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Study of biochemical and phytochemical parameters in local variety of barley (*Hordeum vulgare*) under saline conditions

Naseem Khatoon[1,2], Beena Naqvi[2], Najum-u-Sahar[1,2], Nadir Ali Rind[1,3], Muhammad Rafiq[*1], M Umar Dahot[1]

[1]*Institute of Biotechnology & Genetic Engineering, University of Sindh, Jamshoro, Sindh, Pakistan*

[2]*PCSIR Laboratory Complex, Karachi, Sindh, Pakistan*

[3]*Department of Molecular Biology & Genetics, Shaheed Benazir Bhutto University, Benazirabad, Sindh, Pakistan*

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Abstract

Current research targeted to measure the effect of increasing concentration of (0-200mM) of KCl_2 and $MgCl_2$ salt on *Hordeum vulgare* cultivars, (Jow 83, and Jow 87). Overall, increasing of KCl_2 and $MgCl_2$ stress reduced leaf pigments in both cultivars significantly, with a lesser extent in Jow 83 as compared to Jow 87. The present results justify that a significantly ($p<0.05$ continuously decrease of total sugars, total proteins, antioxidant activity, reducing sugar, phenolic compounds, with increasing of concentration of KCl_2 and $MgCl_2$ as compared to control plants. While significantly ($p<0.05$) increased amount of Proline contents in both cultivars with increasing of salt concentrations as compared to control. Various salt-treated plants showed a decreased amount of various biochemical and phytochemical as compared with control plants. The Jow 87 salt tolerance nature was correlated positively with its better performance in terms of physiological, phytochemical, and biochemical attributes.

* **Corresponding Author:** Muhammad Rafiq ✉ Rafiq_75@yahoo.com

Introduction

Soil Salinity has been posed as a major global problem that may toxically affect more than 20% of irrigated soil, and it also decreases crop production significantly Qadir *et al.* (2014) and Machado and Serralheiro, (2017). Resultantly, rapidly reducing cultivatable land coupled with abnormally growing population is posing threat to agricultural sustainability (Shahabaz, 2013).

It is estimated that every year 1.5 million hectares of irrigated land is going outside of the agriculture production and more than 50% of all arable land will suffer from salinization until the year, 2050 (Wang *et al.,* 2013). According to increasing world population and need for food and water, humans will be encounter lack of soil and water recourses. Therefore, it will be necessary to use marginal lands, waste waters and saline soils. Average production of all major crops has reduced by 20% to 50% in recent years. These losses are mostly due to dryness and high soil salinity. Global climate change is also one of the reasons. The most cultivatable patches of the world will get worse due to climate changes Pooja *et al. (*2015). Salinity postures two main intimidations to plants growth: Osmotic stress and ionic stress (Flowers, 2008) and Acosta-Motos *et al.* (2017). The lethal effect of salinity happens in the results plants physiological and metabolic process. The response to these changes is habitually conducted by the variation features, such as the decline in leaf area, enlargement of leaf thickness and juiciness, abscission of leaves, mortification of root and shoot and downturned of internode length (Parida, 2005). According to the research survey, the $MgCl_2$ despite having major element of salty soil and irrigation water but also effect on countless physiological, biochemical parameters is very dangerous.

Furthermore, $MgCl_2$ is mostly applied even as defroster in numerous countries particularly to read surface and is of important environmental concern, as small concentration of $MgCl_2$ in water and soil could have a significant impact on the plant and also effected close by areas Dougherty, (2006) and Brandenbury, (2011). Magnesium (Mg) and Chloride (Cl) are essential nutrients required for normal growth of plants, butthehigh concentration of $MgCl_2$ accumulate in soil may be fatal or which can prevent up taking of water and nutrients from the soil. The high concentration of $MgCl_2$ may be harmful to plant growth and as the balanced magnesium ion concentration is significantly important in higher physiology of plants, plays an important role in water homeostasis, and closely associated with the process involved in the protein synthesis. In higher plants, potassium and chloride have an impact on the process of photosynthesis and has major function is to regulate the osmotic process at various levels. The adequate source of both chemicals potassium and chloride in plants improve resistance from many diseases in the plants Cakmak, (2005). The potassium can improve drought tolerance in plant by mitigating destructive effect as a result of increasing translocation, and maintain water balance.

Barley (*Hordeum vulgare* L.) is the fourth largest cereal crop high in fiber and rich source of vitamins anfminerls, is one of the most salt tolerant small frain crop in the world. This fodder crop has application of brewing. It belongs to grass family Poaceae, tribe triticale and genus *Hordeum*. It consists of 32 wild and cultivated species in Hordeum out of 350 Barley species. It is one of the important salt-tolerant crop species Jiang *et al.* (2006). Abiotic is main stress which reduces barley yield in arid and semi-arid regions where salt concentration is near equal to seawater (Pareek, 2010). Through the modification of their morphological, physiological and metabolic processes the plants can resist salt stresses (Kalaji, 2008). Increase of, proline content, peroxidase, electrolyteleakage, activity of superoxide dismutase (SOD), ascorbate peroxidase, catalaseand glutathione reductase, and decrease in relative water content and pigmentcontent in barley have been also reported under salinity stress (Mian *et al*., 2011). Salt stress not only decrease the expected yield of crops but also disturbs metabolic processes in plants through loss of water potential of cells, ion toxicity, membrane integrity and function, and uptake of essential mineral nutrients (Arzani and Ashraf, 2016). Research on stress responses of plants have been the focus of the breeders for a long time, but insight into the stress perception and signaling has recently been complemented by the growing evidences for stress-induced biochemical, physiological and epigenetic changes (Kumar and Singh, 2016; Kumar *et al.* Water logging is a prophylactic factor in production of Barley. It is not capable to survive in the environment of water logging than like other some crops. It is come out of recent research that all types of stresses (salinity, drought, and waterlogging) are impacting adversely on grain yield of Barley from 20 to 25% at any stage of growth (Setter L, 2003). Salt stress causes metabolic damage, leaf senescence followed by photosynthetic decline leading to reduced plant productivity. These parameters have been successfully utilized in screening and/or evaluating salt tolerance ability of plants (Kumar *et al.*, 2015; Singh *et al.*, 2015). Barley gives more yields with slighter agriculture. Inputs and it could also replace the damaged soils Naheed *et al.* (2015). Barley likes other crops pretentious by the saline soils, but due to its high resistance it can give well yields than other crops (Shaukat, 2013). It is a primary crop, in the world which is mainly used for animal feed and malt preparation; it is also contemplated as salt tolerant crop. It also used in bread making and other human food and beverages soup stew etc. Barley bread is of innumerable cultures. Gluten. Distilled and base malt beer is main ingredient of barley (Kamali, 2014). Barley is an important grain crop but is not used regularly by our new generation. The barley is the most useful and prehistoric grain contains with more health neuterations. This important source of nuitrituon and food is also affected by salinity due to reduction of production. We need to understand that the salt tolerance mechanism of barley and its relevance to plant survival and yield.

The research will:-

- Identfy, isolate and clone salt tolerance genes.
- Characterize the expression process of the selected genes.
- Screen selected genes for alleles by development of SNPs markers
- Generate transgenic barley lines tolerant to salinity strees.
- Transfer the genes to glycophytes plant and make salt tolerance plants.

The aim of study to compare the effect of KCl_2 and $MgCl_2$ on biochemical and phytochemicals parameters in the present barley cultivativers grown in Pakistan.It may be helpful in devolpng a better understanding and provide addaitianol information on the salt tolerance of the barley plant.

Material and methods

This study was carried out attheInstitute of Biotechnology and Genetic Engineering (IBGE) University of Sindh during 2016-2017. Two barley cultivars Jow 83, and Jow 87, the seeds of barley cultivars were collected from Agriculture Research Institute, Tando Jam Agriculture University was used in this study.

Pot Experiment

Thirty healthy seeds of each barley cultivars were sown using plastic pots. Pots were filled with soil collected from field added sand and yard manure (1:1:1) in the greenhouse of the Department of Biotechnology University of Sindh. Plants were irrigated equally with fresh water in each pot. Four salinity treatments containing 50, 100, 150, 200mM of $MgCl_2$ and KCl_2 along with control were then applied using freshwater maintained for 7th days and all experiments were observed on 8th day after treatment.

Chlorophyll content Determination

0.1g of newly emerging leaf as added in absolute acetone in test tubes separately, crushed with glass rod, airtight with parafilm to prevent evaporation and were incubated in referagerator at 4°C for 48 hours. The absorbance of acetone extracted pigments was measured on UV-visible spectrophotometer at 662nm, 645nm and 470nm. The chlorophyll and carotenoids contents were calculated according to Lichtenthaler and Wellburn (1983).

Chl a (Ca) = 11.75A662¬ - 2.35A645

Chl b (Cb) = 18.61A645¬ - 3.960A662

Carotenoids (Cc) = 1000A470 - 2.270Ca - 81.4Cb/230

Protein Determination

Total protein contents were quantified by lowery *et al.* (1951) method. For this screening, 0.5ml extract of different parts of both plants was added in different test tubes then 2.5ml of freshly alkaline copper reagent added in test tubes in duplicate separately and mixed well at room temperature. After that wait for 10 minutes, 0.25ml of Folin-Ciocalteu reagent (1:1 v/v with distilled water) was added, mixed well and allowed to stand for 30 minutes to complete the reaction at standard room temperature until appearance of the bluish color. The absorbance of each sample was read against blank (distilled water was added instead of sample along with all reagents for the preparation of blank) at 750nm by using UV-visible spectrophotometer. Bovin serum albumin was used as a standard to measure the protein concentration.

Estimation of proline Content

Bates *et al.* (1973) method was applied to estimate the free proline content. Fresh samples (0.5g each) were homogenized in 5ml of 03% (w/v) sulphosalicylic acid. About 2ml of extract was taken in test tube and 2ml of glacial acetic acid and 2ml of ninhydrin reagent were added. The reaction mixture was boiled in a water bath at 100˚C for 30 minutes. After cooling the reaction mixture 04ml of toluene was added. After mixingchromophore containing toluene was separated and absorbance of developed red colour was read against toluene blank.

Determination of Carbohydrate

Total sugar concentration from extract was measured according to the method by Montogomery (1961). To 0.5ml sample solution was added 2.5 mlconc H_2SO_4 and 0.05ml 80% phenol solution. After thoroughly mixing samples wereleft at room temp for 15 minutes. The absorbance wasmeasured against blanked at 485nm.

Determination of reducing sugar

Reducing sugar content from the extract was determined by the method of Miller (1959). According to method, 0.2ml sample was added to 2.0ml of Dintrosalicyclic acid, mixture was heated in a boiling water bath for 5 minutes. After cooling of sample take absorbance against the blank at 540nm. The standard graph was used to calculate the concentration of reducing sugar.

Determination of phenolic content

The total phenolic concentration of the extract was determined by the method of (P. Yaseubi *et al.* 2007). 0.2ml sample added 1ml of Follin and 0.3ml NaCo3 (sodium carbonate). The solution mixture was stand at room temperature for 30 minutes. The absorbance was monitored against the blank at 765nm.

Determination of Antioxidant activity

The antioxidant activity of the sample was determined by the method of Prieto *et al.* (1999). 0.2ml of sample was combined with 2ml of reagent (0.6M Sulfuric acid, 28mM Sodium phosphate and 4mM ammonium molybdate). The test tubes were incubated in the boiling water bath at 95°C for 90 minutes and the sample was cooled at room temperature then absorbance was measured at 695nm against blank.

Determination of total Flavonoids

The total flavonoid concentration of the extract was determined by the method of Kim *et al.* (2003). According to this method, 0.1 samples was added 0.3ml of 05% sodium nitrate and 0.3ml of aluminium chloride, after 05 minutes, add 2ml 1M Sodium hydroxide. The absorbance was noted against blank at 510nm.

Result and discussion

Generally, to understand the response of plants germination, early seedling and stages of vegetative growth are considered as most important in terms elucidating the salt sensitivity or its tolerance mechanism and ultimately their survival under these harsh conditions (Danai-Tamble *et al.*, 2011). Therefore, the present study focused to investigate the biochemical and phytochemical behaviour of *Hordeum vulgare* under different concentration of $MgCl_2$ and KCl_2 stress. Theresults of various biochemical and phytochemicalproducts analyzed from the leaves of control and salt-treated plants of *Hordeum vulgare* are presented in figs 1-09.

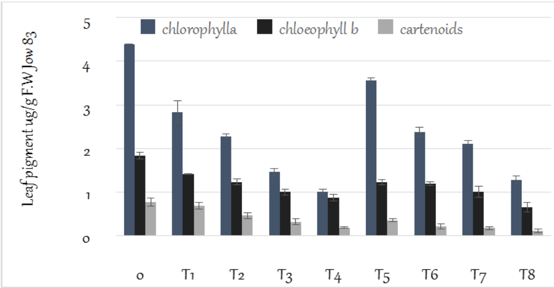

Fig. 1. Chlorophyll a, chlorophyll b and carotenoids contents (µg/g F.W) in *Hordeum vulgare* cultivars Jow 83leaves, treated with different salts against control (Mean ± S.D, p<0.05).

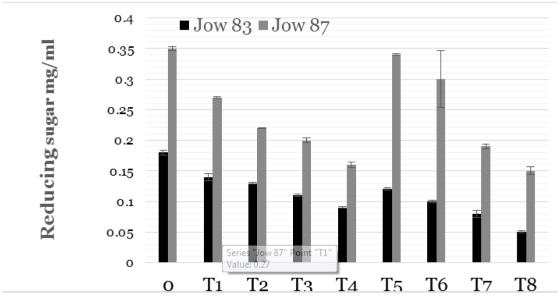

Fig. 5. Effect of different salt treatment on the amount of reducing sugar of *Hordeum vulgare* cultivars leaves (Mean±S.D, p<0.05).

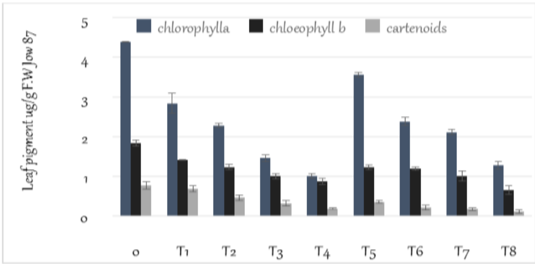

Fig. 2. Chlorophyll a, chlorophyll b and carotenoids contents (µg/g F.W) in *Hordeum vulgare* cultivars Jow 87leaves, treated with different salts against control (Mean ± S.D, p<0.05).

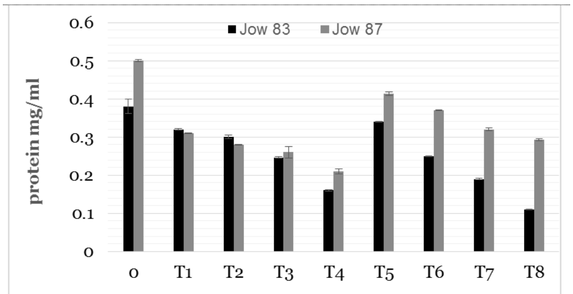

Fig. 6. Effect of different salt treatment on the amount of total protein of *Hordeum vulgare* cultivars leaves (Mean±S.D, p<0.05).

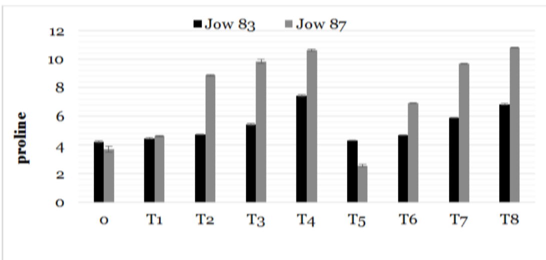

Fig. 3. Proline contents (µg/g F.W) in *Hordeium vulgare* cultivars leaves, treated with different salts against control (Mean ± S.D, p<0.05).

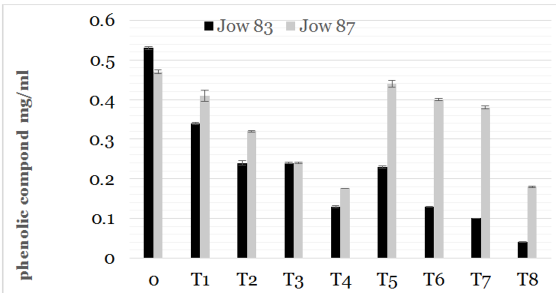

Fig. 7. Effect of different salt treatment on the amount of phenolic compounds of *Hordeum vulgare* cultivars leaves (Mean±S.D, p<0.05).

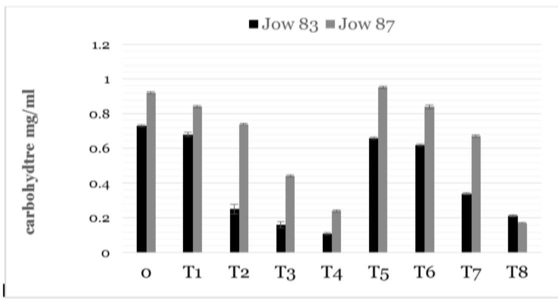

Fig. 4. Effect of different salt treatment on the amount of total carbohydrate of *Hordeum vulgare* cultivars leaves (Mean±S.D, p<0.05).

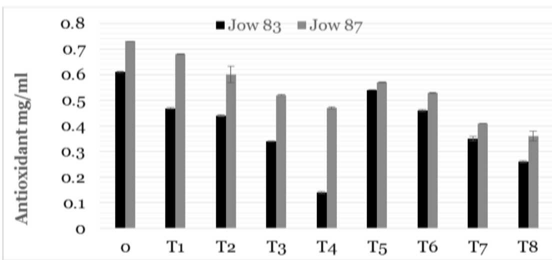

Fig. 8. Effect of different salt treatment on the amount of antioxidant activity of *Hordeum vulgare* cultivars leaves (Mean±S.D, p<0.05).

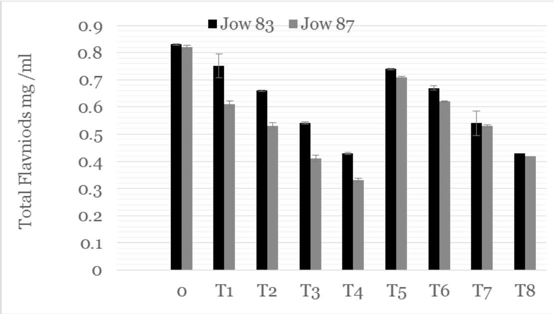

Fig. 9. Effect of different salt treatment on the amount of total flavonoids of *Hordeum vulgare* cultivars leaves (Mean±S.D, $p<0.05$)

The biochemical and phytochemical parameters were clearly showed affected by the increasing of $MgCl_2$ and KCl_2 stress. The various response of intra-specific variety towards $MgCl_2$ and KCl_2 stress evident. In present results and investigation may found to be proved to understand the complexities hidden in the responses tolerance stress evident in present investigation and the results found may be proved to understanding the complexities hidden in the responses tolerance mechanism towards the salinity in the *Hordeum vulgare*. Leaf pigments were continuously decreased with progression in the concentration of $MgCl_2$ and KCl_2. However, contradictory to the other parameters, the degree of reduction in chlorophyll content was higher invariety Jow 83 as compared to Jow 87 (Table 1, 2). Another important biochemical marker is proline, which is considered as a major osmoregulator in plants under different stress conditions, which help plants to counteract and recovery from salt stress Kumar *et al.* (2010). There was Proline content was increase in both genotypes with increasing salinity level from 0mM to 200mM. Amongst two cultivars, Jow 87 showed higher proline content as compared to Jow 83 under maximum salinity level (200mM), against the control plants of the both genotypes (table 3). The results showed a significantly ($p<0.05$) higher amount of total Proteins in Jow 83 and Jow 87 (0.38mg/ml, 5mg/ml D.W.), total carbohydrates in Jow 83 and Jow 87 (0.73mg/ml, 0.92mg/ml D.W.), antioxidant activity in jow 83, and Jow 87 is (0.61mg/ml and 0.73mg/ml D.W), phenolic compounds (0.53mg/ml0.47mg/ml D.W), reducing sugar (0.18mg/ml 0.35mg/ml D.W) were found in control plants of both cultivars. Similar results were also reported other plant species, like Tushar Khare, (2012) treated *Sorghum cultivars* with different concentration of $MgCl_2$and found that amount of proline was increased in salinity and leaf pigments were decreases with increasing of salinity treatment.

Conclusion

In conclusion, results showed that Jow 87 showed better as compared to Jow 83 against the different concentration of $MgCl_2$, but various concentration of KCl_2 showed damaging effect on growth and poor phytochemical both cultivars. *Hordeum vulgare* genotype Jow 87 also showed lower antagonistic effects on biochemical content like chlorophyll and proline content. This cultivar also showed lower level of salt stress-induced in various phytochemicals. All these biochemical and phytochemicals parameters seem to have played an important role in its better $MgCl_2$ than KCl_2 salts.

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Abbreviations

0= Control, T1= 50 mM KCl2, T2= 100mM KCl2, T3=150mM KCl2, T4= 200mM KCl2, T5=50mM MgCl2, T6=100mM MgCl2, T7= 150mM MgCl2, T8= 200 mM MgCl2.

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