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## Drought mitigation potential of *Azospirillum* inoculation in canola (*Brassica napus*)

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### Summary

*Azospirillum* is considered to be a most effective Plant Growth Promoting Rhizobacteria (PGPR), which is responsible for various modifications in plants to cope with stress conditions. Therefore, the present research was planned to evaluate the effect of *Azospirillum lipoferum* (GQ 255949) inoculation on growth, biochemical, yield attributes of canola grown under drought conditions. Two different modes of inoculation were used; i.e., inoculation of seeds directly and exposure of planted seed in the rhizosphere. Drought stress was imposed at flowering stage. *Azospirillum* seed inoculation was helped mitigate stress effects by improving germination percentage up to 12.49%. Root area was increased up to 18.5% and 11.38% with seed and rhizosphere inoculation in drought stress respectively. Chlorophyll contents and water potential were increased 12.21%, and 11.0% in seeds inoculated under drought conditions. Superoxide dismutase activity was decrease up to 24.6% and 12.5% in seed and rhizosphere inoculated plants under well watered conditions. Seed inoculation was most effective, as number of seeds per pod and seed weight per plant was significantly increased up to 25%, and 14.28% as compared to the control. In conclusion, *Azospirillum* can mitigate deleterious effects of drought stress in canola under water deficiency conditions.

**Key words:** PGPRs, Canola, drought

### Introduction

Drought stress is scarcity of available water in soil, which is necessary for optimum growth and reproduction of plants (LICHTFOUSE, 2010). Reduced water supply is one of the most important factors which is responsible for reduction in agricultural productivity (MAHMOOD et al., 2009; ASHRAF, 2010). Deficiency of water causes injurious effects on plants by reducing growth, decreasing nutrient intake and changing water status of plants (ALI and ASHRAF, 2011; SHAHBAZ et al., 2011a). Reduced water supply also causes a decline in leaf development and can alter stomatal conductance (QADERI et al., 2006). In addition to morphology, physiological processes, inhibited by water stress are photosynthesis, cell turgidity and cell growth (TAHIR et al., 2007). In short, every feature of plant growth is affected by water stress including anatomy, morphology, biochemistry, plant physiology and yield (JONES et al., 2003; HAFIZ et al., 2004).

Canola (*Brassica napus* L.) oil productivity stands third after soybean and palm oil crops in the world. It produces as much as 14.7% of the vegetable edible oil and has a high content of unsaturated fatty acids (YASARI et al., 2008). Compared to cereal crops, canola is ranked fifth after wheat, maize, rice and cotton (CARDOZA and STEWART, 2003). Canola oil is a premium cooking oil that has less than 2% erucic acid and is low in saturated fatty acids. It also is rich in mono – poly unsaturated fatty acids which helps to decrease cholesterol level (CARVALOH et al., 2006; OMIDI et al., 2010). Drought is one of the important stress factor which is responsible for reducing production of canola in semi-arid regions of the world.

Almost, 17 to 70% yield reductions have been recorded due to drought (NASRI et al., 2007).

Plant growth-promoting rhizobacteria (PGPR) are able to promote growth and yield of plants under stress conditions. Inoculation of these microorganisms provides higher crop yield without interfering with natural processes in the ecosystem (THAKORE, 2006). During the last two decades, various bacteria (i.e., *Azotobacter* sp., *Azospirillum* sp., *Acetobacter* sp., *Bacillus*, *Pseudomonas* sp.) are used for plant growth promotion under various prevailing biotic and abiotic stresses (TURAN et al., 2006). *Azospirillum* is one of the very effective Plant Growth Promoting Rhizobacteria (PGPR) which act as a general root colonizer to improve crop growth and yield up to 5 to 30%. *Azospirillum* offer inexpensive and easy application while providing minerals, and phytohormones as well as fixed nitrogen, and reduce the synthesis of ethylene thereby Increasing yield (YASARI et al., 2008). *Azospirillum* spp inoculation can improve tolerance to water stress, improve the growth of plants in arid and semiarid regions (ILYAS and BANO, 2010). Various studies have documented the role of *Azospirillum* in improving growth and yield of canola (YASARI et al., 2008; BANIAGHIL et al., 2013). The impact of water deficit conditions on plant physiology has been studied for many years. The present study was conducted in order to evaluate the effect of *Azospirillum* inoculation on growth and yield of canola. Further, physiological and biochemical responses of canola under drought stress and the role of *Azospirillum* in mitigation of drought stress in canola were also studied.

### Materials and methods

To evaluate the effect of *Azospirillum* inoculation on growth and yield parameters of canola plants under drought stress a pot experiment was conducted in a green house, Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi. Two canola varieties (i.e., Rainbow and Con II) were collected from the National Agricultural Research Center (NARC), Islamabad. Seeds of both varieties were surface sterilized in 0.1% mercuric chloride solution for 5 minutes, then washed thoroughly with distilled water. Inoculum was prepared by inoculating 24 h old culture of *Azospirillum* in LB media and kept for 72 h on a shaker, then centrifuged at 10,000 RPM for 10 min. The pellet was diluted to 100 ml with distilled water and the supernatant was discarded. Optical density was calculated to be one. Six hour sterilized seeds were soaked in the inoculum and the heat killed inoculum was prepared by autoclaving the inoculum. Six seeds per pot were sown from 19 November 2013 to 10 February 2014. For rhizosphere inoculation live cultures were added into soil after sowing the sterilized seeds. Heat killed bacteria also were inoculated onto seeds. Each treatment was replicated three times. Plant drought stress was imposed at the flowering stage by restricting water for ten days along with a control for each inoculation.

### Germination analysis

For germination analysis under drought conditions, inoculated and uninoculated seeds were placed in a set of petri plates, which were

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provided with moist filter paper. Seeds were monitored daily for mean daily germination. The seed was considered germinated when its radical emerged 5 mm. Un-inoculated seeds were sown in another set of plates. Following parameters of germination were evaluated.

### Germination percentage

For each treatment, germination percentage was estimated by using the following formula:

$$\text{Germination \%} = \frac{\text{Total seeds germinated}}{\text{Total no of seeds planted}} \times 100$$

### Germination index

Using the following formula (ISTA, 2005), germination index was calculated.

$$\text{Germination Index} = n/d$$

Where, d = days after planting, n = no of seedlings emerged on day 'd'

### Promptness index

By using the method of (NOREEN et al., 2007) Promptness index (P.I) was determined.

$$\text{P.I} = nd2 (1.00) + nd4 (0.75) + nd6 (0.50) + nd8 (0.25)$$

Where (nd2... nd8) are number of emerging seedlings on day 2, 4, 6, 8.

### Seedling vigor index

The method of ABDULBAKI and ANDERSON (1973) was used to determine the Seedling vigor index. Seedling vigor index = germination % × Seedling length (mm)

### Plant growth parameters

After washing root and shoot length were measured for three plants for each treatment.

### Physiological parameters

A Scholander pressure chamber was used for determination of leaf water potential (SCHOLANDER et al., 1965). Chlorophyll content was determined by following the method of BRUINSMA (1963).

### Biochemical parameters

Proline content was determined by using a spectrophotometer (BATES et al., 1973). Soluble sugar was determined by following DUBOIS (1951). For protein determination, an extract of plant samples was prepared by homogenizing 0.2 g of fresh leaf material in 4 ml of sodium phosphate buffer (pH 7) and then centrifuged. In separate test tube 0.5 ml of leaf extract and 0.5 ml distilled water and 3 ml of Coomassie bio red dye were mixed. The reaction mixture was placed undisturbed for 5 minutes and the absorbance recorded at 595 nm (BRADFORD, 1976).

### Antioxidant enzyme assay

#### Superoxide dismutase

For determination of superoxide dismutase, 10 ml sodium phosphate buffer was used to grind 0.5 g leaf material. The solution was allowed to settle and then 0.1ml of extract was added to another set of test tubes along with 0.1 ml riboflavin and 3 ml phosphate buffer. This reaction mixture was placed under a fluorescent lamp for 8 min to start reacting. The same reaction mixture was prepared for

the dark reaction in another set of tubes. Absorbance for both sets of tubes was recorded at 560 nm wavelength (GIANNOPOLITIS and RIES, 1977).

$$\text{SOD } \mu\text{g/ml} = \frac{\text{Absorbance of sample} \times \text{K value} \times \text{dilution factor}}{\text{Weight of the sample}}$$

### Yield parameters:

Various yield parameters of canola plants were analysed such as pod length, number of seeds per pod, number of pods per plant and seed weight per plant, estimated after harvesting.

### Treatments

<b>T0</b>	Well watered and control
<b>T1</b>	Seed inoculated and well watered
<b>T2</b>	Rhizosphere inoculated and well watered
<b>T3</b>	Heat killed-inoculated and well watered
<b>T4</b>	Drought stress and un-inoculated
<b>T5</b>	Seed inoculated and drought stress
<b>T6</b>	Rhizosphere inoculated and drought stress
<b>T7</b>	Heat killed-inoculated and drought stress

### Statistical analysis

The software used for statistical analysis was Statistix 9.1. A two way ANOVA with a factorial block design was carried out for all treatments. Each treatment had three replicates

## Results

### Germination parameters

The effect of plant inoculation with *Azospirillum lipoferum* (Accession no. GQ255949) on germination of two canola varieties was significant ( $p \leq 0.05$ ). Drought stress decreased the germination percentage up to 37% as compared to well-watered conditions (Tab. 1). Seed inoculation, with bacteria resulted in a 10% increase in germination under well-watered conditions and a 12.49% increase under water stress. Heat killed inoculums had no significant effect on germination percentage under both water regimes. Results from the germination index (Tab. 1) showed a similar effect of drought stress. Reduction in germination index was improved by treating the seeds with *Azospirillum*. After inoculation under both well watered and drought conditions an 8.91% and 12.28% increase was observed in plants relative to their respective controls. Effect of the heat killed inoculum remained insignificant under both control and drought conditions.

A significant increase in promptness index was encountered in plants treated with *Azospirillum* inoculum under both well watered conditions and under a water shortage (Tab. 1). There was an increase of 20.87% in seedling vigour index that was recorded in plants inoculated under well watered conditions as compared to un-inoculated plants. A similar trend of inoculation was observed in plants grown under drought conditions; a 12.4% and 4.16% increase in seedling vigour index with both modes of inoculation was observed. There was a significant difference observed for all the parameters of germination in both canola varieties (Tab. 1).

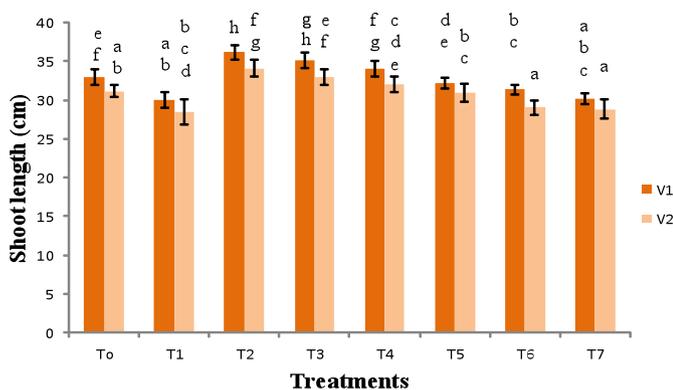
### Effect of inoculation on growth parameters

The effect of bacterial inoculation on growth parameters of plants was significant ( $p \leq 0.05$ ) when compared to control plants in both seed and rhizosphere inoculations. A decrease of 10% was observed in shoot length for drought exposed plants as compared to control plants (Fig. 1A). With a restricted water supply, both seed and rhizosphere inoculations resulted in a 7.2% and 4.5% increase in shoot

**Tab. 1:** Effect of *Azospirillum* inoculation on germination parameters of two canola varieties under drought stress.

Treatments	Seedling vigor index		Promptness index		Germination index		Germination %	
	V1	V2	V1	V2	V1	V2	V1	V2
T0	780±5h	749.91±4i	4.5±0.1f	4.5±0.1g	1.57+ 0.1d	1.57±0.1d	78.57±0.9e	71.42±1e
T1	685.68±5f	650±4h	2.25±0.2e	2.25±0.1e	1.14±0.2ab	1±0.1bc	57.14±0.9d	50±1d
T2	942.81±5b	903.55±4g	6.25±0.2b	6±0.1d	1.71±0.1ab	1.71±0.1bc	85.71±1b	78.57±0.9c
T3	824.98±5a	785.62±4e	4.75±0.1a	3.25±0.1c	1.57±0.1a	1.57±0.1ab	78.57±0.9a	71.42±0.9b
T4	771.36±5k	742.82±4d	3.25±0.1i	2.5±0.1c	1.28±0.1d	1.14±0.2ab	64.28±0.9f	57.14±0.9b
T5	714.25±5j	700±4c	2.5±0.1h	2.25±0.2b	1.14±0.2cd	1±0.1a	57.14±0.9e	50±1a

Where, T<sub>0</sub>= Un-inoculated and well watered, T<sub>1</sub>= Un-inoculated and drought exposed, T<sub>2</sub>= Seed inoculated and well watered, T<sub>3</sub>= Seed inoculated with heat killed inoculums and well watered, T<sub>4</sub>= Seed inoculated and drought exposed, T<sub>5</sub>= Seed inoculated with heat killed inoculums and drought exposed. And V1=Rainbow, V2=Con II.

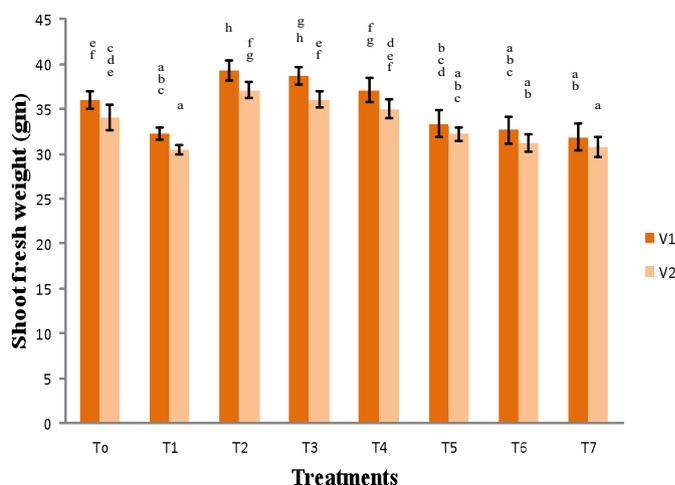
**Fig. 1A:** Effect of *Azospirillum* inoculation on shoot length of two canola varieties (v1 and v2) grown under drought stress.

Where, T<sub>0</sub> = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

length respectively. Data regarding shoot fresh and dry weight also showed the same results with different modes of inoculation (Fig. 1B and 1C). Analysis of data from well watered and drought exposed plants treated with *Azospirillum* showed a significant difference between inoculated and control plants. Seed inoculation resulted in a 23.68% increase in root area compared to controls treated under well watered conditions. Even rhizosphere inoculation resulted in a 18.09% increase in root area compared to regularly watered plants. A similar result was observed in drought exposed plants, where a 18.5% and 11.38% increase in area treated with seed and rhizosphere inoculations respectively. The increase of 9.72% in root area was observed with a heat killed inoculum compared to control plants grown under drought conditions. Rainbow variety showed a better response towards treatments compared to con II.

### Physiological parameters

Drought stress caused a 29.7% decrease in water potential for the leaf in both canola varieties (Fig. 2A). Inoculation tended to reduce the drought effect resulting in a 11.01% and 8.26% increase in water potential with seed and rhizosphere inoculation under drought stress conditions. Effect of heat killed inoculation was not significant un-

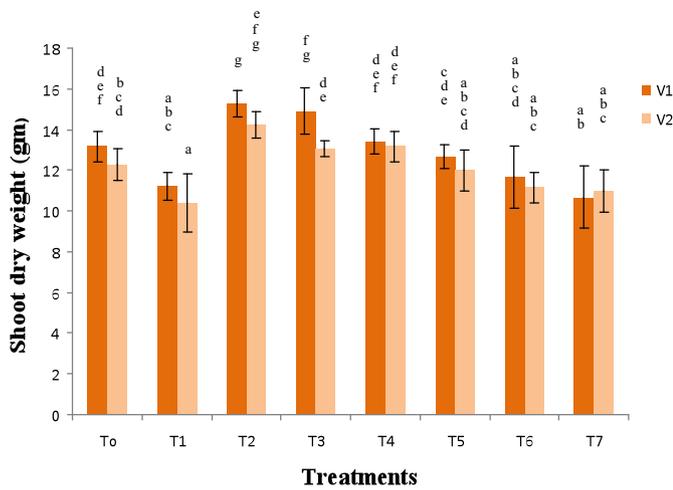
**Fig. 1B:** Effect of *Azospirillum* inoculation on shoot fresh weight of two canola varieties (v1 and v2) grown under drought stress.

Where, T<sub>0</sub> = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

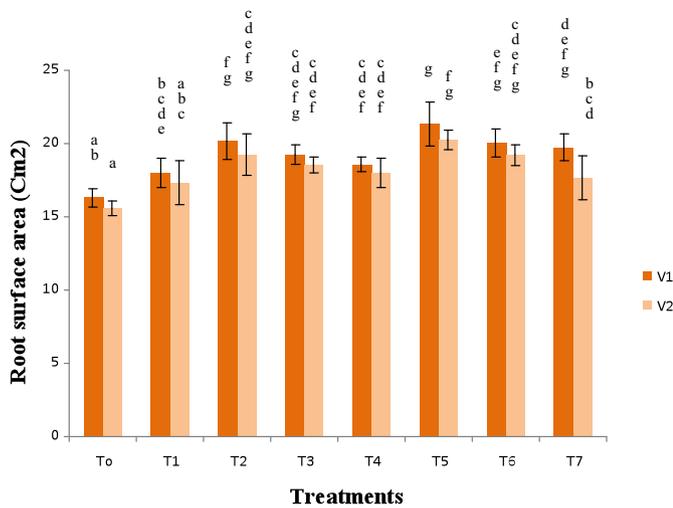
der both well watered and drought conditions. The effect of drought stress on photosynthetic pigments was more pronounced compared to other parameters. Considerable variation was observed in chlorophyll content of well watered and water stressed plants. Water deficit stress resulted in a 23.31% decrease in the total chlorophyll content of water stressed plants as compared to well watered plants (Fig. 2B). Plants with seed and rhizosphere inoculation showed a 20.28% and 9.52% increase of total chlorophyll in leaves compared to controls grown one under well watered conditions. A similar trend was seen in drought exposed plants, where a 12.21% and 5.98% increase was seen in seed and rhizosphere inoculated plants grown under water deficit conditions. Seed inoculation with a heat killed inoculum did not show any significant difference in plants grown under both well watered and water deficit conditions.

### Effect on compatible solutes

A considerable amount of osmolytes was accumulated in water stressed plants. In contrast to well watered plants a 23.29% proline

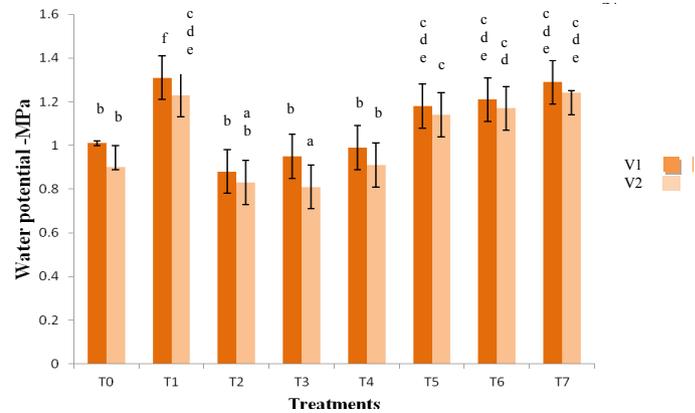


**Fig. 1C:** Effect of *Azospirillum* inoculation on shoot dry weight of two canola varieties (v1 and v2) grown under drought stress. Where, T<sub>0</sub> = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

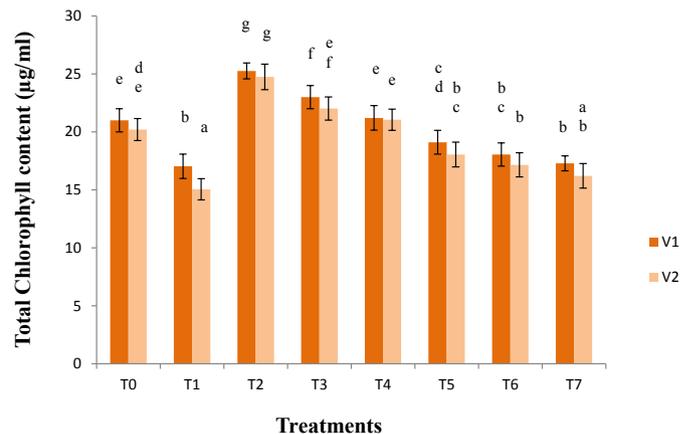


**Fig. 1D:** Effect of *Azospirillum* inoculation on root surface area of two canola varieties (v1 and v2) grown under drought stress. Where, T<sub>0</sub> = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

increase was observed in drought treated plants (Fig. 3A). Seed and rhizosphere inoculation allowed the plants to maintain a high proline level up to 10.34% and 8.62% under water stress compared to plants without inoculation. A similar increase of 21.72% in soluble sugar content was observed in drought exposed plants (Fig. 3B). Inoculation effects remained significant under limited water availability where a 10.69% and 7.28% increase was observed in sugar content of seed and rhizosphere inoculated plants respectively. Results revealed a 36.67% decrease in soluble proteins in drought imposed



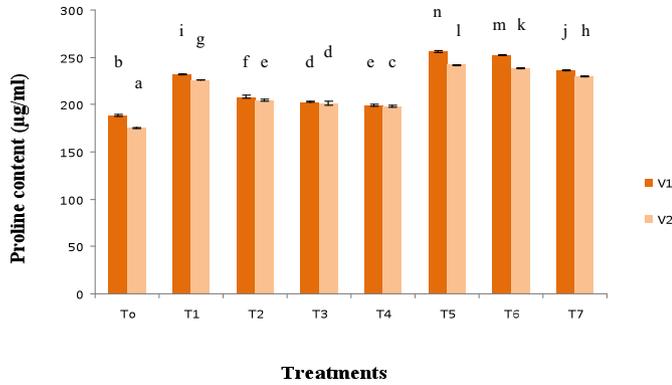
**Fig. 2A:** Effect of *Azospirillum* inoculation on water potential of two canola varieties (v1 and v2) grown under drought stress. Where, T<sub>0</sub> = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.



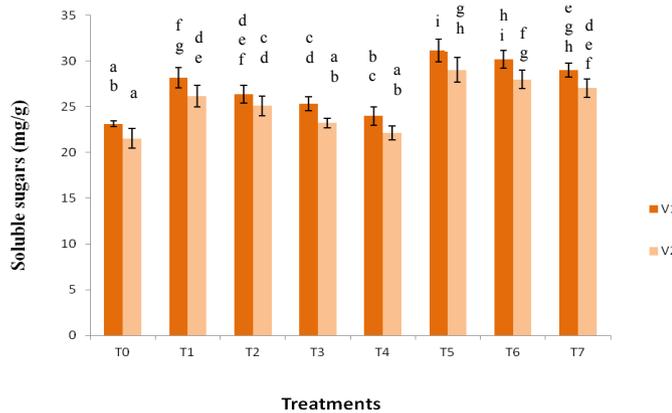
**Fig. 2B:** Effect of *Azospirillum* inoculation on total chlorophyll content of two canola varieties (v1 and v2) grown under drought stress. Where, T<sub>0</sub> = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

plants (Fig. 3C). Inoculation reduces the injurious effect of drought with seed inoculation resulting in a 10.02% and 9.48% decrease in well watered and drought conditions in contrast to their respective controls. Results were found to be similar with rhizosphere inoculation, with a 12.63% decrease in well watered and a 10.70% decline in stressed plants. Response of both varieties was significant following inoculation. Rainbow showed a better response compared to Con II.

An increase in super oxide dismutase (Fig. 4A) was observed in plants grown under drought stress compared to normally irrigated plants. Super oxide dismutase activity was 35.8% increased in drought exposed plants compared to control plants. Data for *Azospirillum* inoculation showed a significant decrease in super oxide dismutase in inoculated plants as compared to untreated plants. Seed and rhizosphere inoculation showed a 24.6% and 12.5% decrease in super



**Fig. 3A:** Effect of *Azospirillum* inoculation on proline content of two canola varieties (v1 and v2) grown under drought stress. Where, T<sub>0</sub> = Un-inoculated and well watered, T<sub>1</sub> = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

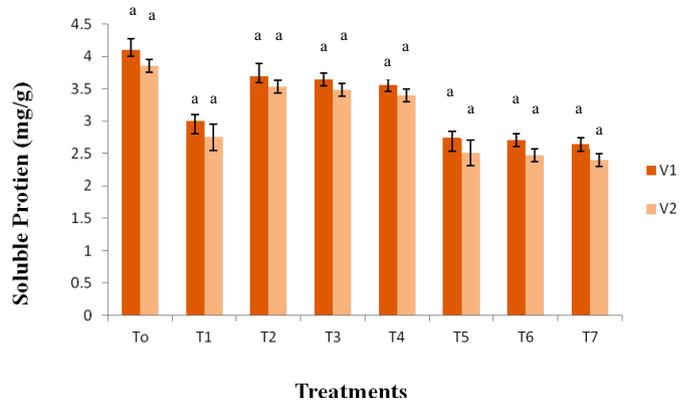


**Fig. 3B:** Effect of *Azospirillum* inoculation on soluble sugar of two canola varieties (v1 and v2) grown under drought stress. Where, T<sub>0</sub> = Un-inoculated and well watered, T<sub>1</sub> = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

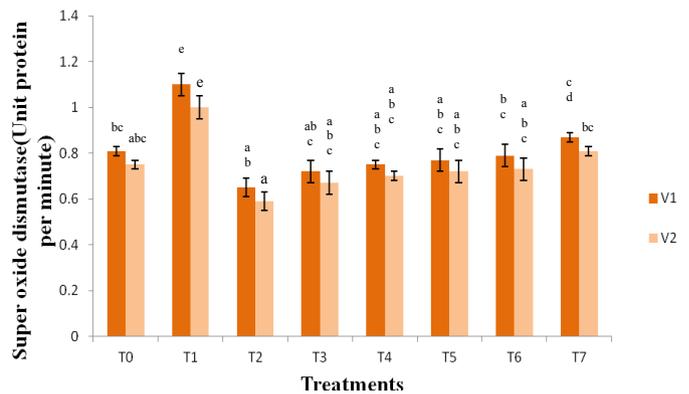
oxide dismutase activity compared to un-inoculated plants under well watered conditions. A similar trend of inoculation was observed in drought exposed plants where a 45% and 43% decrease was observed following seed and rhizosphere inoculation relative to their respective controls. A significant difference was recorded in both varieties. Response of Rainbow was much better as compared to Con II.

#### Yield parameters

Data for yield parameters differs significantly between well watered and drought exposed plants. Drought stress caused a 36.08%, 9.41%, and a 41.42% reduction in the number of seeds per pod, number of pods per plant and seed weight per plant as compared to control. A significant difference was observed in *Azospirillum* inocu-

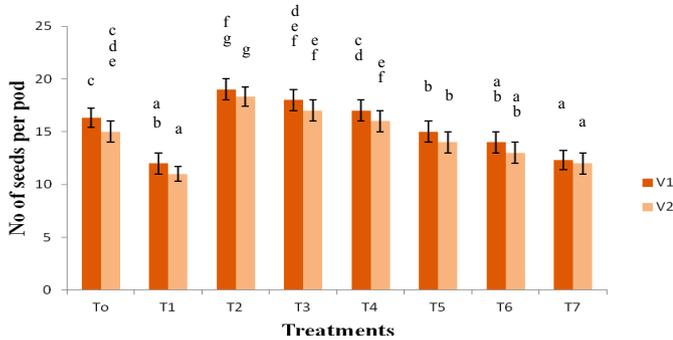


**Fig. 3C:** Effect of *Azospirillum* inoculation on soluble protein of two canola varieties (v1 and v2) grown under drought stress. Where, T<sub>0</sub> = Un-inoculated and well watered, T<sub>1</sub> = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.



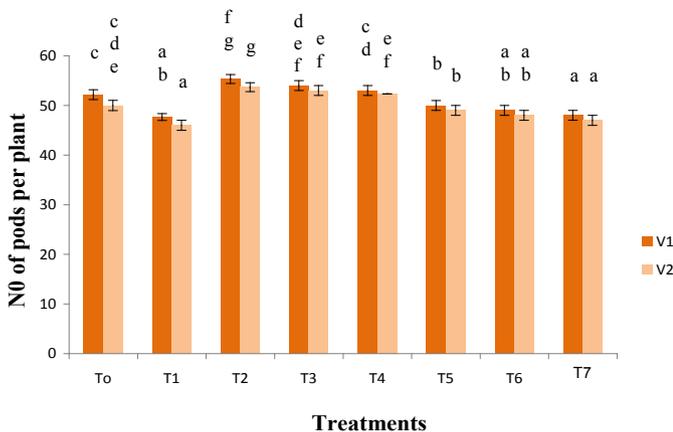
**Fig. 4A:** Effect of *Azospirillum* inoculation on super oxide dismutase of two canola varieties (v1 and v2) grown under drought stress. Where, T<sub>0</sub> = Un-inoculated and well watered, T<sub>1</sub> = Un-inoculated and drought exposed, T<sub>2</sub> = Seed inoculated and well watered, T<sub>3</sub> = Rhizosphere inoculated and well watered, T<sub>4</sub> = Seed inoculated with heat killed inoculums and well watered, T<sub>5</sub> = Seed inoculated and drought exposed, T<sub>6</sub> = Rhizosphere inoculated and drought exposed, T<sub>7</sub> = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

lated plants as compared to un-inoculated plants. The best outcome of inoculation for number of seeds per pod was seen in drought-exposed plants where a 25% and 16.67% increase was observed with two different modes of inoculation (Fig. 5A). Data regarding to number of pods per plant (Fig. 5B) reveals a 9.41% decrease in yield in drought exposed plants compared to control plants. Seed and rhizosphere inoculation resulted in a 6.07% and 4.88% increase in yield compared to control plants grown under well watered conditions. In drought conditions, only a 3.52% and 2.79% increase was observed following seed and rhizosphere inoculation. While in the case of seed weight per plant seed and rhizosphere inoculation resulted in a 14.14% and 6.06% increase in well watered compared to control plants. In drought exposed plants, seed and rhizosphere inoculation resulted in a 14.28% and 7.14% increase with respect to control plants (Fig. 5C).



**Fig. 5A:** Effect of *Azospirillum* inoculation on number of seeds per pod of two canola varieties (v1 and v2) grown under drought stress.

Where, To = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T2 = Seed inoculated and well watered, T3 = Rhizosphere inoculated and well watered, T4 = Seed inoculated with heat killed inoculums and well watered, T5 = Seed inoculated and drought exposed, T6 = Rhizosphere inoculated and drought exposed, T7 = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

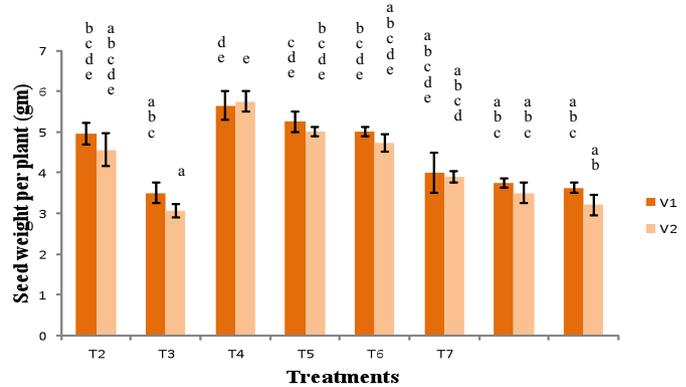


**Fig. 5B:** Effect of *Azospirillum* inoculation on number of pods per plant of two canola varieties (v1 and v2) grown under drought stress.

Where, To = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T2 = Seed inoculated and well watered, T3 = Rhizosphere inoculated and well watered, T4 = Seed inoculated with heat killed inoculums and well watered, T5 = Seed inoculated and drought exposed, T6 = Rhizosphere inoculated and drought exposed, T7 = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

## Discussion

Drought stress causes a significant reduction in germination percentage, germination index and seedling vigor index when compared to control plants. Decline in germination components of water stress is due to less water intake by the seed coat and inhibition of water intake at the initial stage of growth (TURK et al., 2004; BAHRAMI et al., 2012) or reduction in external water potential. Another reason for the reduction in germination is a delay in hydrolysis of storage compounds in the endosperm and cotyledon, or slow transport of water to the developing embryo axis (AYAZ et al., 2000). Under drought stress, enzymatic activity slows down and subsequently a decrease in the germination percentage. Reduction in water absorption decreases cell turgidity, division, and radical length (ZAEFIZADAH et al., 2011). Bacterial inoculation is an effective strategy to enhance germination, improve seedling emergence and respond to external environmental factors (LUGTENBERG et al., 2002). In the present study, a considerable increase in germination parameters (Tab. 1)



**Fig. 5C:** Effect of *Azospirillum* inoculation on seed weight per plant of two canola varieties (v1 and v2) grown under drought stress.

Where, To = Un-inoculated and well watered, T = Un-inoculated and drought exposed, T2 = Seed inoculated and well watered, T3 = Rhizosphere inoculated and well watered, T4 = Seed inoculated with heat killed inoculums and well watered, T5 = Seed inoculated and drought exposed, T6 = Rhizosphere inoculated and drought exposed, T7 = Seed inoculated with heat killed inoculums and drought exposed. And V1 = Rainbow, V2 = Con II.

was observed in seed inoculated plants compared to un-inoculated plants. Results of seed inoculation were also more pronounced compared to rhizosphere inoculation and use of a heat killed inoculum. Similar effects of bacterial inoculation on the germination parameter have been reported in cereals such as sorghum (RAJU et al., 1999), maize (GHAROOBI et al., 2012), wheat (BANGASH et al., 2013) and in sunflower (ZAEFIZADAH et al., 2011). This improvement may be due to synthesis of hormones, and increased activity of certain enzymes like amylase which speed up starch assimilation. Seed vigor index was also improved by an increase in synthesis of auxin by germinating seedlings when inoculated with bacteria (BHARATHI et al., 2004).

The most immediate effect of drought stress is a reduction in growth (SHALHEVET et al., 1995) and a number of morphological processes adversely affected by water deficit (DICKIN and WRIGHT, 2008). During the present study there was a decrease in shoot length (Fig. 1 A) that was observed in drought exposed plants compared to well watered plants because of a reduction in cell division and elongation. A similar reduction in growth parameters was observed by ASHRAF et al. (2013) and SHAFI et al. (2009). Shoot fresh weight and dry weight showed a significant decrease under stress conditions. This reduction in biomass was due to activity of metabolic enzymes being used under stress conditions (HONG and JI-YUN, 2007; XU et al., 2008), a change in metabolism (CHIMENTI et al., 2006) and inhibition in cell division (ARSHAD et al., 2008). Previously reduction in biomass was reported by ASHRAF et al. (2013). Plants with *Azospirillum* inoculation showed an increase in shoot length and biomass (Fig. 1-3 A). Seed inoculation produced maximum results followed by inoculation of the rhizosphere, and use of a heat killed inoculation compared to controls. Phosphate solubilization, production of auxin, the fixation of nitrogen and enhanced nutrient intake are likely responsible for better shoot length and shoot weight (BASHAN and HOLGUIN, 1997; BASHAN et al., 2004).

Data looking at leaf water potential indicates a considerable decrease in water potential under drought stress. Inoculation tends to reduce drought effects. Increase in water potential was observed in seed inoculated plants, although it was not significantly different from control plants (Fig. 1 B). Similar decreases in water potential under drought stress and its mitigation by *Azospirillum* was reported by ILYAS and BANO (2010). KARIMI and MOHSENI (2013) reported a similar decline in chlorophyll content in soybean cultivars grown

under drought stress. The causal agent behind the reduction of chlorophyll content may be a decline in pigment synthesis due to disruption of macro-aggregates of Chl a, Chl b, or production of reactive oxygen species (ROS) (SMIRNOFF, 1993). There was a significant increase in chlorophyll content of plants with *Azospirillum* inoculation under drought stress compared to un-inoculated plants. Seed inoculation showed the best result. A considerable decrease in the chlorophyll content of leaves was observed under water stress in canola plants (KAUSER et al., 2006). In the present study analysis of data regarding chlorophyll content showed a tremendous decrease in chlorophyll content of drought exposed plants compared to well watered conditions (Fig. 2).

Production and accumulation of proline and soluble sugars is a common response in plants exposed to water deficit stress, which plays a role in protection of the cell membrane and macromolecule structure under stress conditions (PRADO et al., 2000). Similar findings were reported by DIN et al. (2011) in canola plants. *Azospirillum* inoculation improves proline and soluble sugar contents as a result of water influx increases in plant cells (Fig. 1-2 C). NOSHEEN et al. (2011) also reported the same result following inoculation of canola. The reason for this increase was the breakdown of polysaccharides, which help to stabilize cell turgor (NAZARLI et al., 2011). Among compatible solutes soluble proteins are quite important for the manifestation of drought stress. Soluble protein content was decreased in plants exposed to drought stress (Fig. 3 C). These findings were supported by GOOD and ZAPLACHINSKI (1994), who observed a reduction in soluble protein content in *Brassica napus* under stress. The decline in soluble protein contents was due to reduction in photosynthesis, absence of raw materials for protein synthesis that cause a decline in or completely stop the process (MOHAMMADKHANI and HEIDARI, 2008). In the present research, a similar decline was observed in protein content, but inoculation of *Azospirillum* mitigated the effects of stress by reducing proportions of plants with which it decreases without inoculation under stress conditions.

The water deficit condition is associated with production of reactive oxygen species (ROS) in the chloroplast, mitochondria and peroxisome leading to oxidative stress. Activation of antioxidant enzyme systems is a common response in plants to reduce oxidative stress (FOYER and NOCTOR, 2003). Plants depend on activities of super oxide dismutase (SOD) and consequently on the activities of other antioxidant enzymes (ALSCHER et al., 2002). In the present study, tremendous increase in super oxide dismutase was noticed in drought exposed plants (Fig. 1). Our results are consistent with those of ABEDI and PAKNIYAT (2010). Data for inoculated plants showed a significant reduction in super oxide dismutase under both well watered and drought conditions. Seed inoculation has shown a better response as compared to the rhizosphere and heat killed inoculation with bacteria. Heat killed inoculation did not show any significant difference in both water regimes. Bacterial inoculation decreased the level of SOD by stimulating the intake of nitrogen and phosphorous, which interacted with carbohydrates as non-enzymic antioxidants. These substances utilize less energy and increase speedily compared to enzymic compounds (DAI et al., 2009).

Drought imposes a negative impact on performance of canola. However this negative effect on yield depends on stage, duration of stress and ability of the plant to cope with stress (TROTEL-AZIZ et al., 2000). Drought stress reduced the yield and yield component in the canola plant (BIRUNARA et al., 2011). Yield parameters of both plant varieties tested were badly affected by drought stress, resulting in a reduction in yield components like number of pods per plant, number of seeds per plant, and seed weight per plant encountered under water deficit conditions compared to well watered conditions (Fig. 1-3 E). Reduction in photosynthesis under water deficit conditions caused pod abortion, consequently a decrease number of pods produced (DIEPENBROCK, 2000). Decline in seed weight is associated with a

decrease in the number of seeds per plant as well as number of seeds per pod. Water deficit had a direct impact on size of sink, decreased storage capacity of source and subsequently caused a reduction in seed weight (SHIRANIRAD et al., 2013). Effects on seed treated in the rhizosphere and with a heat killed inoculation were not very effective compared to direct seed treatment with the bacteria. This may be due to ability of the *Azospirillum* to develop roots, facilitate nutrient and water uptake, displace pathogenic bacteria and help in nitrogen fixation (OKON and ITZIGSOHN, 1995).

From the present research, we conclude that *Azospirillum lipoferum* (Accession no. GQ255950) was able to mitigate the adverse effects of drought stress by improving the morphological, physiological and biochemical aspects of the plant. Among the different modes of inoculation, effects of seed inoculation were more pronounced compared to seed treated in the rhizosphere or with a heat killed inoculate. The strain of bacteria used was isolated from arid soil. Application of this strain can help plants to produce a better yield under stress conditions. There is need to perform similar experiments under field conditions with different types of inocula in the future in order to promote better crop production.

## References

- ABDULBAKI, A., ANDERSON, D.J., 1973: Vigor determination in Soybean seed by multiple criteria. *Crop Sci.* 13, 630-633.
- ABEDI, T., PAKNIYAT, H., 2010: Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). *Czech J. Genet. Plant Breed.* 46, 27-34.
- ALI, Q., ASHRAF, M., 2011: Induction of drought tolerance in maize *Zea mays* L. due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defence mechanism. *J. Agron. Crop Sci.* DOI: 10.1111/j.1439-037X.2010.00463.
- ALSCHER, R.G., ERTURK, N., HEATH, S.L., 2002: Role of superoxide dismutases (SOD) in controlling oxidative stress. *J. Exp. Bot.* 53, 1331-1341.
- ARSHAD, M., SHAHAROONA, B., MAHMOOD, T., 2008: Inoculation with *Pseudomonas* spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.). *Pedosphere* 18, 611-620.
- ASHRAF, M., 2010: Inducing drought tolerance in plants: some recent advances. *Biotechnol. Adv.* 28, 169-183.
- ASHRAF, M., SHABBAZ, M., ALI, Q., 2013: Drought induced modulation in growth and mineral nutrients in canola (*Brassica napus*). *Pak. J. Bot.* 45, 93-98.
- AYAZ, F.A., KADIOGLU, A., URGUT, T.R., 2000: Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Cicer arietinum*. *Can. J. Plant Sci.* 80, 373-378.
- BAHRAMI, H., RAZMJOO, J., JAFARI, O.A., 2012: Effect of drought stress on germination and seedling growth of sesame cultivars (*Sesamum indicum* L.). *Int J. Agri. Sci.* 2, 423-428.
- BANGASH, N., KHALID, A., MAHMOOD, T., SIDDIQUE, T.M., 2013: Screening of rhizobacteria containing ACC- Deaminase for growth and promotion of wheat under water stress. *Pak. J. Bot.* 45, 91-96.
- BANIAGHIL, N., ARZANESH, H.M., GHORBANLI, M., SHAHBAZI, M., 2013: The effect of Plant growth promoting rhizobacteria on growth parameters, antioxidant enzymes and microelements of canola under salt stress. *J. Appl. Environ. Biol. Sci.* 3, 17-27.
- BASHAN, Y., HOLGUIN, G., 1997: *Azospirillum*-plant, relationships: environmental and physiological advances. *Can. J. Microbiol.* 43, 103-121.
- BASHAN, Y., HOLGUIN, G., DE-BASHAN, E., 2004: *Azospirillum*-plant relationships: physiological, molecular, agricultural and environmental advances. *Can. J. Microbiol.* 50, 521-577.
- BATES, L.S., WALDREN, R.P., TEARE, I.D., 1973: Rapid determination of free proline for water stress studies. *Plant Soil.* 39, 205-207.
- BHARATHI, R., VIVEKANANTHAN, R., HARISH, S., RAMANATHAN, A., SAMI-

- YAPPAN, R., 2004: Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. *Crop Protect.* 23, 835-843.
- BIRUNARA, A., SHEKARI, F., HASSANPOURAGHDAM, B.M., KHORSHIDI, B.M., ESFANDYARI, E., 2011: Effect of water deficit stress on yield, yield components and phenology of canola (*Brassica napus* L.) at different growth stages. *J. Food Agri. Environ.* 9, 506-509.
- BRADFORD, M., 1976: A rapid and sensitive method for the quantitation of microgram, Quantitation of proteins utilizing the principle of protein dye-binding. *Anal. Biochem.* 72, 248-254.
- BRUINSMA, J., 1963: The quantitative analysis of chlorophyll a and b in plant extract. *J. Photochem. Photobiol.* 2, 241-249.
- CARDOZA, V., STEWART, N.C., 2003: Increased Agrobacterium-mediated transformation and rooting efficiencies in canola (*Brassica napus* L.) from hypocotyl segment explants. *Plant Cell Rep.* 21, 599-604.
- CARVALOH, I.S., MIRANDA, I., PEREIRA, H., 2006: Evaluation of oil composition of some crops suitable for human nutrition. *Indus Crops Prod.* 24, 75-78.
- CHIMENTI, C.A., MARCANTONIO, M., HALL, J.A., 2006: Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. *Field Crops Res.* 95, 305-315.
- DAI, Q., CHEN, C., FENG, B., LIU, T., TIAN, X., GONG, Y., SUN, Y., WANG, J., DU, S., 2009: Effects of different NaCl concentration on the antioxidant enzymes in oilseed rape (*Brassica napus* L.) seedlings. *Plant Growth Regul.* 59, 273-278.
- DIEPENBROCK, W., 2000: Yield analysis of winter rapeseed oilseed rape (*Brassica napus* L.), a review. *Field Crops Res.* 67, 35-49.
- DICKIN, E., WRIGHT, D., 2008: The effects of winter water logging and summer drought on the growth and yield of winter wheat *Triticum aestivum* L. *Eur. J. Agron.* 28, 234-244.
- DIN, J., KHAN, U.S., ALI, I., GURMANI, R.A., 2011: Physiological and agronomic response of Canola varieties to drought stress. *J. Anim. Plant Sci.* 21, 78-82.
- DUBOIS, M., GILLES, K., HAMILTON, K.J., REBERS, A.P., SMITH, F., 1951: A colorimetric method for the determination of sugar. *Nature* 195, 168-167.
- FOYER, C., NOCTOR, G., 2003: Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Planta.* 119, 355-364.
- GHAROABI, B., GHORBANI, M., NAZHAD, G.M., 2012: Effect of different levels of osmotic potential on germination percentage and germination rate of barley, corn and canola. *Iran. J. Plant Physiol.* 2, 413-417.
- GIANNOPOLITIS, C.N., REIS, K.S., 1997: Superoxide dismutase I. Occurrence in higher plants. *Plant Physiol.* 59, 309-314.
- GOOD, A.G., ZAPLACHINSKI, T.S., 1994: The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. *Physiol. Planta.* 90, 9-14.
- HAFIZ, M.A., MUHAMMAD, I.S., MUHAMMAD, S., ALLAH YAR, A., SAHI, A.K., MUSHTAQ, N.A., 2004: Drought tolerance studies of wheat genotypes. *Pak. J. Biol. Sci.* 7, 90-92.
- HONG, W., JI-YUN, J., 2007: Effects of zinc deficiency and drought on plant growth and metabolism of reactive oxygen species in maize (*Zea mays* L.). *Agri Sci. China.* 6, 988-995.
- ILYAS, N., BANO, A., 2010: *Azospirillum* strains isolated from roots and rhizosphere soil of wheat (*Triticum aestivum* L.) grown under different soil moisture conditions. *Biol. Fertil Soil.* 46, 393-406.
- ISTA, 2005: International rules for seed testing. International Seed Testing Association. Zurich, Switzerland.
- JONES, C.A., JACOBSE, S.J., WRAITH, M.J., 2003: The effects of P fertilization on drought tolerance of Malt Barley. Western Nutrient Management Conference. Salt Lake City U. T. 5, 88-93.
- KARIMI, M., MOHSENI, B.A., 2013: Effect of osmotic stress on soybean varieties. *J. Novel. Appl. Sci.* 2, 101-105.
- KAUSER, R., ATHAR, R.H., ASHRAF, M., 2006: Chlorophyll Fluorescence: A potential indicator for rapid assessment of water stress tolerance in soybean. *Pak. J. Bot.* 38, 1501-1509.
- LICHTFOUSE, E., 2010: Alternative farming system, biotechnology, drought stress and ecological. Fertilisation. Springer Verlag, Berlin, Heidelberg.
- LUGTENBERG, B., CHIN-A-WOENG, T., BLOEMBERG, G., 2002: Microbe-plant interactions: principles and mechanisms. *Antonie van Leeuwenhoek.* 81, 373-383.
- MAHMOOD, T., ASHRAF, M., SHAHBAZ, M., 2009: Does exogenous application of glycinebetaine as a pre-sowing seed treatment improve growth and regulate some key physiological attributes in wheat plants grown under water deficit conditions. *Pak. J. Bot.* 41, 1291-1302.
- MOHAMMADKHANI, N., HEIDARI, R., 2008: Effects of drought stress on soluble proteins in two maize varieties. *Turk. J. Biol.* 32, 23-30.
- NASRI, M., HEIDARI SHARIF ABAD, H., SHIRANI RAD, H.A., MAJIDI HERAVAN, A., ZAMANI ZADEH, R.H., 2007: Effect of drought stress on physiological properties of canola varieties. *J. Agri. Sci.* 1, 127-134.
- NAZARLI, H., FARAJI, F., ZARDASHTI, R.M., 2011: Effect of drought stress and polymer on osmotic adjustment and photosynthetic pigments of sunflower. *Carcet. Agron. Moldova.* 1.
- NOREEN, Z., ASHRAF, M., HASSAN, U.M., 2007: Inter-accessional variation for salt tolerance in pea (*Pisum sativum* L.) at germination stage of plant and screening stage of pea plant for assessment of tolerance potential. *Pak. J. Bot.* 39, 2075-2085.
- NOSHEEN, A., BANO, A., ULLAH, F., 2011: Nutritive value of canola (*Brassica napus* L.) as affected by plant growth promoting rhizobacteria. *Eur. J. Lipid. Sci. Technol.* 113, 1342-1346.
- OMIDI, H., TAHMASEBI, Z., NAGHDI-BADI, H.A., TORABI, H., MIRANSARI, M., 2010: Fattyacid composition of Canola (*Brassica napus* L.) as affected by agronomical, genotypic and environmental parameters. *Comptes Rendas Biologies.* 333, 248-254.
- OKON, Y., ITZIGSOHN, R., 1995: The development of *Azospirillum* as a commercial inoculant for improving crop yields. *Biotechnol. Adv.* 13, 415-424.
- PRADO, F.E., BOERO, C., GALLARODO, M., GONZALEZ, A.J., 2000: Effect of NaCl on germination, growth and soluble sugar content in chenopodium quinoa wild seeds. *Bot. Bull. Acad. Sin. Taipei.* 14, 27-34.
- QADERI, M., KUREPIN, L.V., REID, D.M., 2006: Growth and physiological responses of canola (*Brassica napus*) to three components of global climate change: temperature, carbon dioxide and drought. *J. Plant Physiol.* 128, 710-721.
- RAJU, N.S., NIRANJANA, S.R., JANARDHANA, G.R., PRAKASH, S.H., SHETTY, S.H., MATHUR, S.B., 1999: Improvement of seed quality and field emergence of *Fusarium moniliforme* infected sorghum seeds using biological agents. *J. Sci. Food Agri.* 79, 206-212.
- SCHOLANDER, P., HAMMEL, H., BRADSHOOT, E., HEMMINGSEN, E., 1965: Sap pressure in vascular plants. *Plant Sci.* 148, 339-345.
- SHAFI, M., RAZIUDDIN, J.B., ZHANG, G., 2009: Effect of cadmium and salinity stresses on growth and antioxidant enzymes activity of wheat genotypes. *Bull. Environ. Contam. Toxicol.* 82, 772-776.
- SHAHBAZ, M., IQBAL, M., ASHRAF, M., 2011a: Response of differently adapted populations of blue panic grass (*Panicum antidotale* Retz.) to water deficit conditions. *J. Appl. Bot. Food Qual.* 84, 134-141.
- SHALHEVET, J., HUCK, G.M., SCHOEDER, P.B., 1995: Root and shoot responses to salinity in maize and soybean. *J. Agro.* 87, 512-517.
- SHIRANIRAD, A., ABBASIAN, A., AMINPANAH, H., 2013: Evaluation of rapeseed (*Brassica napus* L.) cultivars for resistance against water deficit stress. *Bulg. J. Agri. Sci.* 9, 266-273.
- SMIRNOFF, N., 1993: The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol. Trust.* 125, 27-28.
- TAHIR, M., ASGHAR, A., NADEM, A.N., TANVEERANS, H., SABIR, M.Q., 2007: Performance of canola (*Brassica napus* L.) under different irrigation levels. *Pak. J. Bot.* 39, 739-746.
- THAKORE, Y., 2006: The biopesticide market for global agriculture use. *Ind. Biotechnol.* 2, 194-208.
- TROTEL-AZIZ, P., NIOGRET, F.M., LARHER, F., 2000: Proline level is partly under the control of abscisic acid in canola leaf discs during recovery

- from hyper-osmotic stress. *Physiol. Plant.* 110, 376-383.
- TURAN, M., ATAOLGU, N., SAHIN, F., 2006: Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. *Sustain. Agri.* 28, 99-108.
- TURK, M.A., RAHMSN, A., TAWAHA, M., LEE, D.K., 2004: Seed germination and seedling growth of three lentil cultivars under moisture stress. *Asian. J. Plant Sci.* 3, 394-397.
- XU, D.Q., HUANG, J., GUO, S.Q., YANG, X., BAO, M.Y., TANG, J.H., ZHANG, S.H., 2008: Over-expression of a TFII-type zinc finger protein gene ZFP252 enhances drought and salt tolerance in rice (*Oryza sativa* L.). *Fed. Eur. Biochem. Soci. Lett.* 582, 1037-1043.
- YASARI, E., PATWARDHAN, M.A., GHOLE, S.V., OMID, C.G., AHMAD, A., 2008: The relationship of different growth parameters and different nutrients uptake with canola plant (*Brassica napus* L.), yield and yield contribution at different nutrients availability levels. *Pak. J. Biol. Sci.* 11, 845-853.
- ZAEFIZADEH, M., JAMAATI-E-SOMARIN, S., ZABIHI-E-MAHMOODABAD, R., KHAYATNEZHAD, M., 2011: Discriminate analysis of the osmotic stress tolerance of different sub-cultivars of durum wheat during germination. *Adv. Environ Biol.* 5, 74-80.

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