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Effect of seed priming with hormonal combinations on morphological and biochemical attributes of maize seedlings

Efecto del tratamiento de semillas con combinaciones hormonales sobre los atributos morfológicos y bioquímicos de plántulas de maíz

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Abstract. Maize (*Zea mays* L., the third most important cereal crop after wheat and rice, is the major kharif (summer) crop of rainfed areas in Pakistan. The yield per hectare and total production of maize in Pakistan is low versus an exponentially increasing population. The objective of this work was to study the interactive effects of phytohormones [Gibberellic acid (GA), Sodium nitroprusside (SNP), Abscicic acid (ABA) and kinetin (KIN)] on morphological, physiological and biochemical parameters during the germination of maize seeds. Seeds were presoaked in different hormones, separately or in combinations. Priming with GA+SNP+ABA+KIN enhanced the total fresh (TFW) and dry weights (TDW) of the seedlings in comparison to the control. Similarly, exogenous application of GA+KN, GA+SNP, GA+SNP+ABA+KIN, SNP+KIN+ABA increased the oxidative enzymes [catalase (CAT) and ascorbate-peroxidase (APX)] of the maize seedlings. Sugar and proteins concentrations were increased in response to the exogenous application of GA+SNP+ABA+KN, KN+ABA, GA+SNP+KIN, SNP+KIN+ABA, GA+ABA and SNP+KIN. A combination of hormones for presoaking seeds is recommended to improve the germination characteristics which will ultimately enhance grain yield.

Keywords: Maize; Hormones; Proline.

Resumen. Maíz (*Zea mays* L.), el tercer cereal de cosecha después de trigo y arroz, es el principal cultivo de cosecha estival en Pakistán en áreas lluviosas. El rendimiento por hectárea y la producción total de maíz en Pakistán es baja. Al mismo tiempo, hay una población de seres humanos que se incrementa en forma exponencial. El objetivo de este trabajo fue estudiar los efectos interactivos de fitohormonas [ácido giberélico (GA), nitroprusiato sódico (SNP), ácido abscísico (ABA), y cinetina (KIN)] en parámetros morfológicos, fisiológicos y bioquímicos durante la germinación de semillas de maíz. Las semillas fueron sumergidas en diferentes hormonas, separadamente o en combinaciones. Este pretratamiento con GA+SNP+ABA+KIN mejoró los pesos frescos (TFW) y secos (TDW) totales de las plántulas en comparación al control. Similarmente, la aplicación exógena de GA+KN, GA+SNP, GA+SNP+ABA+KIN, SNP+KIN+ABA incrementó las enzimas oxidativas [catalasa (CAT) y ascórbico-peroxidasa (APX)] de las plántulas de maíz. Las concentraciones de azúcar y proteínas se incrementaron en respuesta a la aplicación exógena de GA+SNP+ABA+KIN, KN+ABA, GA+SNP+KIN, SNP+KIN+ABA, GA+ABA y SNP+KIN. Se recomienda sumergir las semillas en una combinación de hormonas para mejorar las características de la germinación lo cual finalmente mejorará el rendimiento de grano.

Palabras clave: Maíz; Hormonas; Prolina.

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INTRODUCTION

Maize (*Zea mays* L.), also known as corn, is an important cereal crop that belongs to the Poaceae family. It is an annual plant having an extensive fibrous root system. It is a large, grain plant initially cultivated in Mexico by indigenous people approximately 10000 years ago. Six major kinds of corn are identified which are sweet corn, dent corn, flour corn, flint corn, popcorn and pod corn. The leafy stalk of plant produces pollen grains, and ovuliferous ears or inflorescences that are fruit producing kernels. These kernels are frequently used as starch in cooking. In Pakistan, GDP share of maize is 0.4, and in the agriculture maize contribution is 2.2. During the year 2015-16 the area under maize crop cultivation was increased to 1144 thousand hectares, showing an increase of 0.2 percent to the last year's area of 1142 thousand hectares (GOP, 2017).

Maize is cultivated in mountainous areas of the country, particularly in Azad Jammu and Kashmir, and other northern areas because of the short duration of the growing season. The chilling conditions, less availability of water and snowfall decrease the growth period of cereals in such areas. Regardless of its tropical origin, it has become an extensively cultivated crop in the temperate areas as well. Many pre-sowing treatments have been used to increase maize production after overcoming various constraints. Seed priming is one of the strategies which can improve seed performance, besides providing earlier harmonized germination. It is a simple, low risk and low cost technique that is being recently used in the agricultural lands. It needs partial seed germination by soaking seeds in any solution or water for a specific time, and then re-drying them before the emergence of radicle (Azevedo Neto et al., 2004). This stimulates numerous metabolic processes involved in its early germination phases. It has been observed that seedlings of primed seeds emerge earlier, develop more energetically and show a better performance even in hostile conditions (Miraj, 2005).

MATERIALS AND METHODS

Prior to sowing, surface sterilized seeds (in 0.1% $HgCl_2$) were soaked in a 10^{-4} M solution of the following growth regulators: GA; SNP; KIN; ABA; GA+SNP; GA+KIN; GA+ABA; SNP+KIN; SNP+ABA; KIN+ABA; GA+SNP+ABA; GA+SNP+KIN; GA+KIN+ABA; SNP+KIN+ABA and GA+SNP+KIN+ABA during 4 hours. For conducting the experiment, 48 poly vinyl pots (250 mL volume) were arranged in a Completely Randomized Design. They were filled with sandy soil followed by sowing of three pre-soaked seeds per pot. Samplings were carried out after 15 days of sowing, and kept in a freezer at -50 °C for the further analysis. Seedlings were weighed with an electronic balance and measurements were expressed in grams. **S**eedlings were kept in an oven for 48 hours at 60 °C, in order to calculate the dry weight of the maize seedlings. The activity of the CAT enzyme was assessed according to Aebi (1984). Peroxidase activity was determined by following the method of Bartoli et al. (1999). Sugar concentration of the seedlings was estimated according to Dubois et al. (1956). Protein-Dye Binding assay was used to assess the protein concentration from the samples (Bradford, 1976).

Statistical analysis. Experiments were carried out according to a completely randomized design (CRD) with three replicates. Statistical analysis of data was done by using Statistix 8.1 version and analysis of variance was used to test the significance level at $\alpha \leq 0.05$. The least significant difference (LSD) test was used to compare the difference amongst treatment means (Steel et al., 1997).

RESULTS AND DISCUSSION

Total fresh weight (TFW). There was a significant response of different growth hormones on TFW of the maize seedlings during 2013-14 (Table 1). Maximum TFW (9.4 g) was obtained when seeds were treated with combination of all four growth hormones, significantly followed by the samples treated with GA+SNP+ABA. Minimum TFW (7.4 g) was obtained under control conditions, significantly followed by the samples treated with KIN.

There was a non-significant increase in TFW when seeds were treated with GA and SNP+KIN, but it was significant compared with that in the control. Interactions of KIN+ABA and GA+SNP and SNP showed non-significant increase in TFW, but it was significant compared with that in the control. TFW was increased (non-significantly) when seeds were treated with ABA and GA+ABA, but the increase was significant when compared to that in the control. Non-significant increases in TFW were observed when seeds were treated with GA+SNP+KIN, SNP+ABA and GA+KIN, respectively, but increases in TFW were significant in comparison to that in the control. Interaction of SNP+KIN+ABA and GA+KIN+ABA showed a significant increase in TFW compared to that in the control.

In the next year, significant responses of different growth hormones on TFW of the seedlings of maize were observed (Table 1). Maximum TFW (9.4 g) was achieved when seeds were treated with the combination of all four growth hormones, significantly followed by the samples treated with GA+SNP+ABA. Minimum TFW (7.4 g) was obtained in the control treatment, non-significantly followed by the samples treated with KIN. Total fresh weight was non-significantly increased when seeds were treated with GA and SNP+KIN, but it was significant compared with the control. Interactions of KIN+ABA, SNP and GA+SNP showed non-significant increases in TFW, but they differed from the control. There were non-significant increases in TFW when

Table 1. Interactive effects of hormones i.e GA, SNP, KIN and ABA on some aspects of maize seedlings.
Tabla 1. Efectos interactivos de hormonas (GA, SNP, KIN y ABA) en algunas características de plantas de maíz.

	Treatments	TFW (g/total germinated seedlings)	TDW (g/total germinated seedlings)	CAT activity (unit/g FW)	Proline (unit/ µmole/g FW)	Sugar (µg/g)	Protein (µg/g)
Years 2013-2014	H_2O	7.4 K	3.5 F	2.2 B	0.1 G	1.6 B	3.3 GH
	GA	7.8 I	3.8 DE	1.2 F	0.2 EFG	0.3 J	3.4 FG
	SNP	8.0 H	3.8 EF	0.8 H	0.3 BC	0.1 K	3.5 EF
	KIN	7.6 J	3.9 DE	1.4 E	0.3 BC	0.9 G	4.0 B
	ABA	8.1 G	3.9 DE	1.1 G	0.2EFG	1.6 B	3.3 H
	GA+SNP	8.0 H	3.9 DE	1.8 C	0.3 AB	1.3 D	3.6 D
	GA+KIN	8.4 E	4.1 DE	0.4 I	0.2 FG	1.5 C	3.9 C
	GA+ABA	8.2 FG	4.0 DE	1.7 D	0.3 C	1.0 FG	4.7 A
	SNP+KIN	7.8 I	3.8 DE	0.5 I	0.4 A	0.6 I	3.6 DE
	SNP+ABA	8.4 EF	4.5 BC	0.4 I	0.3 BC	1.6 B	3.6 DE
	KIN+ABA	7.9 E	3.7 EF	0.7 H	0.2 D	1.5 C	3.5 EF
	GA+SNP+KIN	8.3 H	4.2 CD	1.2 F	0.2 D	1.0 EF	3.8 C
	GA+SNP+ABA	9.2 B	3.8 DEF	0.7 H	0.3 BC	0.4 J	3.3 H
	GA+KIN+ABA	8.8 C	4.5 C	1.9 C	0.2 DE	2.0 A	3.9 BC
	SNP+KIN+ABA	8.6 D	4.8 AB	1.2 F	0.2 DEF	1.1 E	3.8 C
	GA+SNP+KIN+ABA	9.4 A	4.9 A	2.7 A	0.3 BC	0.7 H	4.0 B
Years 2014-2015	H_2O	7.4 K	3.5 I	2.2 B	0.1 F	1.6 C	3.3 HI
	GA	7.8 J	3.5 I	1.2 H	0.1 F	0.3 K	3.4 GH
	SNP	8.0 GH	3.7 GHI	0.8 I	0.3 BCD	0.1 L	3.5 G
	KIN	7.5 K	3.9 EFG	1.4 F	0.3 AB	0.8 H	4.0 BC
	ABA	8.1 FG	3.8 FGH	1.1 H	0.2 F	1.6 BC	3.3 I
	GA+SNP	7.9 HI	4.3 CD	1.8 D	0.3 AB	1.4 E	3.6 E
	GA+KIN	8.4 E	4.1 DE	0.4 L	0.1 F	1.4 DE	3.8 D
	GA+ABA	8.2 F	3.9 EFG	1.7 E	0.3 D	1.0 G	4.7 A
	SNP+KIN	7.8 IJ	3.6 HI	0.5 K	0.4 A	0.6 I	3.6 E
	SNP+ABA	8.3 E	4.6 AB	0.4 L	0.3 BCD	1.7 B	3.6 EF
	KIN+ABA	7.9 H	3.6 HI	0.7 IJ	0.2 E	1.5 D	3.5 FG
	GA+SNP+KIN	8.3 E	3.9 EFG	1.2 G	0.2 E	1.0 FG	3.9 D
	GA+SNP+ABA	9.2 B	4.2 D	0.7 J	0.3 CD	0.4 J	3.3 I
	GA+KIN+ABA	8.8 C	4.1 DEF	1.9 C	0.2 E	2.0 A	3.9 CD
	SNP+KIN+ABA	8.7 D	4.9 A	1.2 G	0.1 F	1.1 F	3.9 D
	GA+SNP+KIN+ABA	9.4 A	4.5 BC	2.7 A	0.3 BC	0.8 H	4.0 B

seeds were treated with ABA and GA+ABA, but increases were significant compared to the control. Non-significant increases in TFW were observed when seeds were treated with GA+SNP+KIN, SNP+ABA and GA+KIN, but these increases differ from the control. The interaction of SNP+KIN+ABA and GA+KIN+ABA showed significant increases in TFW compared to values on the control.

Total Dry Weight (TDW). There was a significant response of different growth hormones on TDW of the seedlings of maize during 2013-14 (Table 1). Maximum TDW (4.9 g) was obtained when seedlings were treated with the combination of all four growth hormones, non-significantly followed by the samples treated with GA+SNP+ABA. Minimum TDW (3.5 g) was obtained under control conditions, non-significantly followed by the samples treated with KIN+ABA.

There was a non-significant increase in TDW when seeds were treated with SNP and GA+SNP+ABA. Interactions of SNP+KIN, GA, ABA, KIN, GA+SNP, GA+ABA and GA+KIN showed non-significant increases in TDW, but increases were significant in comparison with the control. TDW was increased non-significantly when seeds were treated with GA+SNP+KIN, GA+KIN+ABA and SNP+ABA, but these increases were significant compared with the control.

Significant responses of different growth hormones on TDW of the seedlings of maize were observed during 2013-14 (Table 1). Maximum TDW (4.9 g) was shown when seedlings were treated with SNP+KIN+ABA, non-significantly followed by the samples treated with SNP+ABA. Minimum TDW (3.5 g) was obtained under control conditions, non-significantly followed by the samples treated with GA.

There was a non-significant increase in TDW when seeds were treated with SNP alone and its combination with KIN and ABA. Interactions of ABA, GA+ABA, GA+SNP+KIN, KIN and GA+KIN+ABA respectively showed non-significant increase in TDW, but significant to that in the control. TDW was increased non-significantly when seeds were treated with GA+KIN, GA+SNP+ABA and GA+SNP, and significant to that of control. Priming of four growth hormones showed significant increase in TDW compared to that in the control.

Catalase (CAT) activity. There was a significant response of different growth hormones on CAT activity of the seedlings of maize during 2013-14 (Table 1). Maximum CAT activity (5.3 unit/g FW) was obtained in those seeds of maize pretreated with GA+KIN, significantly followed by the samples treated with ABA. Minimum CAT activity (1.0 unit/g FW) was observed upon the treatment of the seeds with the combination of SNP+KIN+ABA.

CAT activity was non-significantly decreased by the treatment of the seeds with GA+SNP+ABA and GA, but differences were significant with respect to the control. Non-significant increase in CAT activity was observed when seeds were treated with the combination of all four growth hormones and GA+SNP+KIN. The seeds pretreated with KIN+ABA also exhibited significant increases in CAT activity compared to that under control conditions.

Non-significant increases in CAT activity were achieved when seeds were soaked in SNP and its combination with KIN. Priming of seeds with GA+KIN+ABA significantly enhanced the CAT activity in comparison to that in the control. There was a non-significant increase in CAT activity when seeds were treated with GA+ABA and KIN, but it was significant in comparison to that in the control.

During the next year, there was also a significant response of different growth hormones on CAT activity of the seedlings of maize (Table 1). Maximum CAT activity (5.3 unit/g FW) was obtained in those seeds of maize pretreated with GA+KIN, significantly followed by the samples treated with ABA. Minimum CAT activity (1.0 unit/g FW) was observed when seeds were treated with the combination of SNP+KIN+ABA.

There was a non-significant decrease in CAT activity by the treatment of the seeds with GA+SNP+ABA and GA, but it was significant in comparison to that in the control. Non-significant increase in CAT activity was observed when seeds were treated with the combination of all four growth hormones and GA+SNP+KIN. The seeds pretreated with KIN+ABA exhibited a significant increase in CAT activity in comparison to that in the control. There was a non-significant increase in CAT activity when seeds were primed with SNP and its combination with KIN. Treatment of the seeds with GA+KIN+ABA significantly enhanced the CAT activity in comparison to that in the control. There was a non-significant increase in CAT activity when seeds were treated with GA+ABA and KIN, but these increases were significant in comparison with the control. Interaction of SNP+ABA and GA+SNP also showed significant increases in CAT activity with respect to those in the control.

Ascorbate peroxidase (APX) activity. There was a significant response of different growth hormones on APX activity of the seedlings of maize during 2013-14 (Table 1). Maximum APX activity (3.6 unit/g FW) was observed when seeds were treated with GA+SNP, non-significantly followed by the samples treated with SNP+KIN. Minimum APX activity (0.2 unit/g FW) was noted when seeds were treated with GA+ABA. APX activity decreased non-significantly when seeds of maize were treated with GA+SNP+ABA and GA+SNP+KIN, but it was significant in comparison to the control.

Seeds treated with SNP+ABA showed a significant decrease in APX activity in comparison to that in the control. Interaction of all four hormones and GA+KIN+ABA also showed a non-significant decrease in the APX activity. There was a non-significant increase in APX activity when seeds were treated with ABA and GA, but it was significant compared to that in the control. APX activity was increased non-significantly when seeds were treated with SNP, KIN+ABA and SNP+KIN+ABA, but the increase was significant in comparison to that in the control. Treatment of seeds with GA+KIN and KIN increased the APX activity, but the increase was not significant in comparison to that in the control.

During 2014-15, significant responses of different growth hormones were shown on APX activity of the seedlings of maize (Table 1). Maximum APX activity (3.6 unit/g FW) was observed when seeds were treated with GA+SNP. Although non-significantly, it was followed by the samples treated with SNP+KIN. Minimum APX activity (0.3 unit/g FW) was noted when seeds were treated with GA+ABA, significantly followed by those treated with GA+SNP+ABA.

Seeds treated with GA+SNP+KIN and SNP+ABA showed a significant decrease in APX activity with respect to the control. Interaction of all four hormones and GA+KIN+ABA also showed a non-significant decrease in the APX activity. There was a non-significant increase in APX activity when seeds were treated with GA and ABA, but it was significant as compared to that in the control. APX activity was increased non-significantly when seeds were treated with SNP, KIN+ABA, SNP+KIN+ABA and GA+KIN, but the increase was significant compared to that in the control. Treatment of seeds with KIN increased significantly the APX activity in comparison to that in the control.

There was a significant response of different growth hormones on proline concentrations of the seedlings of maize during 2013-14 (Table 1). Maximum proline concentrations (0.4 unit/μmole/g FW) were obtained when seeds were treated with SNP+KIN, but they were followed non-significantly by the samples treated with GA+SNP. Minimum proline concentrations (0.1 unit/μmole/g FW) were obtained in the control, followed non-significantly by the samples treated with GA+KIN.

There was a non-significant increase in proline concentrations when seeds were treated with ABA and GA but it did not differ from that in the control. Interactions of SNP+KIN+ABA, GA+KIN+ABA, KIN+ABA and GA+SNP+KIN showed non-significant increases in proline concentrations, but these increases were significant compared to those in the control. Interaction of GA+ABA, SNP+ABA, SNP, GA+SNP+KIN+ABA, GA+SNP+ABA and KIN showed non-significant increases in proline concentrations. but they did differ from those in the control.

Different growth hormones showed a significant response on proline concentrations of the seedlings of maize during the next year 2014-15 (Table 1). Maximum proline concentrations (0.4 unit/μmole/g FW) were observed when seeds were treated with SNP+KIN, non-significantly followed by the samples treated with GA+SNP. Minimum proline concentrations (0.1 unit/μmole/g FW) were obtained in the control, but they did not differ from those in the samples treated with GA+KIN.

Non-significant increases in proline concentrations were achieved when seeds were treated with SNP+KIN+ABA, ABA and GA. Interaction of GA+SKIN+ABA, GA+SNP+KIN and KIN+ABA showed non-significant increases in proline concentrations but these increases did differ from those in the control. Proline concentrations were increased non-significantly when seeds were primed with GA+ABA, GA+SNP+ABA, SNP and SNP+ABA, but these increases were significant compared to those in the control.

Interaction of all four growth hormones and KIN showed non-significant increases in proline concentrations but they were significant compared to those in the control.

Sugar concentrations. There was a significant response of different growth hormones on sugar concentrations of the seedlings of maize during 2013-14 (Table 1). Maximum sugar concentrations (2.0 μg/g) were observed when seeds were treated with GA+KIN+ABA, significantly followed by the samples treated under control conditions. Minimum sugar concentrations (0.3 μg/g) were noted when seeds were treated with GA, non-significantly followed by the samples treated with GA+SNP+ABA and SNP.

Sugar concentrations decreased significantly when seeds of maize were treated with SNP+KIN and the combination of all four growth hormones with respect to the control. There was a non-significant decrease in sugar concentrations when seeds were treated with KIN and GA+ABA, but it was significant to that in the control. Interaction of GA+SNP+KIN and SNP+KIN+ABA showed non-significant decrease in sugar concentrations to that of control. Sugar concentrations decreased significantly when seeds were primed with GA+SNP in comparison to that in the control. Non-significant decreases in sugar concentrations were observed when seeds were treated with KIN+ABA and GA+KIN, but decreases were significant in comparison to those in the control. Treatment of seeds with ABA and SNP+ABA enhanced the sugar concentrations non-significantly, but increases were significant in the control.

There was a significant response of different growth hormones on sugar concentrations of the seedlings of maize during 2014-15 (Table 1). Maximum sugar concentrations (2.0 μg/g) were observed when seeds were treated with GA+KIN+ABA, significantly followed by samples treated with SNP+ABA. Minimum sugar concentrations (0.3 μg/g) were observed when seeds were treated with GA, non-significantly followed by samples treated with GA+SNP+ABA and SNP.

Sugar concentrations were decreased significantly when seeds of maize were treated with SNP+KIN in comparison to that in the control. There was a non-significant decrease in sugar concentrations when seeds were treated with the combination of all four growth hormones and KIN, but these decreases were significant to those in the control. Interaction of GA+ABA GA+SNP+KIN and SNP+KIN+ABA showed non-significant decreases in sugar concentrations, but they were significant to those in the control. Non-significant decreases in sugar concentrations were observed when seeds were treated with GA+SNP, GA+KIN and KIN+ABA, but they were significant to those in the control.

Treatment of seeds with ABA enhanced the sugar concentrations non-significantly in comparison to that in the control.

Protein concentrations. There were significant responses of different growth hormones on protein concentrations of seedlings of maize during 2013-14 (Table 1). Maximum protein concentrations (4.7 μg/g) were observed when seeds were treated with GA+ABA, significantly followed by samples treated with a combination of all four growth hormones and KIN. Minimum protein concentrations (3.3 μg/g) were obtained when seeds were treated with ABA, non-significantly followed by the samples treated with GA+SNP+ABA.

Non-significant increases in protein concentrations were observed when seeds were treated with GA, SNP, KIN+ABA, SNP+ABA, SNP+KIN and GA+SNP, but increases were significant in comparison to the control. Protein concentrations were increased non-significantly when seeds were primed with GA+SNP+KIN, SNP+KIN+ABA, GA+KIN and GA+SNP+KIN, but the increases were significant when compared with the control. Significant responses of different growth hormones on protein concentrations of the seedlings of maize were observed during 2014-15 (Table 1). Maximum protein concentrations (3.3 μg/g) were obtained when seeds were treated with GA+SNP+ABA, non-significantly followed by the samples treated with ABA.

Non-significant increase in protein concentrations was observed when seeds were treated with GA, SNP and KIN+ABA, but the increases were significant compared with the control. There was a non-significant increase in protein concentration when seeds were treated with SNP+ABA, SNP+KIN and GA+SNP, but these increases were significant compared with the control. Protein concentrations were increased non-significantly when seeds were primed with GA+KIN, GA+SNP+KIN, SNP+KIN+ABA and GA+KIN+ABA, but the increases were significant compared with the control.

DISCUSSION

Phytohormones are not only required for plant growth and development, but they also play a pivotal role in regulating plants' responses to different conditions. In all morphological parameters, GA, ABA and its combination with other hormones increased the growth of plants.

Biomass is an important parameter to measure the plant growth. In the present study, increases in biomass were observed by priming. Plant fresh weight and dry weight were increased due to hormonal priming (Sabir et al., 2013). The combination of GA, CK, auxin and IAA increased the biomass production of plants (Cabello Conejo, 2015). Exogenous application of ABA increased the weight in barley (Kadlecová et al., 2000). However, increase in dry weight of *K. virginica* seedlings was observed due to NO priming. The present study showed increases in biomass on primed samples in comparison to those in the control. These results are similar to many previous studies. Foliar application of IAA and KIN was observed to increase plant weights.

Environmental stresses result in the generation of reactive oxygen species (ROS) in plants. ROS after accumulation in the cell cause oxidation of chlorophyll, protein and nucleic acids, etc. Cells have complicated defense systems which can be non-enzymatic (such as phenolic compounds, ascorbic acid (ASH), amino acids, alkaloids, and α-tocopherol) or enzymatic systems (CAT, superoxide dismutase and APX) which have the ability to scavenge the generated ROS (Gill & Tuteja, 2010). Priming of seeds with hormones has the ability to increase enzyme activities compared to those in control seeds (Tabatabaei, 2013). The results of our study revealed that priming treatments (i.e GA, SNP, ABA and KIN) caused a significant improvement in antioxidant enzyme activities (CAT, POD, APX and PPO) of maize in comparison to the control. This is in accordance with Rouhi et al. (2012), who reported the increase in free radical scavenging enzyme activities by priming.

In present study, increases in CAT were observed on maize seedlings by priming compared to the control. Increasing activity of CAT in maize suggests a potential process to scavenge H_2O_2 that in turn decrease the oxidative injury on maize (Gangwar et al., 2011). Peroxidase takes part in decomposition of H_2O_2 by oxidation of co-substrates, such as phenolic compounds or antioxidants. Researchers proved that under drought stress conditions, the scavenging system of hydrogen peroxide was more aggressively induced in different wheat genotypes (Hameed et al., 2011).

In the present study maximum proline concentrations were obtained when seedlings were treated with SNP+KIN. This is in agreement with the results of many previous researchers (Li et al., 2008; Grunwald et al., 2009; Sadak & Orabi, 2015). They observed increases of proline concentrations in wheat by SNP. NO scavenger cPTIO reversed the effects of SNP on proline accumulation in wheat leaves. Increased proline levels have an important role in the protection of enzymes that are involved in the antioxidant system. In *Vigna* plants proline concentrations were found to be increased by KIN alone or in combination with spermine (Alsokari, 2011).

Sugars are the regulatory molecules that play an essential role in the life cycle of plants (Weber et al., 2005). Sugar accumulation has important roles in energy preservation and osmoregulation (Cha-um & Kirdmanne, 2007; Kaplan et al., 2007). In the present study, highest sugar concentrations were obtained when seeds were treated with SNP+KIN+ABA. This work is in accordance with previous studies which reported that the application of KIN enhanced the sugar concentrations in plants. There were also increases in soluble sugars in cucumber leaves by exogenous SNP treatments. This is due to enhancement of sugar accumulation by ABA treatment which was due to the rise in the SOX activity (Liu et al., 2011).

The protein synthesis represents distinctive patterns during the germination process, and phytohormones (GA+ABA) play important roles in repressing or inducing its synthesis. This is similar to our results in which maximum protein concentrations were obtained from seedlings treated with GA+ABA. There are reports of increases in protein concentrations of plants after treatments with GA_3 (Khan et al., 2011).

Treatment of plants with GA_3 increased protein concentration compared with untreated plants (Spoel et al., 2009). ABA application on *Pisum sativum* revealed quantitative and qualitative changes in proteins by the disappearance or appearance of some bands (Latif, 2014).

REFERENCES

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology* 105: 121-126.

Alsokari, S. (2011). Synergistic effect of kinetin and spermine on some physiological aspects of seawater stressed *Vigna sinensis* plants. *Saudi Journal of Biological Sciences* 18: 37-44.

Azevedo Neto, ADd, J.T. Prisco & J. Enéas-Filho (2004). Effects of salt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. *Brazilian Journal of Plant Physiology* 16: 31-38.

Bartoli, C.G., M. Simontacchi, E. Tambassi, J. Beltrano, E. Montaldi & S. Puntarulo (1999). Drought and watering dependant oxidative stress: effect on antioxidant content in *Triticum aestivum* leaves. *Journal of Experimental Botany* 50: 375-383.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Cabello Conejo, M.I. (2015). Nickel hyperaccumulating plants: strategies to improve phytoextraction and a characterisation of *Alyssum* endemic to the Iberian Peninsula. Tesis de doctorado, Departamento de Edafoloxía e Química Agrícola, Universidade de Santiago de Compostela, 23 de enero de 2015.

Cha-um, S. & C. Kirdmanne (2007). Minimal growth in vitro culture for preservation of plant species. *Fruit Veg Cer Sci Biotechnol* 1: 13-25.

Dubois, M., K.A. Gilles & J.K. Hamilton (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350-356.

Gangwar, S., V.P. Singh & S.M. Prasad (2011). Differential responses of pea seedlings to indole acetic acid under manganese toxicity. *Acta Physiologiae Plantarum* 33: 451-462.

Gill, S.S. & N. Tuteja (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48: 909-930.

Grunwald, U., W. Guo & K. Fischer (2009). Overlapping expression patterns and differential transcript levels of phosphate transporter genes in arbuscular mycorrhizal, Pi-fertilised and phytohormone-treated *Medicago truncatula* roots. *Planta* 229: 1023-1034.

GOP (2017). Economic Survey of Pakistan. Ministry of Food and Agriculture, Federal Bureau of Statistics Islamabad, Pakistan. 22 p.

Hameed, M., M. Ashraf & F. Al-Quriany (2011). Medicinal flora of the cholistan desert: a review. *Pakistan Journal of Botany* 43: 39-50.

Kadlecová, Z., M. Faltus & I. Prášil (2000). Relationship between abscisic acid content, dry weight and freezing tolerance in barley cv. Lunet. *Journal of Plant Physiology* 157: 291-297.

Kaplan, F., J. Kopka & D.Y. Sung (2007). Transcript and metabolite profiling during cold acclimation of *Arabidopsis* reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. *The Plant Journal* 50: 967-981.

Khan, A.L., M. Hamayun & Y-H Kim (2011). Ameliorative symbiosis of endophyte (*Penicillium funiculosum* LHL06) under salt stress elevated plant growth of *Glycine max* L. *Plant Physiology and Biochemistry* 49: 852-861.

Latif, H.H. (2014). Physiological responses of *Pisum sativum* plant to exogenous ABA application under drought conditions. *Pakistan Journal of Botany* 46: 973-982.

Li, A-L, Y-F Zhu & X-M Tan (2008). Evolutionary and functional study of the CDPK gene family in wheat (*Triticum aestivum* L.). *Plant Molecular Biology* 66: 429-443.

Liu, X., L. Wang & L. Liu (2011). Alleviating effect of exogenous nitric oxide in cucumber seedling against chilling stress. *African Journal of Biotechnology* 10: 4380-4386.

Miraj, G. (2005). Effect of phosphorus and zinc priming on germination, seedling growth and yield of maize. M.Sc. (Hons.) Thesis. Dept. Agric. Chem., NWFP Agricultural University, Peshawar, Pakistan.

Rouhi, H., M. Aboutalebian & S. Moosavi (2012). Change in several antioxidant enzymes activity of Berseem clover (*Trifolium alexandrinum* L.) by priming. *International Journal of AgriScience* 2: 237-243.

Sabir, F., S. Mishra & R.S. Sangwan (2013). Qualitative and quantitative variations in withanolides and expression of some pathway genes during different stages of morphogenesis in *Withania somnifera* Dunal. *Protoplasma* 250: 539-549.

Sadak, M.S. & S.A. Orabi (2015). Improving thermo tolerance of wheat plant by foliar application of citric acid or oxalic acid. *International Journal of ChemTech Research* 8: 333-345.

Spoel, S.H., Z. Mou & Y. Tada (2009). Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. *Cell* 137: 860-872.

Steel, R., J. Torrie & D. Dieky (1997). Principles and procedures of statistics. 3rd (Edition) McGraw Hill Book Co Inc. New York, USA.

Tabatabaei, S. (2013). Effect of osmo-priming on germination and enzyme activity in barley (*Hordeum vulgare* L.) seeds under drought stress conditions. *Journal of Stress Physiology & Biochemistry* 9.

Weber, H., L. Borisjuk & U. Wobus (2005). Molecular physiology of legume seed development. *Annual Review Plant Biology* 56: 253-279.