

COMPARATIVE STUDY ON AGRO-PHYSIOLOGY OF SUGARCANE (*SACCHARUM OFFICINARUM* L.) GENOTYPES AT DIFFERENT IRRIGATION CO-EFFICIENT VALUES

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Abstract

Drought is the primary factor limiting sugarcane growth and physiological development under the climatic conditions of Pakistan; especially in those areas where without supplemental irrigation, productivity is not possible. Lack of detailed information regarding the performance of cane varieties under drought during formative stage and poor selection breeding program played key role in limiting cane productivity. The proposed study was conducted to investigate the genetic response of different cultivars viz., CSSG-676, CSSG-668, HoSG-795, HoSG-529, NSG-59 and HSF-240 (standard) regarding the physiological development of sugarcane and its productivity at different irrigation co-efficient levels (100%, 80% and 60%). This study elucidates that moisture has a pronounced impact on the physiological attributes of sugarcane and proper irrigation scheduling with 20 no. of irrigations were reported best in-term of better germination (69.65%), leaf area index (7.13), crop growth rate (8.44), net assimilation rate (1.06) and chlorophyll contents (5.98). Similarly in case of genomic response, NSG-59 was reported significant best as compared to all other test cultivars in term of better physiological performance, showing significant higher leaf area index, crop growth rate, chlorophyll contents and water use efficiency that maximized the crop growth and resulted in higher net assimilation rate. Higher proline contents (1.59) produced in NSG-59 also made it best under drought conditions.

Key words: Sugarcane, Agro physiology, Irrigation co-efficient.

Introduction

Globally sugarcane (*Saccharum officinarum* L.) is a valuable crop not only as a source of sugar but also a good source of bio-energy, because of its exceptional dry matter producing ability (Afghan, 2003; Babar *et al.*, 2011). Contribution of sugarcane in the economy of sugarcane is 0.7% (Anon, 2013) and is the driving force of Pakistan 2nd largest industry providing raw material to other small industries.

Among the various stresses of sugarcane, water stress is one of the most important abiotic stresses limiting sugarcane production worldwide (Ashraf & Khan, 1993). Therefore, its effective management is desperate, not only in reducing inefficiency, but also in cutting production costs and supporting productivity (Qureshi & Afghan, 2005). Drought is a primary factor limiting crop yield under arid and semi-arid conditions, specifically in the areas without supplemental irrigation (Baligar & Dunean, 1990).

Water stress affects every aspect of plant growth, and worldwide yield losses even temporary drought can cause substantial losses in crop yield (Ashraf & Khan, 1993). Water is essential at every stage of plant growth from seed germination to plant maturation (Ashraf & Naqvi, 1995; Iftikhar *et al.*, 2010). It reduces crop yield regardless of the growth stages at which it occurs (Leigh *et al.*, 2006). So any degree of water imbalance may cause deleterious effects on its growth potentials. With increasing soil moisture stress, the plant height, dry weight and yield per plant decreases (El-Monayeri *et al.*, 1984; Ashraf *et al.*, 1994). Moisture stress retards leaf expansion and so ultimately reduces leaf area (Long *et al.*, 1994; Mosaad *et al.*, 1995). Although reduced leaf area affects plant's photosynthetic capacity it provides a mechanism for reducing water loss. It affects every aspect of plant growth, and worldwide losses in yield from water stress probably exceed the losses from all other causes collectively

because even temporary drought can cause substantial losses in crop yield (Ashraf, 1998) so leaf area limitation can be considered a first line of defense against drought (Taiz & Zeiger, 1991). There is an imperative need to optimize production of sugarcane by efficiently managing water resources and their reliability. The study was aimed to investigate the performance of promising genotypes for tolerance under different moisture regimes and physiological and biochemical phenomenal traits contributing moisture stress in diverse genotypes of sugarcane.

Materials and Methods

The proposed study was conducted in the agro climatic conditions of Jhang, at agronomic research area of Shakarganj Sugar Research Institute, to investigate the genetic response of different cultivars at different irrigation co-efficient levels. The proposed study was laid out in Randomized Complete Block Design in split plot arrangements, replicated thrice. The irrigation co-efficients were kept in main plots and sugarcane varieties were superimposed. Irrigation Co-efficient included following levels I₁=100 %, I₂=80 % and I₃=60 % while sugarcane genotypes include CSSG-676, CSSG-668, HoSG-795, HoSG-529, NSG-59 and HSF-240 (standard).

All others agronomic practices were followed normally during the whole season. The physiological attributes of different genotypes that were examined under varied irrigation co efficient levels described as:

Germination & plant height: Germination (%) was observed 45 days after germination by the total number of buds that sprouted or emerged and total number of known buds sown, however; the plant height was measured between soil surface and apices of randomly selected 05 stalks and converted to the average.

Leaf area index (LAI): Leaf area of five randomly selected stalks from each plot at 30 days intervals was measured. LAI was computed by using the following formula as:

$$\text{LAI} = \text{Leaf area (cm)} / \text{Ground area (cm)}$$

Crop growth rate (g m⁻² day⁻¹): CGR was determined by using the following formula:

$$\text{CGR} = (W_2 - W_1) / (T_2 - T_1)$$

whereas W_1 & W_2 are Shoot dry weight m⁻² at time T_1 & T_2 respectively. T_1 & T_2 = Time of 1st & 2nd harvest respectively.

Net assimilation rate (g m⁻² day⁻¹): The mean NAR was determined by following method as:

$$\text{NAR} = \text{TDM}_{(Final)} / \text{LAD}_{(Final)}$$

whereas NAR=Net Assimilation Rate & TDM=Total dry matter (Final)

$$\text{Chlorophyll a (mg g}^{-1} \text{ f.wt.)} = [12.7(\text{OD663}) - 2.69 (\text{OD645})] \times [V / (1000 \times W)]$$

$$\text{Chlorophyll b (mg g}^{-1} \text{ f.wt.)} = [22.9(\text{OD645}) - 4.68 (\text{OD663})] \times [V / (1000 \times W)]$$

$$\text{Total chlorophyll} = \text{chl a} + \text{chl b}$$

Water use efficiency: Water use efficiency was calculated by striped cane yield and the amount of water applied.

Statistical analysis: The analysis of collected data was done by using the Fisher analysis of variance technique (Steel *et al.*, 1997) and treatment's means was compared by applying Least Significance Difference (LSD) test at 5% probability level.

Results and Discussion

Germination plays significant role in further crop growth and physiology. Germination was reported statistically significant during 2008-09 and 2009-10 regarding irrigation co-efficient (Table 1A). Higher germination (65.39% & 73.90%) was achieved at 100% co-efficient level respectively in both years and successive reduction in germination % was observed from 1st to 3rd irrigation co-efficient. Significant varietal response (Table 1B) elaborated that varieties were showed significantly different germination response during both years. Higher germination (69.55% & 78.60%) was recorded where HSF-240 was sown while CSSG-676 had minimum germination % age (50.22% & 56.75%) than all others in 2008-09 & 2009-10 respectively. The interactive response (Table 1C) elaborated that I_1V_6 had maximum germination count as compared to other treatments and it was statistically higher as compared others in both years. Plant height in sugarcane is a combination of crop growing conditions and varietal character. Data depicted the significant response for plant height under varying irrigation co-efficient, various genetic materials and their interaction. Maximum plant height (4.26 & 4.81 m) was achieved under I_1 (Table 1A), (4.29 & 4.84 m) was gained by HSF 240 that was

Proline concentration estimation: Proline concentration was estimated by using the standard procedure described as to determine the proline concentration; 0.5 g fresh sugarcane biomass was used. 10 ml sulfo-salicylic acid (3%) was added in biomass, grinded it after this the extract obtained was filtered. A mixture was made of 2 ml filtrate, 2 ml acid ninhydrin and 2ml of glacial acetic acid. The mixture was heated at 100 °C for 1 hr. within the water bath followed by its cooling in ice. After this 4ml toluene was added and jiggled it with vortex mixture and finally observed the reading at 520 nm (Bates, 1973).

Pigment contents: Chlorophyll contents were determined by using the method described by Arnon (Arnon, 1949). Fresh leaves (0.5 g) were used. 10 ml acetone was added in fresh leaves and obtained the extract. After this readings were taken at 663 and 645 nm by using this extract. Chlorophyll a & b was calculated by following formula:

statistically higher than any other varieties followed by NSG-59 & HoSG-529 that were statically at par with each other during the both years (Table 1B). The significant interactive response showed that I_1V_6 has statistically higher plant height as compared to other treatments.

Leaf area index (LAI) is an assimilatory system of a crop and have important role in the plant growth and yield. Data elucidated that drought significantly affected LAI, substantial reduction of LAI was reported by increasing drought levels. Maximum LAI (6.66 & 7.60 in 2008-09 & 2009-10 respectively) was achieved where the irrigation co-efficient was kept 100% during the both years as represented in the table 1A. Similarly different varieties produced statistically different Leaf area index according to their genetic makeup as represented in the table 1B. Maximum leaf area index (7.40 & 8.84 in 2008-09 & 2009-10 respectively) was produced by NSG-59 that was statistically higher than any other varieties followed by HSF-240, HoSG-529, HoSG-795, CSSG-668 and CSSG-676 during the both years. However, all cultivars were statistically similar except NSG-59 & HSF-240 during 2008-09, but only HoSG-529 was statistically at par with HoSG-795, CSSG-668 and CSSG-676 but different from HSF-240 during 2009-10. The interaction effect of both factors (Irrigation co-efficient & sugarcane varieties) was also reported significant regarding LAI of sugarcane as represented in the table 1C. I_1V_5 has statistically higher leaf area index (8.28 & 9.74) in 2008-09 & 2009-10 respectively. Leaf area index reduction was more at higher level of drought under the HoSG-529, HoSG-795, CSSG-668 and CSSG-676 genotypes as compared to NSG-59 and HSF-240. These results were found similar with the findings of Naik *et al.* (1993) and Ali (1999).

Table 2. Net assimilation rate, proline concentration and pigment and water use efficiency of diverse sugarcane genotypes (*Saccharum officinarum*) at different drought levels.

Parameter	Net assimilation rate			Proline concentration			Pigment			Water use efficiency			
	Year		Means	Year		mean	Year		Mean	Year		Mean	
	2008-09	2009-10	2008-09	2008-09	2009-10	2008-09	2008-09	2009-10	2008-09	2008-09	2009-10	2008-09	2009-10
Treatments													
A. Irrigation Co-efficient	I ₁ (100%)	1.00A	1.13A	1.06	0.34C	0.36C	0.35	5.61A	6.34A	5.98	1.68A	1.94B	1.81
	I ₂ (80%)	0.82B	0.93B	0.87	0.39B	0.43B	0.41	4.62B	5.23B	4.92	1.68A	2.01AB	1.85
	I ₃ (60%)	0.74B	0.84B	0.79	0.51A	0.53A	0.52	3.87C	4.38C	4.12	1.71A	2.15A	1.93
	LSD	0.04	0.05	0.45	0.03	0.05	0.04	0.04	0.04	0.04	0.04	0.05	0.04
B. Sugarcane Varieties	V ₁ CSSG-676	0.77B	0.86B	0.81	0.33D	0.40CD	0.37	4.30CD	4.86CD	4.58	1.56CD	1.86B	1.71
	V ₂ CSSG-668	0.77B	0.87B	0.82	0.35CD	0.41C	0.38	4.36C	4.93C	4.64	1.44E	1.85B	1.64
	V ₃ HoSG-795	0.75B	0.85B	0.8	0.36CD	0.40CD	0.38	4.23CD	4.78CD	4.51	1.56C	1.85B	1.71
	V ₄ HoSG-529	0.74B	0.84B	0.79	0.37C	0.39D	0.38	4.20D	4.75D	4.47	1.45DE	1.97B	1.71
	V ₅ NSG-59	1.27A	1.44A	1.35	0.57A	0.61A	0.59	6.54A	7.39A	6.97	2.41A	2.69A	2.55
	V ₆ HSF-240	0.82B	0.92B	0.87	0.49B	0.47B	0.48	4.58B	5.18B	4.88	1.73B	1.96B	1.84
	LSD	0.06	0.07	0.07	0.03	0.01	0.02	0.07	0.08	0.08	0.0533	0.1246	0.09
C. I × V	I ₁ V ₁	0.95bc	1.07bc	1.01	0.27j	0.30h	0.29	5.32de	6.01de	5.67	1.61defg	1.85d	1.73
	I ₁ V ₂	0.94bc	1.06cd	1	0.28ij	0.31h	0.30	5.31de	5.99de	5.65	1.58defgh	1.88cd	1.73
	I ₁ V ₃	0.96bc	1.08bc	1.02	0.28ij	0.31h	0.30	5.41d	6.12d	5.76	1.68ede	1.94cd	1.81
	I ₁ V ₄	0.91cd	1.02cde	0.96	0.28ij	0.32h	0.30	5.07e	5.73e	5.4	1.39hi	1.77d	1.58
	I ₁ V ₅	1.28a	1.45a	1.36	0.52cd	0.54c	0.53	7.20a	8.13a	7.66	2.18b	2.30bc	2.24
	I ₁ V ₆	0.96bc	1.08bc	1.02	0.41fg	0.37g	0.39	5.37d	6.07d	5.72	1.62def	1.86d	1.74
	I ₂ V ₁	0.76cde	0.86cdef	0.81	0.33hi	0.40f	0.37	4.27f	4.83f	4.55	1.65cde	1.90cd	1.78
	I ₂ V ₂	0.79cde	0.90cdef	0.84	0.32hij	0.42f	0.37	4.47f	5.05f	4.76	1.50efgh	1.79d	1.64
	I ₂ V ₃	0.71de	0.80def	0.76	0.31hij	0.37g	0.34	4.02g	4.54g	4.28	1.57defgh	1.74d	1.66
	I ₂ V ₄	0.70de	0.79ef	0.75	0.35h	0.37g	0.36	3.99g	4.51g	4.25	1.26i	2.14cd	1.70
	I ₂ V ₅	1.17ab	1.33ab	1.25	0.55bc	0.62b	0.59	6.61b	7.46d	7.03	2.38b	2.62b	2.49
	I ₂ V ₆	0.79cde	0.89cdef	0.84	0.47def	0.41f	0.44	4.41f	4.98f	4.69	1.71cd	1.88cd	1.79
	I ₃ V ₁	0.59e	0.67f	0.63	0.41g	0.50de	0.46	3.31hi	3.73hi	3.52	1.41ghi	1.84d	1.62
	I ₃ V ₂	0.59eE	0.66f	0.62	0.44efg	0.50de	0.47	3.31hi	3.74hi	3.52	1.24j	1.87d	1.55
	I ₃ V ₃	0.58e	0.66f	0.62	0.48de	0.51d	0.50	3.27i	3.70i	3.49	1.41fghi	1.88cd	1.65
	I ₃ V ₄	0.63e	0.71f	0.67	0.48de	0.47e	0.48	3.54h	4.01h	3.77	1.69cde	1.99cd	1.84
	I ₃ V ₅	1.37a	1.55a	1.46	0.63a	0.68a	0.66	5.83c	6.59c	6.21	2.68a	3.17a	2.92
	I ₃ V ₆	0.71de	0.80ef	0.75	0.59ab	0.50de	0.55	3.97g	4.49g	4.23	1.84c	2.15cd	1.99
	LSD	0.11	0.12	0.12	0.05	0.03	0.04	0.12	0.14	0.13	0.09	0.22	0.15

Means followed by different letters in a column are significantly at 0.05 P.

Sugarcane represents the same behavior regarding crop growth rate as in LAI under different irrigations co-efficient as shown in Table 1A. Maximum sugarcane crop growth rate (7.98 & 8.90 in 2008-09 & 2009-10 respectively) was achieved where the irrigation co-efficient was kept 100% during the both years and a gradual reduction in CGR reported as drought level was increased. Similarly varieties also responded substantially different as maximum sugarcane crop growth rate (8.96 & 9.77 in 2008-09 & 2009-10 respectively) was examined by NSG-59 followed by HoSG-795, HSF-240 and CSSG-668 2008-09 & 2009-10. The interaction effect of both factors (Irrigation co-efficient & sugarcane varieties) was also reported significant different regarding sugarcane crop growth rate as represented in the Table 1C. I_1V_5 has statistically higher crop growth rate (9.20 & 10.40 in 2008-09 & 2009-10 respectively). However, I_3V_4 had statistically lowest crop growth rate during 2008-09 & 2009-10.

Net assimilation rate (NAR) is the net gain of photosynthetic assimilates per unit of leaf area and time (Gardner *et al.*, 1988). Data embodied in Table 2, elucidated that different drought levels and various varieties along their combinations affected significantly regarding NAR during both the years. Net assimilation rate showed by I_2 & I_3 were statistically low and similar as compared to I_1 , while maximum NAR (1.00 & 1.13 in 2008-09 & 2009-10 respectively) was achieved where the irrigation co-efficient was kept 100% during the both years as in the Table 2A. Similarly different varieties responded differently and maximum NAR (1.27 & 1.44 in 2008-09 & 2009-10 respectively) was produced by NSG-59 that was statistically higher than any other varieties. However, all other five cultivars were statistically same regarding net assimilation rate. The interaction effect of both factors (Irrigation co-efficient & sugarcane varieties) was also reported significant regarding net assimilation rate of sugarcane as represented in the Table 2C. I_1V_5 & I_3V_5 has higher NAR and was statistically similar during both years.

Maximum proline concentration (0.51 & 0.53 in 2008-09 & 2009-10 respectively) was at 60% co-efficient level, while minimum values (0.34 & 0.36 in 2008-09 & 2009-10 respectively) was at 100% irrigation co-efficient level. The successive reduction in proline concentration was observed from irrigation co-efficient level I_3 to I_1 . The varietal response was also statistically significantly reported in proline concentration (Table 2B). Higher proline concentration (0.57 & 0.61 in 2008-09 & 2009-10 respectively) was recorded where NSG-59 was sown. The inter-active response of irrigation co-efficient levels along with varieties was also statistically significant regarding Proline concentration in both years (Table 2C). I_3V_5 had maximum proline concentration (0.63 & 0.68 in 2008-09 & 2009-10 respectively) as compared to other treatments and it was statistically higher as compared others during both years of experiment. Proline concentration is an osmo-regulator agent that helped regarding drought resistance (Singh *et al.*, 1973; Stewart *et al.*, 1977).

The pigment concentration was substantially affected by irrigation coefficients and varied under different cultivars as presented in Table 2. Sugarcane pigment concentration significantly affected by irrigation levels

that were applied. Sugarcane pigment was drastically reduced where the level of moisture was minimum, maximum sugarcane pigment (5.61 & 6.34 in 2008-09 & 2009-10 respectively) was achieved where the irrigation co-efficient was kept 100% during the both years. Similarly different varieties gained statistically different pigment concentration according to their genetic material as represented in the Table 2B. Maximum chlorophyll pigment (4.29 & 4.84 m) was gained by NSG-59 that was statistically higher than any other varieties followed by HSF-240, CSSG-668, CSSG-676, HoSG-795 and HoSG-529. While under interactive response the I_1V_5 has statistically higher pigment as compared to other treatments. While the minimum sugarcane pigment was reported where the treatment I_3V_3 was executed and it was statistically at par with I_3V_2 and I_3V_1 . Data also presented that sugarcane pigment was low during the year 2008-09 as compared to year 2009-10.

Water use efficiency is the capability of plant with which the plant produced its biomass or economic part after utilizing unit amount of water. Statistical analysis showed that maximum WUE was observed under I_3 as compared to I_1 & I_2 . Cultivars also differed significantly and maximum WUE was recorded where NSG-59 was planted followed by HSF-240. The treatment combinations had significant effects on WUE (Table 2C). Sugarcane in I_3V_5 had substantially maximum water use efficiency during both years of experiment 2008-09 & 2009-10.

Conclusion

Study elucidated the importance of moisture and improved genetic material regarding the efficient growth and the chlorophyll concentration of sugarcane. This study also revealed that under moisture stress the production of proline concentration reported also and its production was also related to genetic source as higher production under I_3 and in NSG-59. The overall results regarding the physiological developmental stages and other physiological attributes of this study guided that I_1 (20 no. of irrigations) is statistically good for higher sugarcane germination (%), leaf area index, crop growth rate, net assimilation rate, and chlorophyll concentration while I_3 had performed better regarding the water use efficiency of sugarcane however; NSG-59 is reported better as compared to all other studied genetic material.

References

- Afghan, S. 2003. A review of irrigation water management practices on sugarcane crop. *Proc. Pakistan Society of Sugar Technologist*.
- Ali, F.G. 1999. Impact of moisture regime and planting pattern on bio-economic efficiency of spring planted sugarcane (*Saccharum officinarum* L.) under different nutrient and weed management strategies. Ph.D. Thesis, Dept. Agron., Uni. Agri., Faisalabad, Pakistan. P: 248-254.
- Anonymous. 2013. *Agricultural Statistics of Pakistan*, 2012-13. MINFAL. Islamabad. Pakistan, pp 4.
- Arnon, D.T. 1949. Copper enzyme in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1-15.

- Ashraf, M.Y. 1998. Photosynthetic efficiency of wheat under water stress conditions. *Pak. J. Sci. Indust. Res.*, 41: 156-163.
- Ashraf, M.Y. and A.H. Khan. 1993. Effect of NaCl on nitrogen status of Sorghum. In: *Current Development in Salinity and Drought Tolerance of Plants*. (Eds.): S.S.M. Naqvi, R. Ansari, T.J. Flower and A.R. Azmi. pp. 84-88.
- Ashraf, M.Y. and S.S.M. Naqvi. 1995. Studies on water uptake, germination and seedling growth of wheat under PEG-6000 induced water stress. *Pak. J. Sci. Indust. Res.*, 38: 130-133.
- Ashraf, M.Y., A.R. Azmi, A.H. Khan and S.S.M Naqvi. 1994. Water relations in different wheat (*Triticum atistivum* L.) genotypes under water deficits. *Acta Physiol. Plant*, 16: 231-240.
- Babar, L.K., T. Iftikhar, H.N. Khan and M.A. Hameed. 2011. Agronomic trials on sugarcane crop under Faisalabad conditions, Pakistan. *Pak. J. Bot.*, 43(2): 929-935.
- Baligar, V.C. and R. R. Dunean. 1990. Crops as enhancers of nutrient use. Academic Press. New York, pp. 453.
- Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207.
- El-Monayeri, M.O., A.M. Hagazi, N.H.. Ezzat, H.M. Saleem and S.M. Tohoun. 1984. Growth and yield of some wheat and barley varieties grown under different moisture stress levels. *Ann. Agri. Sci.*, 20: 23 1-243.
- Gardner, F.P., R.B. Pearce and R.L. Mitchell. 1988. *Physiology of crop plants*. Scientific Publisher, Ratanda Roward, Jhodhpur, India. P: 187-207.
- Iftikhar, T., L.K. Babar, S. Zahoor, N.G. Khan. 2010. Impact of land pattern and hydrological properties of soil on cotton yield. *Pak. J. Bot.*, 42(5): 3023-3028.
- Leigh, A., J.D. Close, M.C. Ball, K. Siebke and A.B. Nicotra. 2006. Leaf cooling curves: measuring leaf temperature in sunlight. *Funct. Plant Biol.*, 33: 515-5.
- Long, S.P., S. Humphries and P.S.J. Falkowskj. 1994. Photo-inhibition of photosynthesis in nature. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 45: 633-662.
- Mosaad, M.G, G. Ortiz-Ferrara, V. Mahalakshmi and J. Hamblin. 1995. Leaf development and phenology of *Triticum aestivum* and 7' durum under different moisture regimes. *Plant and Soil*, 170: 377-381.
- Naik, G.R., R. Somaskekhar and S.M. Hiremeth. 1993. Effect of water stress on growth and stomatal characteristics in sugarcane cultivars. *Indian Sugar*, 43: 645-649.
- Qureshi, M.A. and S. Afghan. 2005. *Sugarcane cultivation in Pakistan*. Sugar Book Pub. Pakistan Society of Sugar Technologist.
- Singh, T.N., L.G. Paleg and D. Aspinall. 1973. Stress metabolism III. Variations in response to water deficit in the barley plant. *Aust. J. Bioi Sci.*, 26: 65-76.
- Steel, R.G.D., J.H. Torrie and D.A. Dicky. 1997. Principles and Procedures of Statistics, A biometrical approach. 3rd Ed. McGraw Hill, Inc. Book Co. N.Y. (U.S.A.). PP: 352-358.
- Stewart, C.R., S.F. Bogess, D. Aspinall and L.G. Paleg. 1977. Inhibition of proline oxidation by water stress. *PI. Physiol.*, 59: 930-932.
- Taiz, L. and E. Zeiger. 1991. *Plant Physiology*. The Benjamin/Cummings Publishing Co. Inc., California, pp. 346-350.

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