ZnGA concentrations varied from $1x10^{-6}$ to $1x10^{-5}$%. Seedling height treated by $1x10^{-6}$ZnGA increased by $1x10^{-5}$% of the control. Root length with $1x10^{-6}$% or $1x10^{-5}$% ZnGA application was significantly longer than control. Seedling fresh and dry weight treated by $1x10^{-6}$% ZnGA increased by 22 and 14% of the control, respectively.

Table 1.Effects of ZnGA on seed germination percentage and growth of wheat seedlings.

ZnGA concentration	Germination percentage	Seedlingheight	Root length	Freshweight	Dry weight
0	84.1d	13.13±0.55b	10.04±0.43b	2.004±0.32b	0.201±0.03 b
$5x10^{-7}$	92.1b	14.54±0.54b	10.98±0.31b	2.011±0.12b	0.208±0.01 b
$1x10^{-6}$	95.2a	18.34±0.33a	14.13±0.45a	2.213±0.22a	0.234±0.02 a
$1x10^{-5}$	91.3a	16.02±0.41a	14.04±0.64a	2.105±0.17a	0.228±0.02 a
$5x10^{-5}$	90.1ab	13.65±0.37b	11.12±0.23b	2.065±0.13b	0.219±0.05 b
$1x10^{-4}$	88.5bc	13.02±0.28b	10.34±0.53b	2.011±0.53b	0.213±0.03 b
$1x10^{-3}$	85.8cd	14.45±0.55b	09.43±0.54b	2.008±0.23b	0.205±0.03 b

Different letters indicate significant differences at $P < 0.05$

Table 2.Correlation coefficient (r) between seedling and chlorophyll (Chl) content, photosynthesis rate (Pn) and stomatal conductance (Gs).

Parameter	Chl content	Pn	Gs
Seedling height	0.83	0.81	0.85

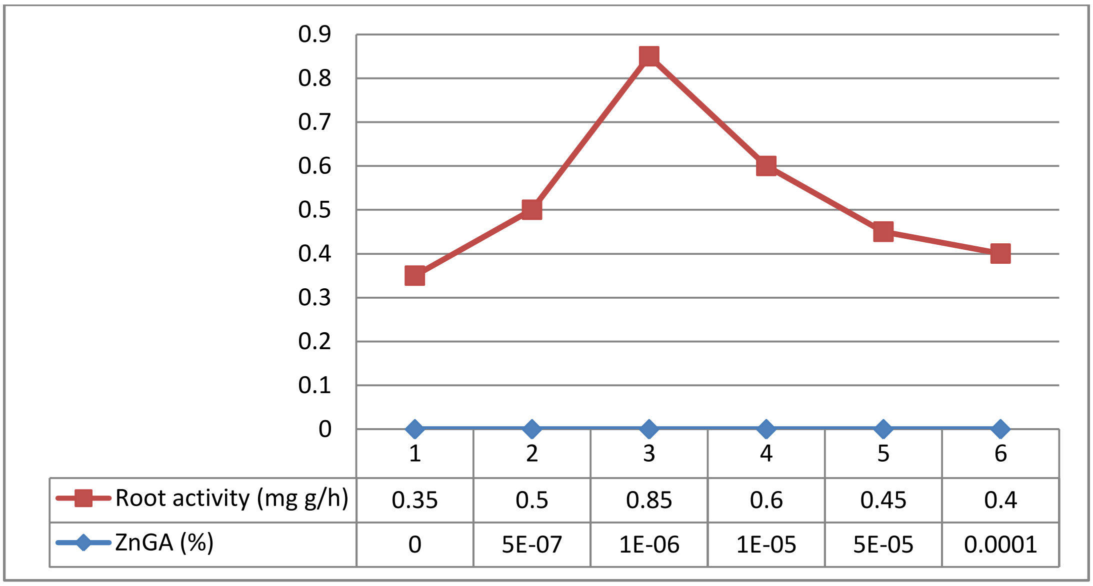
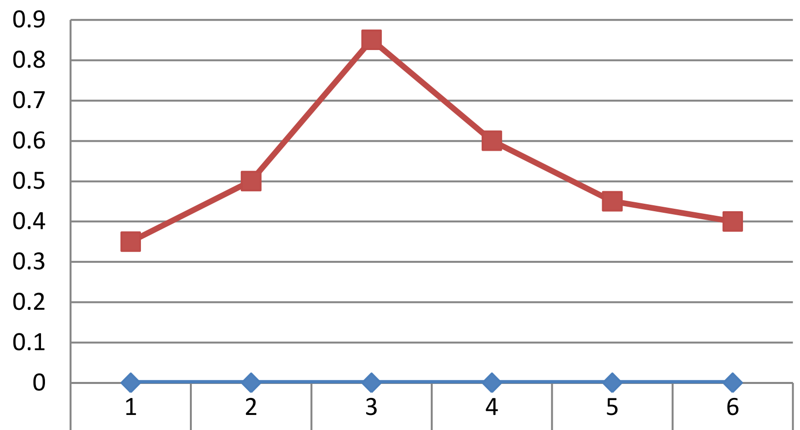


Fig. 1. Effects of ZnGA on wheat root activity. Each value represents the mean ± SD. (Different letters indicate significant differences at P < 0.05).

Root Activity

Triphenyltetrazolium chloride (TTC) indicated roots dehydrogenase activity as a proton receptor [15]. Root activity was significantly increased in comparison to control (Fig. 1) when ZnGA concentration varied from $1x10^{-6}$ to 0.5% and a maximum of increase was obtained at $1x10^{-6}$% ZnGA in root activity. Triphenyltetrazolium chloride (TTC) indicated roots dehydrogenase activity as a proton receptor.

Root activity was significantly increased in comparison to control (Fig. 1) when ZnGA concentration varied from $1x10^{-6}$ to 0.5% and a maximum of increase was obtained at $1x10^{-6}$ % ZnGA in root activity.
Chlorophyll Contents and Photosynthetic Characters.
Chlorophyll content of plants treated with ZnGA increased (Fig. 2). Chlorophyll content showed a significant difference (p＜0.05) in comparison to control when ZnGA concentration varied from $1x10^{-7}$ to 0.25%. Chlorophyll content of $1x10^{-6}$ % ZnGA was the highest with increase of 112% of the control. Chlorophyll content gradually decreased with ZnGA concentration varying from $1x10^{-6}$ to 1%. Positive correlation was found between seedlings and chlorophyll content (r=0.83) (Table 2).



	1	2	3	4	5	6
ZnGA (%)	0	5E-07	1E-06	1E-05	5E-05	0.0001
Chlorophyll content (mg g-1 FW)	0.35	0.5	0.85	0.6	0.45	0.4

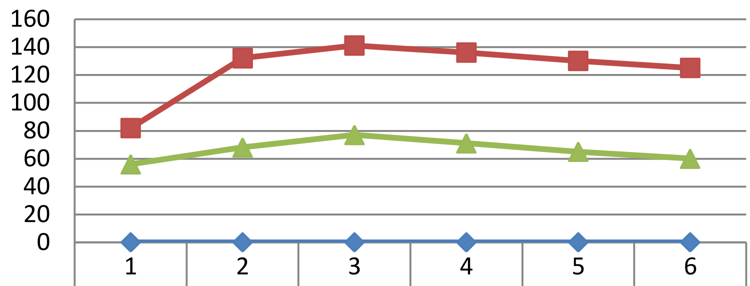
Fig. 2. Effects of ZnGAZnGA on Chl content of wheat leaves. Each value represents the mean ± SD. (Different letters indicate significant differences at P < 0.05).

ZnGA induced a remarkably increase in photosynthetic rate (Pn) (Fig. 3a). Significant differences were noted in Pn from $1x10^{-7}$ to 0.24% ($p < 0.05$). Photosynthetic rate with $1x10^{-6}$ % ZnGA reached the highest to 14.6 μ mol m^{-2} s^{-1}. Stomatal conductance (Gs) response tendency to ZnGA were consistent with Pn (Fig. 3b). A linear positive correlation existed between seedlings and Pn (r=0.83) and Gs (r=0.85), respectively (Table 2).

Antioxidant Enzymes
Activities Superoxide dismutase activity was higher than control under different ZnGA concentrations (Fig. 4a). A peak in SOD activity was observed at $1x10^{-6}$ % but decreased gradually afterward. Superoxide dismutase activity variation of different concentrations of ZnGA was not apparent. The response trend of CAT activity was similar to SOD activity (Fig. 4b). Except for $1x10^{-5}$ and 1% ZnGA, CAT activity of wheat leaves showed significant difference compared with control. Peroxidase activity (POD) increased when ZnGA concentrations was above $1x10^{-6}$ %, but gradually decreased below $1x10^{-6}$ %. Thus POD activity of $1x10^{-6}$ % ZnGA treatment showed the highest value (Fig. 4c).

DISCUSSION
ZnGA promoted wheat growth in terms of germination capacity, root length and seedling height (Table 1), and increase in root activity (Fig. 1). (Li, Y., et all., 2008) reported increased dry weight of B. napus, Zhang and Lin (Ma, et all., 2010) improved seedling vigor and uniform roots in Salvia miltiorrhiza Bge. Chlorophyll content of wheat seedlings treated with ZnGA increased (Fig. 2) and increased photosynthetic rate (Pn) and Gs were found (Fig. 3). Increase of Pn might promote wheat leaves to enhance its assimilation, which increased dry matter accumulation (Li, Y., et al., 2008) . Increased root activity (Fig. 1), chlorophyll content, Pn and Gs led to improve the growth and interestingly, increased biomass after all the ZnGA concentrations treatment was found (Table 1).

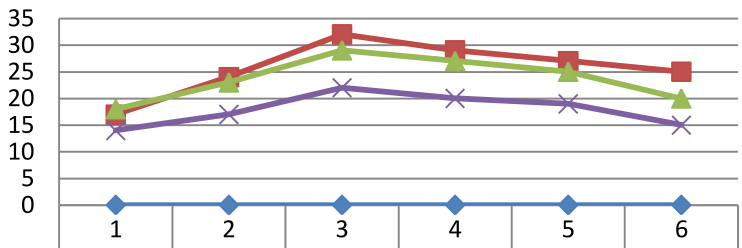
Increase of antioxidant enzymes activities might be beneficial to eliminate excess of reactive oxygen species. In present study, we also found that antioxidant enzymes activities were changed in wheat leaves treated by different concentrations of ZnGA. Superoxide dismutase (SOD) activity in wheat leaf significantly increased from $1x10^{-3}$ to $1x10^{-6}$ % ZnGA in comparison with control (Fig. 4a). Superoxide dismutase activity increased protection of wheat seedling from oxidant damage is consistent with our earlier findings in wheat under salinity suggesting the participation of SOD in the defense mechanism during seedlings development (Ma, L.J., et all., 2012). Catalase subsequently scavenged H_2O_2 produced by SOD against oxidative stress. Except for $1x10^{-7}$ and 1% ZnGA, CAT activity in wheat leaves showed significant difference compared with control after ZnGA treatment (Fig. 4b). Peroxidase is also considered to scavenge H_2O_2 and keep H_2O_2 balance in plant tissues. It was shown that POD activities of $1x10^{-6}$ and $1x10^{-5}$ % ZnGA increased in wheat leaves compared with control (Fig. 4c). Thus, increased CAT and POD activity helped wheat seedling development.



	0	5E-07	1E-06	1E-05	5E-05	0.0001
ZnGA (%)	0	5E-07	1E-06	1E-05	5E-05	0.0001
Pn (mmol m-2 g-1)	82	132	141	136	130	125
Gs (mmol m-2 g-1)	56	68	77	71	65	60

Fig. 3.Effects of ZnGA on photosynthesis rate and stomatal conductance of wheat leaves. Each value represents the mean ± SD.

(Different letters indicate significant differences at P < 0.05)



ZnGA (%)	0	5E-07	1E-06	1E-05	5E-05	0.0001
SOD activity (U mg-1 protein)	17	24	32	29	27	25
CAT activity (U mg-1 protein)	18	23	29	27	25	20
POD activity (U mg-1 protein)	14	17	22	20	19	15

Figure 4. Effects of ZnGA on antioxidant enzymes activities of wheat leaves. Each value represents the mean ± SD. (Different letters indicate significant differences at *P* < 0.05).

Effect of low concentration ZnGA was better than high concentration in the growth-regulatory activity which acts as an antioxidant and stimulated the plant growth. The character of ZnGA was similar to plant hormone IAA. Among different ZnGA concentrations, $1x10^{-6}$ % ZnGA was the most suitable to promote wheat seedling growth than $1x10^{-5}$ and 1%. Low concentration ZnGA had less effect on plant growth. Nonetheless, ZnGA functions as signals to promote the growth of wheat seedling. In conclusion, ZnGA effected growth and development, defense and other interactions of wheat plants with the environment via changing physiological and biochemical process. The most suitable ZnGA concentration was $1x10^{-6}$ % when wheat (*Triticum aestivum* L.) seeds were pre-soaked with different ZnGA levels.

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