INFLUENCE OF OSMOPROTECTANTS AND THEIR DIFFERENT APPLICATION METHODS ON WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS UNDER WATER DEFICIENCY IN SOUTH PUNJAB REGION OF PAKISTAN

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Abstract. Water deficiency in Agriculture sector is the main factor that limits the production of crops. During the cropping years 2016-17 and 2017-18, a field experiment was conducted at Central Cotton Research Institute, Multan, Pakistan with the objective to minimize the adverse impacts of water deficiency on wheat. In this study, two wheat cultivars Johar-2016 (Jr) and Gold-2016 (Gd) were tested with three types of osmoprotectants with control treatment (Cl = Control, Gb = Glycine betaine, Th = Trehalose, Pl = Proline) along with three different application methods (Sp = Seed priming, As = Application on Anthesis stage, Sp + As = Seed priming + Application on Anthesis stage) of osmoprotectants. All the osmoprotectants were applied at the rate of 150 mg/l of water while in control only water was sprayed. The crop was sown under RCBD with the split-split-plot arrangement. Water scarcity was enforced on the crop by giving 25.4 cm (62.5%) depth of water out of 40.64 cm (100%) of crop water requirement. Data was collected regarding crop growth and yield, crop water relation, grains quality, antioxidant contents and osmoprotectant contents. Mean data of both years were statistically analyzed. Results showed that interactive treatment Jr × Gb × Sp + As performed better in most of the parameters (e.g. 1000 Grain weight = 35.905 g, Grain yield = 4.6883 t ha^{-1}, Biological yield = 10.367 t ha^{-1}) however interactive treatments Jr × Th × Sp + As (Starch contents = 58.14%) and Jr × Pl × Sp + As (Protein contents = 13.79%) were also remained fruitful.
Keywords: *glycine betaine (Gb), trehalose (Th), proline (Pl), crop water relation, antioxidants*

Introduction

Wheat is consumed worldwide as well Pakistan in the form of grain, flour and bread (Wetherhell, 2019). According to Pakistan Grain and feed annual (2019-20) Pakistan produced about 25.6 million metric tons of wheat grains. 9 million ha (40%) cultivable land area in Pakistan is under wheat cultivation (Statista, 2020). As the population is increasing very fast in Pakistan, 8 million metric tons more of wheat grains required every year to feed the population of Pakistan by 2030 (Archibald and Watts, 2011). Like the increase in population, climate change is also a very critical problem, especially in Pakistan. Due to the uneven use of resources, the climate is changing rapidly causing water scarcity in most of the regions in Pakistan (Khan et al., 2020). In Pakistan, unequal use of water will also cause more water scarcity in the future (IRSA, 2019-20).

Water is the major resource for successful completion of crop, any fluctuation in the availability of water causes a reduction in the growth and yield of crops. A restricted amount of water causes serious growth and yield problems as it creates hurdles in light reactions, dark reactions, etc. (Wahid and Rasul, 2005). The deficiency of water causes disturbance in the defense system of plants (Reddy et al., 2004). Plant leaf, tillers, leaf

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area, and bud formation reduces due to limited water supply (Taiz and Zeiger, 2006). Yield parameters like spikes, dry matter accumulation and grain yield are largely affected by water deficiency (Cattivelli et al., 2008).

Nowadays osmoprotectants are used to assist plants in hard conditions, especially under water scarcity. These compounds serve as moisture-conserving bodies and maintain growth and development in hard environmental conditions. These are suitable to plant cells and tissues as they do not create problems in plant functions. They maintain the osmotic potential of the membrane, optimize the cell membrane, assist proteins, and maintain cell wall dryness (Wani et al., 2013). They are allocated in three types regarding composition. Ammonium osmoprotectants (chlorine-Osulfate, dimethyl-sulfonio propionate and glycine betaine, etc., alcoholic and sugar osmoprotectants (Trehalose, mannitol and Sorbitol), and amino acid osmoprotectants (Ectoine, Proline) (Reguera et al., 2012).

Glycine betaine is categorized as ammonium osmoprotectants and plays a vital role in regards to plant growth maximization in water deficit conditions (Chaitanaya et al., 2009). In intense environmental conditions, glycine betaine can be used to optimize antioxidant activity (Wang et al., 2010). The exogenous application of glycine betaine is helpful to improve plant growth and crop yield (Ma et al., 2006; Farooq et al., 2008). The separation of sodium and potassium ions during salt accumulation is done by glycine betaine (Hamida and Shaddad, 2010). Farmers are using glycine betaine to decrease stresses (water, salt and heat stress) in plants and get desired results (Ma et al., 2007). Significant results were found in the wheat crop when treated with glycine betaine under stress (Raza et al., 2014).

Trehalose is a sugar-containing compound and is available in very small quantities. A variety of crop plants generate trehalose during stress conditions, it is involved in embryo production and the emergence of flowers (Iturriaga et al., 2009). It performs a variety of functions in the cell (Paul et al., 2008). Trehalose accelerates photosynthesis and C-metabolism, it performs functions of energy source, protein protectant and carbohydrate storage compounds during stress (Lunn et al., 2014). In various species of higher plants, it is involved in signaling compounds and responds against stress conditions (Yang et al., 2014).

Proline is an amino acid compound which involves in metabolic activities. It accumulates in plant tissue therefore it can be very helpful for plants to overcome stress conditions (Hayat et al., 2012). It is responsible to fight against harsh conditions including water stress and heat stress furthermore it stabilizing plant growth (Ali et al., 2007). If proline is exogenously applied to plants in stress conditions, it can be a healthy approach to increasing plant growth because it improves the inside conditions of plants (Ashraf and Foolad, 2007; Ali et al., 2008). It works as a storage compartment for carbon and nitrogen. Also, it develops turgor and optimizes the Stress, maintains the shape of the protein, and retains lipid membrane peroxidation (Trovato et al., 2008). In this experiment our main objective is to study the response of osmoprotectants applied by different application methods during the water deficient condition in wheat crop.

Materials and methods

Experimental site, design and metrological data

The research was conducted for two continual years (2016-17 and 2017-18) by the side of the Agronomic Trial Area of CCRI (Central Cotton Research Institute) Multan

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(30° 10’ 53.2524” N and 71° 29’ 31.7652” E with altitude 122 m), south Punjab region of Pakistan. The crop was sown on the 18th of November and harvested on the 17th of April during both years. In this experiment, two different wheat cultivars Johar-2016 and Gold-2016 were tested with three types of osmoprotectants with control treatment (Cl = Control, Gb = Glycine betaine, Th = Trehalose, Pl = Proline) along with three different application methods (Sp = Seed priming, As = Application on Anthesis stage, Sp + As = Seed priming + Application on Anthesis stage) of osmoprotectants. The Crop was sown under RCBD (Randomized Complete Block Design) in conjunction with the split-split-plot arrangement. Cultivars were kept in the main plot, osmoprotectants were kept in the sub plot and application methods were kept in the sub-sub plot. All the treatments were replicated three times and the net plot size of each treatment was 16 m^2.

The texture as well as the physio-chemical characteristics of the research zone was inspected. The texture of soil was inspected by the hydrometer technique, the method of Dewis and Freitas (1970). Physio-chemical features of soil were examined employing method of Jackson (1962). Data regarding texture and the physio-chemical characteristics is presented in *Table 1*. Meteorological data (mean temperature, mean relative humidity and rainfall) for the months were recorded and shown in *Figure 1*.

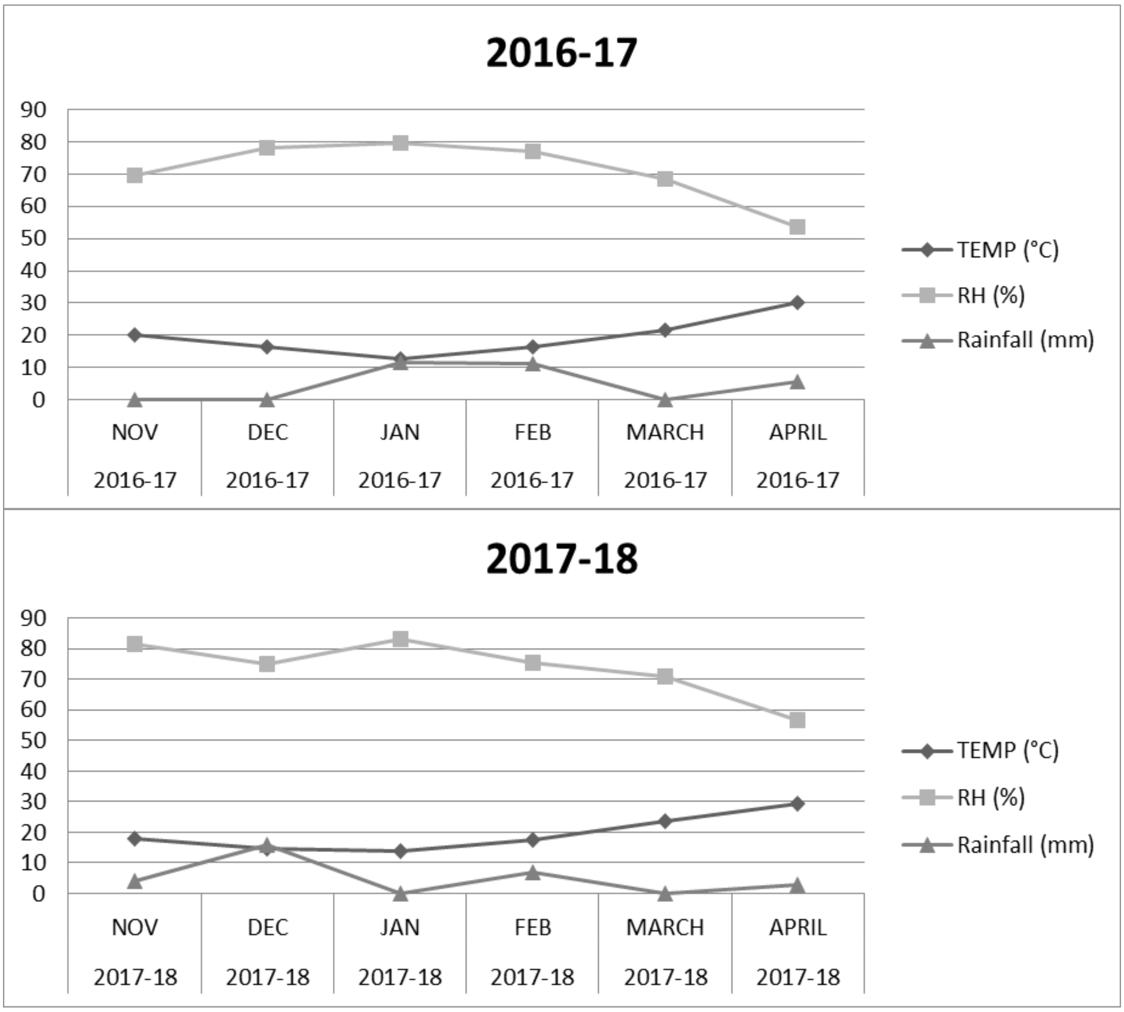

Figure 1. *Meteorological data for cropping years. Temp = Temperature and RH = Relative Humidity*

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Table 1. *Soil texture and physio-chemical features*

Properties	2016-17	2017-18
Ph.	7.61	7.52
EC (dS m^{-1})	0.51	0.51
Soil saturation (%)	39.2	40.3
Organic matter (%)	0.61	0.63
Nitrogen %	0.03	0.03
Phosphorus (ppm)	7.92	7.14
Potassium (ppm)	221	231
Soil textural class	Clay loam	Clay loam

Treatments preparation and application time

In both cultivars (Jr = Johar-2016 and Gd- = Gold-2016), all the osmoprotectants (Cl = Control, Gb = Glycine betaine, Th = Trehalose and Ph = Proline) were applied via three application methods (Sp = Seed priming, As = Application on Anthesis stage and Sp + As = Seed priming + Application on Anthesis stage) at the rate of 150 mg/l of water while in the control treatment only distilled water was used.

Creation of water deficit condition

Water scarcity was enforced on the crop by giving 25.4 (62.5%) of water out of 40.64 mm (100%) of crop water requirement. This 25.4 mm of water was applied at the seedling, tillering, milking and dough stage, 6.35 mm of water is applied on each mentioned stage however irrigation on the anthesis stage was completely skipped.

Crop husbandry

The crop was sown via a hand drill by following recommended row-to-row distance (22 cm), using recommended seed rate 120 kg ha^{-1}, and applying a recommended dose of fertilizers (Nitrogen = 125 kg ha^{-1}, Phosphorous = 110 kg ha^{-1} and potassium = 60 kg ha^{-1}) using Urea, DAP (Di-ammonium Phosphate) and SOP (sulphate of potash) as the source. ½ of the nitrogen, full dose of phosphorus and potassium were applied just earlier than the sowing whereas the remaining ½ dose of nitrogen was applied along with the first irrigation. The crop was secure from insects, pests, weeds and diseases via cultural (agronomic) practices and adaptation of plant protection measures in all treatments.

Parameters of the study

Crop growth parameters (plant height cm, number of fertile tillers m^{-2}, number of lateral roots/plant and root length cm)

Plant height cm (centimeter) of each treatment was determined at the maturity time of the crop via rod meter. The numbers of fertile tillers m^{-2} (per square meter) were determined with the help of quadrates. The number of fertile tillers was counted from five different positions in each treatment and averaged. Averaged roots length cm (via meter tape) and number of lateral roots per plant (via counting) were concluded after uprooting, washing as well drying of collected samples (five plants) from each treatment.

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Crop yield parameters (biological yield t ha^{-1}, grain yield t ha^{-1} and 1000 grain weight g)

As the crop fully matured, a five square meter crop was harvested from different positions for each treatment. Harvested samples were dried and weighted to obtain biological yield (obtained values were converted m^2 to t ha^{-1}). For grain yield, theses dried samples were threshed and grains of each sample were weighted (obtained values were converted m^2 to t ha^{-1}). For a thousand grain weight (g) of each treatment, five different samples were counted, weighted and averaged.

Crop water relation parameters (water potential and relative water contents)

Water potential -MPa (megapascals) in leaves were examined at the anthesis stage of the crop. At early morning (07:00 AM – 08:00 AM) water potential of each treatment was measured in four fully expended leaves by the use of a water potential device (Chas W. Cook Div., England).

As concerned RWC (Relative water contents) %, five leaves from each applied treatment were selected, separated, weighted (get fresh leaves weight) and soaked in distilled water for the period of 14 h and weighted again to get water soaked leaves weight. Moreover, samples were oven dried (at 80°C for 48 h) to get dried leaves weight, RWC (%) of samples concluded via given below formula:

$$\text{RWC} = \frac{\text{Fresh leaves weight} - \text{Dried leaves weight}}{\text{water soaked leaves weight}} \times 100$$

Grains quality parameters

Protein contents (%) and Starch contents (%) in grains were concluded. Starch contents concluded via the procedure defined in AACC (2000) Method No. 76-13. Protein contents (%) were determined using the method of Bradford (1976).

Antioxidant contents (u/mg protein)

As the crop promoted over anthesis stage (irrigation skipped stage) as well as got at milking stage, firstly, leaves from all treatments were collected, protein contents in leaves take out thru the method of Bradford (1976). Furthermore, the method designated in Bhutta (2011) aimed for the calculation of antioxidant contents (Catalase, Peroxidase and Superoxide Dismutase u/mg protein) in take-out protein contents of collected samples.

As concerning CAT (Catalase u/mg protein), a 3 ml mixture containing 1.5 ml of (100 mmol) potassium phosphate buffer with pH value 7.2, 0.5 ml of (75 mmol) H_2O_2 and 0.05 ml enzyme abstraction is used. The desired volume of 3 ml was created using the distilled water. When H_2O_2 is added reaction gains ground. The exhaustion is noted in a reduction on 240 nm designed for 60 s. The enzyme action was taken into account via calculating the amount of decayed H_2O_2.

Peroxidase (u/mg protein) was assessed using 3 ml mixture, enclosed 0.1 mmol EDTA, 1 ml of 0.2 mol/m^3 potassium phosphate, 0.1 ml of 2 mmol NADPH, 0.5 ml of 3 mmol DTNB, 0.1 ml enzyme extraction. Adding the distilled water, 2.9 ml of closing volume was produced. One unit of POD activity added to start the reaction. Over the period of 5 min, a spectrophotometer at 25°C recorded an increase in absorbance at 412 nm.

Superoxide Dismutase (u/mg protein) predicted thru recording the deterioration in the absorbance of superoxide nitro blue tetrazolium compound through the enzyme. To prepare the 3 ml combination, the following amounts were used: 0.1 ml of 3 mmol EDTA,

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0.1 ml of 200 mmol methionine, 1.5 ml of 100 mmol potassium phosphate buffer, 0.01 ml of 2.25 mmol nitro-blue tetrazolium (NBT), 0.05 ml of enzyme extraction, and 1 ml of distilled water. As a control, without any enzyme two tubes were used. The effect produced as 0.1 ml of riboflavin (60 mol) was added, and these tubes were placed beneath the light of lamps for 20 min. The light was turned off to stop any reaction. These tubes remained protected using black cloth. Tubes used as a control generated its most color. A non-irradiated complete reaction mixture which did not develop color served as blank. Absorbance was recorded at 560 nm and one unit of enzyme movement was taken as the amount of enzyme. The tubes lacking enzymes compact the absorbance reading of samples up to 50%.

Osmoprotectant contents (umol/5 g fresh weight)

Glycinebetaine contents calculation carried out via method of Grieve and Grattan (1983). Five gram of fresh leaves was grinded in 10 ml distilled water. Following filtration, 1 ml of extraction was given 1 ml of 2 N HCl treatment. Then 0.2 ml of potassium tri-iodide solution was then added to 0.5 ml of this mixture. This mixture was mixed vigorously for 90 min while cooling in cold water and shaking occasionally. 20 ml of 1,2-dichloromethane (cooled to 10°C) and 2 ml of ice-cooled distilled water were incorporated into the mixture. The bottom organic layer's OD was measured at 365 nm, and the upper aqueous layer was removed. At the end a standard curve was used to calculate the amount of glycinebetaine.

Proline contents were estimated via the method of Bates et al. (1973). Samples (5 g of fresh leaves) were chewed with 6 ml of 3% (w/v) sulfosalicilic acid aqueous solution, then filtered via filter paper (Whatman No. 1). For the analysis, 2 ml of the sieved extract was acquired, to which 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added, incubated in a boiling water bath (1 h) and the reaction was complete in an ice bath. Four ml of toluene was added to the reaction mixture and the organic phase was extracted, in which a toluene soluble reddish chromophore was obtained that was read at 520 nm using toluene as blank by UV-visible spectrophotometer.

Trehalose contents were calculated via the method described in Iqbal et al. (2012). Trehalose was analyzed by HPLC (SCL-10A, Shimadzu) using column (Rezex RCM-monosccharide Ca+2, Phenomenex) for separation of the sugars and refractive index detector for the detection. Quantitative determination of Trehalose was achieved by comparing peak surface areas with those obtained with pure Trehalose standard solutions in the range 0-5 mM. The mobile phase was DD-H2O with 1 mL/min as flow rate at 80°C.

Statistical analysis

Average data of both years (2016-17 and 2017-18) was almost equal in all the attributes. Mean data of both years were statistically assessed by Fisher's analysis of variance technique on $P < 0.05$ to observe the variances amongst treatments result (Steel et al., 1997). Statistix software (version 9.0) was used to analyze the obtained data.

Benefit–cost ratio

Economic analysis of the experiment was calculated thru the benefit-cost ratio (BCR) formula:

$$\mathrm{BCR} = \frac{\text{Gross income}}{\text{Total costs}}$$

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Results

Obtained results of all the growth and yield parameters (plant height cm, number of fertile tillers m^{-2}, number of lateral roots, root length cm, 1000 grain weight g, grain yield t ha^{-1} and biological yield t ha^{-1}) were noted significant. Among all the 24 interactive treatments, the interaction of Jr × Gb × Sp + As (Johar-2016 × Glycine betaine × Seed priming + Athesis stage) gave fruitful results in all the growth and yield parameters of the study with the highest values of Plant height (89.35 cm), Number of fertile tillers (271.20 m^{-2}), Number of lateral roots (26.753), Root length (16.737 cm), 1000 Grain weight (35.905 g), Grain yield (4.6883 t ha^{-1}), Biological yield (10.367 t ha^{-1}). Whereas among all the interactive treatments, the interaction of Gd × Cl × Sp (Gold-2016 × Contrl × Seed priming) gave the lowest values of Plant height (68.54 cm), Number of fertile tillers (203.96 m-2), Number of lateral roots (18.733), Root length (10.077 cm), 1000 Grain weight (27.948 g), Grain yield (1.9850 t ha^{-1}), Biological yield (5.647 t ha^{-1}). Results of growth and yield parameters are shown in *Table 2 and 3*.

Results regarding crop water relation parameters (Water potential –MPa and Relative water contents %) were noted significant. Discussing water potential (-MPa) highest value (1.336-MPa) was recorded in cultivar Gold-2016 (Gd) with the combination of control (Cl) among osmoprotectants, seed priming (Sp) among application methods i.e. Gd × Cl × Sp. The minimum value (0.836-MPa) of water potential was recorded in cultivar Johar-2016 (Jr) with the combination of Glycine betaine application (Gb) among osmoprotectants, seed priming + application at Anthesis stage (Sp + As) among application methods i.e. Jr × Gb × Sp + As. As discussed Relative water contents (%) highest value (82.53%) obtained in treatment Jr × Gb × Sp + As while the minimum value (59.47%) was obtained in treatment Gd × Cl × Sp. Results of growth and yield parameters are shown in *Figure 2*.

Grain quality parameters (Protein contents % and Starch contents %) of the experiment were affected significantly by all the interactive treatments. Among all the treatments, protein contents (%) in grains examined maximum in Jr × Pl × Sp + As (Johar-2016 × Proline × Seed priming + Anthesis stage) treatment combination with the value of 13.79 (%) while minimum protein contents (%) examined in Gd × Cl × Sp treatment combination with the value of 9.91 (%). As concern starch contents (%) in grains, the maximum concentration found in treatment Jr × Th × Sp + As (Johar-2016 × Trehalose × Seed priming + Anthesis stage) with the value of 58.14 (%) whereas minimum concentration of starch contents (%) founded in treatment Gd × Cl × Sp with the value of 41.30%. Results of grain quality parameters (Protein contents % and Starch contents %) are shown in *Figure 3*.

Obtained results regarding antioxidant (catalase, peroxidase and superoxide dismutase) contents (u/mg protein) production were also influenced significantly by interactive treatments. Production of all the antioxidant contents (u/mg protein) was examined maximum in treatment Jr × Gb × Sp + As (Johar-2016 × Glycine betaine × Seed priming + Athesis stage) with the values of catalase (85.50 u/mg protein), peroxidase (103.10 u/mg protein) and superoxide dismutase (119.33 u/mg protein) whereas minimum antioxidant contents (u/mg protein) production were examined in treatment Gd × Cl × Sp with the values of catalase (80.35 u/mg protein), peroxidase (93.39 u/mg protein) and superoxide dismutase (107.56 u/mg protein). Results regarding antioxidants are shown in *Figure 4*.

As regards osmoprotectant (Glycine betaine, Trehalose and Proline) contents (umol/5 g fresh wt.) production, results of all osmoprotectant contents production noted

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significant. Discussing Glycine betaine (umol/5 g fresh wt.) contents production, the highest value (79.13 umol/5 g fresh wt.) was recorded in cultivar Johar-2016 (Jr) with the combination of Glycine betaine (Gb) among osmoprotectants, seed priming + application at anthesis stage (Sp + As) among application methods i.e. Jr × Gb × Sp + As. and minimum value (63.88 umol/5 g fresh wt.) was recorded in treatment Gd × Cl × Sp. Likewise Trehalose (umol/5 g fresh wt.) contents production, highest value (87.33 umol/5 g fresh wt.) obtained in treatment Jr × Th × Sp + As (Johar-2016 × Trehalose × Seed priming + Anthesis stage) while minimum value (70.19 umol/5 g fresh wt.) obtained in treatment Gd × Cl × Sp. Furthermore, Proline contents (umol/5 g fresh wt.) production, maximum value (73.55 umol/5 g fresh wt.) was recorded in treatment Jr × Pl × Sp + As (Johar-2016 × Proline × Seed priming + Anthesis stage) while minimum proline contents produced in treatment Gd × Cl × Sp with the value of 61.17 umol/5 g fresh weight and their results are shown in *Figure 5*.

The benefit-cost ratio of individual treatments was also calculated. Among cultivars, Johar-2016 remained beneficial with a value of 7.33 as compared to Gold-2016 (7.05). As discussed osmoprotectants, Glycine betaine (7.82) remained more profitable against Proline (6.94), Trehalose (6.62) and Control (5.29). Results are shown in *Table 4*.

Table 2. *Statistical summary indicating the impact of cultivars, osmoprotactants, application methods and their interaction of studied parameters*

Parameter	Cultivars (Cr)	Osmoprotactants (Os)	Application methods (Am)	Cr × Os × Am
Plant height (cm)	0.0167*	0.0000**	0.0000**	0.0000**
No. of fertile tillers (m^{-2})	0.0056**	0.0000**	0.0000**	0.0000**
No. of lateral roots ($plant^{-1}$)	0.0030**	0.0000**	0.0000**	0.0000**
Root length (cm)	0.0001**	0.0000**	0.0000**	0.0000**
1000 grain weight (g)	0.0005**	0.0000**	0.0000**	0.0000**
Grain yield (t ha^{-1})	0.0037**	0.0000**	0.0000**	0.0000**
Biological yield (t ha^{-1})	0.0036**	0.0000**	0.0000**	0.0000**
Water potential (-MPa)	0.0123**	0.0000**	0.0000**	0.0000**
Relative water contents (%)	0.0041**	0.0000**	0.0000**	0.0000**
Starch contents (%)	0.0149*	0.0000**	0.0000**	0.0000**
Protein contents (%)	0.0004**	0.0000**	0.0000**	0.0000**
Catalase (u/mg protein)	0.0097**	0.0000**	0.0000**	0.0000**
Peroxidase (u/mg protein)	0.0010**	0.0000**	0.0000**	0.0000**
Superoxidase dismutase (u/mg protein)	0.0143*	0.0000**	0.0000**	0.0000**
Glycine betaine contents (umol/5 g fresh wt.)	0.0002**	0.0000**	0.0000**	0.0000**
Trehalose contents (umol/5 g fresh wt.)	0.0000**	0.0000**	0.0000**	0.0000**
Proline contents (umol/5 g fresh wt.)	0.0008**	0.0000**	0.0000**	0.0000**

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Table 3. *Results regarding growth (plant height cm, number of fertile tillers m^{-2}, number of lateral roots/plant and root length cm) and yield parameters (1000 grain weight g, grain yield t ha^{-1} and biological yield t ha^{-1})*

Treatments			Parameters						
Cultivars	**Osmoprotectants**	**Application methods**	**Plant height (cm)**	**Number of fertile tillers (m^{-2})**	**Number of lateral roots/plant**	**Root length (cm)**	**1000 grain weight (g)**	**Grain yield (t ha^{-1})**	**Biological yield (t ha^{-1})**
Johar-2016 (Jr)	Control (Cl) (Only distilled water was used)	Seed priming (Sp)	74.94 i	207.62 q	20.087 n	10.737 r	29.905 r	2.3750 p	6.020 m
		Foliar application at Anthesis stage (As)	75.27 i	212.47 p	20.137 n	10.787 qr	29.955 q	2.4350 nop	6.203 m
		Seed priming + Foliar application at Anthesis stage (Sp) + (As)	76.38 i	215.31 o	20.227 n	10.877 p	30.045 p	2.4983 n	6.740 l
	Glycinebetaine (Gb)	Seed priming (Sp)	81.55 b-e	251.53d	23.827b-e	14.477 d	33.645 c	3.7217 cd	8.517 cd
		Foliar application at Anthesis stage (As)	81.79 bc	254.39c	23.917 bc	14.567 bc	33.735 b	3.8083 b	8.607 bc
		Seed priming + Foliar application at Anthesis stage (Sp) + (As)	89.35 a	271.20a	26.753 a	16.737 a	35.905 a	4.6883 a	10.367 a
	Trehalose (Th)	Seed priming (Sp)	78.76 h	233.28 m	22.797 lm	13.447 n	32.615 n	3.1983 lm	7.393 j
		Foliar application at Anthesis stage (As)	79.25	235.67 l	22.987 kl	13.637 m	32.805 l	3.3017 jk	7.507 ij
		Seed priming + Foliar application at Anthesis stage (Sp) + (As)	79.69 d-h	240.38 jk	23.237 ij	13.887 k	33.055 j	3.3850 ij	7.777 gh
	Proline (Pl)	Seed priming (Sp)	80.02 b-h	242.82 i	23.417 ghi	14.067 i	33.235 h	3.4717 hi	7.960 fg
		Foliar application at Anthesis stage (As)	80.82 b-g	245.61 gh	23.617 efg	14.267 g	33.435 f	3.5650 fg	8.103 ef
		Seed priming + Foliar application at Anthesis stage (Sp) + (As)	81.34 b-e	249.33 ef	23.707 c-f	14.357 ef	33.525 e	3.6350 def	8.310 de
Gold-2016 (Gd)	Control (Cl) (Only distilled water was used)	Seed priming (Sp)	68.54 j	203.96 r	18.733 o	10.077 s	27.948 s	1.9850 q	5.647 n
		Foliar application at Anthesis stage (As)	75.11 i	208.75 q	20.117 n	10.767 r	29.935 qr	2.4050 op	6.067 m
		Seed priming + Foliar application at Anthesis stage (Sp) + (As)	75.66 i	213.27 o	20.187 n	10.837 pq	30.005 p	2.4683 no	6.537 l
	Glycinebetaine (Gb)	Seed priming (Sp)	8.39 b-e	250.95de	23.757 b-e	14.407 e	33.575 d	3.6617 de	8.360 d
		Foliar application at Anthesis stage (As)	81.67 bcd	253.80 c	23.857 bcd	14.507 cd	33.675 c	3.7483 bc	8.600 bc
		Seed priming + Foliar application at Anthesis stage (Sp) + (As)	81.72 b	258.28 b	23.957 b	14.607 b	33.775 b	3.8283 b	8.800 b
	Trehalose (Th)	Seed priming (Sp)	76.69 i	231.27 n	22.647 m	13.297 o	32.465 o	3.1417 m	7.003 k
		Foliar application at Anthesis stage (As)	79.01 gh	235.67 l	22.927 kl	13.577 m	32.745 m	3.2450 kl	7.470 ij
		Seed priming + Foliar application at Anthesis stage (Sp) + (As)	79.56 e-h	239.04 k	23.117 jk	13.767 l	32.935 k	3.3283 jk	7.617 hi
	Proline (Pl)	Seed priming (Sp)	79.78 c-h	242.17 ij	23.347 hi	13.997 j	33.165 i	3.4317 i	7.930 fg
		Foliar application at Anthesis stage (As)	80.46 b-h	245.29 h	23.527 fgh	14.177 h	33.345 g	3.5183 gh	8.020 f
		Seed priming + Foliar application at Anthesis stage (Sp) + (As)	81.12 b-f	247.50 fg	23.647 def	14.297 fg	33.465 e	3.5950 efg	8.137 ef

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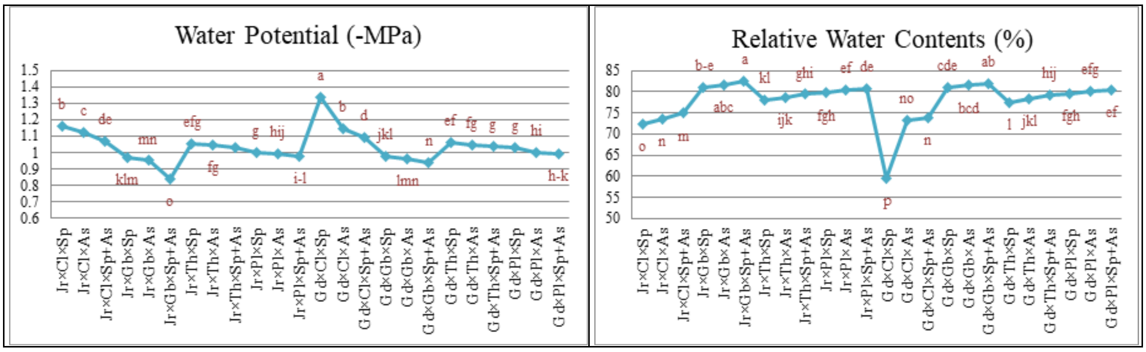


Figure 2. *Results regarding crop water relation parameters*

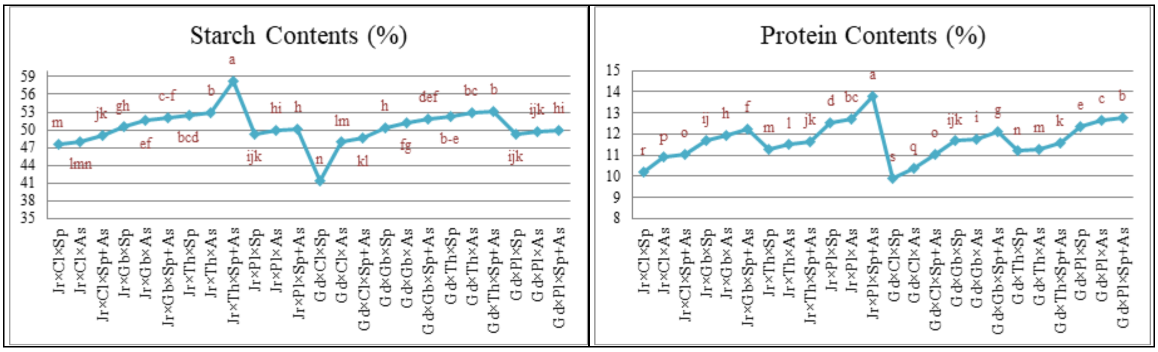
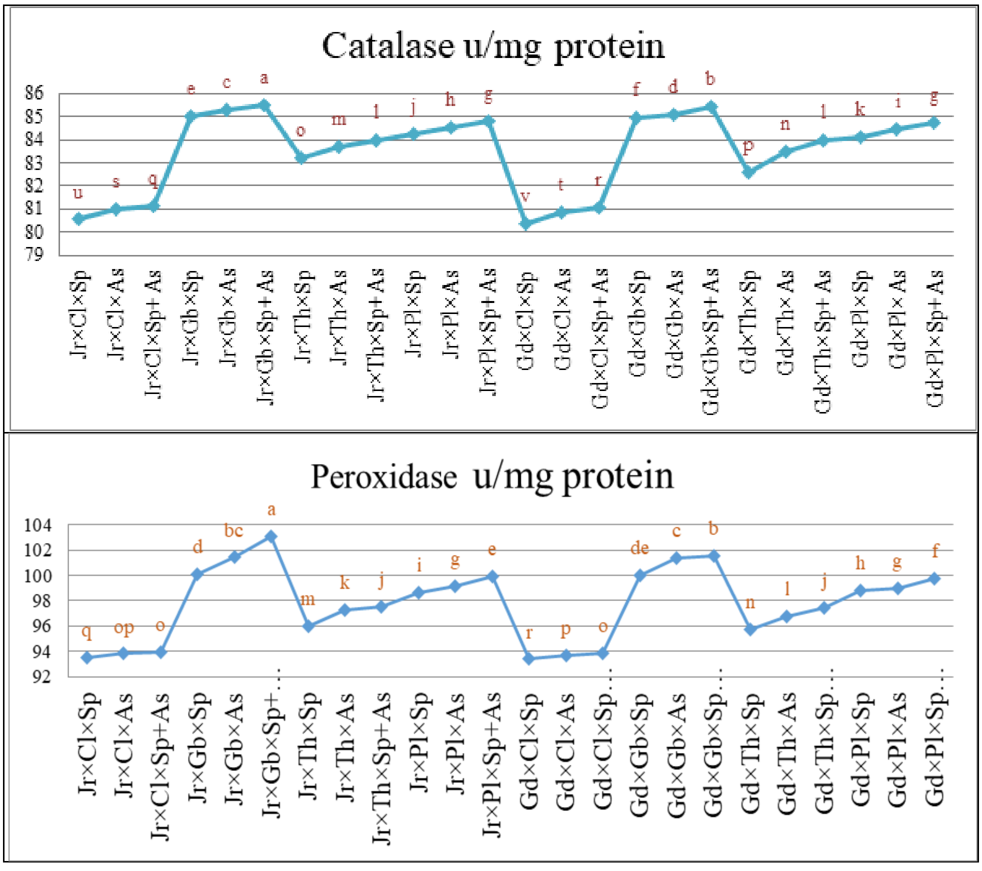


Figure 3. *Results regarding grain quality parameters*



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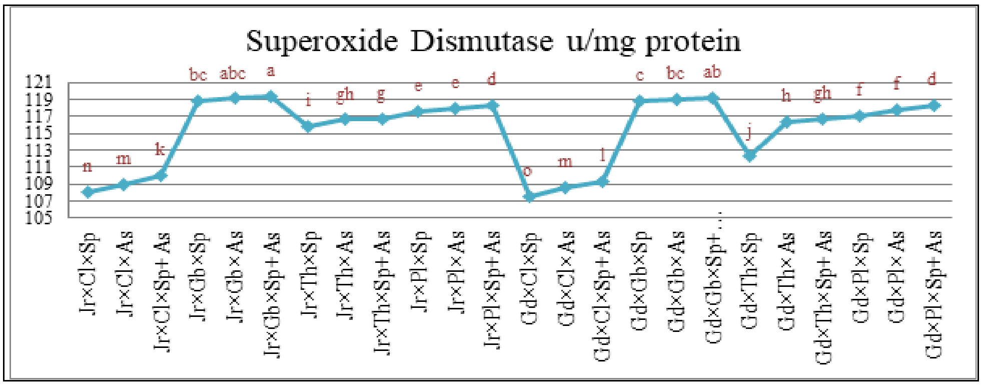

***Figure 4.** Results regarding antioxidants contents (u/mg protein)*

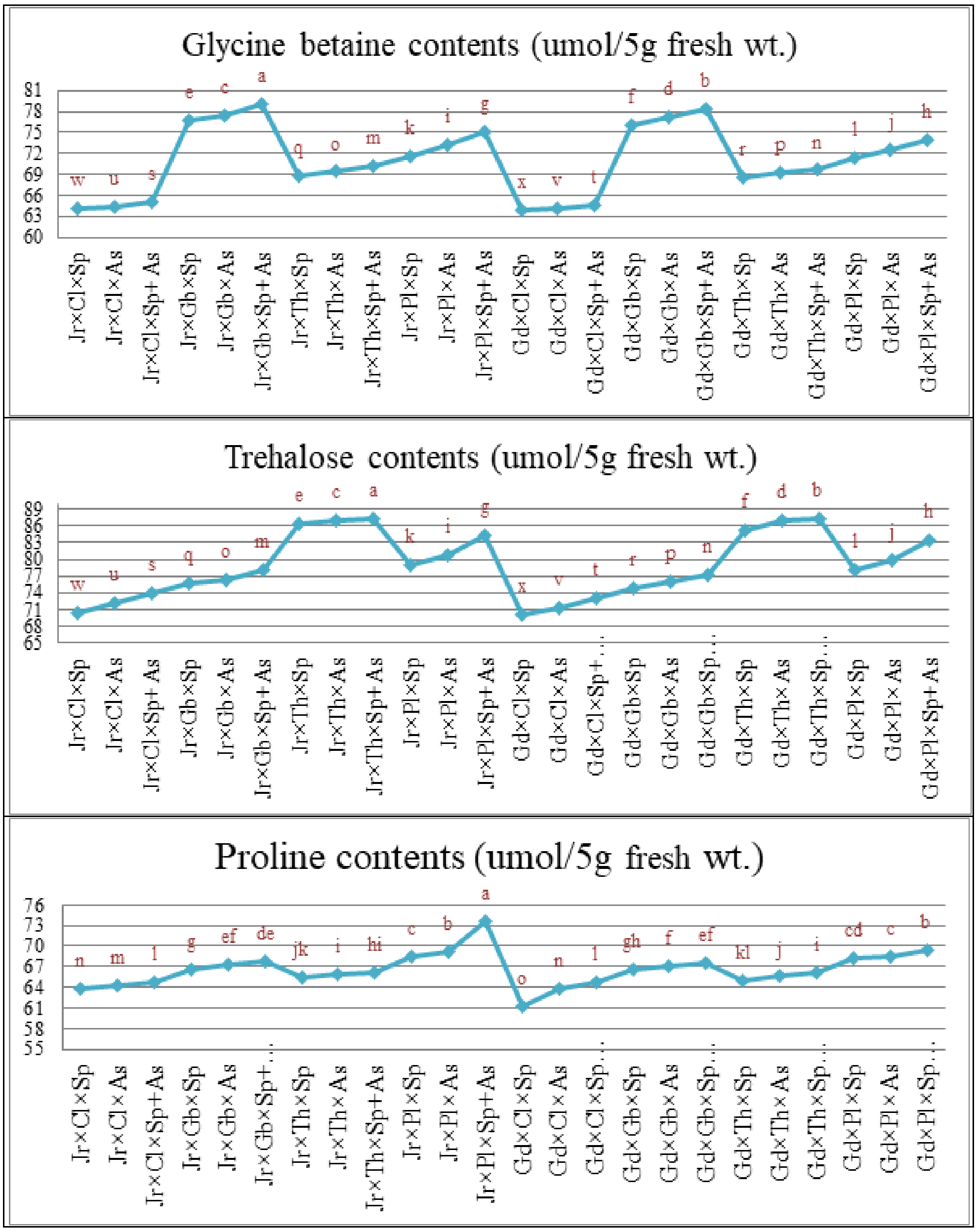

***Figure 5.** Results regarding osmoprotectant contents (umol/5 g fresh wt.)*

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Table 4. *Benefit–cost ratio*

	Treatments	Grain yield t/ha	Grain yield Rs/ha	Gross value	Total cost	Net return	BCR
Cultivars	Johar-2016	3.335	88377	106397	14500	91897	7.33
	Gold-2016	3.195	84667	102319	14500	87819	7.05
Osmoprotectants	Control	2.355	62407	76804	14500	62304	5.29
	Glycinebetaine	3.905	103482	123623	15800	107823	7.82
	Trehalose	3.265	86522	104445	16000	88445	6.52
	Proline	3.535	93677	112559	16200	96359	6.94

* Grain yield Rs 26.5/kg

Discussion

A proper quantity of water is crucial to obtaining the maximum yield of crop. Various abiotic stresses such as inequality in nutrients application, salt stress as well as water stress affect crop production (Rashid et al., 2010). From these stresses, water deficiency is the main factor in the reduction of crop production in Pakistan (Ahmed et al., 2010). Osmoprotectants application is the better option to reduce the water stress on crops. In this study, all the concerned parameters gave fruitful results by application of osmoprotectants via different application methods under water deficiency.

As we talk over the results of growth and yield parameters of the study (Plant height cm, Number of fertile tillers m^{-2}, Number of lateral roots and Root length cm, 1000 grain weight g, Grain yield t ha^{-1} and Biological yield t ha^{-1}), it was noticed by obtained results that water shortage has adverse impacts on growth parameters. Results observed regarding plant height, interactive treatment Jr × Gb × Sp + As (Johar-2016 × Glycinebetaine × Seed priming + Application at Anthesis stage) remained most vital to acquiring maximum plant height than all other applied treatments. Among all the applied interactive treatments, Gd × Cl × Sp remained inferior regarding plant height results. Among osmoprotectants application of glycine betaine (Gb) plays a significant role in enhancing the functions of the cell organelles which assisted the plants to achieve more height (Sakamoto and Murata, 2002). The number of fertile tillers is a vital yield parameter and maximum number of fertile tillers gave maximum yield but the water stress plants are incapable of producing more fertile tillers. Results regarding the number of fertile tillers (m^{-2}) indicated that cultivar Johar-2016 with the combination of Glycine betaine along with the application method of seed priming + anthesis stage (Jr × Gb × Sp + As) produces more fertile tillers (m^{-2}) while treatment Gd × Cl × Sp produces least fertile tillers (m^{-2}) than all other applied treatments. It is determined that exogenous application of glycine betaine accelerates flowering which produces maximum fertile tillers (Miri and Armin, 2013). No. of lateral roots and root length (cm) are also affected by water shortage. However, both parameters were detected more in interactive treatment Jr × Gb × Sp + As (Johar-2016 × Glycinebetaine × Seed priming + Application at Anthesis stage) while both parameters were detected less in interactive treatment Gd × Cl × Sp (Gold-2016 × Contrl × Seed priming). It is also highlight by an experiment that water shortage affects the roots badly but osmoprotectants application assist plants to gain better root status in such adverse condition (Rezaei et al., 2012). 1000 grain weight (g) is considered an efficient characteristic of crops which is linked to the economic yield of crops. Statistical observation revealed that 1000 grain weight was noted more in cultivar

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Johar-2016 with combination Gb × Sp + As and less in cultivar Gold-2016 with the combination of Cl × Sp among all the applied treatments. It is also revealed by a previous study that the application of Glycine betaine assists plants to increase grain weight as Glycine betaine contributes to assimilating storage (Rezaei et al., 2012; Gupta and Thind, 2017). As regards, grain and biological yield (t ha^{-1}), among all the treatments highest value of grain yield and biological yield (t ha^{-1}) were obtained in Jr × Gb × Sp + As and the lowest values were perceived in Gd × Cl × Sp. It was also determined by previous study that the application of osmoprotectants especially Glycine betaine increases the yield of crops under water stress (Manaf, 2016).

As concern plant water relation parameters (Water potential -Mpa and Relative water contents %), superior results of water potential (-MPa) and relative water contents (%) were recorded in a combination with Jr × Gb × Sp + As while results of treatment Gd × Cl × Sp were noted inferior among all the treatments. Previous studies also give details that foliar application of osmoprotectants empowers plants to combat abiotic stresses and hold the proper amounts of water inside plant system (Rezaei et al., 2012; Ahmad et al., 2019).

As we discuss grain quality parameters (Protein contents % and Starch contents % in grains), statistical results disclosed that the deficit water conditions had a bad effect on the quality of grains. Thus, the osmoprotectants application gave prolific results as compared to the control treatment. Protein contents (%) in grains were acquired more in treatment Jr × Pl × Sp + As (Johar-2016 × Proline × Seed priming + Application at Anthesis stage) and minimum Protein contents (%) acquired in treatment Gd × Cl × Sp (Gold-2016 × Control × Seed priming) while results of other applied treatments were between of above mention treatments (Jr × Pl × Sp + As and Gd × Cl × Sp). Osmoprotectants application assists the plants to produce better protein contents (Dawood, 2016). Starch contents (%) in grains reached their top by the application of interactive treatment Jr × Th × Sp + As (Johar-2016 × Trehalose × Seed priming + Application at Anthesis stage) and minimum starch contents (%) in grains perceived in treatment Gd × Cl × Sp (Gold-2016 × Control × Seed priming) among all other applied treatments. Osmoprotectants are involved in the improvement of starch contents (%) in grains of crops (Borger et al., 2010; Ahmad et al., 2013).

Antioxidant (Catalase, Peroxidase and Superoxide dismutase u/mg protein) contents production was also examined in this study. Antioxidant contents production scrutinized higher in treatment Jr × Gb × Sp + As (Johar-2016 × Glycinebetaine × Seed priming + Application at Anthesis stage) and minimum antioxidants (u/mg protein) produced in treatment Gd × Cl × Sp i.e. Gold-2016 × Control × Seed priming among all the applied treatments. The application of osmoprotectants is beneficial under adverse conditions as osmoprotectants enhanced antioxidant (u/mg protein) content production in crops (Dong et al., 2015; Ahmad et al., 2019).

As concerns production of osmoprotectants inside the body of plants under water deficiency, maximum Glycinebetaine contents (umol/5 g fresh wt.) were produced where treatment Jr × Gb × Sp + As was applied while minimum Glycinebetaine contents (umol/5 g fresh wt.) were produced in treatment Gd × Cl × Sp. Maximum Trehalose contents (umol/5 g fresh wt.) were produced where treatment Jr × Th × Sp + As was applied while minimum Trehalose contents (umol/5 g fresh wt.) were produced in treatment Gd × Cl × Sp. Maximum Proline contents (umol/5 g fresh wt.) were produced where treatment Jr × Pl × Sp + As was applied while minimum Proline contents (umol/5 g fresh wt.) were produced in treatment Gd × Cl × Sp. Closely results are founded by Hasegawa et al. (2000), Fu et al. (2012), Abdallah et al. (2016) and Mahboob et al. (2016).

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Conclusion

Obtained results of the study showed that a limited supply of water to crops has adverse effects on growth, yield and quality of crops but choosing drought-resistant genotypes and application of osmoprotectants via an efficient method is a good option to overcome the adverse effects of water deficiency. Statistical results of the study were in the fever of Johar-2016 among cultivars, Glycine betaine among osmoprotectants and application of osmoprotectants via seed priming + foliar application at anthesis stage among application methods.

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