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Effects of drought stress on the morphological, physiological, biochemical, and phytochemical characteristics of Capsicum annuum cultivars Pusa Jwala and Pusa Sadabahar.

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Abstract: This study examines how drought stress influences the morphological, physiological, biochemical, and phytochemical traits of two *Capsicum annuum* cultivars: Pusa Jwala (PJ) and Pusa Sadabahar (PSB). Under induced drought conditions, relative water content decreased, with PSB maintaining the highest level (82.7%). Notable variations in carbohydrate content were observed between the genotypes at both 30 and 90 days after sowing (DAS) under mannitol treatment. PSB consistently showed the highest average carbohydrate content, recording 23.81 mg/g DW at 30 DAS and 29.88 mg/g DW at 90 DAS. Both cultivars showed a general decline in protein levels at both sampling stages, but PSB maintained higher protein content than PJ at both 30 and 90 DAS.

Keywords: DAS, biochemical, phytochemical

1. INTRODUCTION

Capsicum annum is an important crop (Castonera *et al*., 2003). This crop is more prone to drought stress because the leaves of chilli are bigger having more transpiration rate (Alvino *et al*., 1994; Delfine *et al*., 2002). Drought (Abiotic stress) reduces the crop yield by up to 70% economic loss (Fernandez *et al*., 2005). Due to this abiotic stress irrigation is essential for chilli production due to its sensitive nature towards drought stress (Bosland and Votava, 2000). Different environmental factors like light, temperature, water stress and soil nutrients affect the contents of capsaicinoids (Murakami *et al*., 2006). Chilli plants are subjected to drought stress during the initial stages (30 to 45 days) i.e. from flowering to the fruiting stage which is a critical period for yield development Drought stress results in to accumulation of a large amount of capsaicinoid content as compared to normal irrigated water (Estrada *et al*., 2000). Drought is a major risk to world food security due to the limited availability of water (Somerville and Briscoe, 2001). The severity of water stress is unpredictable because it differs with a number of agents like evaporation rate, moisture holding capability of the soil, and distribution of rainfall (Werry *et al*., 1994). It has a number of beneficial compounds like fatty acids, fatty oils, capsaicinoids, carotenoids, vitamins, steam volatile oils, fibre and minerals (Bosland and Votava, 2000). Fruits of *Capsicum* are used as bactericidal and fungicidal agents to kill a number of germs. Chillies are cholesterol-free, having retinol, ascorbic acid and also folic acids. Chilli contains the major level of alkaloids that include dihydrocapsaicin and capsaicin (Hornero-Mendez *et al*., 2002; Perej-Galvez *et al*., 2004; Manjula *et al*., 2011) Per 100 gm of powdered chilli contains: carbohydrate 31.6gm, fibre 30.6gm, protein 15.9gm, mineral 6.1gm and vitamin C 50 mg. In India, chillies are cultivated on approximately 801,500 ha with the overall production of 6800t of fresh chillies and 1.3 million tones of dry chilli fruits.

Abiotic stress can be defined as any unfavourable condition that affects plant metabolism rate and overall development. It can also be designated an ecological state that prevents the plant from reaching its heredity

perspective. If the abiotic stress remains temporary & for a short duration then the damage might be short-term and the plant might improve itself. These are like as temperature stress, water stress, light stress, heavy metal stress, salinity stress and drought stress whereas water stress is more pronounced as compared to the other stress. Water stress can be of two types: one is due to excess water and second during scarcity of water i.e. known as drought stress. Because of aridness crop yield decreases to 50-30 % during drought due to low humidity in plant growth which increases the rate of evaporation and temperature (Ghodsi *et al*., 1998). The severity of drought stress can not be predicted because it depends upon on number of factors like evaporative conditions, distribution of precipitation and wetness absorbing capability of soil (Werry *et al*., 1994).

2. MATERIAL AND METHODS

Two Capsicum varieties PSB (Pusa Sadabahar) and PJ (Pusa Jwala) were procured from the Indian Agricultural Research Institute, New Delhi. Both genotypes were grown under natural and drought stress conditions created by different mannitol concentrations (100, 150, 200, 250 mM) at the herbal garden of the Department of Botany, Maharshi Dayanand University, Rohtak in the season of 2017-18 and 2018-19. Before sowing seeds were surface sterilized with sodium hypochlorite and ten seeds were sown in each earthen pot with three replicates containing a mixture of loamy and sandy soil.

Chemicals used

Analytical grade chemicals were used for the present investigation (AR) and procured from sigma Che. Co., USA, Hi-Media, SRL & E. Merck.

Culture Conditions- Sand filled plastic pots, each having 12 kg dune sand and one sapling were saturated with the solution of respective concentration of mannitol and control along with nutrients (Hoagland and Arnon,1950).

Soil chemical composition

Nitrogen, Phosphorus, Potassium, organic carbon electrical conductivity (EC) and PH were analyzed from the soil at the time of sowing.

Table (1): Chemical composition of soil:

Particulars	Values	Methods
Organic carbon (%)	0.08	Walkly and Black's rapid titration method (Jackson, 1973)
Available Nitrogen ($kgha^{-1}$)	62.0	Alkaline permanganate method (Subbiah and Asija, 1956)
Available phosphorus ($kgha^{-1}$)	8.9	Olsen's method (Olsen *et al*., 1954)
Available potassium ($kgha^{-1}$)	129.0	Flame photometric method, (Richards, 1954)
pH (1:25 soil water ratio)	8.4	Glass electrode pH meter (Jackson, 1973)
Electrical conductivity (dSm^{-1} at 25 ºC)	0.31	Conductivity bridge method (Richards, 1954)

Table (2): Concentration of mannitol used in experiment

S.NO	Treatment	Composition
1.	**Control**	**Distilled water + Hoagland solution**
2.	**100mM**	**Distilled water + 18.2 gL^{-1}mannitol**
3.	**150mM**	**Distilled water + 27.3 gL^{-1}mannitol**
4.	**200mM**	**Distilled water + 36.4 gL^{-1}mannitol**
5.	**250mM**	**Distilled water + 45.5 gL^{-1}mannitol**

Stock Solution

TABLE (3): Composition of Hoagland and Arnon (1950) nutrient solution.

S.NO.	Chemical Name	Chemical Formula	Concentration
Solution A (Macronutrients)			
1.	**Calcium nitrate**	**$CaNO_3$**	**364 g/l**
2.	**Potassium nitrate**	**KNO_3**	**221 g/l**
3.	**Magnesium sulphate**	**$MgSo_4$**	**217 g/l**
4.	**Potassium hydrogen phosphate**	**KH_2PO_4**	**62.1 g/l**
Solution B (micronutrients)			
5.	**Copper sulphate**	**$CuSO_4$**	**0.035 g/l**
	Manganese chloride	**$MnCl_2$**	**0.609 g/l**
	Zinc sulphate	**$ZnSO_4$**	**0.097 g/l**
	Boric acid	**H_3BO_3**	**1.269 g/l**
	Molybdic acid	**$MoO_3.H_2O$**	**0.400 g/l**
Solution C (Trace nutrient)			
6.	**Tartaric acid**	**$C_4H_6O_6$**	**0.4 %**
7.	**Ferric acid**	**H_2FeO_4**	**0.5%**

Nutrients solution was prepared by dissolving 62.5 ml each of stock solution from serial number one to five and 15 ml each of stock solution from serial number six and seven and finally diluting to 25 litre with tap water.

2.1 Morpho-physiological parameter

Relative water content:

Relative water content (RWC) was estimated by the method given by Barrs & Weatherley, (1962). Leaf samples were removed from the plant, weighed immediately for FW (fresh weight) and placed in petri plates comprising 20 ml of distilled water for 6 hours. When leaves became fully turgid, the samples were taken out from Petri plates; adhered water was blotted off with rough filter paper and reweighed for the turgid weight (TW). Then samples were kept for oven 48 hours at 70˚C and dry weight (DW) was taken.

RWC was calculated by the following formula:

$$\mathbf{RWC}\ (\%) = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})} \times 100$$

FW- Fresh weight

TW- Turgid weight

DW- Dry weight

2.2 Biochemical assay

2.1.1 Carbohydrate

Carbohydrate contents were measured by the proposed method of Hedge and Hofreifer (1962).

Reagent Used

1. **Anthrone reagent-** Dissolve 200mg anthrone/ 100ml 95% H_2SO_4 (ice cold)
2. **2.5 N- HCL**
3. **80% Ethanol**
4. **Conc. H_2SO_4**

Extraction:

Soluble carbohydrates are extracted according to Barnett and Nayler (1966). 100mg of fresh plant sample was homogenised with 2ml of 80% ethanol (v/v) It was cooled and centrifuged at 10000 rpm for 30 minutes. The supernatant (extract) was kept a side and the pallet re-extracted twice with 80% ethanol. The final 5ml volume was made by using 80% ethanol.

Procedure

Extract measuring 0.1 ml was evaporated to dryness in a test tube. After cooling, the residue was dissolved in 1 ml of distilled water, and to it, 4 ml of anthrone reagent was added. The mixture was then heated in a

water bath for ten minutes. After cooling, optical density was recorded at a wavelength of 620 nm against a blank reagent. For standard a range of concentration (20-100µg/ml) of D-glucose was used.

2.1.2 Total protein content

Total protein content was estimated by the method of Lowry *et al.,* (1951).

Reagents used:

1. Phosphate buffer (pH 7.4)
2. 20 % TCA
3. Folin - Ciocalteau reagent
4. Alkaline sodium carbonate
5. Alkaline copper solution.

6. Standard protein

Extraction

Sample material of 1 g was homogenised with 5 ml of 0.1 M phosphate buffer (pH 7.4) in mortar and pestle, centrifuged content at 8000 rpm for 20 min. collected the supernatant, repeated the procedure four times, combined the supernatants and made the volume to 50 ml with 0.1 M phosphate buffer (pH 7.4).

Procedure

To 1 ml of the supernatant, 1 ml of 20 % TCA was added, kept for half an hour, and centrifuged at 8000 rpm for 20 min. washed the pellet with acetone twice, centrifuged it again, and discarded the supernatant. The pellet was dissolved in 5 ml of 0.1 N NaOH and the content was mixed thoroughly till the pellet was dissolved. Took a suitable aliquot (1 ml) of the above solution, added 5 ml of freshly prepared alkaline copper reagent, mixed properly, added 0.5 ml of Folin - Ciocalteau reagent after 10 min. mixed the contents instantaneously, allowed to develop colour and absorbance was read at 660 nm. The amount of protein in sample was determined from the standard curve of BSA (10 – 100 µg).

2.2 Secondary metabolite analysis:

Preparation of extract

The plant sample was ground with the help of a grinder at low heat generation. 10 gm of powdered sample was taken in a soxlet apparatus and extracted with 100ml of different solvents (according to polarity basis) like hexane, butanol, ethanol, chloroform and water. After this extract was dried at room temperature until the moisture evaporated and till the extract became solid. The solid extract was used with a suitable concentration of solvent for further analysis.

Screening: Different solvent extracts of chilli samples were used for qualitative phytochemical analysis like sugars, alkaloids, saponins, flavonoids, and tannins.

2.2.1 Sugars

The amount of sugars was estimated using either anthrone method developed by Barnett and Nayler (1966). 0.1 ml of plant extract was evaporated to dryness in a test tube. After cooling, the residue was dissolved in 1 ml of distilled water and added 4 ml of anthrone. The reaction mix was heated in a water bath for about ten minutes. After cooling, optical density was recorded at a wavelength of 620 nm against the reagent blank. For standard a range of concentration (20-100µg/ml) of D-glucose was used.

2.2.2 Alkaloids

Alkaloids in the sample were tested by the method of Oguyemi, 1979. About 2 mL of Methanolic filtrate was dissolved in 1.5 mL of 1% HCl. Heating the solution in a water bath for 15 min. After that 6 drops of Dragendroff reagents/Wagner's reagent/ Mayors reagent was added. Orange precipitate showed the presence of alkaloids in the sample tested.

2.2.3 Flavonoids

2 g of tissue was extracted in 10 mL alcohol & water. Take 2 mL filtrate and add drops of concentrated HCl with 0.5 g of magnesium or zinc. After 3 minutes change in colour to magenta red or pink colour indicated the presence of flavonoids (Parekh and Chanda, 2007).

2.2.4 Saponins

Saponins content in sample tissue was determined by the proposed method of Obadoni and Ochuko, 2001). About 1000 µl of the plant was mixed with 1ml of H_2O and shaken well. The test solution changes to foamy leather that shows the presence of saponins

3. RESULTS

The present investigation was carried out to study morpho, physiological, biochemical antioxidant, phytochemical and yield potential of *Capsicum* spp. (L) under drought stress condition. Two genotypes (PSB and PJ) were evaluated for physical, biochemical, phytochemical and antioxidant activity under different mannitol concentration (100mM, 150mM, 200mM and 250mM) and control condition (Water + Hoagland solution) at 30 and 90 DAS of sowing in pots with three replications at the experimental area of herbal garden in MDU, Rohtak. The results pertaining on the study undertaken are given below under the following heads:

3.1 Morpho- physical parameter

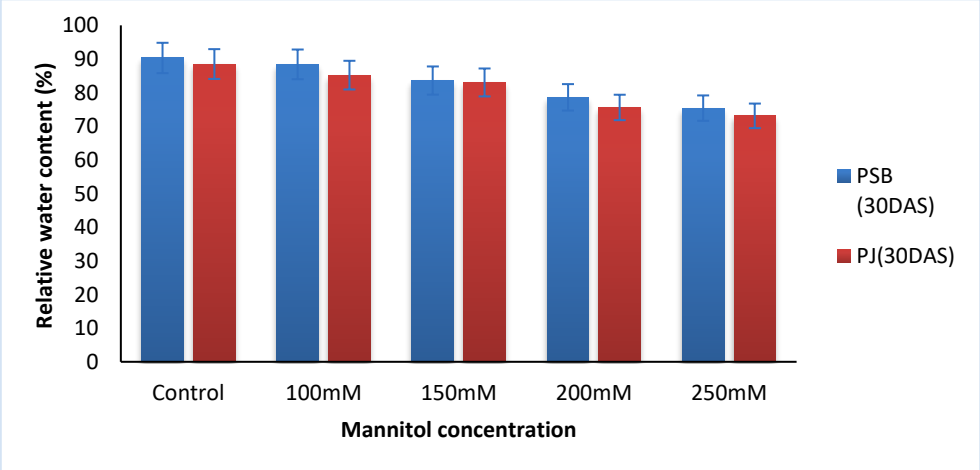
The morpho- physical parameter like germination, relative water content, shoot length, root length, plant fresh weight and plant dry weight were observed during to two rabi crop season of 2016-17 & 2017-18 and pooled mean data are presented.

Relative water content (RWC %)

The data presented in table showed a decline in relative water content under drought stress condition created by mannitol on both tested genotypes (Table 14). The average mean reduction in relative water content for genotypes was ranged between 81.7 % to 82.7.0% whereas for treatment at was varied from 74.4% to 89.4%. Both genotypes (Pusa Sadabhar and Pusa Jwala) showed maximum relative water content under control condition followed by 100mM mannitol concentration whereas minimum relative water content under 250mM concentration of mannitol. Both varieties at 30 days after sowing showed variation in Relative water content from 90.30 % in (PSB) and 88.50 % in (PJ) whereas, at 250mM concentration of mannitol it was found 75.6 % in PSB and 73.1 % in PJ. Relative water content decreased from control to induced drought condition. With the application of mannitol concentration, a sharp reduction was observed in both genotypes as compared to the control. Genotype, treatments and interaction among genotype and treatment were found significant at every level of stress.

Table (4) Effect of mannitol concentration on relative water content (%) after sowing Capsicum genotypes (PSB & PJ):

Treatment	Control	100mM	150mM	200mM	250mM	Mean (G)
PSB	90.30	85.20	83.60	78.60	75.60	82.70
PJ	88.50	88.40	83.00	75.40	73.10	81.70
Mean (T)	89.40	86.80	83.30	77.00	74.40	
CD at 5%	Genotype (G)= 0.25		Treatment (T)= 0.40		GxT= 0.60	



GRAPH 1 - Application of mannitol concentration on Relative water content (%) at 30 days after sowing on Capsicum genotypes (PSB & PJ):

Graphical representation in figure 12 showed a graphical representation of relative water content under different drought condition (100mM, 150mM, 200mM and 250mM) at 90 DAS after sowing. Variance in relative water content at various concentration of mannitol was found. A continued decline was seen from 100 mM to 250 mM concentration of mannitol compared with the controlled condition.

3.2 Biochemical parameters

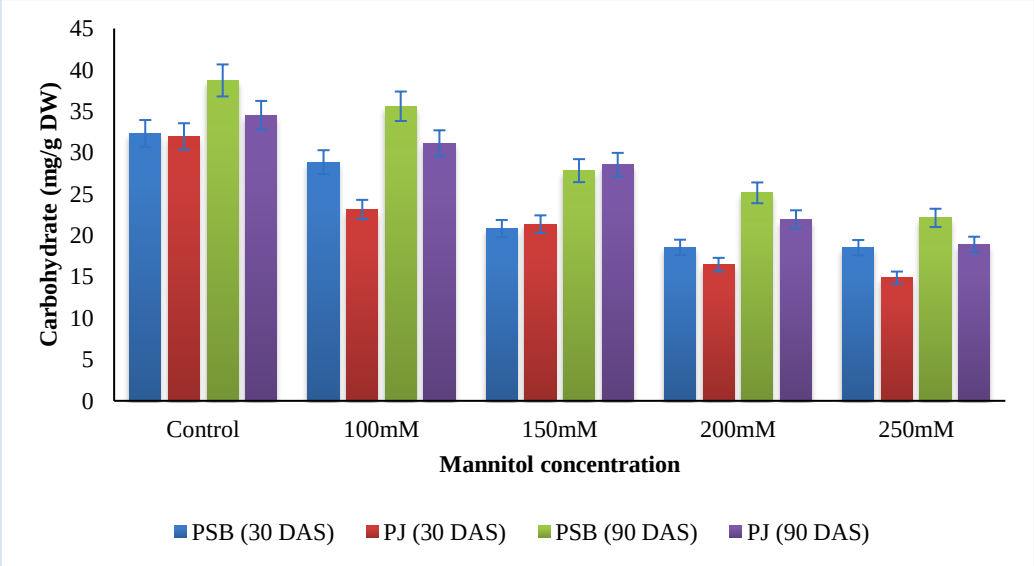
3.2.1 Carbohydrate (mg/g DW)

The data represented in the table (21) showed an increase in carbohydrate content at 90 days after sowing in both genotypes but reduced with the application of mannitol (100mM, 150mM, 200mM and 250mM) as compared to control. Average carbohydrate content for induced drought condition for genotypes ranged from 23.81 (mg/g DW) to 21.55 (mg/g DW) (30 DAS) and 29.88(mg/g DW) to 27.01 (mg/g DW) (90 DAS), whereas carbohydrate content for treatment varied between 34.38 (mg/g DW) to 18.60 (mg/g DW). Both varieties at 30 days after sowing showed variation in carbohydrate content from 32.32 (mg/g DW) to 18.51 (mg/g DW) (PSB) and 31.95 (mg/g DW) to 14.88 (mg/g DW) (PJ) whereas, at 90 days after sowing PSB carbohydrate content ranged between 38.72 (mg/g DW) to 22.11 (mg/g DW) and 34.52 (mg/g DW) to 18.89 (mg/g DW) for PJ. Carbohydrate content showed increased from 30 to 90 DAS after sowing but with the application of mannitol concentration a sharp reduction was observed in both genotypes as compare to control.

A significant difference in carbohydrate content was found with different induced drought condition i.e. 100mM, 150mM, 200mM, 250mM as compare to control. The genotype PSB 23.8 (mg/g DW) and 29.88 (mg/g DW) had maximum carbohydrate content at 30 & 90 days after sowing.

Table (5) - Effect of Mannitol concentration on Carbohydrate (mg/g DW) at 30 and 90 days after sowing:

Treatment	Control	100mM	150mM	200mM	250mM	Mean (G)
PSB (30 DAS)	32.32	28.85	20.82	18.55	18.51	23.81
PJ (30 DAS)	31.95	23.13	21.35	16.46	14.88	21.55
PSB (90 DAS)	38.72	35.60	27.82	25.14	22.11	29.88
PJ (90 DAS)	34.52	31.14	28.54	21.93	18.89	27.01
Mean (T)	34.38	29.68	24.63	20.52	18.60	
CD at 5%	Genotypes= 0.36		Treatments= 0.40		T×G= 0.81	



GRAPH 2- Application of different mannitol concentration on Carbohydrate (mg/g DW) at 30 and 90 days after sowing:

Graphical representation of figure 17 showed a significant reduction in carbohydrate content under different drought condition at 30 and 90 days after sowing respectively. A significant decline in carbohydrate content was observed among both (PSB & PJ) of the genotypes. Genotype PSB was found to have maximum

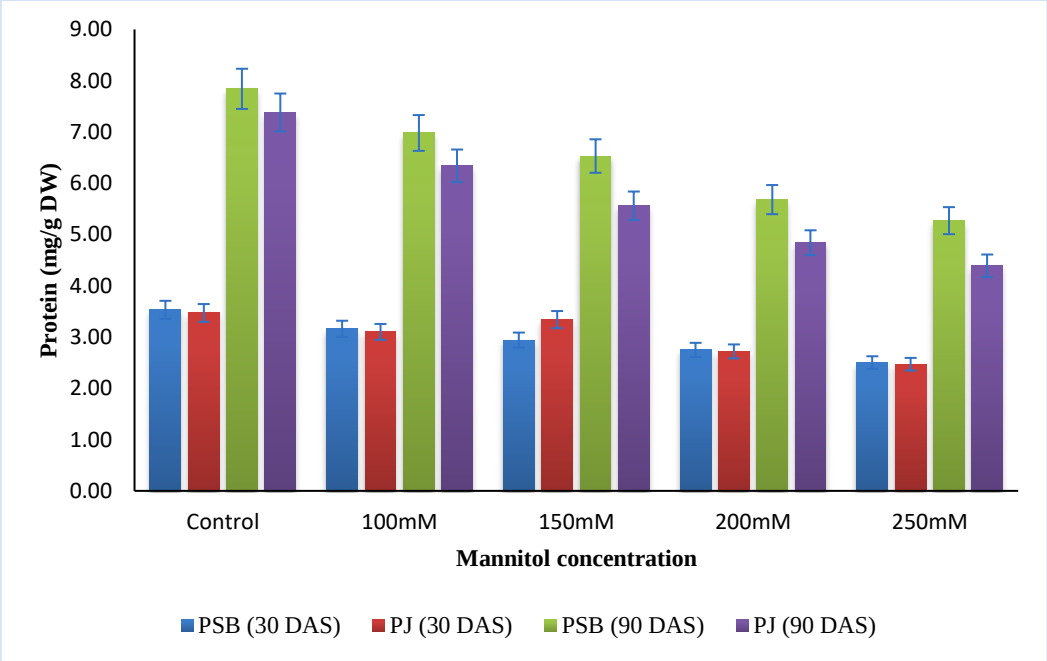
carbohydrate content at 90 DAS of sowing under control condition while it continues decreased up to 250mM concentration of mannitol.

3.2.1 Protein (mg/g DW)

The decrease in protein content was observed in the table (22) at 30 and 90 DAS after sowing with the application of different mannitol concentration in both genotypes whereas it was more at 90 DAS as compare to 30 DAS. Application of mannitol showed a reduction in protein content at both stages of observation. Average protein content for genotype (PSB) ranged from 3.53 (mg/g DW) to 2.50 (mg/g DW) (PSB) and 3.47(mg/g DW) to 2.47 (mg/g DW) (PJ) at 30 DAS after sowing whereas it was 7.84 (mg/g DW) to 5.27 (mg/g DW) (PSB) and 7.38 (mg/g DW) to 4.39 (mg/g DW) (PJ) at 90 DAS. The genotype PSB had maximum of 6.46 (mg/g DW) protein content under different mannitol concentration at 90 DAS after sowing. Both varieties at 30 days after sowing showed variation in protein content from 3.53 (mg/g DW) to 2.50 (mg/g DW) (PSB) and 3.47 (mg/g DW) to 2.47 (mg/g DW) (PJ) whereas, at 90 days after sowing PSB protein content ranged between 7.84 (mg/g DW) to 5.27 (mg/g DW) and 7.38 (mg/g DW) to 4.39 (mg/g DW) for PJ. Protein content showed increased from 30 to 90 DAS after sowing but with the application of mannitol concentration a sharp reduction was observed in both genotypes as compare to control.

Table (6) Effect of mannitol concentration on Protein (mg/g DW) at 30 and 90 days after sowing:

Treatment	Control	100mM	150mM	200mM	250mM	Mean (G)
PSB (30 DAS)	3.53	3.16	2.94	2.75	2.50	2.97
PJ (30 DAS)	3.47	3.10	3.34	2.72	2.47	3.02
PSB(90 DAS)	7.84	6.98	6.53	5.68	5.27	6.46
PJ (90 DAS)	7.38	6.34	5.56	4.84	4.39	5.70
Mean (T)	5.55	4.89	4.59	4.01	3.66	
CD at 5%	Genotypes= 0.16		Treatments= 0.18		T×G= 0.36	



GRAPH 3 - Application of different mannitol concentration on Protein (mg/g DW) at 30 and 90 days after sowing:

Graphical representation (figure 18) indicates a significant reduction in protein content under control and treated with mannitol at 30 and 90 days after sowing. A statistically significant decline was observed in protein content was observed among both genotypes PSB & PJ. Genotype PSB was found to have maximum protein content at 90 DAS of sowing under control conditions while it continues to decrease up to 250mM concentration of mannitol.

TABLE (7)- Mean sum of the square of capsicum genotypes for carbohydrate and protein at 30 and 90 days after sowing:

Source of variation	df	Carbohydrate	Protein
Genotype (G)	3	597.37**	146.94**
Treatment (T)	4	2,033.40**	26.67**
interaction G X T	12	93.76**	6.91**
Error	40	9.57**	1.93**

** Significant at 1% of significance

The mean sum square for carbohydrate and protein at 30 and 90 DAS after sowing in table 23 indicated significant differences due to genotypes (G), different mannitol concentrations (T) i.e. 100mM, 150mM, 200mM and 250mM. Interaction effects between genotypes, drought and sowing time were also found significant. This indicated that genotypes differed in their response to drought conditions and sowing time of the traits under study.

3.3 Phytochemical attributes

The data represented in Table (40) revealed the qualitative analysis of the various aqueous extracts of leaf samples of *Capsicum annum* (PSB) containing sugar, alkaloids, flavonoids, tannins and saponins. Phytochemical evaluation of different secondary metabolites was done on genotype Pusa Sadabhar (PSB). The maximum concentration of sugar content was estimated in hexane as compared to other solvents and a remarkable amount of alkaloids was found in water whereas, the maximum content of flavonoids was noticed in ethanol. Great variability was noticed in tannin and Saponin, both metabolites showed their presence in hexane and ethanol but the maximum concentration of tannin was found in hexane as compared to ethanol whereas, saponin was found in ethanol as compared to water and hexane solvent.

Table (8) Qualitative analysis of the various aqueous extract of leaf sample of *Capsicum annum* (PSB):

S.N	Name of constituent	Hexane	Butanol	Water	Ethanol
1.	**Sugar**	**+++**	**-**	**-**	**-**
2.	**Alkaloids**	**-**	**-**	**++**	**-**
3.	**Flavonoid**	**-**	**-**	**-**	**++**
4.	**Tanin**	**++**	**-**	**-**	**+**
5.	**Saponin**	**+**	**-**	**+**	**++**

+ indicates the presence of a constituent

- indicates absence of constituent

4. DISCUSSION

Capsicum annum is a crop of chief economic importance and it is cultivated almost all over the world and used as a spice and is highly sensitive to water stress because of a wide range of transpiring leaf surface area (Alvino *et al*., 1994). Fruits of the chilli are a rich source of antioxidant compounds (Marin *et al*., 2004; Howard *et al*., 2000). These antioxidant compounds are beneficial for multiple diseases like cancer, anaemia and cardiovascular disease. Vitamin C is a crucial dietary nutrient required in our daily diet plan and capsicum are rich source of vitamin C help reduce the risk of cardiovascular disease and cancers (Harris, 1996). Drought is a major stress that confines plant production, performance of the yield and its growth and development (Shao *et al*., 2009). Plants experience drought stress either when the water supply to roots becomes tricky or when the transpiration rate becomes very high. This stress effects growth, yield, membrane integrity, pigment content, osmotic adjustment water relation and photosynthetic activity (Benjamin and Nielsen, 2006 ; Praba *et al*., 2009). Insufficient supply of water plant leads to adaptive change in plant growth, biochemical processes & low yield b disrupting the leaf gas exchange properties that often leads to change in growth rate and plant structure etc (Farooq *et al*., 2009c). It inhibits the production of dry matter by inhibiting the extension of leaf & its development (Nam *et al*., 2001). At flowering stage water stress result in to bareness.

During drought stress in case of Maize plant it caused reduction in grain yield, Kernel weight, harvest index & biological yield (Anjum *et al*., 2011a).

Out of the total world almost 45% of the agricultural lands are affected by drought and it can affect growth of the plant, development and its total yield (Bot *et al*., 2000). It leads to perturbation of most of the biochemical and physiological process of the plant that results in to lesser yield (Boutraa, 2010). In all vegetable crop flower is major limiting factor which undergoes reduced under drought stress (Wien *et al*., 1989). During these stress abscission of floral organ has been associated with changes in physiological development (Aloni *et al*., 1996). In tomato plant reduction in photosynthesis, abscission of flower and flower bud were high as compare to drought tolerant variety (Bhatt *et al*., 2009). In case of Soyabean plant fruit set and flower retention are highly sensitive to these abiotic stress (Kokubun *et al*., 2001).

Drought suppressed the rate of photosynthesis by decreasing the protein content in leaf area (Xu and Zhou, 2006). Under water deficit condition SPS activity get decreased during leaf desiccation in susceptible genotypes (Foyer *et al*., 1998). Different type of abiotic stress (drought, salinity, temperature, cold and oxidative stress) reduced the growth and yield of chilli plants (Kumar and Arumugam, 2013; Wu *et al*., 2018; Nouri *et al*., 2015). Sezen *et al*., 2006 reported the yield loss in chilli plants under drought stress. Many countries of the world have become extremely vulnerable to the impacts of the climate change (Rosmaina *et al*., 2018). The scarcity of the water is a serious problem for food security of these countries. Optimal irrigation gives a healthy plant with maximum yield and high-quality fruits (Wu *et al*., 2018). Water defilicit condition allows a crop to tolerate some degree of water stress to increase income and reduces costs (English and Raja, 1996).

Different abiotic environmental stress degrades the major components of photosynthesis including increase accumulation of carbohydrate, destruction of lipid, stomatal control of CO_2 etc (Allen & Ort, 2001). Drought also leads to a reduction in relative water content and plant height in three genotypes of pepper plants. During drought stress in *Capsicum annum*, proline content gets increased while relative water content of chilli leaves get reduced (Estrad *et al*., 2000; Loreto *et al*., 1994). In *Capsicum annum*, antioxidant and phenolic compounds were present that may reduce the many heart diseases (Narmin *et al*., 2012). Presence of antioxidant compounds in chilli protects the lipid peroxidation to get rid of free. Drought causes a reduction in yield of the crop in arid and semi- arid areas of the world. Indued drought caused a reduction in germination rate and germination percentage. Pepper plants require 85.6% of irrigation within 3 days to obtain 98.3cm of plant height (Khan *et al*., 2005). Different plants have different mechanism under drought stress like drought avoidance, escape and tolerance while drought escape is a widely used method (Levitt, 1972). Low availability of soil moisture decreases seed germination and seedling growth (Gamze *et al*., 2005).

4.1 Morpho-physical Parameter

Relative water content (RWC)

The water status of the plant or relative water content was reduced under drought stress condition that result in to decreased CO_2 assimilation **(**Anjum *et al*., 2012). It also used as indicator for declining the sensitivity of plants to drought stress (Kavas *et al*., 2013). In *Capsicum annum* RWC decreased under stressed condition of increasing drought (E-I Sayed, 1992). *Tomato* plants showed a continuous decline in RWC with increased stress condition of drought (Thakur *et al*., 1993). RWC (relative water content) get decreased in *Zarin* cultivar as response to drought stress. Present study showed that (Table 14) Relative water content reduced significantly in Capsicum genotypes from 100mM to 250mM concentration of mannitol at 30 days after sowing. Maximum declining rate was observed at 250mM concentration of mannitol. Genotype PSB has maximum relative water content under control and induced condition as compare to genotype PJ. Our result is lined with the study of (Ramnjulu & Sudhakar,1997., Farooq & Azam, 2006) they also found that drought stress resulting in to decline of RWC that were reported by several investigators. Plants having high relative water content beyond 30% they are found dehydrated tolerant variety (Ramanjulu *et al*., 1996). Due to lack of water drought causes major change in RWC (Ramanjulu and Sudhakar, 1997; Chakraborty *et al*., 2013). Drought facing plants experience decreased leaf water potential, transpiration rate and enlarge in leaf temperature (Siddique *et al*., 2001). It was affected by duration of drought stress & species of the plant and also found declined in Poplar plant when submitted to drought stress (Yang & Miao, 2010). As this content under goes water defilicit condition it leads to stomata closure which leads to low content of CO_2 assimilation (Anjum *et al*., 2012). It is a superior indicator of water importance as compare than water potential (Sinclair

and Ludlow, 1995). Relative water content get decreased with increase of drought condition in *Dracocephalum moldavica* and its value was 77.6% under mild stress while it found 65.4% under severe stress (Rahbarian *et al*., 2010).

4.2 Biochemical Parameter

4.2.1 Carbohydrate

Under water deficiency different carbohydrates like glucose, sucrose and fructose accumulate & they play a major role towards osmotic adjustment, Osmo protection and radical scavenging. Total soluble carbohydrates increased under better irrigation in *Savina barbata* (Tavili, 2008) while total soluble sugar was not affected by the irrigation period in case of *Matricaria chamoilla*. The results of the present investigation indicated that (table 21) carbohydrate content show an increasing pattern in Capsicum genotypes from 100mM to 250mM concentration of mannitol at 30 and 90 days after sowing. Maximum increase was observed at 250mM concentration of mannitol. Genotype PSB has maximum Carbohydrate content as compare to genotype PJ under control conditions. After 25 days of stress in *Radix astragali* Mongolia genotype soluble sugar content get decreased (Yong *et al*., 2006). Among the different plants accumulation of sugar found to be different with different level of drought intensities & it was found maximum under moderate stress in *Platycarya fortuneana, Cinnamomum bodinieri and Broussonetia papyrifera* (Liu *et al*., 2011). In *poplus cathayana* sugar content in leaves get increased significantly at early drought stage as compare to later stages (Xiao *et al*., 2008).

4.2.2 Protein

Protection or disfolding of protein structure is one of the important mechanisms alleviating the detrimental effects of water stress (Sankar *et al*., 2007). Abiotic stress strongly affects senescence and degradation of chloroplast proteins (Fellar *et al*; 2008; Wingler and Roitsch, 2008). Accumulation of amino acids under abiotic stress conditions were also observed (Barnett and Naylor, 1966; Krasensky and Jonak, 2012). Under stress period accumulation of amino acids results in to cell damage in different species (Widodo *et al*., 2009; Krasensky and Jonak, 2012). The results from the present investigation indicated that (Table 22) decreased pattern of protein concentration in Capsicum genotypes from 100mM to 250mM concentration of mannitol at 30 and 90 days after sowing. The highest decrease was observed at 250mM concentration of mannitol. Genotype PSB has maximum Protein content as compared to genotype PJ under control conditions. Our results lined with **(**Anjum *et al*., 2012; Salekjalali *et al*., 2012) found that protein content gets reduced under drought stress in *Zea mays* and *Hordeum vulgare.* It was also found to be get reduced in different varieties of *Piper i.e. P.nigrum, P.colubrinum, P.longum and P.hymenophyllum*. When *Cicer* plants were exposed to drought stress stress-soluble protein decreased at both the flowering and vegetative stage (Mafakheri *et al*., 2011). During mild and severe stress in *Lycopersicon escculentum* total protein gets reduced (Ghorbanli *et al*., 2013). By increasing drought quantity protein content of *Oryza sativa* declined (Sikku *et al*., 2010). When *Gossypium hirsutum* plants were subjected to drought stress protein content was found to be reduced (Parida *et al*., 2007).

4.3.Phytochemical Attributes

Secondary metabolites are produced by explants like leaves, stem and roots from commercial medicinal plant (Jian Zhaoa *et al*., 2005). They play a major role in plant defence against herb-ivory and interspecies defenses (Samuni-Blank *et al*., 2012). They have the ability to slow down the spore germination and cleanse the environment for competing micro-organism during germination (Demain *et al*., 2000). Due to presence of these bioactive compounds plants are used by food consumers as well as to treat a number of diseases (Temple *et al*., 2012). Plants containing bioactive components have the anticancer properties (Mans *et al*., 2000). They are able to hunt free radical immediately (Liu Rh *et al*., 2003) and create the signals in response to chemical or electrophillic stress that activate proteins associated to diverse cellular signalling pathways (Finley *et al*., 2011). The results of the present study in (Table 40) shows qualitative analysis of different solvents extracts in chilli samples. The maximum concentration of sugar content was estimated in hexane as compared to other solvents and a remarkable amount of alkaloids was found in water whereas, the maximum content of flavonoids was noticed in ethanol. Great variability was noticed in tannin and Saponin, both metabolites showed their presence in hexane and ethanol but the maximum concentration of tannin was

found in hexane as compared to ethanol whereas, saponin was found in ethanol as compared to water and hexane solvent. The absence of these metabolites does not lead to immediate death but in the long term, they play a role in the survival of plant cells and these organic compounds are produced in a very narrow range of species within a phylogenetic group (Chemical Plants, Retrieved 2008-19). During the past decade, humans used these metabolites as medicines, flavourings and for the synthesis of drugs. They play a major role in plant defence against herb-ivory and interspecies defences (Samuni- Blank *et al*., 2012). They have different activities like antiviral, antifungal, and antibiotic due to which they have the ability to protect our plants from different types of pathogens (Kossel *et al*., 1981).

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Conclusion:

Relative water content declined under induced drought conditions and were highest in PSB (82.7%). A significant difference in carbohydrate content was observed in both genotypes under mannitol concentration at 30 and 90 DAS. Genotype PSB had maximum mean carbohydrate content both at 30 DAS (23.81 mg/g DW) and 90 DAS (29.88 mg/g DW). An average reduction was observed in protein content at both stages of sampling in both genotypes; maximum protein amount was found in PSB compared with PJ at 30 & 90 days after sowing.

This study can be used to examine plant breeding programmes and to study morphological and physiological changes during drought.

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