PHYSIOLOGICAL AND BIOCHEMICAL ALTERATIONS IN GLYCINE MAX.L PLANTS UNDER CONTINUOUS STRESS CONDITIONS

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ABSTRACT - The present study was aimed at evaluating the effects of progressive and continuous abiotic stress conditions on four varieties of Glycine max. L. In order to emphasize the main effects of different continuous stress conditions on physiological and biochemical changes in Glycine max. L, pot experiments were carried out. After germination, seedlings were well watered until two complete weeks, then the plants were subjected to one week of heavy metal treatment (50µM) followed by one week of salinity treatment (150mM) and finally left for drought treatment for a week. Several parameters known as indicators of plant status were monitored which include plant shoot length, root length, protein, chlorophyll, soluble carbohydrates, ascorbic acid, proline and glycine betaine levels. However significant changes were observed in physiological parameters and decrease in total chlorophyll, protein and starch. Apart from that, there was a significant increase in sugars, proline and glycine betaine levels as a consequence of stress induced to plants. This fact reveals the inefficient osmotic adjustment as a consequence of continuous stress applied to the plants.

Key words - Glycine max. L., Abiotic stress, Reactive oxygen species, Ascorbic acid, Osmotic adjustment

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1. INTRODUCTION:

An extensive loss to the agricultural productivity worldwide is mainly caused due to abiotic stress conditions. Stress conditions such as salinity, heavy metals, high/low temperatures, pH and drought have been individually the subject of intense research. But, in the fields, the crops routinely encounter a combination of different abiotic stress conditions [1]. Major problem encountered by many agriculturally important crops, is the combination of drought and other stresses, such as heat or salinity [2]. It is clear from the recent studies, that the physiological, biochemical, molecular and metabolic responses of plants individually to each of these different stresses is completely different to that of combination of stresses [1].

Plant cells adopt many strategies in response to different abiotic stress conditions such as salinity, drought, cold, heat and excessive osmotic pressure.

To resist to all these abiotic stress conditions, cells adopt themselves by undergoing different mechanisms including changes in morphological and developmental pattern as well as physico-biochemical processes. Adaptation to stress is associated with metabolic adjustments which lead to the accumulation of several organic solutes and osmolytes. Osmolytes such as proline, Glycine betaine, sugars, polyols and amino acids are synthesized in response to continuous stress conditions [3].

Salinity or heavy metal stress might pose a problem to plants when compared with drought because enhanced transpiration could result in enhanced uptake of salt or heavy metal. Thus these conditions generate oxidative stress due to an increase in the levels of reactive oxygen species (ROS) within subcellular compartments. ROS include the superoxide radical (O2-), hydrogen peroxide (H2O2), and the hydroxyl radical (OH-), all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids [4]. ROS are also known to damage cell membranes by inducing lipid peroxidation, causing membrane damage, and inactivation of enzymes and alteration of DNA activity [5]. Under steady state conditions, the ROS molecules are scavenged by various antioxidative defense mechanisms. The equilibrium between the production and the scavenging of ROS may be perturbed by various abiotic stress factors such as heavy metals, salinity, heat, cold and drought. The major example of antagonism between different abiotic stresses is drought and heavy metal stress, which exaggerate the effects of each other [6]. The simultaneous exposure of crops to different abiotic stress conditions will result in the coactivation of different stress response pathways. Those might have a synergistic and antagonistic relationships between different pathways exist. Tolerance to a combination of different stresses is likely to be complex trait involving multiple pathways and cross talk between different sensors and signal transduction pathways. Many plant species naturally accumulate protein, proline and Glycine betaine as major organic osmolytes when subjected to different abiotic stresses. These compounds play an adaptive role in mediating osmotic adjustment and protecting sub cellular structures in stressed plants [7]. Thus, different approaches have been contemplated to increase the concentrations of these compounds in plants grown under stress conditions to increase their stress tolerance.

Soybean is an important economical, nutritive and medicinal plant. However, the yield of this plant is reduced due to stress conditions in the soil. Therefore, reduction in plant growth under stress conditions could be an outcome of altered hormonal balance. Moreover it presents the possibility of using the carbohydrates as energy source under severe stress conditions. When compared to other tropical legumes, such as Vigna unguiculata and Phaseolus vulgaris, Soyabean is considered to be a sensitive species to the several abiotic stress conditions [8, 9, 10]. Hence, for the enhanced tolerance of crops and plants to combined stress conditions, it is important to bridge the gap between molecular, physiological and metabolic aspects of stress combination. In the present study, attention has been focused to investigate the impact of salinity, heavy metal and drought continuously on physiological and biochemical attributes of Glycine max. L.

2. MATERIALS AND METHODS:

2.1 Plant material and growth conditions:

The certified seeds of Glycine max.L were collected from Regional Agricultural Research Station (RARS), Adilabad, Andhra Pradesh, India. The four varieties of Soyabean used for the present study are Basar, DSB-20, JS-335 and JS-93.05. The seeds were surface sterilized with commercial bleach 0.1% HgCl2 solution for 5 min and washed thoroughly three times with distilled water. The seeds were propagated in clay pots containing air-dried black soil and vermicompost with 70:30 ratio respectively. Seeds of all the four bean cultivars were sown in this soil medium and were maintained under natural photoperiod and then watered regularly.

2.2 Treatments and Sample Collection:

Germination was observed on 3rd day from the day of sowing of seeds. The plants were grown normally by supplying water regularly for 14 days. Based on the preliminary experiment results, 50µM lead acetate, 150mM NaCl were selected as the heavy metal and saline stress concentrations respectively for subsequent experiment. The experimental design used was carried out at randomized scheme, with 2 regimes (stress and control). The 14 days old Soya plants were first subjected to heavy metal treatment for 7 days followed by salinity treatment for 7 days and finally left for drought treatment for 7 days. Simultaneously, Controls were maintained by regular watering throughout the period. After 35 days seedlings were harvested, washed with double distilled water, plotted and used for the following measurements. Triplicates were maintained for all the experiments performed in the study. The samples can be stored at -80^{o}c for further use.

2.3 Morphological Parameters:

The plants were removed carefully with root system and washed thoroughly. 10-15 plantlets from each treatment were taken at random; the shoots length (cm) and roots length (cm) per plant were measured by meter scale. Immediately the plants were weighed for fresh weight, Plants were dried in oven by setting at low heat (100°C) over night. Then plants were cooled in a dry environment. After the complication of cooling, the plants were weighed for dry weight.

2.4 Biochemical Analysis:

Shoots were taken, 500 mg of both control and stressed leaves were taken, washed thoroughly with tap water followed by distilled water and blotted to dry in between filter paper folds. The midribs of leaf samples were removed, cut into bits and macerated with respective buffers for all biochemical experiments. For protein estimation, the leaves were homogenized using 70% (v/v) ethyl alcohol and precipitated by adding 20% (w/v) trichloroacetic acid. The precipitate was then dissolved in 1% (w/v) sodium hydroxide (NaOH) solution. Quantitative estimation of protein was done employing the method of Lowry et al. [11].Photosynthetic pigments such as chlorophyll a, chlorophyll b and total chlorophylls were extracted and estimated adopting the methods described by Arnon [12]. The estimation of total soluble sugars in leaves were determined by the method of Dubois et al. [13] and total starch content by method of Mc Cready et al., [14]. Ascorbic acid was estimated by colorimetric method of Sadasivam and Manickam [15]. Proline was determined according to the method of Bates et al. [16] and Glycine betaine was estimated by the method of Barak and Tuma [17].

2.5 Statistical Analysis:

All the Experiments were carried out in triplicates and mean of three values was calculated. The standard errors were calculated for each point, as well as in the results were applied the variance analysis (ANOVA) and were compared by the T- test at the (P≤0.05) significance level using the software SPSS.

3. RESULTS AND DISCUSSION:

3.1 Physiological Alterations:

After the exposure of plantlets to three different successive abiotic stress conditions, well defined morphological changes were observed in all four Glycine max.L. varieties studied in the experiment. The growth rate and height of the plant was observed to be decreased slowly with the increasing time period of stress treatment. Continuous abiotic stress treatments significantly (P≤0.05) decreased shoot and root length when compared to those plantlets grown under control conditions (Table 1). Shoot length was reduced in all cultivars at the end of all three abiotic stress treatments. Basar displayed 55.22% shoot length reduction over the control (100%), while the reduction percentage was 42.86% in DSB-20, 55.38% in JS-335 and 43.10% in JS-93.05 when compared with the control plants. On the other hand, root length was retarded in all cultivars of soyabean. Root length showed more pronounced decrease in Basar (60%) compared to other three varieties DSB-20 (52.38%), JS-93.05 (33.33%) and JS-335 (21.43%). Number of roots was also observed to be decreased. The fresh weight of plant decreased by 67.53% in Basar, 70.97% in DSB-20, 72.36% in JS-335 and 59.35% in JS-93.05. Similarly, with respect to decrease in dry weight of plant it was 74.16%, 70.79%, 75.32 % and 73.74 % in Basar, DSB-20, JS-335 and JS-93.05 respectively. In all the physiological parameters studied, shoot length, plant fresh and dry weights were found to be highly decreased in JS-335, whereas root length was found to be greatly decreased in Basar (Supplementary data provided).

The reduction in the plant height, fresh weight and dry weight may be attributed to the water deficiency in the soil. During photosynthesis, water losses occur through stomatal mechanism and the water assimilation rate is negatively affecting during drought stress [18]. The significant decrease in plant height may also be due to change in the concentrations of available elements and nutrients in the soil, which thereby disturbs the carbohydrate metabolism.

The root growth is a strategy used by the plants to capture water in substrate under water deficit conditions, in which the growth and development of plant depends on the cell turgor. Shoot and root growth has also been considered as a very sensitive indicator to combined stress conditions. Heavy metals, high salts have been shown to cause many morphological, physiological and biochemical changes in plants, such as growth inhibition [19]. The inhibitory action of excess concentrations of NaCl and lead in shoots and roots may be due to reduction in cell division, and toxic effect of salt and heavy metal on photosynthesis. These contributed to the retardation of normal growth demonstrated that, higher concentrations of salt and heavy metal along with water deficit decreased the growth, fresh and dry matter production.

3.2 Biochemical Alterations:

3.2.1 Total Protein Content:

Total protein content decreased due to abiotic stress conditions. Two soyabean cultivars exhibited decline in the total protein content whereas other two varieties showed slight increase (Fig 1). Basar and JS-335 showed increase in the protein content by 32.63% and 21.10% respectively. DSB-20 and JS-93.05 showed reduction by 10.76% and 2.28% respectively. The reduction in the total soluble proteins showed in the plants under stress is due to probable increase of the protease enzyme activity, which promotes the breakdown of the proteins and consequently decreases the protein amount present in the plant under abiotic stress conditions [20]. Under inadequate conditions, the plants activate the pathway of proteins breakdown, and use the proteins for the synthesis of nitrogen compounds as amino acids that might accelerate the plant osmotic adjustment [21]. Similar results on reduction in the proteins were found by Ramos et al. [22] investigating the effects of the water stress in Phaseolus vulgaris.

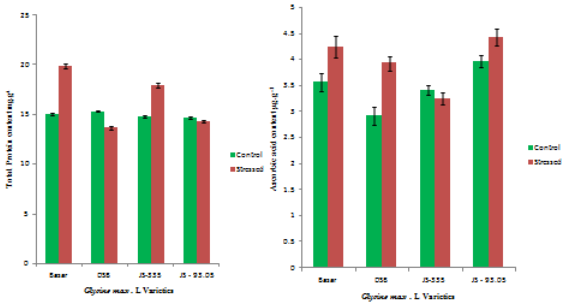

Fig.1. Total protein content and Ascorbic acid content in four cultivars of Glycine max.L during stress conditions when compared to control plants

3.2.2. Chlorophyll Content:

From the data in (Table.2), it is clear that abiotic stress treatments caused significant reduction (P≤0.005) in the content of Chlorophyll a, chlorophyll b and total chlorophyll in all Glycine max.L cultivars studied. In Basar, Chl a, chl b and total chlorophyll decreased by 3.49%, 12.78% and 7.31% respectively with respect to control plants (100%). Whereas in DSB-20, the decrease was 66.21% for chl a, 85.82% for chl b and 66.57% for total chlorophyll levels. JS-335 showed a decrease of 26.75% in chl a, 22.71% in chl b and 15.81% in total chlorophyll. In JS-93.05, chl a, chl b and total chlorophyll contents decreased by 56.1%, 64.57% and 40.62% respectively with respect to controls. The reduction in chlorophyll content may be characterized as a means to reduce photooxidation threat. Similar results were obtained by Thalooth et al., [23] which demonstrated that the limiting of water supply diminish the chl a, chl b and total chlorophyll content in the leaves of Vigna radiata at any stage of plant development. The same study explained this decrease may be due to the decrease of magnesium that is one of the principal chlorophyll constituents. It was also revealed that this can be attributed to the high level of chlorophyll degradation more than to its biosynthesis limitation under water stress conditions [24].

3.2.3 Total sugars and starch:

In all Glycine max.L cultivars studied, stressed plants showed a higher soluble sugar content with respect to control plants. Total soluble sugars increased by 14.67% in Basar, 11.17% in DSB-20, 8.42% in JS-335 and 9.30% in JS-93.05(Fig.2). Highest increase of soluble sugars was found in Basar. Earlier studies also indicate that, an increase of soluble sugars is accompanied with increased activity of acid invertase and sucrose synthesis. In addition to the role of sugars in osmoregulation, the soluble sugars allow the plants to maximize carbohydrates storage reserves to support basal metabolism under stressed environment [25].

The total starch contents were decreased in stressed plants by 1.02%, 39.94%, 8.88% and 1.82% in Basar, DSB-20, JS-335 and JS-93.05 respectively when compared to control plants (fig 2). The increase and decrease carbohydrates levels in plants under stress conditions occur due the two biochemical events that simultaneously happen in plants under these conditions. The starch pathway is the main, because the starch is degraded, which is promoted by the amylase action [26]. Besides this, the sucrose pathway is also disturbed, because the sucrose suffers from invertase enzyme actions and there by liberates hexoses that may be utilized in anabolic or catabolic processes and if not used they may contribute to the reducing carbohydrates accumulation [27]. There are several reports on carbohydrate accumulation during various abiotic stresses in the temperate grasses and cereals where long term carbohydrate storage occurs during reproductive development [28]. Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses

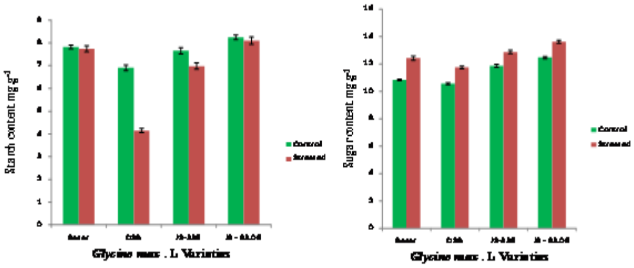

Fig.2. Total soluble sugars & starch in four cultivars of Glycine max.L during stress conditions when compared to control plants

3.2.4 Ascorbic acid content:

The ascorbic acid content was increased in all cultivars of soyabean studied. When compared to control plants, stressed plants showed an increase of 19.38% in Basar, 34.54% in DSB-20 and 11.59% in JS-93.05 (Fig 1). Whereas, JS-335 showed decreased ascorbic acid content by 4.68%. Ascorbic acid (Vit C) is a water soluble antioxidant which acts as scavenger of ROS to prevent or at least alleviate deleterious effects caused by ROS. It plays an important role in minimizing the damage caused by oxidative processes. Decreased ascorbate content under heavy metal stress has been observed in the roots and nodules of Glycine max.L [29].

3.2.5 Proline and Glycine betaine:

The most interesting observation made during the study was drastic increase in the proline and glycine betaine contents in all the soyabean varieties studied in the experiment. It clearly shows significant increase ($P \leq 0.05$) in the accumulation of proline and glycine betaine in all stressed plants. The percentage of proline content was significantly higher by 57.56% in Basar, 74.19% in DSB-20, 41.84% in JS-355 and 46.58% in JS-93.05 when compared to controls. The proline level was significantly increased in DSB-20 and this accumulation is a response characteristic of plants under abiotic stresses, which it works as osmotic adjustor and reduces the negative effects in the plants under adverse conditions, besides of this, it promotes higher resistance in cells under these circumstances. In higher plants, proline is considered to play an important role in defense mechanism of stressed cells providing carbon, nitrogen and energy source after stress by degradation [30]. Earlier studies reported that stressed cells have lower protein synthesizing capacity increasing lipid and carbohydrate metabolism. Similar results on the proline accumulation in plants under water deficiency were showed by Sarkar et al. [31] working with cultivars of Triticum aestivum and Costa [32] studying Vigna unguiculata.

Glycine betaine is the principle solute in highly salt tolerant found in plants under stress conditions. In the present study, Glycine betaine content increased with increase in continuous stress conditions (Fig. 3). In Basar, it was increased by 28.15%, in DSB-20 by 28.07 %, in JS-335 by 33.33% and in JS-93.05 by 29.55% with reference the controls studied. Among all cultivars, JS-335 showed highest increase in Glycine betaine content. Glycine betaine and proline increase the cytoplasmic volume and free water content and permit cell proliferation under unfavorable conditions [33]. However glycine betaine preserves thylakoid and plasma membrane integrity when exposed to adverse conditions [34].

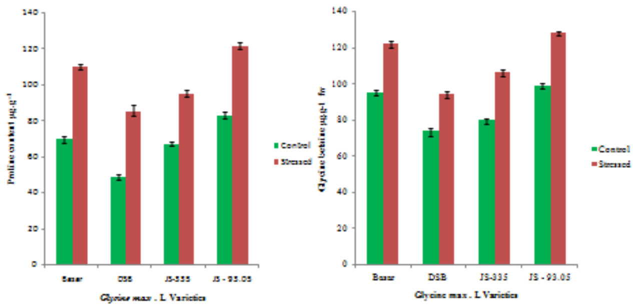

Fig.3 Proline & Glycine betaine content in four cultivars of Glycine max.L during stress conditions when compared to control plants

4. CONCLUSION:

The effect of stress of salinity (NaCl), heavy metal (Lead acetate) and drought successively on four Glycine max L. cultivars showed, reduction in shoot and root lengths, plant fresh and dry weights and chlorophyll a, chlorophyll b and Total chlorophyll. However, total protein content was observed to be fluctuating which showed increased in two varieties (Basar & JS-335) whereas decreased in the other two varieties (DSB20 & JS-93.05). While significant increase in soluble sugars, production of proline and glycine betaine was observed in all soyabean varieties studied. These beneficial properties indicate that, adaptation of plants to stress conditions can be characterized by the accumulation of osmolytes like carbohydrates, proline and glycine betaine.

From the results obtained in the present investigation, it could be concluded that, JS-335 showed better response to stress conditions studied followed by Basar, DSB-20 and JS-93.05 respectively. All the biochemical parameters studied, may serve as important biochemical markers for stress tolerance trait in plants. These types of continuous abiotic stress impose considerable constraints on crop production, thereby affect agricultural productivity. This preliminary study may pave way for further investigation to improve our understanding of the effect of combined stress on biochemical parameters during plant growth and to select a plant variety having high tolerance to these stress conditions. Some stress responsive enzymes are activated during stress conditions, such enzymes and their corresponding genes may be manipulated at molecular level and thereby stress tolerant plants for combined or continuous abiotic stress conditions are produced.

Table 1. Shoots length, Roots Length , Plant Fresh weight & Plant Dry weight in four Glycine max.L cultivars grown under continuous abiotic stress conditions for 21 days.

Glycine max.L Cultivars	Shoot Length			Root Length			Plant Fresh Wt			Plant Dry Wt		
	Control (Cms)	Stressed (Cms)	Per Diff (%)	Control (Cms)	Stressed (Cms)	Per Diff (%)	Control (gms)	Stressed (gms)	Per Diff (%)	Control (gms)	Stressed (gms)	Per Diff (%)
BASAR	67±1.54	30±1.47	55.22	25±3.11	10±2.13	60.00	11.15	3.62	67.53	9.83	2.54	74.16
DSB – 20	49±1.43	28±1.41	42.86	21±2.34	10±1.45	52.38	8.75	2.54	70.97	6.71	1.96	70.79
JS – 335	65±1.25	29±1.53	55.38	14±2.61	11±3.02	21.43	10.71	2.96	72.36	8.63	2.13	75.32
JS – 93.05	58±2.07	33±1.73	43.10	18±3.05	12±2.21	33.33	11.98	4.87	59.35	10.51	2.76	73.74

Table 2. Chlorophyll a (Chl a) , Chlorophyllba (Chl b) & Total Chlorophyll (Chl) in shoots of four Glycine max.L cultivars grown under continuous abiotic stress conditions for 21 days.

Glycine max.L Cultivers	CONTROL			STRESSED		
	Chl a (mg g-1 f.w)	Chl b (mg g-1 f.w)	Total Chl (mg g-1 f.w)	Chl a (mg g-1 f.w)	Chl b (mg g-1 f.w)	Total Chl (mg g-1 f.w)
BASAR	206.91±0.13	175.52±0.12	287.368±0.17	199.69±0.24(3.49)	153.088±0.18(12.78)	266.35±0.13(7.31)
DSB – 20	240.07±0.16	392.36±0.17	484.97±0.23	81.13±0.18(66.21)	55.62±0.15(85.82)	162.11±0.18(66.57)
JS – 395	223.45±0.21	200.32±0.09	311.86±0.14	163.67±0.12(26.75)	154.82±0.2(22.71)	262.54±0.17(15.81)
JS – 93.05	201.17±0.18	170.316±0.36	281.88±0.19	88.31±0.07(56.1)	60.349±0.12(64.57)	167.38±0.23(40.62)

REFERENCES

1. Ron Mittler, "Abiotic Stress, the field environment and stress combination," Trends in Plant science, 11:15-19, 2006.
2. E.G. Heyne, A. M. Brunson, "Genetic studies of heat and drought tolerance in maize," J. Am. Soc. Agro, 32:803–814, 1940.
3. M.A. Hoque E. Okuma, M.N.A. Banu, Y. Nakamura, Y. Shimoishi, Y. Murata, "Exogenous proline mitigates the detrimental effects of salt stress more than the betaine by increasing antioxidant enzyme activities," J Plant Physiol., 164:553-561, 2007.
4. L. Brahim, M. Mohamed, "Effects of copper stress on antioxidative enzymes, chlorophyll and protein content in Atriplex halimus," Afr J Biotech,. 10:10143-10148, 2011.
5. De Vos CHR, W.M. Ten Bookum, R. Vooijs, H. Schat, L. J De Kok, "Effect of copper on fatty acid composition and peroxidation of lipids in roots of copper tolerant and sensitive Silene cucubalus," Plant Physiol Biochem., 31:151-158, 1993.
6. J. Barcelo, C. Poschenrieder, "Plant water relations as affected by heavy metal stress: A review," J. Plant Nutr., 13:1–37, 1990.
7. M. Ashraf and M.R. Foolad, "Role of Glycine Betaine and Proline in Improving Plant Abiotic Stress Resistance," Env. Exp. Bot., 59:206-216, 2007.
8. P.D.R. Van Heerden, G.H.J. Krüger, "Photosynthetic limitation in soybean during cold stress," South African. J. Sci., 96:201–206, 2000.
9. H. Roy-Macauley, Y. Zuily-Fodil, M. Kidric, A.T. Pham Thi, J.V. Silva, "Effects of drought stress on proteolytic activities in Phaseolus and Vigna leaves from sensitive and resistant plants," Physiol. Plant, 8590-96, 1992.
10. J.A.G. Silveira, R.C.L. Costa, R.A. Viegas, J.T.A. Oliveira, M.V.B. Figueiredo, "N-Compound accumulation and carhydrate shortage on N2 fixation in drought-stressed and rewatered cowpea plants," Spanish J. Agric. Res., 1:65-75, 2003.
11. O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, "Protein Measurement with Folin Phenol Re-agent," J. Bio. Chem, 193:265-275, 1951.
12. D.I. Arnon, "Copper enzymes in isolated chloroplast polyphenoloxidase in Beta vulgaris," Plant Physiol, 24:1-15 1949.
13. M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, "Colorimetric method for determination of sugars and related substances," Anal. Chem, 28:350-356, 1956.
14. R. Mccready, M.J. Goggle, V. Silviera, H.S. Owens, "Determination of starch and amylase in vegetables," Analytical Chem, 22:1156-1158, 1950.
15. S. Sadasivam, A. Manickam, "Biochemical methods," New age international (P) Limited, Publishers, II (ed), New Delhi, pp.152-160, 1996.
16. L.S. Bates, R.P. Waldeen, I.D. Teare, "Rapid estimation of free proline for water stress studies," Plant Soil. 39:205-208, 1973.
17. A.J. Barak, D.J. Tuma, "Determination of choline, phosphorylcholine and betaine," In: J.M. Lowstein (ed.), methods of Enzymology, Academic Press, New York, pp. 287-292, 1981.
18. P.E. Verslues, M. Agarwal, S. Katiyar-Agarwal, J. Zhu, J.K. Zhu, "Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status," The Plant J. 45: 523-539, 2006.
19. S. Farouk, A.A. Mosa, A.A. Taha, H.M. Ibrahim, A.M. EL-Gahmery, "Protective effect of humic acid and chitosan on radish (Raphanus sativus, L. var. sativus) plants subjected to cadmium stress," J Stress Physio Biochem, 7:99-116, 2011.
20. M. Debouba, H. Gouia, A. Suzuki, M.H. Ghorbel, "NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato "Lycopersicon esculentum" seedlings," J. Plant Physiol, 163:1247-1258, 2006.
21. B. Sankar, C.A. Jaleel, P. Manivannan, A. Kishorekumar, R. Somasundaram, R. Panneerselvam, "Drought induced biochemical modifications and proline metabolism in Abelmoschus esculentus (L.)," Moench. Acta Bot. Croat, 66:43-56 2007.
22. M.L.G. Ramos, R. Parsons, J.I. Sprent, "Differences in ureide and amino acid content of water stressed soybean inoculated with Bradyrhizobium japonicum and B. elkanii," Pesq. Agropec. Bras, 40:453-458, 2005.

23. A.T. Thalooth, M.M. Tawfik, H. Mohamed Magda, “A Comparative Study on the Effect of Foliar Application of Zinc, Potassium and Magnesium on Growth, Yield and Some Chemical Constituents of Mungbean Plants Grown under Water Stress Conditions,” World J. Agricul. Sci., 2:37-46, 2006.
24. J. Yang, J. Zhang, Z. Wang, Q. Zhu, L. Liu, “Water deficit-induced senescence and its relationship to the remobilization of pre-stored carbon in wheat during grain filling,” Agron. Journal, 93:196-206, 2001.
25. R.K Dubey, T.N Singh, “Influence of pH on mechanical properties of sandstone – An experimental approach,” J. Sci. Ind. Res, 59:583-586, 2000.
26. J.T. Chaves Filho, E. Stacciarini-Seraphin, “Changes in osmotic potential and soluble carbohydrates levels in Solanum lycocarpum St.- Hil. in response to water stress,” Rev. Bras, Bot. 24:199-204, 2001.
27. A.H. Kingston-Smith, R.P. Walker, C.J. Pollock, “Invertase in leaves: conundrum or control point,” J. Exp. Bot. 50:735-743, 1999.
28. H. Meier, J.S. Reid, “Reserve polysaccharides other than starch in higher plants,” In: Encyclopedia of Plant Physiol, New series. Loewus FA., Tanner W (Eds.). Springer- Verlag, Berlin, 13a pp. 418-471, 1982.
29. P. Ahmad, C. Abdul Jalel, M. M. Azooz, G. Nabi, “Generation of ROS, and Enzymatic antioxidants during Abiotic stress in plants,” Bot. Res. Int. 2:11-20, 2009.
30. G. Szekely, “The role of proline in Arabidopsis thaliana osmotic stress response,” Acta Biol Szeged 48: 81 -85, 2004.
31. A.M. Sarker, M.S. Rahman, N.K. Paul, “Effect of soil moisture on relative leaf water content, chlorophyll, proline and sugar accumulation in wheat,” J. Agron. Crop Sci, 183:225-229, 1999.
32. R.C.L. Costa, “Nitrogen assimilation and osmotic adjustment in nodulated plants of bean [Vigna unguiculata (L.) Walp] under water stress,” Ph.D Thesis. Universidade Federal do Ceara, Brasil, 1999.
33. M. T. Record, E. S. Couternay, S. Cayley, J. H. Guttman, “Biophysical compensation mechanisms buffering E. coli proteins, nucleic acid interactions against changing environment,” Trend Biochem. Sci. 23:190-194, 1998.
34. D. Rhoades, A.D. Hanson, “Quaternary ammonium and tertiary sulphonium compounds in higher plants,” Annu. Rev. Plant Physiol. Plant Mol. Biol., 44: 357-384 1993.
35. R.C.L. Costa, “Nitrogen assimilation and osmotic adjustment in nodulated plants of bean [Vigna unguiculata (L.) Walp] under water stress,” Ph.D Thesis. Universidade Federal do Ceara, Brasil, 1999.

Supplementary Data

Morphological changes in four varieties of *Glycine max* L after treatment for 35 days.

Control and treated plants of *Glycine max* L. varieties after treatment for 35 days under stress conditions