# Influence of Exogenous Application of Proline On Some Physio-Biochemical Parameters of Maize (*Zea mays* L.) Under Drought Stress

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## ABSTRACT

**Background**: In nature, the plants are exposed to various biotic and abiotic stress factors. In abiotic factor soil salinity, water deficit, heavy metal and extreme temperature are included are the major factors which directly inhibit growth and development of crop plants (Ali, M, H, & Athar, 2007). In these abiotic stresses, the drought/water deficit is major abiotic stress that limits biological yield (Ashraf & Foolad , 2007).Among abiotic stresses drought is a major detrimental factor all over the world.

**Objective**: The present study was designed to investigate the influence of seed priming with Proline under drought stress.

**Material and Methodology**: The seeds of two varieties of maize (SG 2002 F-Goi & Hay Corn) were grown in control and drought stress condition under the exogenous application of Proline as seed priming with varying regimes (0, 200 ppm, 400 ppm). Both varieties differ in terms of drought tolerance.

**Results**: The biomass production was reduced due to drought stress, but Proline enhanced the biomass production in terms of shoot and root fresh and dry organs at the 400 ppm as compared to other regimes. The chlorophyll “a”, “b”, “a/b” and “a+b” content was reduced under drought stress while 400ppm pre-soaked Proline enhanced the chlorophyll “a”, “b”, “a/b” and “a+b” content in shoot organ of both varieties. Total Protein and amino acid content in leaf was greatly affected due to drought stress but seed primed with Proline @400ppm enhanced the production of total protein and amino acids in leaf organ of both varieties. SDS –PAGE Protein.

**Conclusion**: Profiling showed that 200ppm concentration of Proline was most effective in both varieties. Among both varieties the variety “Hay Corn” is most tolerant toward the drought stress

**Keywords**: Abiotic stress, Drought, Free amino acid, Proline, Proteins, Maize

## 1. INTRODUCTION

In nature, the plants are exposed to various biotic and abiotic stress factors. In abiotic factor soil salinity, water deficit, heavy metal and extreme temperature are included are the major factors which directly inhibit growth and development of crop plants (Ali, M, H, & Athar, 2007). In these abiotic stresses, the drought/water deficit is major abiotic stress that limits biological yield (Ashraf & Foolad , 2007)Drought stress causes the changes at anatomical, physiological, biochemical and molecular level in plants at all stages of life cycle (Ali, M, H, & Athar, 2007). All these metabolic processes are determined the plant health. Disturbance in one of these can affect the plant

growth and development. Drought or water deficit condition severely effects on seed germination and cell growth of plants. (Ali & Komatsu, 2006). The activity of meristematic cell division and other expansion of newly develop cell increases the growth of plant depends upon turgor pressure of plant cell. Under drought stress, growth is retarded in higher plant due to disturbance in water movement from xylem to the make longer cell ( (Harris, D, Tripathi, R, & Joshi, A, 2011).

Drought stress reduced the shoot and root fresh weight (Neisiani, et al., 2009), reduced ion uptake like Potassium and Calcium enhanced the uptake of Sodium decreased the accumulation of protein and amino acids (CHAUM & Kirdmanee, 2010), decrease the chlorophyll content and causes the photo-oxidation of chlorophyll. Water deficit decrease the chlorophyll a, b, a/b and over-all chlorophyll (Jaleel, P, & G.M, 2008). Photosynthetic efficiency of plant decreases chlorophyll contents in water deficit condition. Drought change a variety of plant responses that ranges from cellular metabolism as result of this growth rates and crop yields are reduced.

To save the plant from the harms of drought or other stresses plant accumulate the low molecular weight enzymatic or non-enzymatic antioxidants. Superoxide dismutase, Peroxides, Catalase (CAT) and ascorbate peroxides (APX) are enzymatic antioxidants (CHAUM & Kirdmanee, 2010).While glutathione, and ascorbate and carotenoids are non-enzymatic antioxidants. These both enzymatic and non-enzymatic antioxidants work together in scavenger mechanism to ROS. The osmolyte may be sucrose, soluble carbohydrates, glycine betaine and other solutes. The drought tolerant plant may have increased level of these osmolytes. While their level decreases in sensitive plants (Anjum, et al., 2011).

Among these osmolytes the Proline which is water soluble amino acid is the most important in the protection of plant facing drought stress. Under drought stress, the Proline is first osmolytes that protect the plant from the injury to cell (Anjum, et al., 2011). So, to overcome the drought stress condition, the exogenous application of Proline is a smart tool.

Proline could be turned as a signaling molecule to modify plant physiological functions in termns of osmotic adjustment, upgrade photosynthetic enhanced ion uptake (Abdelhamid, et al., 2013), enhanced antioxidant activity and reduce ROS production improve biological yield (Ali Q. F., 2013).and also effects on cell explosion or cell death and cause gene expression, that can be vital for plant rescue from osmotic stress. Proline accumulation was observed in many stress tolerant plants like maize, rice, wheat etc (Ashraf & Foolad , 2007).

Maize (Zea mays L.) is cereal crop belong to family *Poaceae* (Chen, Zhong, Fan, & Li, 2015). Maize is 3rd significant crop among cereals and it is consumed by man as food also forage for cattle and poultry ( (Rahi, et al., 2013). Due to over population food demand is increasing with the passage of time (Muhammad, A, J. L, Meade, & Regmi, 2011),and this crop is gaining an imperative position in crop cultivation farming due to its high yield prospective, high nutritious worth, short growth period, consumption in industry to make corn silk, corn sugar, and corn flask. Thus, the present study was designed to find out the physiological, biochemical and molecular aspects of maize under drought stress treated with exogenous application of Proline (Reid, et al., 2015).

## 2. MATERIALS AND METHODS

The experiment was carried out in Botanical Garden of Bahauddin Zakariya University Multan. The experiment was arranged in Complete Randomized Design with four replicates. The pots were filled with soil. The seeds of two varieties of maize SG 2002 F-Goi and Hay corn were pre-soaked in 0ppm, 200ppm and 400ppm Proline. Plants of three weeks were treated with drought stress by skipping the irrigation. After a week of stress, the plants were harvested for further morphometric, physio- biochemical and proteomic profile.

### 2.1 Morphometric Attributes

#### 2.1.1 Shoot and Root length

The shoot and root length was measured by using the tape-meter.

#### 2.1.2 Fresh and dry weight of root and shoot

The fresh weight of root and shoot weight was calculated by using the electric balance. Then the plants root and shoot were kept in oven at 50ºC for a week. After one week, the plants were completely dried. Then the weight of samples was done

### 2.2 Biochemical assays

#### 2.2.1 Chlorophyll estimation

The chlorophyll was measured by the method of Arnon, Allen, and Whatley (1956). The leaf sample about 1g was ground into 3ml acetone, filtered and raised up to 10ml with acetone. The values were taken by using double beam spectrophotometer (U-Hiteach 2900) at 663nm, 652nm and 645nm.

#### 2.2.2 Proteins quantification

Proteins quantification was done following the procedure of Bradford (1976).

#### 2.2.3 Total soluble proteins

The leaf samples about 200g was ground into sodium phosphate buffer having pH 6.8 in chill condition and centrifuged at 15000rpm. Supernatant was kept for further quantification of proteins. The 30µl supernant was mixed in 1500µl Bradford reagent in dark condition. Then the reading was taken by using double beam spectrophotometer (U-Hitachi) at 595nm.

#### 2.2.4 Total free amino acids

The 1ml supernant was mixed with 1ml ninhydrin and 1ml pyridine. Then the mixture was heated in water bath for 30minutes at 100ºC and raised up to 100ml by using distal water. Then the read was taken in double beam spectrophotometer at 570nm.

### 2.3 Molecular investigations

#### 2...3.1 Proteins profiling

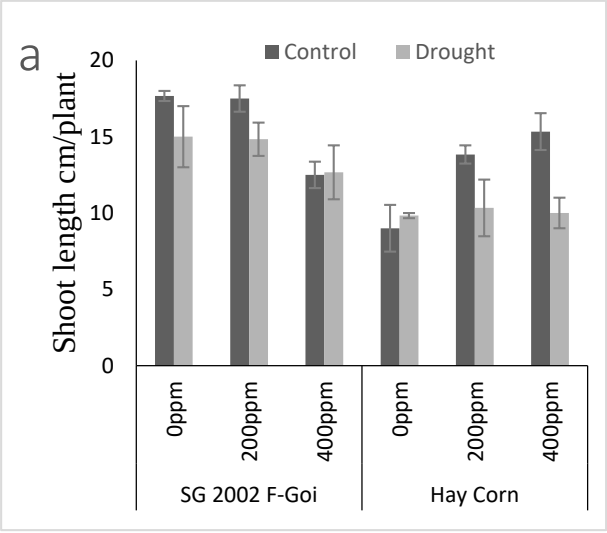
Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed to resolve the proteins. It was performed by using the protocol of Laemmli (1970).

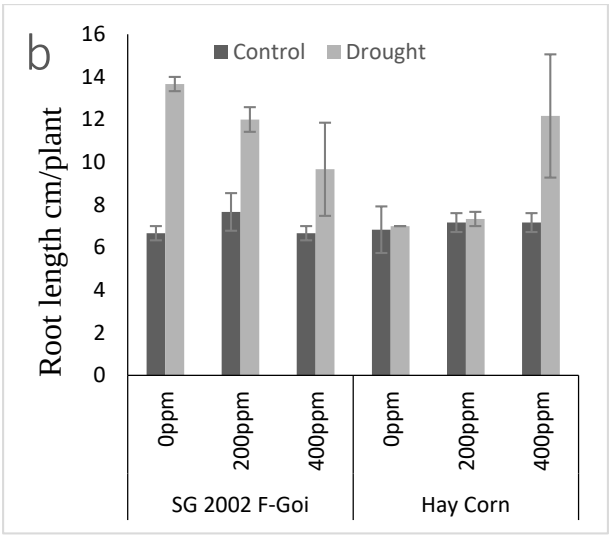
## 2.4 Statistical analysis

The analysis of variance (ANOVA) was done by using the SPSS software.

## 3. RESULTS

The analysis of variance for the shoot length had been shown in table 1. The significance results were observed when maize is subjected to pre-soaked Proline under drought stress. The maximum shoot length was recorded in SG 2002 F-Goi varieties in control condition without the pre-soaked with Proline. While the minimum shoot length was recorded in Hay corn in control condition as shown in figure 1(a). Under control condition 0ppm Proline pre-soaked, the variety Hay corn showed minimum shoot length than other variety. The analysis of variance of root length was shown in table 1. The analysis showed non-significance interaction between the drought stresses in different pre-soaked level of Proline. While the both two varieties showed the significance results with pre-soaked proline and without pre-soaked Proline in control condition. The Hay Corn in 0ppm Proline was showed highest root length.





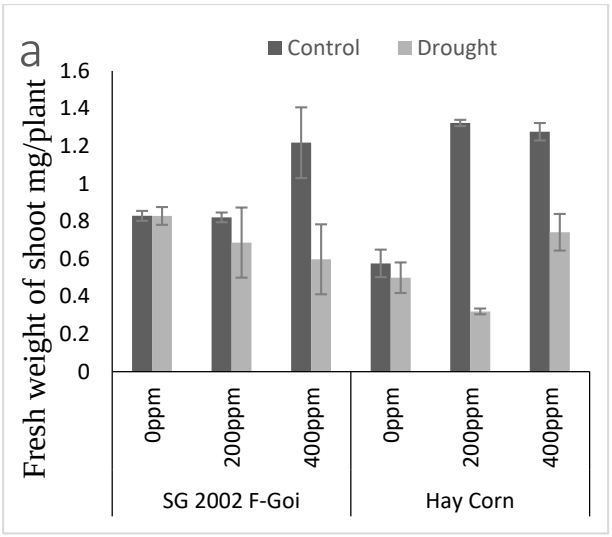
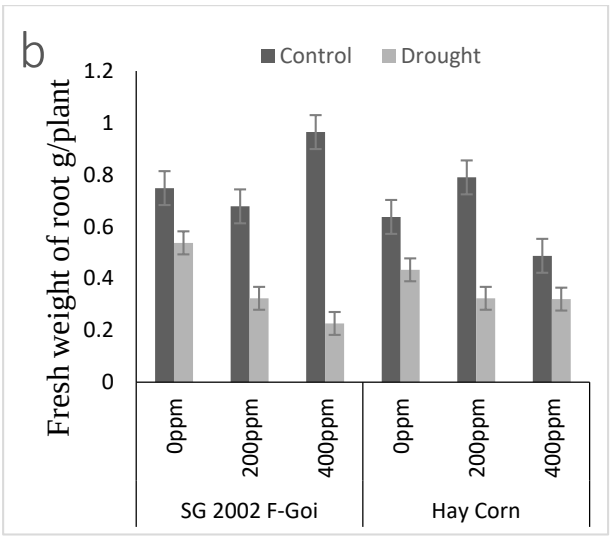
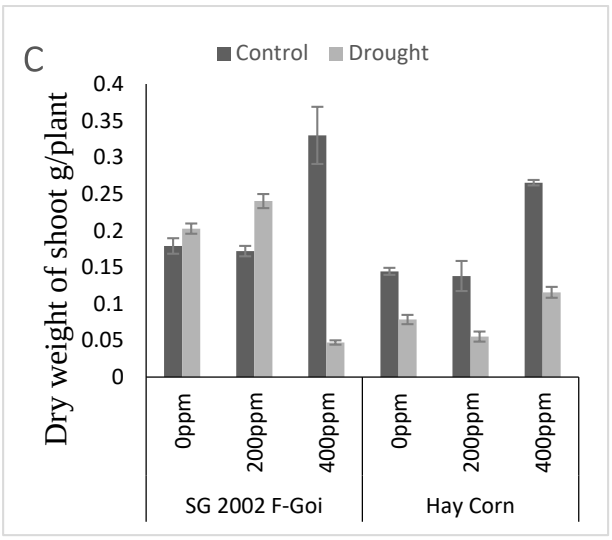




**Figure 1**: Shoot (a) and Root (b) length of maize varieties pre-soaked with 0ppm, 200ppm and 400ppm Proline grown under control and drought condition.

The analysis of variance for fresh weight of shoot has been shown in table 1. The maximum fresh weight shoot was seen in Hay Corn with 200ppm and 400ppm Proline treatment in control condition and minimum with Proline 200ppm under drought (figure in 2(a)). The analysis of variance for the fresh weight of root has been shown in table 1. The fresh weight of root was decreased in both varieties in drought condition without and with pre-soaked Proline. The variety SG 2002 F-Goi was showed highest fresh weight of root in control condition with pre-soaked 400ppm Proline as shown in figure 2(b). The analysis of variance for the dry weight of shoot had been presented in table 1. From the table it has shown application of Proline shown significance enhancement of dry weight of shoot in 0 and 200ppm in SG 2002 F-Go under drought stress. The dry weight of shoot was decreased in pre-soaked in Hay Corn variety under drought condition as shown in figure 2(c).



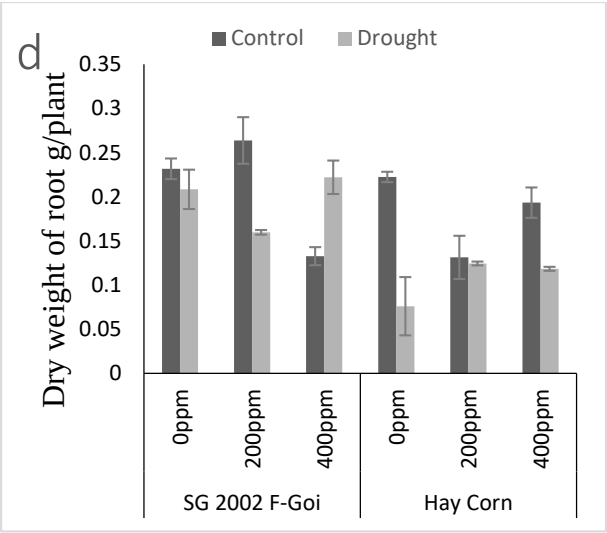
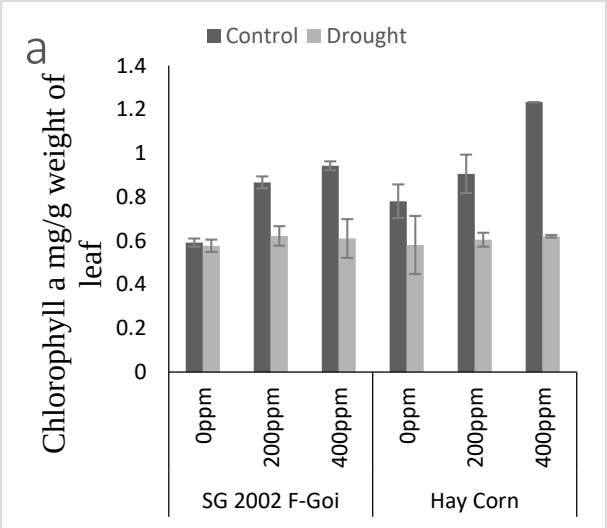
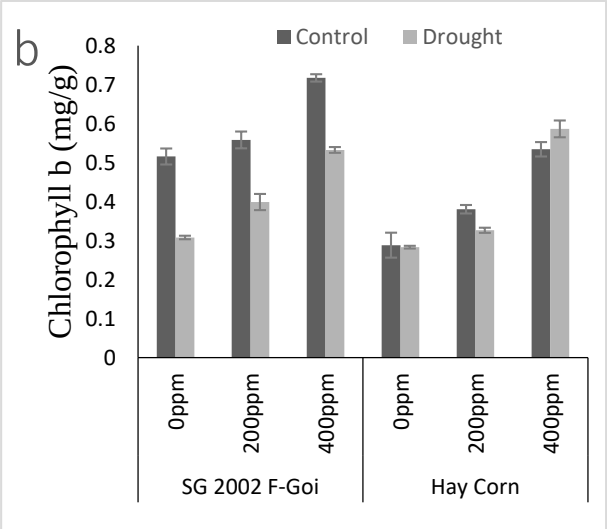


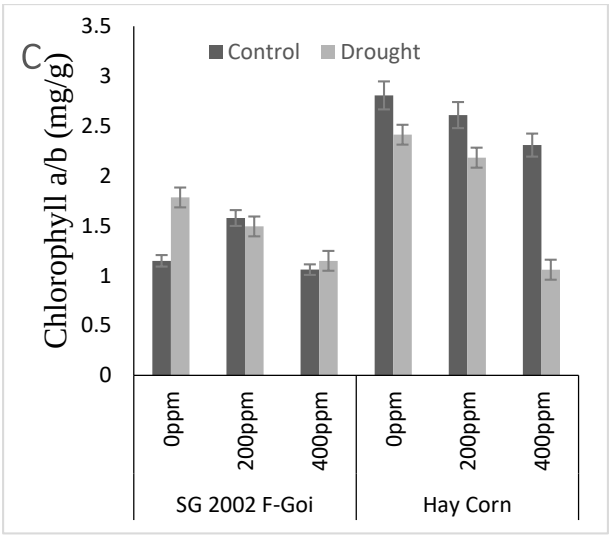

**Figure 2**: Fresh weight of shoot (a), Fresh weight of root (b), Dry weight of shoot (c) and Dry weight of root (d) of maize varieties pre- Soaked with 0ppm, 200ppm and 400ppm Proline grown under control and drought condition.

From the table and figure it has been cleared that the drought has negative effect on the dry weight of root. The variety SG 2002 F-Goi was showed better performance under drought and control condition with and without pre-soaked Proline. In control condition with 200ppm pre-soaked Proline. The variety SG 2002 F-Goi was showed maximum dry weight of root in control condition with pre-soaked 200ppm Proline as shown in figure 2(d).

The chlorophyll a content was shown in table 1 and figure 3(a). From the figure it is cleared that chlorophyll a contents were reduce in drought stress as compared to control except 0ppm. Under control condition with pre-soaked with 400ppm showed highest chlorophyll contents. While under drought stress all pre-soaked with 0ppm, 200ppm and 400ppm was showed equal chlorophyll a contents. The chlorophyll b contents are presented in figure 3(b). From the figure it is cleared that the chlorophyll b contents were reduced in drought stress as compared to control condition in variety SG 2002 F-Goi. Under drought condition, the pre-soaked 400ppm was highest chlorophyll b contents in Hay corn variety. In variety SG 2002 F-Goi, the a/b was increased 0ppm under drought stress. Under drought stress, the a/b of chlorophyll was decreased in all treatment of Proline The total chlorophyll contents were shown in figure 3(d) and table 1. Under control and drought condition pre-soaked 400ppm proline was showed highest chlorophyll contents. While under control and drought condition 0ppm was showed lowest chlorophyll contents.





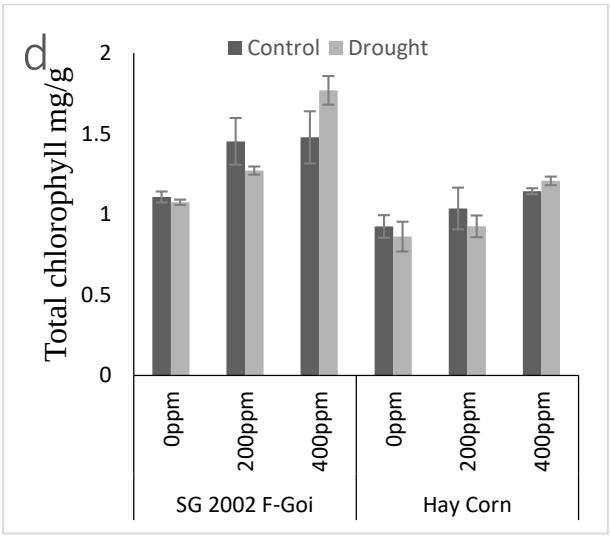




**Figure 3.** Chlorophyll a (a), b (b), a/b (c) and Total chlorophyll (c) contents of maize varieties pre- Soaked with 0ppm, 200ppm and 400ppm Proline grown under control and drought condition

**Table No. 1: Analysis of variance for shoot length, root length, fresh weight of shoot and root, Dry weight of shoot and root, Chlorophyll contents, total soluble proteins and total free amino acids**

| Source of Variance (SOV) | Shoot Length (cm/plant) | Root length (cm/plant) | Shoot Fresh Weight (g/plant) | Root Fresh Weight (g/plant) | Shoot Dry Weight (g/plant) | Root Dry Weight (g/plant) | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Chlorophyll a/b (mg/g) | Total Chlorophyll (mg/g) | Total Soluble Proteins (mg/g) | Total Free Amino Acids (mg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Varieties** | 25.729 *** | 23.761*** | 0.446 ns | 1.695 ns | 57.517 *** | 32.420 *** | 6.917 * | 14.730 *** | 14.474 *** | 0.012 ns | 0.350 ns | 0.918ns |
| **Drought** | 9.357 ** | 4.614* | 42.34*** | 32.789 *** | 98.193 *** | 18.525 *** | 32.931 *** | 7.736 * | 1.110 ns | 45.274 *** | 16.332 *** | 19.085 *** |
| **Proline Priming** | 1.673 ns | 0.138ns | 6.991 ** | 0.713 ns | 9.647 *** | 1.1576 ns | 5.351 * | 19.303 *** | 3.235 ns | 21.441 *** | 1.466 ns | 9.138 ** |
| **Varieties * Drought** | 0.433 ns | 4.976* | 5.559 * | 1.555 ns | 4.737 * | 9.461 ** | 7.062 * | 7.410 * | 3.980 ns | 0.374 ns | 2.324 ns | 0.749ns |
| **Varieties * Proline Priming** | 8.030 ** | 0.616ns | 4.281 * | 1.360 ns | 16.360*** | 3.389 ns | 2.329 ns | 0.827 ns | 0.516 ns | 3.542 * | 0.086 ns | 6.043 ** |
| **Drought * Proline Priming** | 0.833 ns | 4.875* | 8.633 ** | 1.474 ns | 67.406*** | 6.921 ** | 3.246 ns | 0.149 ns | 0.802 ns | 2.162 ns | 0.125 ns | 1.756 ns |
| **Varieties * Drought * Proline Priming** | 3.280 ns | 3.783* | 5.915 ** | 2.853 ns | 27.528*** | 15.46*** | 1.848 ns | 0.273 ns | 0.421 ns | 0.734 ns | 0.060 ns | 5.35* |
| **Error** | 4.632 | 4.069 | 0.033 | 0.0350 | 6.080 | 9.573 | 0.018 | 0.009 | 0.461 | 0.023 | 701.354 | 2632026.9 |

The analysis of variance for the total soluble proteins has been presented in table 1. The both varieties showed non-significance results in control with and without pre-soaked Proline. These result also non-significance between two varieties under drought stress as shown in figure 4(a) and table 1. The analysis of variance for total free amino acids has been presented in table 1. From the table and figure, it has been shown that the both varieties in 0ppm and 200ppm showed non-significance results in control condition. The maximum value of total free amino acids has been recorded in SG 2002 F-Goi variety under drought stress pre-soaked with 400ppm Proline as shown in figure 4(b).

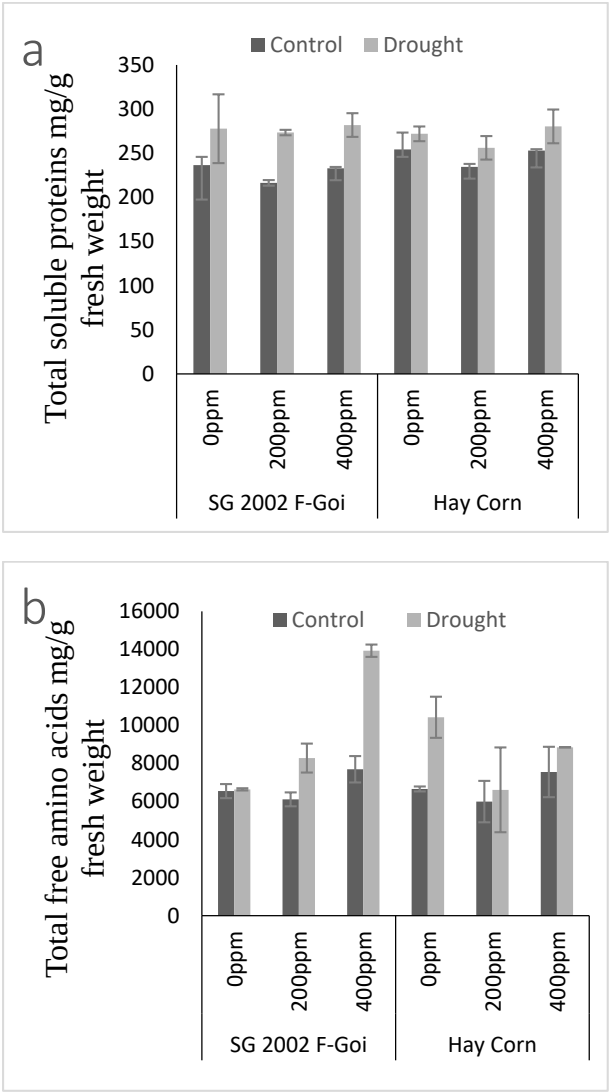



**Figure 4.** Total soluble proteins (a) and Total free amino acids (b) of maize varieties pre-soaked with 0ppm, 200ppm and 400ppm Proline grown under control and drought condition.

The figures 5 (A, B, C and D) showed the different banding pattern under reducing and non-reducing condition of maize varieties pre-soaked with Proline 0, 200 and 400 ppm concentration. Under reducing condition in both varieties, the bands are clearer and reflect the expression of proteins under drought and pre-soaked condition.

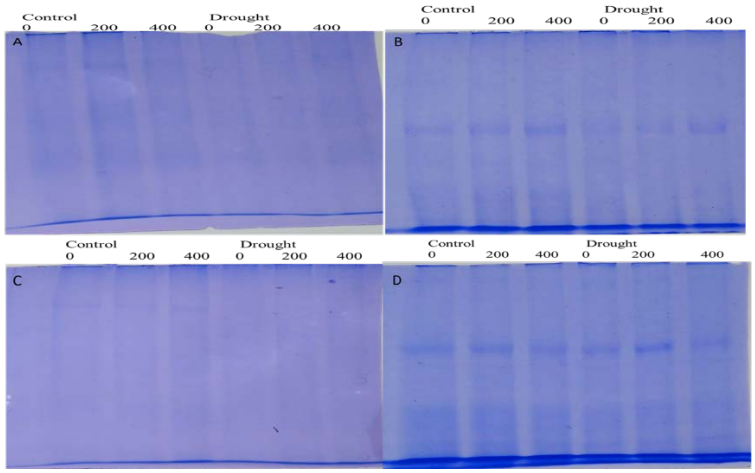



**Figure 5.** SDS-PAGE gel stain with Coomassie brilliant blue dye G-250 in non-reducing condition (A) and Reducing (B) of maize variety SG 2002 F-Goi while C and D non-reducing and reducing respectively for Hay corn maize variety with 0ppm, 200ppm and 400ppm Proline grown under control and drought condition.

**Figure 5**: SG 2002 F-Goi maize variety pre-soaked with 0ppm, 200ppm and 400ppm Proline grown under control and drought condition.

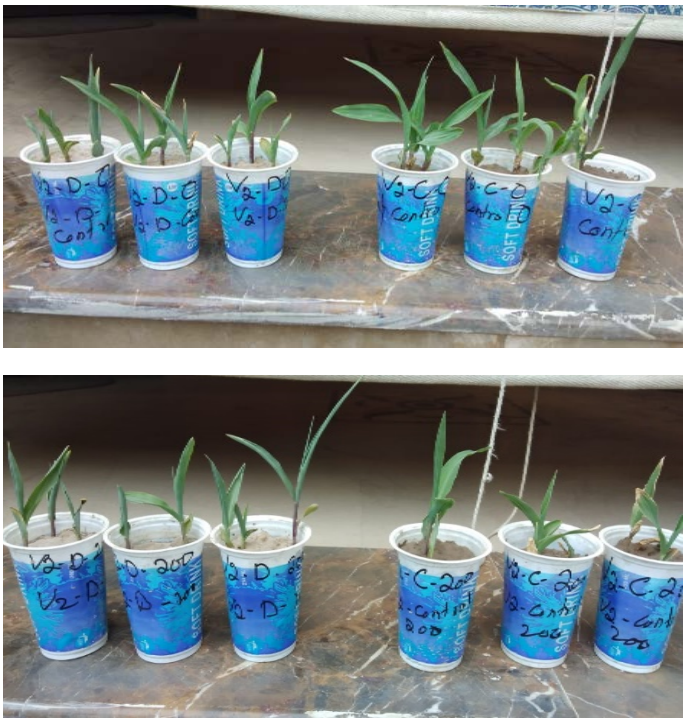


**Figure 6**: Hay Corn maize variety pre-soaked with 0ppm, 200ppm and 400ppm Proline grown under control and drought condition.

## 4. DISCUSSION

Drought is major abiotic stress that decreased the plant growth, development and yield of crop plant. Plant accumulates different osmolytes such as Proline. The exogenous application of Proline relief the plant under stress condition ( (Aldesuquy, et al., 2012).

Biomass production of Maize was decreased when it is subjected to drought in comparison to control condition and increased while in the plants treated with Proline concentrations. Same happened with root and shoot length. The reason for reduction in shoot and root length as well as reduction in their biomass like may be due to enhance in osmotic potential by cumulative salts, which centrals to dehydration, ionic imbalance in developing leaves that caused reduction in meristem activity and cell elongation.These results are similar to previous studies by (Kausar, A, M, & Niaz, 2014), (Harris, N, V, Tester, & M, 2010) and (Noreen, Athar, U, & Ashraf, 2013), exhibited reduction in biomass production by the imposition of salt in barley, wheat, pigeon pea and cotton. Under drought stress, the plant usually goes towards programmed cell death due to unavailability of water for a long time.

When exogenous application Proline is done on plants, this compatible solute usually ameliorate the consequences of drought stress by doing osmotic adjustment as in maize (Ali, et al., 2013).These results are similar to the findings of (Aldesuquy, et al., 2012). In the present study, the chlorophyll contents were decreased under drought stress and showed high graph in treatment with Proline. Stress avoids the plant to set off working of pigments like chlorophyll and causes the production of protein (Davison, P, Hunter, C, & Horton, P, 2002). Similar findings were observed in the chlorophyll contents of maize. Proline is a compatible solute and it enhances the chlorophyll content of crop plants as it maintains the integraty of light harvesting complex proteins which usually hold chlorophyll molecules. These results are compatible to (Cha-um, T. Samphumphuang, & C. Kirdmanee, 2013), who respoted maintainance of chlorophyll content in rice under drought stress.

Drought stress has significance effect on proteins and amino acids. The current study indicates that the drought stress increased the total soluble proteins and total free amino acids. The pre-Soaked Proline varieties showed more proteins and amino acids under drought stress. These results are similar to previous studies. The increment in soluble protein by the application of Proline is because of de novo synthesis of proteins and amino acid for cell defence (Teixeira, M & Carvalho, I. S. d, 2009). Literature says that the proteins content improves in the plants facing stress and this increased amount of proteins tries to protect the plant from unadorned stress (Stich, B, et al., 2010). Under drought stress, proteins and amino acids content increase in crop plants to ameliorate the adverse effects of drought but Proline foliar application further causes increase in total soluble proteins and total free amino acids. These results are favorable with (Talat, et al., 2013), who reported increase in TSP and total free amino acids in wheat crop by foliar application of proline.

Protein Profiling is usually done for the quantification of the disintegration of proteins under different stresses. Thus clear band of increase in proteins are present by the exogenous application of Proline under drought stress. These results are compatible to (Moharramnejad, et al., 2015).

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