# EVALUATION OF PHYSIOLOGICAL AND BIOCHEMICAL TRAITS IN SORGHUM (*Sorghum bicolor* L.) VARIETIES DURING SUMMER SEASON

**BY**

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# CERTIFICATE–I

This is to certify that this thesis entitled, **“Evaluation of physiological and biochemical traits in sorghum (*Sorghum bicolor* L.) varieties during summer season”** submitted for the degree of **Master of Science** in the subject of **Plant Physiology** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Ms. Gayatri Kumari** Admn. No. **2019BS36M** under my supervision and guidance and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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This is to certify that this thesis entitled **“Evaluation of physiological and biochemical traits in sorghum (*Sorghum bicolor* L.) varieties during summer season”**, submitted by **Ms. Gayatri Kumari** Admn. No. **2019BS36M** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfilment of the requirement for the degree of **Master of Science** in the subject of **Plant Physiology**, has been approved by the Student’s Advisory Committee, after an oral examination on the same.

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*(Gayatri Kumari)*

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# ABBREVIATIONS

$NH_4OAC$ : Ammonium oxaloacetate
CAT : Catalase
C : Control
$dS\ m^{-1}$ : Deci siemen per meter
DW : Dry weight
DAS : Days after sowing
EC : Electrical conductivity
FW : Fresh weight
g : Gram
ha : Hectare
HJ : Haryana jowar
HC : Haryana chari
hr : Hour
L : Litre
m : Meter
MPa : Mega pascal
µl : Microliter
ml : Milliliter
mM : Millimolar
mg : Milligram
min : Minute
µg : Microgram
RSI : Relative stress injury
ppm : Parts per million
PS II : Photosystem II
POX : Peroxidase
RWC : Relative water content
ROS : Reactive oxygen species
$NaHCO_3$ : Sodium bicarbonate
SOD : Superoxide dismutase
$H_2SO_4$ : Sulphuric acid
NaCl : Sodium chloride
TW : Turgid weight
TSS : Total soluble solids
q : Quintal
Fv/Fm : Quantum yield
V : Volume
W : Weight

# CHAPTER-1

# INTRODUCTION

Sorghum, (*Sorghum bicolor L.*) popularly known as great millet, Indian millet or jowar, belongs to grass family *Poaceae*. In terms of production and planted area sorghum is the fifth most important crop subsequent to wheat, rice, maize and barley. Sorghum is primarily grown for grain and forage during summers in arid and semi-arid regions of the world. Sorghum is an annual $C_4$ crop. It is used as a staple food in arid regions of Africa, China and India. World covers around 39.6 mha area, producing 57.79 million tones with the efficiency of around 1400 kg per hectare of sorghum yield. Maikasuwa and Ala (2013) estimated high yield in American continent, while low in India. With 373 million bushels produced in 2020, the United State becomes the world's major producer of grain sorghum. India and Nigeria are also the largest sorghum producers. India contributes 9.45% of the global sorghum production on 5.82 mha area with 5.39 million tonnes of total production (Gite *et al*., 2015).

In India sorghum is known as jowar, *cholam*, or *jonna*, in West Africa as Guinea corn, and in China as kaoliang. Out of 28 only 4 species are grown in India (*Sorghum purpureosericeum*, *Sorghum propinquum*, *Sorghum controversum* and *Sorghum bicolor)*. The total sorghum production in India was 34, 75,410 tonnes (FAO, 2019). According to APEDA, the maximum production of sorghum in the year (2017-18) was in the state of Maharashtra (1,810 tonnes) followed by Karnataka then Madhya Pradesh, Tamil Nadu, Andhra Pradesh, Rajasthan, Uttar Pradesh, Gujarat and other states. Punjab, Haryana, Delhi, western and central Uttar Pradesh, and bordering parts of Madhya Pradesh are the main producers of forage sorghums. In Haryana, sorghum is grown as feed in 40.3 thousand hectare, and total production of sorghum is 21.3 thousand tones with average yield of 528 kg per hectare (DOA, Haryana). The major constraints that reduce sorghum productivity are abiotic and biotic stress. Sorghum is somewhat tolerant up to 6-8 dS $m^{-1}$ of salt stress, which can also produce high biomass and yield than other crops (Greenway and Munns, 1980). Sorghum is especially valued for its resistance to drought, heat and waterlogged conditions as compared to other cereal crops. Different species of sorghum have different economic values, used for grain and hay production, silage, grazing pasture and green crop for livestock feeding. The crop is being produced most frequently at high temperature, low rainfall and saline area.

Average plant height ranges from 0.6 to 2.5 m but sometimes it reaches upto a height of 4.6 m. Stem is made of series of nodes and internodes. The stem is slender to very stout, measuring 0.5 to 5 cm in diameter, becoming narrower at the upper end. Leaves are broad and coarse and borne at different angles. The number of leaves vary greatly and they may be as long as 1m and vary in width from 10 to 15 cm. White wax was coated on the leaves and stalks. Certain varieties stalks are sweet and juicy at the portion of pith and centre. The very small flowers are formed in panicles. In each flower, 800-3000 kernels are present. Shape, size and color of its seed varied widely. Sorghum is made up of 75% complex carbohydrates (rich in fibre content), have 10.4% of protein and 3.1% of fat content in it and a small quantity of vitamin $B_1$. Per 100g, the seed is reported to contain 22 mg calcium, 3.9 mg niacin, 3.8 mg iron and 0.18 mg riboflavin. Sorghum grain has a crude protein content ranging from 9 to 13% of dry matter. It can be grown in both the Kharif and Rabi seasons. Food (grain), feed (grain and biomass), fuel (ethanol production), fibre (paper), fermentation (methane production), and fertiliser (utilization of organic byproducts) are all potential applications for sorghum. Forage sorghum is most commonly used as silage for livestock feed. In India, during the rainy season, sorghum grain is primarily used for animal/poultry feed, whereas after the rainy season, sorghum grain is primarily used for human use. Sorghum can reduce the use of $N_2$-fertilizer by inhibiting biological nitrification. Both syrup and sugar can be manufactured from sorghum as its juicy stem contains 10% of sucrose.

Plants growth and development is adversely affected due to environmental stresses, such as UV-radiation, high and low temperature, flooding, metal toxicity, abundance of light, soil hardness etc. which restrict plant growth and development. Abiotic stresses such as high salinity, drought, cold and heat also have been shown to reduce the survival, biomass production and yield of staple food crops by up to 70% (Ahmad *et al.*, 2012). Salt stress is major threat to modern agriculture and is one of the key factors limiting crop production. Dehnavi *et al.* (2020) reported that approximately 19.5% of irrigated and 2.1% of dry land is affected by salt stress and every year about 10% additional area is getting salt affected (Kumar and Sharma, 2020) and it is expected that by the year 2050, 50% of the world's arable area will be salt affected. Approximately 76 mha of the total are affected by human-induced salinization and sodification (Hossain, 2019). In India, about 6.7 mha of the cultivated land are affected by salt stress and in Haryana alone, it is 0.50 mha.

Salt stress caused due to the elevated levels of soluble salt, particularly NaCl in soil and water. Many field crops, including rice, wheat, maize, sorghum, cotton, and sugarcane, are hampered by salt stress. Soil salinization has been identified as a major cause of land degradation that makes the lands unsuitable for cultivation of crops. (Almodares *et al.*, 2008)**.** Salt stress hazard exist when the salt ions are too high. Salt stress adversely affect the plant growth and development by inducing water stress, cytotoxicity due to excessive accumulation of ionic contents and causes nutritional imbalance which may result in death of the plant (Isayenkov *et al.*, 2019). Salt stress also has a negative impact on physiological and metabolic processes in plants. Symptoms of these alterations include decreased leaf area, increased leaf thickness and succulence, abscission of leaves, root and shoot necrosis and shorter internode lengths. (Parida and Das, 2005). Oxidative stress facilitated by reactive oxygen species (ROS) which result in reduction of assimilation rate and finally affect the plant growth and yield. The competitive absorption of ions or insolubility, according to Greenway and Munns (1980), influence the balance of plant nutrition. ROS harm chloroplasts and mitochondria by destroying their cellular structure. Plants engage their antioxidant defense mechanism, which produces antioxidative enzymes, to combat reactive oxygen species. The increase in osmotic pressure in the cytosol occurs when the plant absorbs a large amount of salt (Munns, 2002). Cell homeostasis is maintained by osmotic adjustment mechanisms in these settings. Compatible osmoprotectants, such as proline and soluble carbohydrates, are produced in many species to protect cells from the negative effects of salt stress.

Development of salt tolerant, high yielding sorghum genotypes is the finest way to increase productivity under salty soils. Large genotypic variations are found for tolerance to salt stress in sorghum (Krishnamurthy *et al*., 2007).

Research works on salt tolerance in sorghum are few and far between. The reason for selecting sorghum for this study is being a dual crop grown for both food and fodder. Therefore, the present study was carried out to achieve the following objectives:

1. To evaluate the effect of salt stress on physiological and biochemical traits in summer season
2. To find out the suitable salinity tolerant single cut forage sorghum variety for summer season

# CHAPTER-II

# REVIEW OF LITERATURE

Sorghum (*Sorghum bicolor* L.*)* is facing extreme saline conditions which results in decreased production and seed yields. Morpho-physiological and biochemical trait profiling of sorghum crop may boost our knowledge about the effects of salt stress on sorghum varieties. The research work relevant to the present study in India and abroad has been reviewed under the following heads:

2.1 Germination studies
2.2 Growth studies
2.3 Plant water relation
2.4 Physiological and biochemical studies
2.5 Antioxidative enzymes
2.6 Soil analysis

## 2.1 Germination studies:

Germination and seedling growth are most vital phases of plant development that determines the yield (Dehnavi *et al.*, 2020). Tolerance to salt stress at the germination and seedling establishment stage determines better plant growth in saline soils (Bojovic *et al.*, 2010; Keshavarizi *et al.*, 2012). It was reported that the forage yield of sorghum decreases by 5.2 units with the enhanced level of salt stress (Aishah *et al.*, 2011). Nimir *et al.* (2014, 2015) and Ali *et al.*, (2020) reported that seedling emergence gets decreased with the increasing levels of salinity in sorghum but the responses varied with the genotype. Salinity reduces the germination rate by altering the imbibition of water by seeds due to the lower osmotic potential of germination media, which delays water absorption by seed and hence delays germination (Jamil *et al.*, 2006; Khan *et al.*, 2008). Similar results were also noticed by Kaveh *et al.* (2011) in *Solanum lycopersicum.* Toxicity caused by salinity, which alters the activities of nucleic acid metabolism enzymes (Gomes-Filho *et al.*, 2008), disrupt protein metabolism (Dantas *et al.*, 2007), and reduces seed reserve utilisation (Othman *et al.*, 2006). Germination percentage in *Brassica napus* reduced significantly at 150 and 200 mM of NaCl. Germination rate decreased as salinity levels increased at 200 mM NaCl, germination percentage and germination speed were reduced by 38 and 33 percent, respectively, over control (Bybordi, 2010).

Khodarahmpour *et al.* (2012) observed a significant reduction in germination rate (32%), length of radicle (80%) and plumule (78%), seedling length (78%), and seed vigor (95% ) over control when *Z. mays* seeds were exposed to 240 mM NaCl of salt stress. Sun *et* *al*. (2019) investigated the adaptation ability of seedlings (*Sorghum bicolor* L. Moench.) in coastal saline alkaline environment. Seedlings were treated by different salt and alkali stress. Seedlings of sorghum showed good adaptability to salt stress.

## 2.2 Growth studies:

Growth is one of the best parameter for finding out plant responses under environmental stresses. Seredo and Serena (Kenyan sorghum varieties) were grown in a greenhouse under different levels of salt stress (0, 50, 100, 150, 200, and 250 m*M*). The highest concentration of salt (250 m*M*) decreased plant growth by 73 and 75% and dry weight of stem i.e. 53% and 75%, respectively in Seredo and Serena. Leaf blades of young leaves of both varieties being reduced by 67% while sheaths were reduced by 83 and 87% for Serena and Seredo, respectively (Netondo *et al.,* 2004). According to the results obtained from Almodares *et al.* (2008) leaf area index, shoot fresh and dry weight and chlorophyll content decreased by 37.38, 43, 47, and 54.62%, respectively with the onset of salt stress. Speedfeed and KFS4 are the two forage sorghum varieties that showed the significant decreased forage dry biomass i.e. 44.09 g/plant under control to 32.76 g/plant at 15 dS $m^{-1}$ of salt stress (Roy, 2017). Similar results were also seen by Manish (2018) and Devi *et al.* (2019) and Satpal et al., 2020 in sorghum genotypes under salt stress.

Sadak *et al.* (2020) observed significant differences in adaptability in two mungbean varieties (Pusa vishal and Pusa ratna) under salt stress conditions. Plants were found to be more tolerant at early vegetative stage under salt stress conditions than late vegetative and reproductive stages under salinity. Osmotic stress caused by salt stress, high temperature, and salinity incredibly restricted plant growth, morpho-physiological traits, and yield attributes during the summer. Plant height, total chlorophyll and carotenoid contents, plant length, leaf area, rate of photosynthesis, number of pods per plant, and grain yield were less affected under high salinity levels during the spring season.

## 2.3 Plant water relation

### 2.3.1 Osmotic potential (Ψs):

Morgan *et al.* (1991) studied the declined osmotic potential which decreases the pressure and water potential, finally creates physiological drought conditions under saline stress. Plants maintain their water potential through osmotic adjustment. Jones *et al.* (1980) also studied the osmotic status in sorghum under stressed (-1.15 MPa) and control conditions (-0.90 MPa). Two sorghum genotypes (*Sorghum bicolor*), one salt tolerant (CSF 20) and the other salt sensitive (CSF 18) were grown in nutrient solution containing 0, 50 and 100 mmol $L^{-1}$ NaCl for seven days and osmotic potential (Ψs) was determined in the leaves and roots. It was observed that salinity reduced the Ψs in both genotypes, mainly in the salt sensitive one. Osmotic potential in salt tolerant genotype decreased from (-1.039 to -1.530 MPa) from control to 100 mmol$L^{-1}$, while in sensitive genotype osmotic potential ranges from -1.032 to -

1.878 MPa in control to 100 $mmolL^{-1}$ respectively (Lacerda *et al.,* 2003). Duarte and Souza (2016) noticed that osmotic potential of leaf sap of *Capsicum annuum* L. decreased with the increase of salt levels. It was because of the accumulation of ions and organic solutes, since a potential gradient between the soil and plant is necessary for the absorption of water and nutrients. Osmotic potential also decreased in leaves of *Atriplex portulacoides* under salt stress (Benzarti *et al.,* 2014) Osmotic potential at full turgor ($\Psi_\pi^{100}$) ranges from -2.52 MPa to -5.51 MPa from control to 1000 mM osmotic potential at turgor loss point ($\Psi_\pi^{0}$) ranges from -3.13 MPa to -6.30 MPa in control to 1000 mM.

### 2.3.2 Relative water content (RWC %):

Water facilitates all the physio-biochemical processes that contribute to plant growth and development (Xiong *et al.,* 2002). Saline conditions impede the absorption and transport of water from the soil to the plants by lowering root water potential and declined relative water content at the cellular level (Saha *et al.,* 2015). Salinity stress clearly showed the reduction in relative water content and photosynthates (Polash *et al.,* 2018). When plants are exposed to salinity, they first face ionic and osmotic stress, which reduces water uptake by roots. Furthermore, ABA-mediated stomatal closure influences transpiration pull, resulting in reduced water absorption by roots and low relative water content in the cell. The response of two wheat cultivars, Giza 168 and Gimeza 9, to NaCl stress (0-14 dS $m^{-1}$) was investigated and changes in relative water content (RWC), polyamines, amino acids, ethylene and lipid peroxidation were determined in absence and presence of NaCl. It was observed that salt stress decreased the RWC of both cultivars, the effect increased with increasing salinity level. Giza 168 showed greater reduction (14%) in the RWC than Gimeza 9 (12%) (Bassiouny *et al.,* 2005). Sairam *et al.* (2002) also reported a greater reduction in the RWC of salt sensitive wheat cultivar as compared to tolerant one under salt stress.

Significant decrease in RWC and shoot dry matter was observed when 7 days old rice plants were exposed to salt stress of 100 mM NaCl. It was observed that at 100 mM of NaCl RWC declined by 6.6% over their respective control. Other varieties also observed percent decline in RWC i.e. Ashfal (8.1), Benapol (11.5), Jamainaru (12.1), Gunshi (14.7), Mohini (18.3) and BRRIdhan 29 (21.7%) respectively in comparison to control (Polash *et al.,* 2018). Stepien and Klobus (2006) found the declined RWC in cucumber (*Cucumis sativus*) leaves when cucumber plants were exposed to 14-day period of salinity (0, 50, 100 mM NaCl). After 8 day of salinity the RWC decreased by 13 and 27 % in plants treated with 50 and 100 mM NaCl, respectively.

## 2.4 Physiological and biochemical studies

### 2.4.1 Relative stress injury (RSI %):

According to Sullivan and Ross (1979), the degree of cell membrane injury was induced by any stress may be easily calculated via measurement of relative stress injury of the cells. Three barley genotypes were grown under field conditions with three salinity levels (2, 10, and 18 dS $m^{-1}$). It was observed that electrolyte leakage percentage increased due to high salinity. Under 10 and 18 dS $m^{-1}$ salinity, Khatam (salt-tolerant) had minimum electrolyte leakage, whereas Morocco (salt-sensitive) showed maximum electrolyte leakage. In khatam, percent electrolyte leakage increased from 32 to 37% from control to 18 dS $m^{-1}$, while in Morocco electrolyte leakage % increased from 33 to 45% from control to 18 dS $m^{-1}$ (Mahlooji *et al.*, 2018).

Latrach *et al.* (2014) studied the adverse effects of salinity on growth, nodulation of alfalfa-rhizobia symbiosis under different salinity levels and observed the low percent electrolyte leakage (<15%) in symbiotic combination under control condition. In all symbiotic combinations tested, electrolyte leakage increased in roots under 100 mM NaCl. When these combinations were compared, their electrolyte leakage percentages were found to be significantly different. The lowest values were obtained after inoculating the plants with strain rhLAr 4, while the highest values were obtained after inoculating the plants with rhizobial strain rhLAr 1; the recorded values were 42 and 36% for strains D-rhLAr 1 and T-rhLAr 1, respectively.

### 2.4.2 Proline content:

Proline is an osmolyte that plays an important role in plant tolerance to stress (Liang *et al.*, 2013). It aids in ROS (reactive oxygen species) scavenging and cellular redox potential maintenance. Its higher accumulation under stress conditions strengthens the cell's ability to make ionic adjustments (Jaarsma *et al.,* 2013). Osmolytes enhances the activity of defense enzymes such as SOD (superoxide dismutase) and POD (peroxidase) (Devnarain e*t al.*, 2016). Salt stress can cause an increase in proline levels in the cell cytosol (Hu *et al.,* 2016). Increasing the salt concentration significantly affected proline and MDA content of shoots. With increased salinity, proline content increased continuously, with a 343% increase at 100 mM NaCl. Chaparzadeh and Hosseinzad-Behboud (2015) also studied the increased (9.2 and 8.5 µmol $g^{-1}$ FW) proline content in leaves and roots of radish with salt stress as compared to non-stressed plants (7.9 and 7.4 µmol $g^{-1}$ FW). Sorghum (*Sorghum bicolor*) plants were treated with 50, 100 and 150 mM NaCl. Proline content was increased significantly at 100mM and 150mM NaCl in leaves and roots. The increase in root was much greater than in leaves; at high concentration (150mM NaCl ); being approximately 2 and 3 folds in leaves and roots, respectively (Omari *et al.*, 2015). Payam, Kimia, and Jambo sorghum varieties with varying salt tolerance were grown under 0, 50, 100, 150, and 200 mM salt stress. At each NaCl concentration, there was a significant increase in proline content in the root and leaf tissues of all varieties. The variety Jambo had significantly higher proline concentration compared to variety Kimia and variety Payam under salt stress (Bavei *et al.*, 2011).

### 2.4.3 Glycine betaine content (GB):

Glycine-betaine (GB) is involved in turgor maintenance via osmoregulation, as well as maintaining and regulating the performance of PSII protein complexes. (Orlovsky *et al.,* 2016). Glycine-betaine accumulates in some crops of family *Poaceae* and *Chenopodiaceae* under stressed conditions (Khan *et al.,* 2019). Sorghum, spinach, wheat, barley, and sugar beets naturally accumulate GB. Wang *et al.* (2000) studied that in *Amaranthus tricolor* under salt stress (300mM), out of 7 days salt stress treatment, GB content enhancement and accumulation was observed at 3rd day of treatment. Glycine betaine content showed declining trend when these plants were transferred to non-stressed conditions. According to Moharramnejad *et al.* (2015), both proline and glycine betaine concentration were increased in the leaves of two maize inbred lines (B73 and MO17) under drought stress. But the concentration of proline and glycine betaine was more in MO17 inbred line.

### 2.4.4 Total soluble solids (TSS):

Carbohydrates present in plant leaves and roots are progressively depleted due to salinity. Younger leaves accumulate more hexose and starch than older leaves. The sucrose content of salt-tolerant genotypes is higher than that of salt-sensitive genotypes (Shahid *et al.,* 2020). Increased carbohydrate accumulation in leaves under salt stress aids in osmotic adjustment (Ali *et al.,* 2018). Massaretto *et al.,* 2018 observed that tomato varieties Negro Yeste (NY) and Moneymaker (MM), grown under (control) and with salt stress (100 mM NaCl) for 70 days displayed significantly higher TSS contents while in ripe fruits this was only observed in NY. Total soluble solids content induced by salinity were significantly higher in both landraces (60 and 78% in NY and V, respectively) compared with MM (34%). Studies were carried out in two sweet sorghum (Soave and Sofra) under salt stress of 0, 30, 60, and 90 mM NaCl along with nutrient solutions. Salt stress raises carbohydrate content, particularly in salt-tolerant plants. Sucrose (52.66 %), glucose (44.98 %), and fructose (24.94 %) levels were highest in the shoot, leaf, and root, respectively. Plant organs and cultivars had different distributions of sucrose, glucose, and fructose content. Sofra had the highest levels of sucrose (57.15%), fructose (29.08%), and glucose (54.85%). Soave had less sucrose and more glucose than Sofra in the shoot. Soave, on the other hand, had more sucrose and less glucose in the leaf than Sofra (Almodares *et al.,* 2008).

### 2.4.5 Total chlorophyll content:

Photosynthesis and chlorophyll pigmentation in pepper plants have been shown to be lower when the entire root system is exposed to salt stress on comparison to partial root exposure, and other gaseous exchange parameters were also affected by salt stress. (Lycoskoufis *et al.,* 2005). Radi *et al.* (2013) also studied declined chlorophyll content in wheat and bean. Changes in photosynthetic pigments i.e. chlorophyll and carotenoids and photosynthetic enzymatic activities altered photosynthesis at maximum salt levels (Zhang *et* *al.,* 2010). Mbinda *et al.* (2019) evaluated the salt tolerant *Sorghum bicolor* L. under salt stress (100, 200 and 300 mM NaCl) for chlorophyll content. At initial stage, the values for chlorophyll content varied from 9.37 to 9.44 mg/g FW. A slight decrease in chlorophyll content ranging from 5.87 mg/g FW for Serena to 8.15 mg/g FW for Gadam with the augmentation of 100 mM NaCl was observed.

**2.4.6 Chlorophyll stability index (CSI %):**

*In vitro* grown *Lasiurus* seedlings were exposed to three different levels of NaCl (25,50 and 100mM). After the seventh day of treatment, the CSI percentage decreased as the NaCl level increased (Gadi *et al.,* 2016). Reduction in CSI indicates salt sensitivity of Lasiurus, as previously supported by studies on *Momordica charantia* and *Vigna radiata* (Agarwal and Shaheen, 2007). Ahmad *et al.* (2012) discovered a decrease in CSI percentage in *Pisum sativum*, with CSI ranging from 72.12% (control) to 69.38% (25mM), 62.02% (50 mM), and 53.19% (100Mm). According to Dogra *et al.* (2018), the CSI data revealed that the genotypes with maximum CSI showed tolerance of drought as compared to unstressed plants. The CSI decreased with drought stress in all genotypes and the values ranged from 68.32 % to 54.11 %.

**2.4.7 Photochemical quantum yield (Fv/Fm):**

PS II maximum quantum efficiency reduces due to salt stress (Acosta-Motos *et al.,* 2017). Latrach *et al.* (2014) investigated the effects of salinity on the growth and nodulation of the alfalfa-rhizobia symbiosis under different salinity levels and found that, in the absence of salt treatment, all symbiotic treatments showed the same behavior by exhibiting the maximum quantum yield of photosystem II (Fv/Fm). Imposition of salt stress had a negative impact on PS II activity by lowering the maximum quantum yield. Under this limitation, the Fv/Fm ratio in T-rhLAr 4 and D-rhLAr 4 were 0.74 and 0.73, respectively. Decreased photochemical quantum yield with increasing salt stress was also reported by Ghassemi-Golezani and Lotfi (2015).

**2.4.8 Assimilation rate ($P_N$):**

The rate of leaf $CO_2$ assimilation ($P_N$) was significantly reduced when *Ricinus communis* seedlings were exposed to salt stress. Greater reductions in photosynthetic rate (78%) was noticed under 150 mM NaCl when compared to control plants (Rodrigues *et al.,* 2014). Sperling *et al.* (2014) and Manish (2018) also studied the reduced assimilation rate in *Phoenix dactylifera* L. and *Sorghum bicolor* L. under salt stress respectively. Assimilation rate was also declined in the leaves of *Torreya grandis* under salt stress but was enhanced with the SA application (Li. *et al.,* 2014). Salt stress (100 mM NaCl) significantly reduced photosynthetic rate in all the maize genotypes compared to control. The Indigenous yellow and Hybrid decreased higher amount of photosynthesis (81.05 and 83.49%, respectively); whereas Indigenous yellow pure line and Indigenous white decreased the same by 59.55 and

63.57%, respectively under salt stress condition (Uddin *et al.,* 2019). Babar *et al.* (2014) also observed the declined photosynthetic rate under NaCl stress.

### 2.4.9 Transpiartion rate (E):

Leaf transpiration (E) rate was significantly reduced by increasing NaCl levels in *Ricinus communis*. Under mild stress (50 mM NaCl), transpiration rate decreased by 18% and by 38% in the 100 mM treatments and 72% in the 150 mM treatments (Rodrigues *et al.,* 2014). Salt stress (100 mM NaCl) significantly reduced transpiration rate in maize genotypes. The relative decrease in transpiration rate over control was 83.80, 75.47 and 78.20% in Indigenous yellow pure line, Indigenous yellow and Hybrid respectively (Uddin *et al.*, 2019). Acosta-Motos *et al.* (2017) also observed decreased transpiration rate under salt stress. Mani, (2015) observed in pea (*Pisum sativum*) that higher transpiration rate under control condition in comparison to salt stress (75 mM).

### 2.4.10 Stomatal conductance ($g_S$):

Yan *et al*. (2012) showed that under salt stress photosynthetic activity declined mainly due to decrease in stomatal conductance. Salinity reduced the stomatal conductance in two rice varieties, Basmati-370 and Basmati-4048, according to Khan and Abdullah (2003). In tolerant varieties, stomatal conductance remains constant (KS-282, Nona Bokra and NIAB-6) (Khan and Abdulllah, 2003. Latrach *et al.* (2014) studied the adverse effects of salinity on growth, nodulation of alfalfa-rhizobia symbiosis under different salinity levels and observed that without salt treatment, the average stomatal conductance values were between 14 and 16 mmol $m^{-2}s^{-1}$. Salt stress resulted in a significant decline in stomatal conductance. When *Ricinus communis* seedlings were exposed to salt stress, the leaf stomatal conductance ($g_S$) was significantly reduced with increasing salt stress (Rodrigues *et al.,* 2014). At 50 mM of salt stress $g_S$ reduced by 22% whereas at 100 and 150 mM NaCl, the values of $g_S$ was reduced by 70% and 94% respectively. Salt stress (100 mM NaCl) significantly decreased stomatal conductance in maize genotypes compared to control. The relative decrease in stomatal conductance under salt treated genotypes compared to control was 88.64, 80.85, 66.67 and 43.59% respectively (Uddin *et al.,* 2019).

### 2.5 Antioxidative enzymes:

Plants defend themselves against ROS using both enzymatic and non-enzymatic mechanisms. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione-synthesizing enzymes are among the enzymes used to scavenge ROS (Shanker *et al.,* 2011; Maleka *et al.,* 1999; Xia *et al.,* 2014). Das *et al.* (2016) and Sairam *et al.* (2004) reported that in wheat, during abiotic stress, activities of SOD, APX, CAT, and GR increases significantly.

### 2.5.1 Superoxide dismutase (SOD):

Almost all plant cell types have superoxide dismutase (SOD) activity. It causes singlet oxygen to disproportionate to molecular oxygen and hydrogen peroxide (Shahid *et al.,* 2020). Conversely, SOD activity gets declined in potato cultivars under salt stress (Hu *et al.,* 2016). Chernane *et al.* (2015) showed the declined SOD activity in wheat under salt stress. Ebrahimian and ByBybordi (2012) investigated the specific activity of SOD in barley under salt stress, which was found to be approximately 52% higher than in unstressed barley. The specific activity of SOD was increased from 250 to 550 mg$^{-1}$ protein min$^{-1}$ with an increase in salt level (0 to 3 mM NaCl), and silicium element was added to significantly increase the activity of superoxide dismutase enzyme. According to Biju *et al.* (2017), the activity of antioxidant enzymes such as APX, CAT, and SOD increased significantly under drought stress when compared to normal lentil seedlings. Manish (2018) also studied the specific activity of SOD in sorghum genotypes. SOD activity decreased with the increasing levels of salt stress but it increased after foliar application of salicylic acid.

### 2.5.2 Catalse (CAT):

Catalases (CATs) are enzymes that are synthesised in peroxisomes and glyoxysomes and are responsible for the conversion of hydrogen peroxide into water and oxygen (Shahid *et al.,* 2020). The antioxidant defence systems of two bean genotypes (Tema and Djadida) were studied under salinity. With increasing NaCl concentrations in the growth medium, both genotypes showed a significant increase in antioxidative enzymes. CAT activity increased fourfold in the high-yielding genotype Tema and twofold in the low-yielding genotype Djadida (Taibi *et al.,* 2016). Catalase activity was enhanced under low salt stress but declined in higher salt stress in chickpea (Kukreja *et al.,* 2006). Same was recorded in sorghum by Manish (2018).

### 2.5.3 Peroxidase (POX)

Peroxidase is another antioxidative enzyme that scavenges $H_2O_2$. The specific activity of peroxidase increases with $H_2O_2$ content under salt stress. Kukreja *et al.* (2006) also reported increase in peroxidase activity under salt stress in chickpea plant. Biju *et al.* (2017) observed observed increased specific activity of peroxidase in *Lentil* under drought stress. Similar results were also noticed in sorghum under salt stress (Manish, 2018).

### 2.6 Soil Analysis:

Soil texture helpe to determine how much water will be able to pass through the soil. Soil pH is a predictor of various chemical activities within the soil and a rough indicator of the plant availability of nutrients in the soil (Neina, 2019). The electrical conductivity (ECe) of soil is a measurement of the amount of salts in the soil (salinity of soil). It is a key indicator of soil health because it influences crop yields, crop suitability, plant nutrient availability and the activity of soil microorganisms, all of which influence key soil processes. Excess salts impede plant growth by interfering with the soil-water balance. Excess salts hinder plant growth by affecting the soil-water balance. Irrigation water contains the dissolved salts such as $Cl^-$, $SO_4^{2-}$, $CO_3^-$, $HCO_3^-$ of $Ca^{2+}$ and $Mg^{2+}$. Saline irrigation could not improve soil health but it also adversely affect plant growth and development. Water storage in the soil also depends on the texture of the soil that how much amount of water will be able to pass and capability of binding of $Na^+$ ions to the soil particles.

# CHAPTER-III

## MATERIALS AND METHODS

The present study was carried out to evaluate the effect of salt stress on physiological, biochemical traits, in sorghum genotypes. Seeds of six single cut varieties (HC-136, HC-171, HC-260, HC-308, HJ-513 and HJ-541) were collected from Forage Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana). Seeds of sorghum varieties were grown in pots under screen house conditions on 26th February 2021. All analytical work was done at the stress physiology laboratory of Department of Botany and Plant physiology.

### 3.1 Experimental details are as follows:

**Experiment: The effect of salt stress on physiological and biochemical parameters in sorghum varieties**

Seeds of six sorghum varieties were surface sterilized in 1% sodium hypochlorite (NaOCl) solution for 5 minutes and was sown in plastic pots containing 10 kg of dune sand. Before sowing pots were saturated with desired levels of salt i.e. Control (0), 5, 8 and 10 dS $m^{-1}$. The control pots were irrigated with canal water. Nutrient solution was given at different intervals (Hoagland and Arnon, 1950).

**Chemical composition of Hoagland nutrient solution**

**Major salts**

| | | | |
|---|---|---|---|
| I | - | $Ca(NO_3)_2$ | 364.0 g $l^{-1}$ |
| II | - | $MgSO_4$ | 217.6 g $l^{-1}$ |
| III | - | $KH_2PO_4$ | 62.1 g $l^{-1}$ |
| IV | - | $KNO_3$ | 221.3 g $l^{-1}$ |

Each stock solution I-IV was prepared by dissolving salts separately in distilled water and total volume was made up to one L in four different bottles.

**Minor salts**

| | | |
|---|---|---|
| V - | $ZnSO_4$ | 0.097 g $l^{-1}$ |
| | $H_3BO_3$ | 1.269 g $l^{-1}$ |
| | $Na_2MoO_4$ | 0.400 g $l^{-1}$ |
| | $CuSO_4.5H_2O$ | 0.035 g $l^{-1}$ |
| | $MnSO_4$ | 0.60 g $l^{-1}$ |

All these salts were added in distilled water and the total volume was made one L.

**Iron source**

| | | |
|---|---|---|
| Freshly prepared | Tartaric acid | 0.06 g $l^{-25}$ |
| | $FeSO_4$ | 0.075 g $l^{-25}$ |

Nutrient solution was made by mixing 62.5 ml of each of stock solution I to V for 25 L and then freshly prepared iron source was added.

**Treatments:**

Four different treatments were given to each variety of sorghum (HC 136, HC 171, HC 260, HC 308, HJ 513 and HJ 541).

**T1:-** Control

**T2:-** Salt stress by NaCl 5 dS $m^{-1}$

**T3:-** Salt stress by NaCl 8 dS $m^{-1}$

**T4:-** Salt stress by NaCl 10 dS $m^{-1}$

**Sampling Stages**

The sampling was done at following stages:

1. At 30 days after sowing (DAS)
2. At 60 days after sowing (DAS)
3. At the time of 50% flowering

**Statistical analysis**

Statistical analysis of data was done by complete randomized design (CRD) with OPSTAT programme. Treatments were compared with CD values at 5% level of significance.

**Replications:** For each parameter three replications were sampled at a time.

**Observations recorded at the time of germination were:**

### 3.2 Germination studies

3.2.1 Germination percentage (%)

3.2.2 Days to germination

**Observations recorded at 30 and 60 DAS and at the time of 50% flowering were:**

### 3.3 Growth studies

### 3.3.1 Plant height (cm):

Plant height was measured using a meter scale from the surface of the soil to the tip of the apical shoot at 30, 60 days after sowing (DAS) and at the time of 50% flowering. The average height of the plants was calculated.

### 3.3.2 Fresh weight (g $plant^{-1}$):

The weight of the plant was measured on weighing machine right after they were removed from the pots.

### 3.3.3 Dry weight (g $plant^{-1}$):

The plant dry weight was measured using a weighing machine after they were removed from the pots and samples were then dried in an oven at 60°C for 72 hours.

### 3.3.4 No. of leaves per plant:

Number of leaves per plant were counted and the data was expressed on average number of leaves per plant basis.

### 3.3.5 Leaf area per plant (cm$^2$ plant$^{-1}$):

With the use of a leaf area meter (Model LI 3000, LI COR Ltd., Nebraska, USA), the leaf area of the removed leaves of each sample was measured and expressed as cm$^2$ plant$^{-1}$.

### 3.3.6 Salinity Susceptibility Index (SSI %):

Salinity susceptibility index was calculated by the formula given by Fischer and Maurer, 1978.

$$SSI = (1 - Y_s / Y_p) / SI$$

Where,

$SI = (1 - \bar{Y}_s / \bar{Y}_p)$

**Observations recorded at the time of 50% flowering were:**

## 3.4 Plant water relation

### 3.4.1 Osmotic potential (Ψs) (-MPa):

The psychrometric approach was used to determine the osmotic potential (Model 5199-B vapour Pressure Osmometer, Wescor Inc. logan, Utah, USA). The third leaf from the top was preserved in airtight eppendorf tubes. The leaves were crushed at room temperature. A filter paper disc was quickly dipped in sap and inserted into the concave depression of the sample holder, avoiding contact with the wet disc on the sample holder's exterior surface. The sample slide was gently put into the device, and the chamber was sealed by turning the knob clockwise. A beep tone was heard after approximately a minute. On the digital meter, the osmotic potential reading (mmol kg$^{-1}$) was recorded. The osmometer was calibrated using sodium chloride osmolarity reference standards (Wescor Inc, USA) and the following calculations were made:

1000 mmol kg$^{-1}$ = 2.5 MPa

2.5 MPa = 25 bars

### 3.4.2 Relative water content (%):

To determine the fresh weight (FW) of the sample, 200 mg leaf discs were cut and weighed right away. The leaf discs were then hydrated to full turgidity by floating for 3-4 hours at room temperature in de-ionized water in a closed petri plate. The leaf discs were removed from the water after 3-4 hours and any surface moisture was swiftly removed using filter paper before being weighed to obtain fully turgid weight (TW). The leaf discs were dried in an oven at 60°C for 48 hours before being weighed (after cooling in a desiccator) to determine their dry weight (DW). Weatherly's algorithm was used to determine the RWC (%) (1950).

$$RWC\ (\%) = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100$$

### 3.5. Physiological and biochemical studies

### 3.5.1 Relative stress injury (%):

The relative stress injury was estimated according to the method of Sullivan and Ross (1979). The electrical conductivity of the external medium was used to calculate the percentage of ion leakage into the external aqueous medium compared to the total ion concentration in the stressed tissue.

**Procedure:**

200 mg of leaves were stored in 20 ml test tubes with 10 ml de-ionized water at room temperature. The electrical conductivity (EC) of the solution was measured with an EC metre after 5 hours and labelled as EC1. After that, the samples were placed in a boiling water bath for 50 minutes to accomplish total tissue disruption. The EC of the solution was measured again after cooling and labelled as EC2. The following formula was used to calculate the membrane injury:

$$\text{RSI (\%)} = (\text{EC1}/\text{EC2}) \times 100$$

### 3.5.2 Proline content (µg $g^{-1}$ DW):

Bates *et al.* (1973) approach was used to calculate the proline content of cell free extract.

**Reagents**

1. Acid-ninhydrin was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M ortho-phosphoric acid, with agitation, until dissolved and kept cool (stored at 4°C). The reagent remains stable for 24 hours.
2. Extraction buffer: 3% (w/v) aqueous sulfosalicylic acid.
3. Glacial acetic acid
4. Toluene
5. Proline

**Extraction:**

300 mg of fresh leaves were homogenised in 5 ml of 3% sulphosalicylic acid, centrifuged for 15 minutes at 5000 rpm, and the supernatant was collected.

**Procedure:**

In a test tube, 2 ml of the supernatant was taken, followed by 2 ml acid-ninhydrin and 2 ml glacial acetic acid. The reaction was started in a water bath at 100°C for 1 hour and then stopped in an ice bath. 4 ml toluene was added to the reaction mixture, which was vigorously stirred and kept at room temperature for 30 minutes until the two phases separated. The chromophore-containing toluene (upper phase) was warmed to room temperature and its optical density was measured at 520 nm using toluene as blank. The proline concentration was determined from a standard curve using L-Proline.

### 3.5.3 Glycine betaine content (µmole $g^{-1}$ DW):

Glycine betaine content was determined according to the method of Grieve and Grattan (1983).

**Reagents:**

1. 0.05% toluene
2. 2 N HCl
3. Potassium tri-iodide solution
4. 1,2-dichloroethane

**Procedure:**

Leaf extract was made by cutting 0.5 g leaves in 5 ml of 0.05 % toluene in 20 ml test tubes. All of the tubes were kept at 25°C for 24 hours. After filtering, 0.5 ml of extract was mixed with 1 ml of 2 N HCl solution and 0.1 ml of potassium tri-iodide solution (containing 7.5 g iodine and 10 g potassium iodide in 100 ml of 1 N HCl) and shaken in an ice cold water bath for 90 minutes, after which 2 ml of ice cold water was added after gentle shaking and 10 ml of 1, 2-dichloroethane (chilled at $-10^0$C) was pour in it. Two layers were separated by blowing a continuous stream of air for 1-2 minutes, the upper aqueous layer was removed, and the optical density of the organic layer was measured at 365 nm. The data was represented as µmole $g^{-1}$ DW of the tissue and the standard curve was made using a graded concentration of glycine betaine.

### 3.5.4 Total soluble solids (%):

Total soluble solids were determined with the method Yemm and Willis (1954).

**Reagents:**

i) Anthrone reagent: Anthrone reagent was prepared by dissolving 0.4 gm of anthrone in 100 ml concentrated $H_2SO_4$

ii) 80% Ethanol

**Extraction:**

The procedure suggested by Bernett and Naylor (1966) for extraction was used. Fresh leaf samples weighing 200 mg were homogenized individually in 80 % ethanol, then refluxed for 15 minutes on a steam bath prior to centrifugation. The residue was refluxed with 40% ethanol. Extraction was repeated thrice, with same procedure. The supernatants from extractions were pooled and volume was made to 5 ml with 80% ethanol. TSS was calculated using anthrone reagent after the extraction.

**Procedure:**

In a boiling water bath, a 0.2 ml aliquot of extract was evaporated to dryness in a test tube. After cooling, the residue in the tube was dissolved in 1 ml distilled water and added with 4 ml anthrone reagent, which was then heated in a water bath for 10 minutes. Spectrophotometer was used to measure absorbance at 620 nm after cooling (Systronic India

Spectrophotometer 117). The data was represented in percentage and the standard curve was created using a graded concentration of glucose.

### 3.5.5 Total chlorophyll content (mg $g^{-1}$ FW):

As detailed by Sawhney and Singh (2002), leaves were washed, blotted dry, then cut into 200 mg discs and dipped in test tubes containing 5 ml dimethyl sulfoxide (DMSO) overnight. The absorbance of the extracted chlorophyll in DMSO was measured at 663 and 645 nm, respectively, and its content was determined using the formula:

$$\text{Chl “a”} = \frac{12.3\ A_{663} - 0.86\ A_{645}}{a \times W} \times V$$

$$\text{Chl “b”} = \frac{19.3\ A_{645} - 3.6\ A_{663}}{a \times W} \times V$$

Total chlorophyll = Chl ‘*a*’ + Chl ‘*b*’

Where, V= Volume of DMSO, a = Path length, W = Weight of tissue taken (mg)

### 3.5.6 Chlorophyll stability index (CSI %):

CSI was determined according to Sairam *et al.* (1997) and calculated as follows:

CSI = (Total chlorophyll under stressed condition / Total chlorophyll under control)×100

### 3.5.7 Photochemical quantum yield (Fv/Fm):

At mid-day, the fluorescence of chlorophyll was measured in intact plants using a chlorophyll fluorometer (OS-30p, Opti-Science, Inc., Hudson, USA). By attaching a clip to the completely expended leaf, it was initially acclimated to dark for at least two minutes. An array of three light emitted diodes in the sensor then constantly illuminated the dark adapted leaf for one second (1500 mol $m^{-2}s^{-1}$). The variable fluorescence (Fv) was calculated by subtracting the initial (F0) and maximum (Fm) fluorescence. The Fv/Fm ratio was then used to compute photochemical quantum yield.

### 3.5.8 Assimilation rate (µmole $CO_2$ $m^{-2}s^{-1}$), Transpiration rate (mmole $H_2O$ $m^{-2}s^{-1}$) and Stomatal conductance (mmole $H_2O$ $m^{-2}s^{-1}$):

Third leaf from top was used to record the observations on transpiration rate, assimilation rate and stomatal conductance by Infra Red Gas Analyzer (IRGA, LCi-SD, ADC Bioscience, USA). Clean and dry leaves with no sign of diseases or damage were selected from three plants in each plot for the measurements. Leaf was held in the leaf chamber of photosystem facing towards sunlight and data was recorded on stabilization of values.

## 3.6 Antioxidative enzymes

### Extraction:

500 mg of leaves were rinsed in chilled distilled water and homogenised separately in 5 ml of extraction buffer with a chilled pestle and mortar (0.1 M phosphate buffer, pH 7). The

extract was then centrifuged for 20 minutes at 15000 rpm at 4°C. Following that, the supernatant was used to determine the presence of the following enzymes:

### 3.6.1 Superoxide dismutase (SOD):

Specific activity of SOD was estimated by the method of Giannopolitis and Ries (1977) with little modifications.

**Reagents**

1. 60 µM riboflavin
2. 17 mM methionine
3. 0.5 mM nitrobluetetrazolium (NBT)
4. 1.5 M $Na_2CO_3$
5. 3 mM EDTA

**Procedure:**

The reaction mixture contained 1 ml of enzyme extract, 0.5 ml of each of methionine, NBT, EDTA, and $Na_2CO_3$, and a total volume of 4 ml was prepared with buffer in each set, adjusting the pH to 10.2, adding 0.5 ml of riboflavin to each set in the last. The tubes were shaken before being placed 30 cm away from the light source (8 x 20 W fluorescent lamps). After allowing the reaction to run for 10 minutes, the light was turned off. A black cloth was draped over the tubes right away. At 560 nm, the absorbance was measured. As a control, a non-irradiated reaction mixture that did not develop colour was used. However, in the presence of SOD the reaction was inhibited and the amount of inhibition was used to quantify the enzyme. Log $A_{560}$ was plotted as a function of enzyme extract used in reaction mixture. The amount of enzyme extract corresponding to 50% inhibition of the photochemical reaction was calculated from the graph and was considered as one enzyme unit expressed as $mg^{-1}$ protein $min^{-1}$.

### 3.6.2 Catalase:

Catalase (CAT) activity was estimated by the method of Aebi (1983)

**Reagents**

1. 0.1 M $H_2O_2$
2. 0.05 M potassium phosphate buffer (pH 7.0)

**Procedure**:

500 µl of enzyme extract was mixed with 0.2 ml of 0.1 M $H_2O_2$ and 1.5 ml of 50 mM potassium phosphate buffer. At the time of recording the absorbance, the enzyme sample was added and incubated for 3 minutes. For 1.5 minutes, the change in absorbance was measured at 240 nm at 15-second intervals. The enzyme's specific activity was measured in units of $mg^{-1}$ protein $min^{-1}$.

### 3.6.3 Peroxidase:

The procedure of Siegel and Siegel (1986) was followed for estimating peroxidase activity.

**Procedure:**

Three ml of reaction mixture contained of 0.1 M phosphate buffer (pH 7.0), 0.1 mM guaiacol, and 100 ml of cell free extract. With the addition of 0.1 mM $H_2O_2$, the reaction was initiated and the increase in absorbance at 470 nm was measured for 2 minutes. The extinction coefficient value of 26.6 $mM^{-1}$ $cm^{-1}$ for guaicol was used to compute the activity.

## 3.7 Soil analysis:

Before sowing and at the time of 50% flowering, samples were taken from various pots. The soil samples were tested for ECe and pH using the method outlined below.

### 3.7.1 Electrical Conductivity (ECe):

The electrical conductivity of soil saturation extract (ECe) was measured with a Conductivity Bridge and expressed as dS $m^{-1}$ at $25^0$C.

### 3.7.2 pH:

The pH of the soil saturation percentage or extract was determined using an Elico glass electrode on a pH metre.

# CHAPTER-IV

# RESULTS

The results of experiment entitled, **"Evaluation of physiological and biochemical traits in sorghum (*Sorghum bicolor* L.) varieties during summer season"** conducted during the summer season of 2021 (February-May) are presented in this chapter. The data are supplemented with different tables and graphs under following headings:

**4.1 Germination studies**

**4.2 Growth studies**

**4.3 Plant water relations**

**4.4 Physiological and biochemical studies**

**4.5 Antioxidative enzymes**

**4.6 Soil analysis**

## 4.1 Germination studies:

Table 1 showed the effect of salt stress on germination percentage, days to germination and days to 50% flowering in all the varieties.

### 4.1.1 Germination percentage (%):

Data presented in Table 1 clearly showed that germination percentage was significantly influenced due to salt stress. Germination (%) decreased with increasing salt levels from control to 10 dS $m^{-1}$. It was also observed that out of all the six varieties (HC 136, HC 171, HC 260, HC 308, HJ 513 and HJ 541) only one variety i.e. HJ 541 showed germination upto 10 dS $m^{-1}$. At 8 dS $m^{-1}$, maximum germination was observed in HJ 541 (85%) followed by HJ 513 (81.3%) while minimum in HC 260 (63.3%) with respect to control.

Maximum percent decline was observed in HC 260 (32.1%) while minimum in HJ 541(8.9%) at 8 dS $m^{-1}$ of salt stress. The interaction between the treatments and varieties was found statistically non-significant.

### 4.1.2 Days to germination:

*Sorghum* plants grown in control condition exhibited normal days to germination which was almost at par with plants grown under 5 dS $m^{-1}$ of salt stress while delayed germination was noticed at 8 dS $m^{-1}$ and 10 dS $m^{-1}$ of salt stress. Maximum days to germination were observed in HC 308 (13) and minimum in HJ 541 (9) at 8 dS $m^{-1}$ of salt stress. The interactions values were statistically non-significant (Table 1).

### 4.1.3 Days to 50% flowering:

Days to 50% flowering were significantly influenced due to both the factors i.e. salt stress and varieties. Significantly, more number of days to 50% flowering was taken by the varieties under salt stress conditions as compared to the control. Among the treatments, maximum number of days to 50% flowering was observed at 8dSm$^{-1}$. At 8 dS m$^{-1}$ of salt stress, least number of days to 50% flowering was observed in HJ 541 (85) which was at par with HC 171 (85) followed by HJ 513 (86) while highest number of days to 50% flowering was observed in HC 260 (97).

**Table 1: Effect of salt stress on germination percentage (%), days to germination and days to 50% flowering in sorghum varieties**

| Salt Levels (dS m$^{-1}$) | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|
| | Germination percentage (%) | | | | | | |
| | HC 136 | HC 171 | HC 260 | HC 308 | HJ 513 | HJ 541 | Mean |
| 0 | 89.7 | 90.3 | 93.3 | 90.0 | 93.3 | 93.3 | 91.7 |
| 5 | 87.2 | 86.6 | 80.0 | 87.9 | 89.1 | 90.5 | 86.9 |
| 8 | 76.7 | 70.3 | 63.3 | 80.0 | 81.3 | 85.0 | 76.1 |
| Mean | 84.5 | 82.4 | 78.8 | 85.5 | 88 | 89.6 | |
| CD at 5% | S = 4.1, V = 5.6, S×V = N.S. | | | | | | |
| Days to Germination | | | | | | | |
| 0 | 5 | 6 | 6 | 7 | 7 | 5 | 6 |
| 5 | 7 | 7 | 7 | 8 | 9 | 6 | 7 |
| 8 | 10 | 10 | 10 | 13 | 13 | 9 | 11 |
| Mean | 7.5 | 7.7 | 7.5 | 9.5 | 9.7 | 6.8 | |
| CD at 5% | S = 1.3, V = 1.9, S×V = N.S. | | | | | | |
| Days to 50% Flowering | | | | | | | |
| 0 | 71 | 68 | 76 | 69 | 71 | 71 | 71 |
| 5 | 76 | 74 | 88 | 72 | 75 | 74 | 77 |
| 8 | 87 | 85 | 97 | 76 | 86 | 85 | 86 |
| Mean | 77.6 | 75.6 | 87.1 | 72.4 | 77.2 | 76.5 | |
| CD at 5% | S = 1.7, V = 2.46, S×V = 2.09 | | | | | | |

*S = Salt levels, V = Varieties, CD =Critical difference, DAS = Days after sowing and N.S.= Non significant

**Note:** Out of all the six varieties, only HJ 541variety survived upto 10 dS m$^{-1}$ with the germination percentage of 50.7 %, 12 days to germination and displayed 106 days to 50% flowering.

### 4.2 Growth studies:

Table 2-7 showed the effect of salt stress on growth parameters of all varieties at 30, 60 DAS and 50% flowering.

### 4.2.1 Plant height (cm):

Data presented in Table 2 depicted that plant height decreased significantly with increasing levels of salt stress (control to 8 dS m$^{-1}$) at 30, 60 DAS and at 50% flowering in all varieties. At 8 dS m$^{-1}$ of salt stress, HC 260 had a highest percent decline in height (67.1%) while least in HJ 541 (52.1%) over their respective control. Among all the varieties, maximum plant height was observed in HJ 541 (11.3, 100.3 and 107.3 cm) and minimum in HC 260 (8.0, 56.7 and 60.0 cm) at 8 dS m$^{-1}$ respectively at 30, 60 DAS and at 50% flowering.

The interaction values at 30 DAS was found non-significant, whereas significant at 60 DAS and at 50% flowering.

**Table 2: Effect of salt stress on plant height (cm) at 30, 60 DAS and at the time of 50% flowering in sorghum varieties**

| Salt Levels (dS $m^{-1}$) | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|
| | Plant Height (cm) At 30 DAS | | | | | | |
| | HC 136 | HC 171 | HC 260 | HC 308 | HJ 513 | HJ 541 | Mean |
| **Control** | 20.8 | 20.0 | 23.3 | 22.3 | 23.7 | 24.2 | 22.4 |
| **5** | 14.7 | 12.3 | 15.0 | 14.3 | 14.8 | 15.2 | 14.4 |
| **8** | 9.3 | 9.0 | 8.0 | 9.7 | 10.3 | 11.3 | 9.6 |
| **Mean** | 14.9 | 13.7 | 15.3 | 15.4 | 16.3 | 17.1 | |
| **CD at 5%** | S = 1.3, V = 1.8, S×V = N.S. | | | | | | |
| **At 60 DAS** | | | | | | | |
| **Control** | 117.7 | 131.0 | 126.3 | 137.0 | 130.7 | 139.3 | 130.3 |
| **5** | 101.3 | 107.0 | 110.3 | 109.3 | 116.7 | 121.0 | 110.9 |
| **8** | 69.3 | 77.7 | 56.7 | 86.7 | 97.0 | 100.3 | 81.3 |
| **Mean** | 96.1 | 105.2 | 97.7 | 111.1 | 115 | 120 | |
| **CD at 5%** | S = 2.4, V = 3.4, S×V = 5.9 | | | | | | |
| **At 50% flowering** | | | | | | | |
| **Control** | 123.7 | 135.3 | 138.0 | 141.0 | 142.7 | 149.3 | 138.3 |
| **5** | 110.7 | 112.3 | 119.0 | 114.7 | 122.3 | 129.0 | 118.0 |
| **8** | 71.7 | 88.0 | 60.0 | 89.7 | 102.0 | 107.3 | 86.5 |
| **Mean** | 101.6 | 111.8 | 105.8 | 115.1 | 122.3 | 128.4 | |
| **CD at 5%** | S = 2.2, V = 3.0, S×V = 5.3 | | | | | | |

*S= Salt levels, V = Varieties, CD=Critical difference NS=Non-significant and DAS=Days after sowing

**Note:** Out of all the six varieties of sorghum, only HJ 541variety survived upto 10 dS $m^{-1}$ with plant height of 5.7, 43.2 and 49.6 cm at 30, 60 DAS and at 50% flowering respectively.

### 4.2.2 Fresh weight (g/plant):

Table 3 illustrated the significant differences between the fresh weights of plants among all the treatments. It was observed that with increasing levels of salt stress (control to 8 dS $m^{-1}$), fresh weight of plant reduced in all varieties. The genotypic mean value of the fresh weight in all varieties ranges from 5.2 to 8.7 g. Among all the varieties at 30 DAS, HJ 541 (4.6 g/plant) showed maximum fresh weight whereas minimum in HC 136 (2.4 g/plant).

Similar trend were observed at 60 DAS and 50% flowering. Interaction among the treatments (salt stress and varieties) was found non-significant at 30 DAS but significant at 60 DAS and at 50% flowering.

**Table 3: Effect of salt stress on fresh weight (g/plant) at 30, 60 DAS and at the time of 50% flowering in sorghum varieties**

| Salt Levels (dS $m^{-1}$) | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Fresh weight (g/plant) At 30 DAS** | | | | | | |
| | **HC 136** | **HC 171** | **HC 260** | **HC 308** | **HJ 513** | **HJ 541** | **Mean** |
| **Control** | 9.5 | 7.3 | 10.1 | 9.7 | 11.5 | 11.9 | 10.0 |
| **5** | 7.1 | 5.7 | 7.3 | 7.0 | 9.3 | 9.7 | 7.7 |
| **8** | 2.4 | 2.6 | 2.8 | 2.5 | 3.3 | 4.6 | 3.0 |
| **Mean** | 6.3 | 5.2 | 6.7 | 6.3 | 8.6 | 8.7 | |
| **CD at 5%** | S = 0.7, V = 1.1, S×V = N.S. | | | | | | |
| **At 60 DAS** | | | | | | | |
| **Control** | 50.2 | 59.6 | 50.6 | 57.0 | 60.4 | 65.1 | 57.2 |
| **5** | 39.9 | 34.5 | 40.4 | 41.6 | 42.1 | 45.6 | 40.7 |
| **8** | 16.6 | 15.4 | 21.4 | 21.2 | 26.1 | 28.1 | 21.5 |
| **Mean** | 35.5 | 36.5 | 37.4 | 39.9 | 43.9 | 46.9 | |
| **CD at 5%** | S = 2.3, V = 3.3, S×V = 5.8 | | | | | | |
| **At 50% flowering** | | | | | | | |
| **Control** | 56.3 | 60.9 | 58.9 | 61.1 | 70.7 | 76.9 | 64.1 |
| **5** | 45.4 | 36.8 | 35.1 | 40.8 | 45.5 | 47.5 | 41.9 |
| **8** | 26.1 | 24.7 | 25.4 | 20.2 | 28.7 | 30.2 | 25.9 |
| **Mean** | 42.6 | 40.8 | 39.8 | 40.6 | 47.1 | 50.8 | |
| **CD at 5%** | S = 1.5, V = 2.1, S×V = 3.7 | | | | | | |

*S= Salt levels, V= Varieties, CD=Critical difference, NS=Non-significant and DAS=Days after sowing

**Note:** Out of all the six varieties of sorghum, only HJ 541variety survived upto 10 dS $m^{-1}$ with fresh weight of 2.3, 12.1 and 17.5 gram at 30, 60 DAS and at 50% flowering respectively.

### 4.2.3 Dry weight of plant (g):

Data presented in table 4 depicted that dry weight (g/plant) at 30 DAS, decreased remarkably with the increasing salt levels (control to 8 dS $m^{-1}$) in all the varieties. At 30 DAS, the maximum dry weight was observed in HJ 541 (0.7 g/plant) followed by HJ 513 (0.5 g/plant) while minimum in HC 171 (0.1 g/plant) at 8 dS $m^{-1}$ of salts stress. Genotypic mean value of dry weight ranges from 0.9 to 1.8 g/plant. A similar trend was noticed at 60 DAS and at 50% flowering. Significant interaction was found at all sampling stages, i.e. 30, 60 DAS, and 50% flowering.

**Table 4: Effect of salt stress on dry weight (g/plant) at 30, 60 DAS and at the time of 50% flowering in sorghum varieties**

| Salt Levels ($dS\ m^{-1}$) | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|
| | Dry weight (g/plant) At 30 DAS | | | | | | |
| | HC 136 | HC 171 | HC 260 | HC 308 | HJ 513 | HJ 541 | Mean |
| Control | 3.0 | 2.3 | 3.5 | 2.2 | 2.8 | 3.7 | 2.9 |
| 5 | 1.7 | 1.6 | 1.7 | 1.3 | 1.8 | 2.4 | 1.8 |
| 8 | 0.2 | 0.1 | 0.2 | 0.2 | 0.5 | 0.7 | 0.3 |
| Mean | 1.6 | 1.3 | 1.8 | 1.2 | 1.7 | 2.3 | |
| CD at 5% | S = 0.12, V = 0.17, S×V = 0.3 | | | | | | |
| At 60 DAS | | | | | | | |
| Control | 15.4 | 18.7 | 16.2 | 16.4 | 24.1 | 24.2 | 19.2 |
| 5 | 12.7 | 11.9 | 10.6 | 11.7 | 12.8 | 16.0 | 13.3 |
| 8 | 4.9 | 4.1 | 4.0 | 4.9 | 4.1 | 7.1 | 4.8 |
| Mean | 11 | 11.5 | 10.2 | 11 | 13.6 | 17.1 | |
| CD at 5% | S = 1.1, V = 1.5, S×V = 2.6 | | | | | | |
| At 50% flowering | | | | | | | |
| Control | 16.0 | 18.7 | 16.2 | 18.4 | 24.1 | 27.7 | 20.2 |
| 5 | 14.7 | 12.9 | 11.6 | 15.5 | 18.2 | 20.0 | 14.5 |
| 8 | 4.9 | 6.7 | 5.1 | 4.8 | 7.2 | 9.1 | 6.3 |
| Mean | 11.8 | 12.6 | 10.9 | 12.8 | 16.5 | 17.3 | |
| CD at 5% | S = 1.5, V = 2.1, S×V = 3.7 | | | | | | |

*S= Salt levels, V = Varieties, CD=Critical difference and DAS=Days after sowing

**Note:** Out of all the six varieties of sorghum, only HJ 541variety survived upto 10 dS $m^{-1}$ with plant dry weight of 0.5, 1.5 and 2.5 gram at 30, 60 DAS and at 50% flowering respectively

### 4.2.4 Number of leaves per plant:

Leaf number varies differently with imposition of salt stress among all the varieties. The number of leaves per plant decreased with increment in salt concentration form control to 8 dS $m^{-1}$ at 30 DAS in all the varieties (Table 5). The percent reduction in leaf number was found higher in HC 308 (77.8%) while the minimum in HJ 541 (21.05%) over their respective control. Among all the varieties leaf number was found higher in HJ 541 (5) at 8 dS $m^{-1}$ of salt level. Similarly, declining trend was also noticed at 60 DAS and 50% flowering, but overall mean values of leaf number was maximum at 50 % flowering stage. The interaction between both the factors was non-significant at 30, 60 DAS and at 50% flowering.

**Table 5:** **Effect of salt stress on number of leaves per plant at 30, 60 DAS and at 50% flowering in sorghum varieties**

| Salt Levels (dS $m^{-1}$) | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Number of leaves per plant At 30 DAS** | | | | | | |
| | **HC 136** | **HC 171** | **HC 260** | **HC 308** | **HJ 513** | **HJ 541** | **Mean** |
| **Control** | 6.0 | 5.0 | 4.7 | 6.0 | 6.7 | 6.3 | 5.8 |
| **5** | 5.0 | 4.7 | 4.3 | 4.7 | 6.7 | 6.7 | 5.4 |
| **8** | 4.0 | 3.1 | 3.5 | 1.3 | 4.7 | 5.0 | 3.6 |
| **Mean** | 5 | 4.2 | 4.2 | 4.0 | 6.0 | 6.0 | |
| **CD at 5%** | S = 0.6, V = 0.9, S×V = N.S. | | | | | | |
| **At 60 DAS** | | | | | | | |
| **Control** | 8.7 | 6.7 | 6.0 | 8.0 | 7.0 | 8.0 | 7.4 |
| **5** | 6.3 | 6.0 | 5.0 | 6.3 | 5.7 | 7.7 | 6.2 |
| **8** | 5.0 | 5.0 | 5.3 | 4.7 | 5.7 | 6.3 | 5.3 |
| **Mean** | 6.6 | 5.8 | 5.4 | 6.3 | 6.1 | 7.3 | |
| **CD at 5%** | S = 0.9, V = N.S., S×V = N.S. | | | | | | |
| **At 50% flowering** | | | | | | | |
| **Control** | 10.3 | 9.0 | 9.3 | 9.0 | 9.3 | 11.3 | 9.7 |
| **5** | 8.7 | 7.3 | 6.7 | 7.3 | 7.0 | 9.7 | 7.8 |
| **8** | 5.7 | 6.0 | 5.5 | 5.3 | 6.3 | 7.3 | 5.9 |
| **Mean** | 8.2 | 7.4 | 7.2 | 7.2 | 7.5 | 9.4 | |
| **CD at 5%** | S = 0.9, V = 1.3, S×V = N.S. | | | | | | |

*S= Salt levels, V = Varieties, CD=Critical difference NS=Non-significant and DAS=Days after sowing

**Note:** Out of all the six varieties of sorghum, only HJ 541variety survived upto 10 dS $m^{-1}$ with number of leaves of 9, 4 and 6 at 30, 60 DAS and at 50% flowering respectively.

### 4.2.5 Leaf area ($cm^2$/plant):

Leaf area per plant decreased remarkably with the increasing salt levels from control to 8 dS $m^{-1}$. The variety HC 260 (75.7%) had the highest decrease in leaf area per plant whereas least in HJ 541 (54.6%) followed by HC 513 (60.3%) over control. Likewise, decreased leaf area per plant was also noticed at 60 DAS and at 50% flowering. The overall mean value of leaf area per plant varied from 294.5 to 473.9 $cm^2$/plant at 50% flowering. Significant interaction was calculated at 30, 60 DAS, and 50% flowering.

### 4.2.6 Salinity Susceptibility Index (SSI %):

Salinity susceptibility index (SSI %) was used to test the sensitivity of different varieties under salt stress. The values less than one showed the tolerance of variety towards salt stress. It was clearly indicated from the Table 7 that only the variety HJ 541 had the values less than one at all the salt levels. The value less than one were also noticed at 5 dS $m^{-1}$ of salt stress in all varieties at 60 DAS and 50% flowering stage.

**Table 6: Effect of salt stress on leaf area (cm$^2$) at 30, 60 DAS and at the time of 50% flowering in sorghum varieties**

| Salt Levels (dS m$^{-1}$) | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|
| | Leaf area At 30 DAS | | | | | | |
| | HC 136 | HC 171 | HC 260 | HC 308 | HJ 513 | HJ 541 | Mean |
| Control | 354.5 | 231.3 | 290.8 | 289.0 | 479.2 | 466.9 | 352.0 |
| 5 | 246.4 | 180.1 | 170.3 | 220.5 | 361.1 | 383.7 | 260.4 |
| 8 | 88.5 | 91.6 | 70.8 | 73.6 | 190.3 | 211.9 | 121.1 |
| Mean | 229.8 | 167.6 | 177.3 | 194.3 | 343.5 | 354.1 | |
| CD at 5% | S = 5.4, V = 7.6, S×V = 13.3 | | | | | | |
| At 60 DAS | | | | | | | |
| Control | 784.9 | 577.0 | 551.0 | 549.8 | 841.8 | 894.3 | 699.8 |
| 5 | 534.1 | 360.0 | 280.9 | 447.3 | 506.1 | 623.6 | 458.7 |
| 8 | 160.0 | 133.5 | 135.2 | 204.5 | 254.6 | 270.5 | 193.1 |
| Mean | 401.3 | 356.8 | 322.3 | 400.5 | 534.1 | 596.1 | |
| CD at 5% | S = 9.0, V = 12.7, S×V = 22.1 | | | | | | |
| At 50% flowering | | | | | | | |
| Control | 791.9 | 577.0 | 669.0 | 670.8 | 837.2 | 904.0 | 741.7 |
| 5 | 557.4 | 363.6 | 389.6 | 517.3 | 607.5 | 617.9 | 508.9 |
| 8 | 264.3 | 237.5 | 240.5 | 207.2 | 261.0 | 278.5 | 248.2 |
| Mean | 537.8 | 392.7 | 433.1 | 465.1 | 568.5 | 600.1 | |
| CD at 5% | S = 6.0, V = 8.4 S×V = 14.5 | | | | | | |

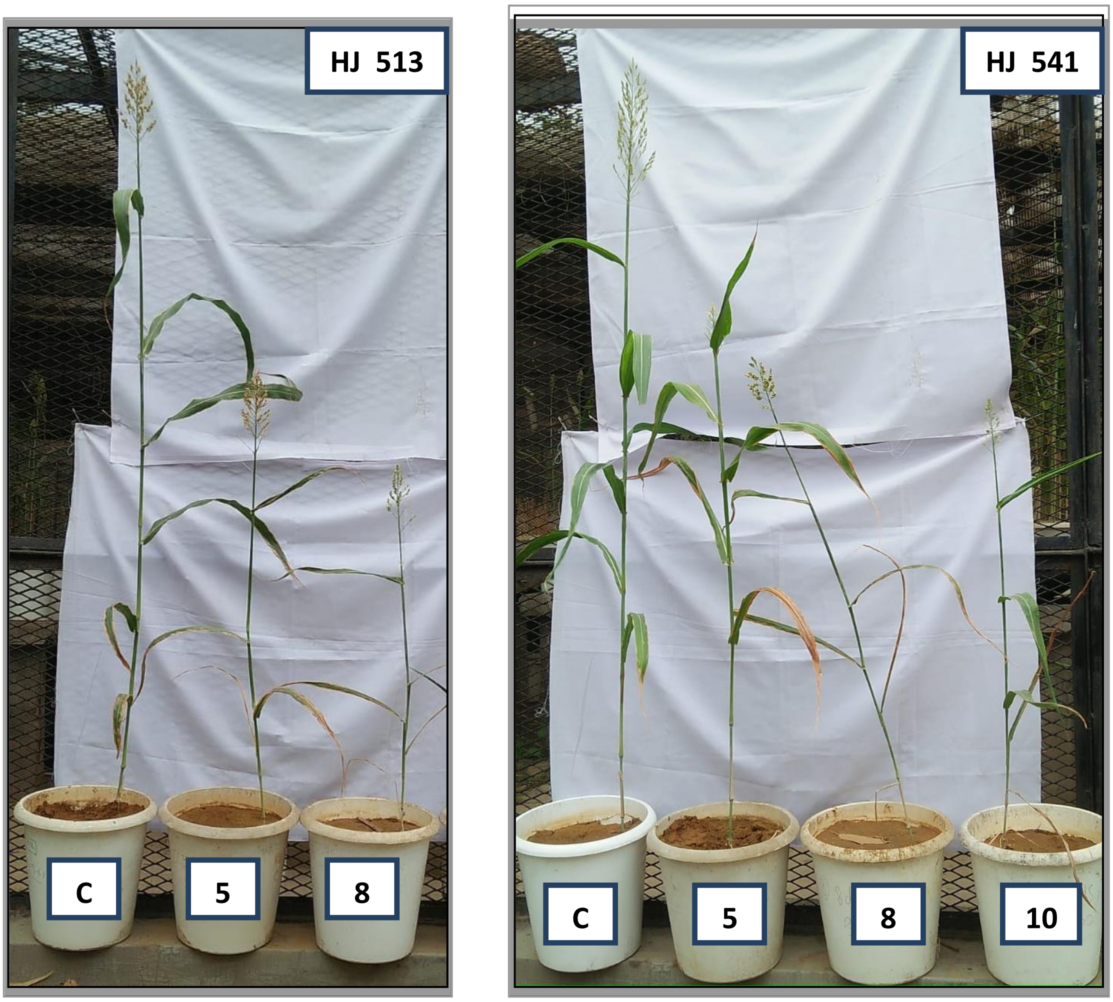
*S= Salt levels, V= Varieties, CD=Critical difference and DAS=Days after sowing

**Note:** Out of all the six varieties of sorghum, only HJ 541variety survived upto 10 dS m$^{-1}$ with leaf area of 44.5, 88.7 and 95.1 cm$^2$ at 30, 60 DAS and at 50% flowering respectively.

**Table 7: Effect of salt stress on salinity susceptibility index (%) at 30, 60 DAS and at the time of 50% flowering in sorghum varieties**

| Salt Levels (dS m$^{-1}$) | Varieties | | | | | | |
|---|---|---|---|---|---|---|---|
| | SSI (%) At 30 DAS | | | | | | |
| | HC 136 | HC 171 | HC 260 | HC 308 | HJ 513 | HJ 541 | Mean |
| 5 | 0.94 | 0.66 | 1.12 | 0.89 | 0.78 | 0.76 | 0.86 |
| 8 | 1.05 | 1.07 | 1.06 | 0.87 | 1.04 | 0.91 | 1.00 |
| Mean | 0.99 | 0.86 | 1.09 | 0.88 | 0.91 | 0.83 | |
| At 60 DAS | | | | | | | |
| 5 | 0.38 | 0.79 | 0.75 | 0.89 | 1.02 | 0.38 | 0.70 |
| 8 | 0.77 | 0.88 | 0.52 | 0.79 | 0.93 | 0.79 | 0.78 |
| Mean | 0.57 | 0.83 | 0.63 | 0..84 | 0.97 | 0.58 | |
| At 50% flowering | | | | | | | |
| 5 | 0.58 | 0.91 | 0.75 | 0.82 | 0.93 | 0.92 | 0.82 |
| 8 | 0.78 | 0.72 | 0.77 | 0.83 | 0.79 | 0.75 | 0.77 |
| Mean | 0.68 | 0.81 | 0.76 | 0.82 | 0.86 | 0.83 | |

**Plate 1:** **The figure shows the effect of salt stress (Control, 5, 8 and 10 dS m$^{-1}$) on growth of sorghum varieties (HJ 513 and HJ 541) at the time of 50% flowering**



### 4.3 Physiological and biochemical studies: At the time of 50% flowering

#### 4.3.1 Osmotic potential (ψs) of leaf (-MPa):

It was clearly indicated from the Fig. 1 that osmotic potential of leaf (-MPa) followed a declining trend (from less -ve to more –ve) with the increasing salt levels from control to 8 dS m$^{-1}$. Less –ve values of osmotic potential was observed in HJ 541 (-1.0 MPa) followed by HC 260 (-1.1 MPa) and more negative in HC 308 (–1.4 MPa) at 8 dS m$^{-1}$ of salt level. At 10 dS m$^{-1}$ of salt stress, HJ 541 showed osmotic potential of -1.53 MPa where other varieties failed to survive. The genotypic mean value ranged from -0.8 to -1.1 MPa. The interaction values were statistically non-significant.

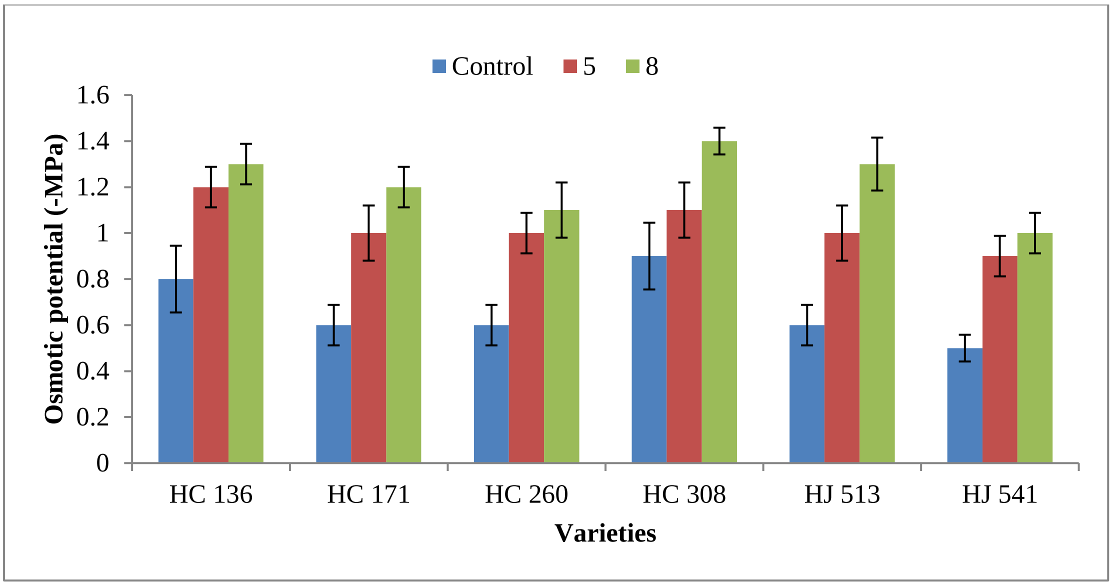


**Figure 1:** **Effect of salt stress (Control, 5, and 8 dS m$^{-1}$)on osmotic potential (-MPa) at the time of 50% flowering in sorghum varieties (CD at 5% = S = 0.12, V = 0.17, S×V = N.S.)**

### 4.3.2 Relative water content (RWC %):

Results presented in Fig. 2 shows that relative water content (%) decreased with every increment of salt stress from control to 8 dS m$^{-1}$. Plants grown in control conditions exhibited normal relative water content. At 8 dS m$^{-1}$of salt stress maximum relative content was maintained by HJ 541 (71.5%) followed by HJ 513 (69.3%) while minimum by HC 260 (57.1%). Among all the treatments, higher percent reduction was calculated in HC 260 (22.4%) at 8 dS m$^{-1}$ over control. At 10 dS m$^{-1}$ of salt stress, HJ 541 showed RWC of 49.1% where other varieties failed to survive. The genotypic mean value of the RWC of all the varieties varied from 70.3 to 79.1%. The interaction among the treatment was found statistically significant.

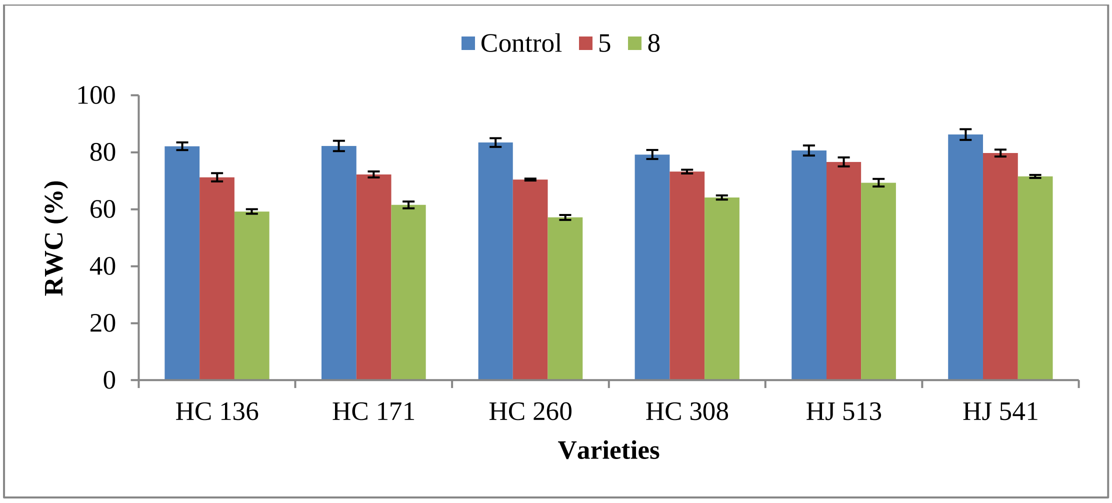


**Figure 2:** **Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on relative waster content (%) at the time of 50% flowering in sorghum varieties (CD at 5%= S = 1.5, V = 2.1, S×V = 3.7)**

### 4.3.3 Relative stress injury (RSI %):

RSI was calculated higher under stressed conditions as compared to unstressed conditions. In general, relative stress injury showed an increasing trend with increasing levels of salt stress from control to 8 dS m$^{-1}$. Maximum RSI (%) was estimated at 8 dS m$^{-1}$ in all the varieties. Minimum RSI (%) was shown by HJ 541 (19.2%) followed by HJ 513 (20.0%) whereas maximum in HC 260 (34.7%) at 8 dS m$^{-1}$ of salt stress. At 10 dS m$^{-1}$ of salt stress, HJ 541 showed RSI of 37.1% where other varieties failed to survive.The genotypic mean value for RSI ranged from 13.1 to 20.5%. Differences and interaction between two treatments was statistically significant.

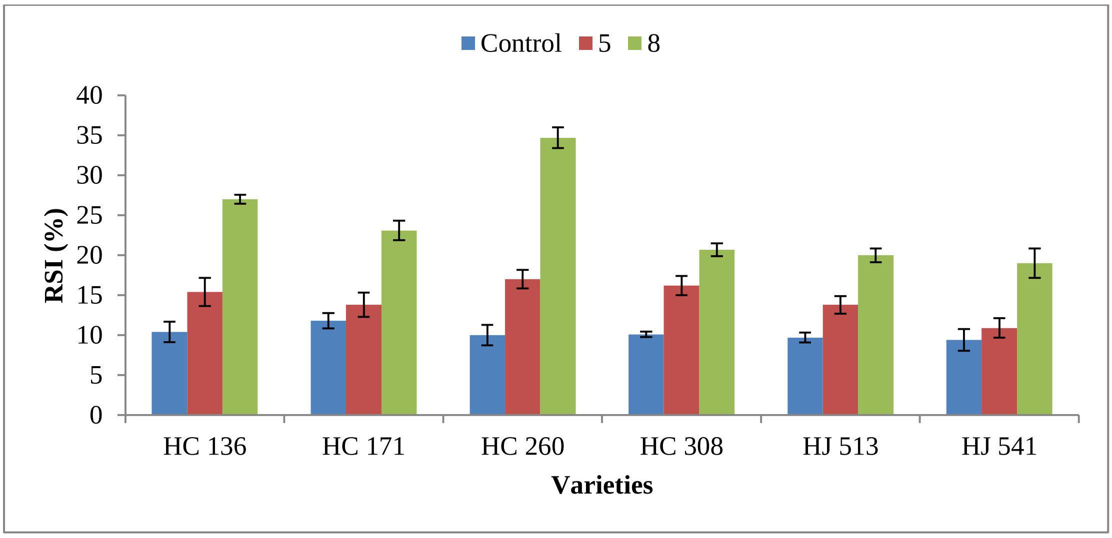


**Figure 3: Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on relative stress injury (%) at the time of 50% flowering in sorghum varieties (CD at 5% = S = 1.4, V = 1.9, S×V = 3.4)**

### 4.3.4 Proline content (μg g$^{-1}$ DW):

The data pertaining to proline content depicted that significant differences were observed in proline content among all the varieties under salt stress. Salt stress enhanced the level of proline content in all the varieties with imposition of salt stress. Maximum proline content was observed in HJ 541(73.7 μg g$^{-1}$ DW) followed by HJ 513 (69.7 μg g$^{-1}$ DW) while minimum was observed in HC 308 (63.7 μg g$^{-1}$ DW) at 8 dS m$^{-1}$. At 10 dS m$^{-1}$ of salt stress, HJ 541 showed proline content of 85.7 μg g$^{-1}$ DW where other varieties failed to survive. Interaction among the treatment was statistically significant.

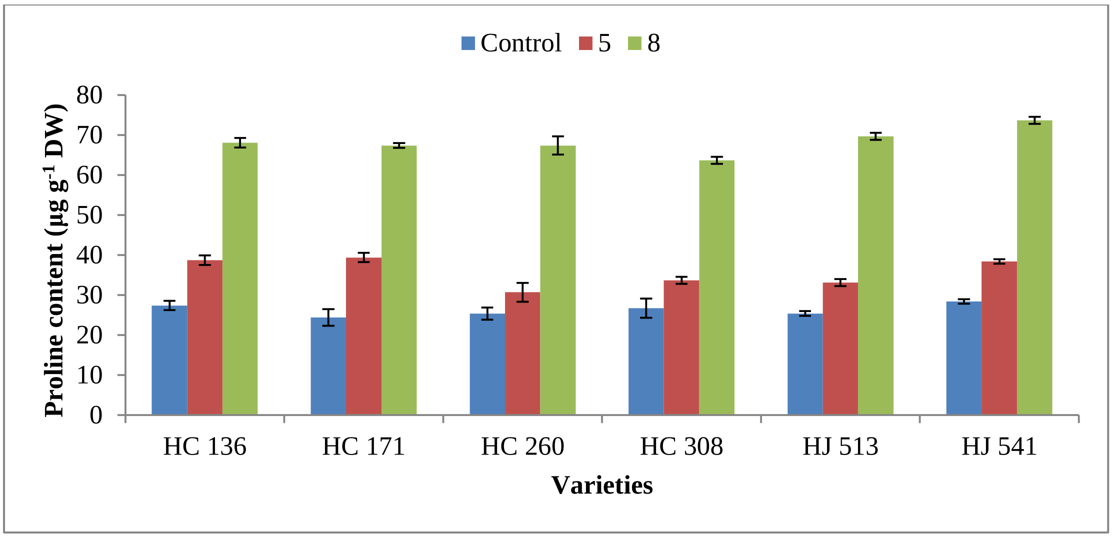


**Figure 4: Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on proline content at the time of 50% flowering in sorghum varieties (CD at 5%= S = 1.6, V = 2.2, S×V = 3.95)**

### 4.3.5 Glycine Betaine Content (µmole g$^{-1}$ DW):

Perusal of data presented in Fig 5 showed that the glycine betaine content of plants grown under stress conditions exhibited more values as compared to the control. Glycine betaine content increased significantly with increasing salt stress from control to 8 dS m$^{-1}$. Glycine betaine content was maintained on higher side in HJ 541(175.6 µmole g$^{-1}$ DW) followed by HC 171 (169.5 µmole g$^{-1}$ DW), HC 136 (169.0 µmole g$^{-1}$ DW) whereas lower in HC 308 (147.1 µmole g$^{-1}$ DW). At 10 dS m$^{-1}$ of salt stress, HJ 541 showed GB content of 191.8 µmole g$^{-1}$ DW where other varieties failed to survive. Genotypic mean value for glycine betaine content ranged from 98.4 to 114.8 µmole g$^{-1}$ DW. Significant interaction between the treatments was noticed.

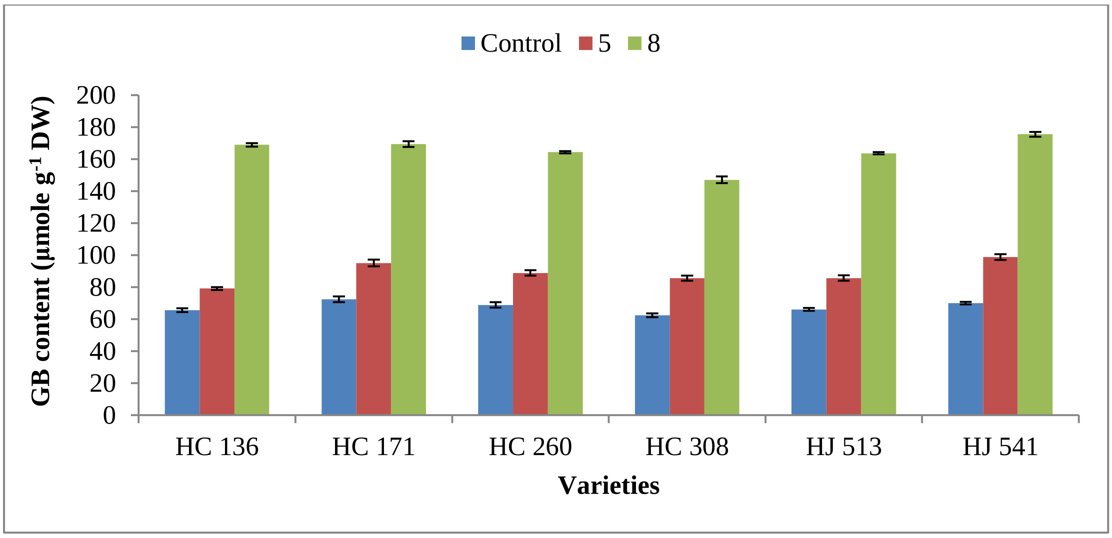


**Figure 5: Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on glycine betaine content at the time of 50% flowering in sorghum varieties (CD at 5% = S = 1.7, V = 2.5, S×V = 4.3)**

### 4.3.6 Total soluble solids (%):

Significant differences were observed in total soluble solids among all the varieties under salt stress (Fig 6). Decreasing trend was noticed in total soluble solids with every increment of salt levels from control to 8 dS m$^{-1}$. Among all the treatments maximum reduction in total soluble solids was observed at 8 dS m$^{-1}$ (21.5%). Maximum total soluble solids was observed in HJ 541 (55.4%) followed by HC 260 (52.3%) while minimum in HC 136 (40.9%). At 10 dS m$^{-1}$ of salt stress, HJ 541 showed TSS % of 44.6 where other varieties failed to survive. Overall, genotypic mean value for total soluble solids varied from 48.1 to 62.4%. Interaction among the treatment was statistically non-significant.

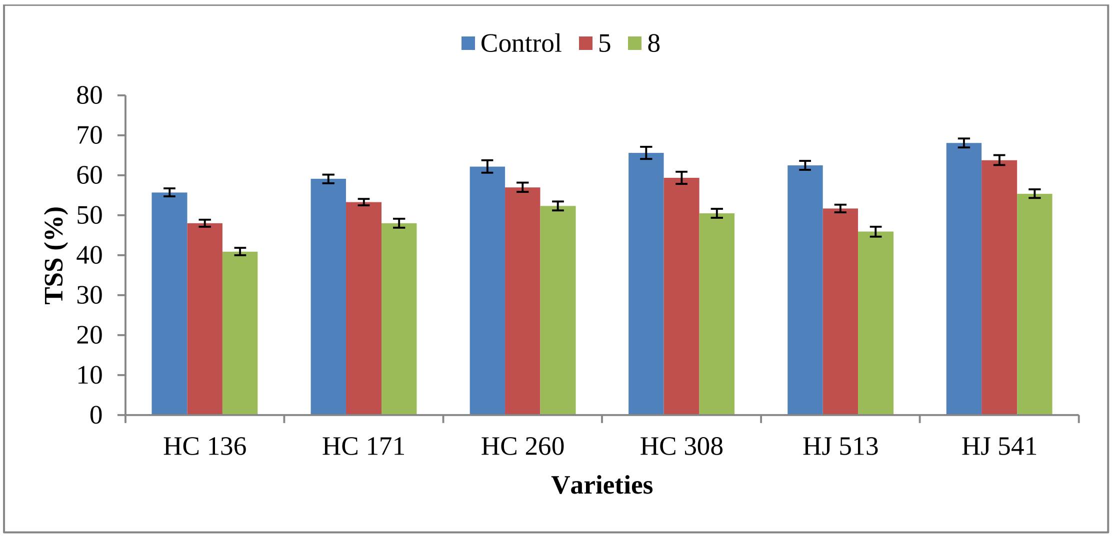


**Figure 6: Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on total soluble solids (%) at the time of 50% flowering in sorghum varieties (CD at 5%= S = 1.4, V = 1.9, S×V = N.S.)**

### 4.3.7 Total chlorophyll content (mg g$^{-1}$ FW):

Fig 7 illustrates that salt stress had significant effect on the chlorophyll content in the varieties. It was observed that chlorophyll content decreased with the increasing levels of salt stress from control to 8 dS m$^{-1}$. Among all the treatments, maximum decrease was observed at 8 dS m$^{-1}$ i.e. 22.8% with respect to control. At 8 dS m$^{-1}$, higher chlorophyll content was observed in HJ 541 (3.6 mg g$^{-1}$ FW) followed by HJ 513 (3.4 mg g$^{-1}$ FW) and minimum in HC 136 (1.8 mg g$^{-1}$ FW). At 10 dS m$^{-1}$ of salt stress, HJ 541 showed chlorophyll content of 1.7 mg g$^{-1}$ FW where other varieties failed to survive. Interaction among the treatments (salt stress and varieties) was found significant.

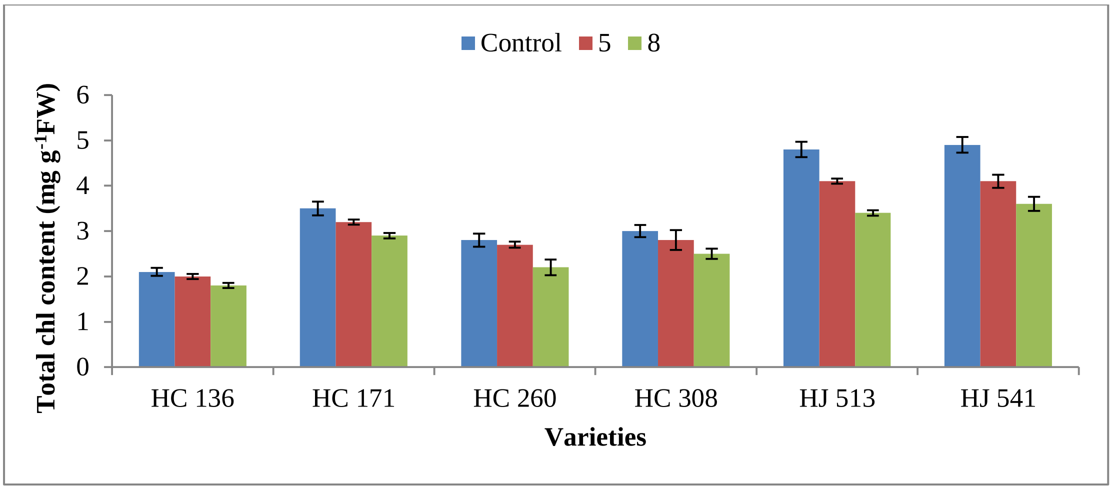


**Figure 7: Effect of salt stress (Control, 5, and 8 dS $m^{-1}$) on total chlorophyll content at the time of 50% flowering in sorghum varieties (CD at 5% = S = 0.15, V = 0.2, S×V = 0.36)**

### 4.3.8 Chlorophyll Stability Index (%):

Significant differences were also observed in chlorophyll stability index (%) among the treatments (Figure 8). All the varieties under salt stress exhibited reduced chlorophyll stability index as compared to the control. Among the treatments, minimum chlorophyll stability index was observed at 8 dS $m^{-1}$. The genotypic mean value for the chlorophyll stability index ranges from 39.1 (HJ 513) to 45.2 (HJ 541 and HC 136).

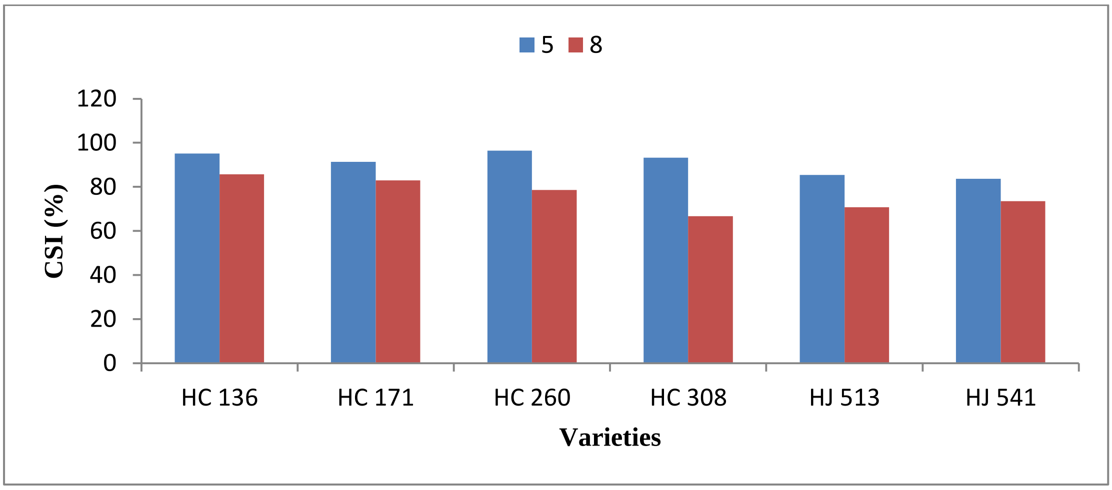


**Figure 8: Effect of salt stress (5, and 8 dS $m^{-1}$) on chlorophyll stability index (%) at the time of 50% flowering in sorghum varieties**

### 4.3.9 Photochemical Quantum Yield (Fv/Fm):

Data presented in Fig. 9 shows significant variations in the photochemical quantum yield among the treatments. It showed the decreasing trend with increasing salt stress from control to 8 dS $m^{-1}$. Maximum chlorophyll fluorescence was observed in HJ 541 (0.69) followed by HJ 513 (0.68) while minimum in HC 260 (0.58) at 8 dS $m^{-1}$ of salt stress. At 10 dS $m^{-1}$ of salt stress, HJ 541 showed quantum efficiency of 0.57 where other varieties failed to survive. Interaction among the treatments was statistically non-significant.

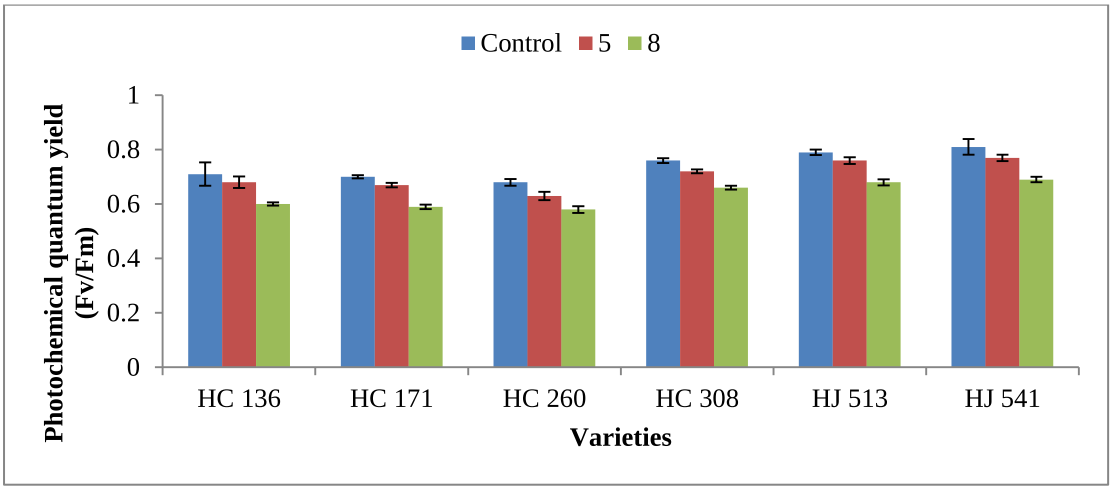


**Figure 9: Effect of salt stress (Control, 5, and 8 dS $m^{-1}$) on photochemical quantum yield at the time of 50% flowering in sorghum varieties (CD at 5% = S = 0.02, V = 0.02, S×V = N.S.)**

### 4.3.10 Assimilation rate (µmole $CO_2$ $m^{-2}s^{-1}$):

From the data showed in Fig. 10, significantly decline in assimilation rate with increasing salt stress from control to 8 dS $m^{-1}$ was noticed. Among all the treatments, assimilation rate was found minimum at 8 dS $m^{-1}$. Maximum assimilation rate was noticed in HJ 541 (7.6) followed by HJ 513 (6.9) and minimum in HC 171 (4.2) at 8 dS $m^{-1}$ of salt stress. At 10 dS $m^{-1}$ of salt stress, HJ 541 showed assimilation rate of 3.6 µmole $CO_2$ $m^{-2}s^{-1}$ where other varieties failed to survive. Genotypic mean value for assimilation rate varied from 6.5 to 9.9 µmole $CO_2$ $m^{-2}s^{-1}$. Interaction among the treatments was statistically significant.

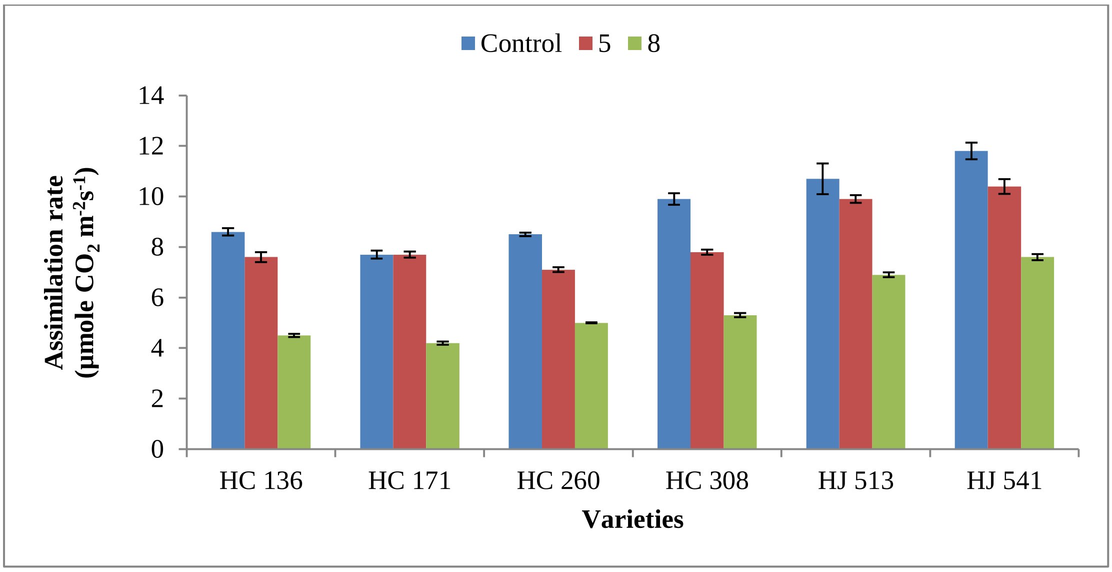


**Figure 10: Effect of salt stress (Control, 5, and 8 dS $m^{-1}$) on assimilation rate at the time of 50% flowering in sorghum varieties (CD at 5% = S = 0.2, V = 0.3, S×V = 0.6)**

### 4.3.11 Transpiration rate (mmole $H_2O$ $m^{-2}s^{-1}$):

Perusal of data revealed that there was a progressive decline in rate of transpiration with every increment of salt levels i.e. from control to 8 dS $m^{-1}$. Maximum reduction in transpiration rate was noticed in HC 136 (60.9%) while minimum in HJ 541 (39.1%) at 8 dS $m^{-1}$ over their respective controls. Genotypic mean value for transpiration rate ranges from 8.7 to 10.3 mmole $H_2O$ $m^{-2}s^{-1}$. At 10 dS $m^{-1}$ of salt stress, HJ 541 showed transpiration rate of 2.7 m mole $H_2O$ $m^{-2}s^{-1}$ where other varieties failed to survive. Among all the treatments, maximum decline in transpiration rate was observed at 8 dS $m^{-1}$ (50.8%) with respect to control. (Fig. 11) Interaction between treatments (salt stress and varieties) was statistically significant.

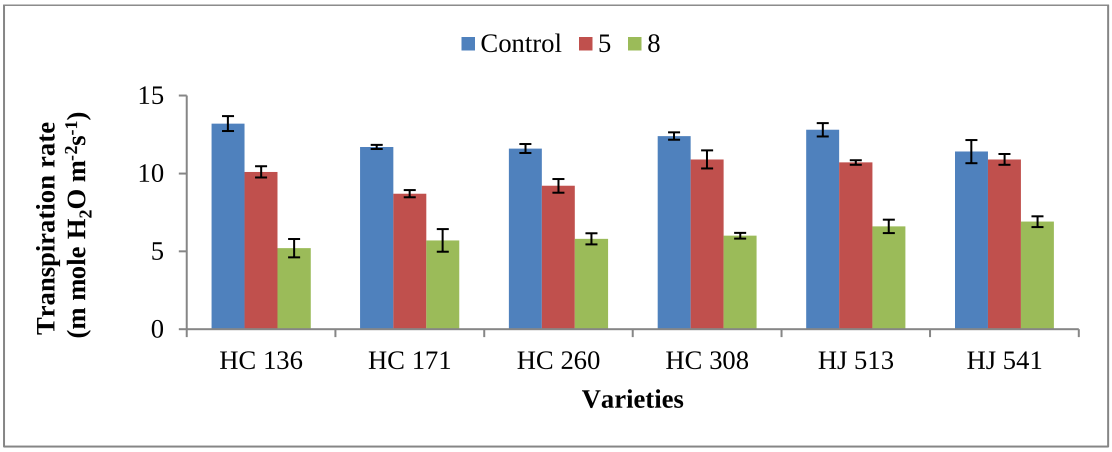


**Figure 11: Effect of salt stress (Control, 5, and 8 dS $m^{-1}$) on transpiration rate at the time of 50% flowering in sorghum varieties (CD at 5% = S = 0.5, V = 0.7, S×V = 1.2)**

### 4.3.12 Stomatal conductance (mmole$H_2O$ $m^{-2}s^{-1}$):

Fig. 12 depicts that there was also a progressive decline in stomatal conductance with every increment of salt levels i.e. from control to 8 dS $m^{-1}$. At 8 dS $m^{-1}$, maximum stomatal conductance was observed in HJ 513 i.e. 0.05 which is at par with HC 171 while the minimum stomatal conductance was observed in HC 308 i.e. 0.02. Genotypic mean value for stomatal conductance in all varieties ranges from 0.04 to 0.08 mmole$H_2O$ $m^{-2}s^{-1}$. At 10 dS $m^{-1}$ of salt stress, HJ 541 showed stomatal conductance of 0.02 m mole $H_2O$ $m^{-2}s^{-1}$ where other varieties failed to survive. Interaction between treatments (salt stress and varieties) was statistically non-significant.

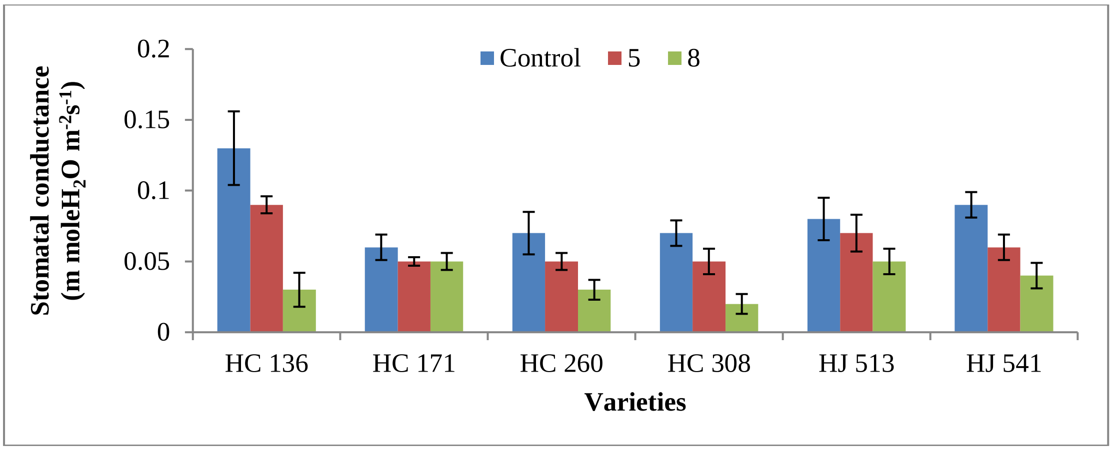


**Figure 12:** **Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on stomatal conductance at the time of 50% flowering in sorghum varieties (CD at 5% = S = 0.013, V = 0.018, S×V = N.S.)**

## 4.4 Antioxidative enzymes:

### 4.4.1 Superoxide dismutase (SOD unit mg$^{-1}$ protein min$^{-1}$):

Results presented in Fig. 13 illustrate that specific activity of superoxide dismutase increased significantly with the increasing salt level from control to 8 dS m$^{-1}$. At 8 dS m$^{-1}$, maximum SOD activity was observed in HJ 541 (212.4 mg$^{-1}$protein min$^{-1}$) which was at par with HC 308 and HC 171. The minimum SOD activity was observed in HC 136 (198.9 mg$^{-1}$protein min$^{-1}$). Among all the treatments the maximum percent increase in SOD activity was observed at 8 dS m$^{-1}$ (45.04%) with respect to control. At 10 dS m$^{-1}$ of salt stress, HJ 541 showed specific activity of 230.4 unit mg$^{-1}$ protein min$^{-1}$ where other varieties failed to survive. Interaction between treatments (salt stress and varieties) was statistically significant.

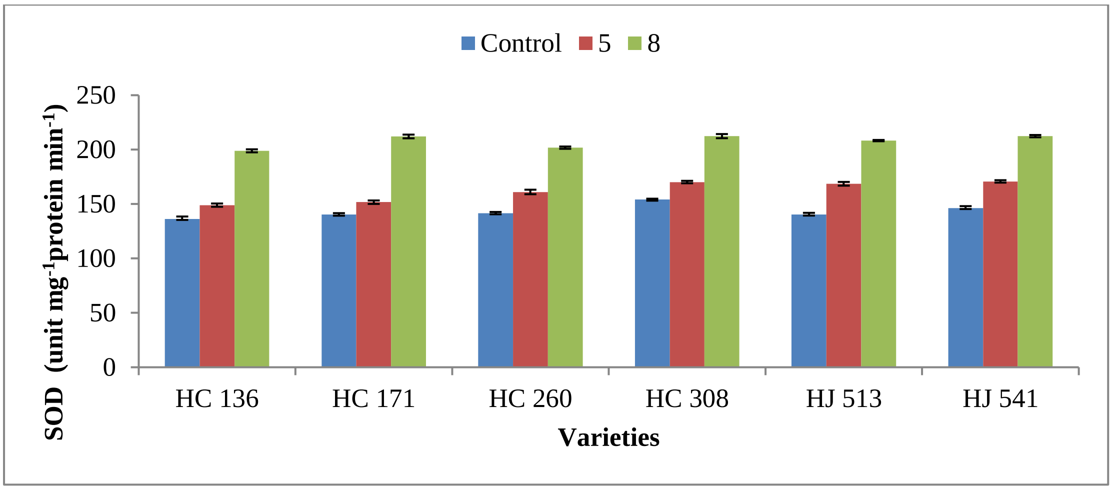


**Figure 13:** **Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on superoxide dismutase at the time of 50% flowering in sorghum varieties (CD at 5% = S = 1.7, V = 2.4, S×V = 4.2)**

### 4.4.2 Catalase (unit mg$^{-1}$ protein min$^{-1}$):

From the Fig. 14, it was observed that specific activity of catalse enhanced with the augmentation of salt level from control to 8 dSm$^{-1}$. Maximum catalase activity was observed in HJ 541 (28.6 mg$^{-1}$ protein min$^{-1}$) followed by HJ 513 (27.3 mg$^{-1}$ protein min$^{-1}$) while minimum in HC 260 (21.1 mg$^{-1}$ protein min$^{-1}$) and treatment mean values varied from 7.3 to 25.0 mg$^{-1}$ protein min$^{-1}$. At 10 dS m$^{-1}$ of salt stress, HJ 541 showed specific activity of 19.9 unit mg$^{-1}$ protein min$^{-1}$ where other varieties failed to survive. Genotypic mean value for catalase activity ranged from 13.4 to 16.6 mg$^{-1}$ protein min$^{-1}$. Interaction between treatments (salt stress and varieties) was statistically significant.

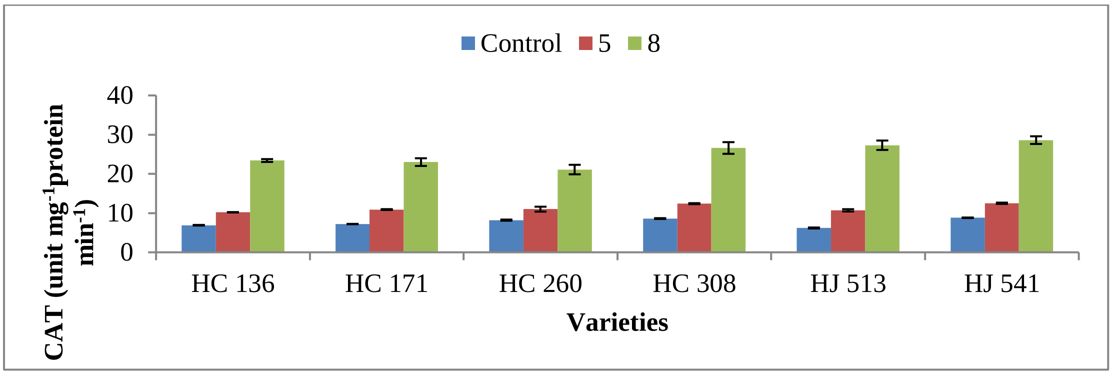


**Figure 14: Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on catalase at the time of 50% flowering in sorghum varieties (CD at 5% = S = 0.7, V = 1.1, S×V = 1.9)**

### 4.3.3 Peroxidase (unit mg$^{-1}$ protein min$^{-1}$):

Data presented in Fig. 13 showed significant enhancement in specific activity of peroxidase with imposition of salt stress. At 8 dS m$^{-1}$, maximum activity of peroxidase was observed in HJ 541 (60.5 mg$^{-1}$ protein min$^{-1}$) followed by HJ 513 (59.6 mg$^{-1}$ protein min$^{-1}$) and minimum in HC 260 (43.6 mg$^{-1}$ protein min$^{-1}$). Among all the treatments the maximum percent increase in peroxidase activity was calculated at 8 dS m$^{-1}$with respect to control. At 10 dS m$^{-1}$ of salt stress, HJ 541 showed specific activity of 70.1 unit mg$^{-1}$ protein min$^{-1}$ where other varieties failed to survive. Interaction between treatments (salt stress and varieties) was statistically significant.

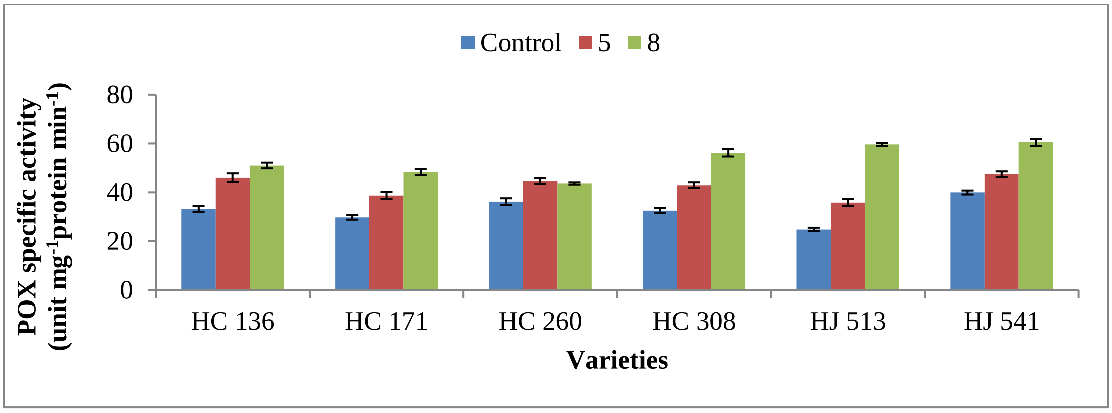


**Figure 15: Effect of salt stress (Control, 5, and 8 dS m$^{-1}$) on peroxidase at the time of 50% flowering in sorghum varieties (CD at 5% = S = 1.4, V = 1.9, S×V = 3.4)**

## 4.5 Soil Analysis

Soil analysis was done before sowing and at the time of 50% flowering. ECe and pH parameters were used for the analysis of soil property. Before sowing, ECe values ranged from 0.3 to 8.3 and pH ranged from 7.6 to 7.8 from control to 8 dS $m^{-1}$ of salt level (Table 8).

Values of ECe varied with the treatments and varieties. On comparison with before sowing ECe declined at the time of 50% flowering. At 8 dS $m^{-1}$ of salt stress, HJ 541 showed ECe of 6.3. It was observed that pH value remains almost same on comparison with before sowing values. Interaction between salt stress and varieties was non-significant.

**Table 8: Effect of salt stress on ECe and pH before sowing and at the time of 50% flowering in sorghum varieties**

| **Salt Levels (dS $m^{-1}$)** | **Before Sowing** | **Varieties** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **ECe (at the time of 50% flowering)** | | | | | | |
| | | **HC 136** | **HC 171** | **HC 260** | **HC 308** | **HJ 513** | **HJ 541** | **Mean** |
| **0** | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| **5** | 5.1 | 4.2 | 4.2 | 4.1 | 4.3 | 4.2 | 4.2 | 4.2 |
| **8** | 8.3 | 6.5 | 6.4 | 6.4 | 6.3 | 6.4 | 6.3 | 6.4 |
| **Mean** | 4.6 | 4.3 | 4.3 | 4.3 | 4.4 | 4.3 | 4.2 | |
| **CD at 5%** | S = 0.12, V = N.S., S×V = N.S. | | | | | | | |
| **pH** | | | | | | | | |
| **0** | 7.6 | 7.5 | 7.7 | 7.8 | 7.8 | 7.8 | 7.6 | 7.7 |
| **5** | 7.8 | 7.8 | 7.8 | 7.8 | 7.9 | 7.9 | 7.7 | 7.8 |
| **8** | 7.8 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 7.9 | 8.0 |
| **Mean** | 7.8 | 7.8 | 7.8 | 7.9 | 7.9 | 7.9 | 7.7 | |
| **CD at 5%** | S = 0.08, V = N.S., S×V = N.S. | | | | | | | |

* S = Salinity, V = Varieties and CD=Critical difference

Note: Out of all the six varieties of sorghum, only HJ 541variety survived upto 10 dS $m^{-1}$ with ECe and pH of 9.2 and 8.1 at 50% flowering respectively.

# CHAPTER-V

# DISCUSSION

Abiotic stresses had a negative impact on physiological processes, resulting in altered metabolic activities and declined plant growth (Dwivedi *et al.,* 2020). Salinity has emerged as a major abiotic challenge to crop production, productivity, and food security. Salt stress has a negative impact on seed germination and growth, as well as physiological processes and membrane properties. It reduces photosynthetic carbon assimilation, stomatal conductance and the efficiency of photosynthetic electron transport. The results obtained from this study are discussed in the light of available literature with following heads:

## 5.1 Delayed germination by salt stress:

Better seed germination and seedling development stage clearly depicted the plant growth and tolerance ability under salt stress (Keshavarizi *et al.,* 2012). A perusal of the data on germination percentage and days to germination in Table 1 depicted that germination percentage decreased with increasing salt levels. Reversibly, days to germination increases significantly. Only HJ 541 survived up to 10 dS $m^{-1}$ whereas other varieties (HC 136, HC 171, HC 260. HC 308 and HJ 513) showed the delayed germination or no germination. Maximum germination percentage and minimum days to germination was observed in HJ 541. Salt stress reduces the rate of seed germination rate by the first step of absorption from soil i.e. altering imbibition of water due to the low osmotic potential (-ve) of soil hence delayed germination (Jamil *et al.,* 2006 ; Khan *et al.,* 2008). Reduced germination percentage and speed with the imposition of salinity in *Brassica napus* and *Z. mays* was confirmed by Bybordi (2010) and Khodarahmpour *et al.* (2012) respectively.

Days to 50% flowering also showed increasing trend with salt stress. Devi *et al.,* 2019 also reported the increased days to 50% flowering with the augmentation of salt stress in sorghum.

## 5.2 Growth inhibition caused by salt stress:

Growth is one of the best tools for finding out plant response under different environmental stresses. A remarkable decline in plant height, fresh and dry weight, leaf number and leaf area per plant was observed under salt stress in all the varieties of sorghum (HC 136, HC 171, HC 260, HC 308, HJ 513 and HJ 541) (Table 2-6). The percent decrease in all the growth parameters was on lower side in HJ 541 followed by HJ 513 while maximum percent decrease was observed in HC 260.

Similar results were also obtained by Niu *et al.,* (2012) in maize genotypes under salt stress respectively. Osmotic stress caused by salt stress and high temperature incredibly restricted plant growth, morphology, physiology, and yield characteristics during summer

(Sadak *et al.,* 2020). Decrease in growth may be due to reduced or no water uptake by the plant under saline conditions that cause changes in the cell metabolism, which was responsible for reducing the plant growth and development. This decrease could be attributed to negative effects on physiological processes such as plant water status, ion homeostasis, and photosynthesis (Babar *et al.,* 2014). Our results are also in concomitant with the findings of Manish (2018) and Devi *et al.* (2019) in sorghum under salt stress.

### 5.3 Salt stress affects the plant water status:

A perusal of the data on osmotic potential (ψs) and relative water content (RWC %) (Figs. 1 and 2) in leaves shows that salt stress had significant decreasing effects on these parameters in all varieties. The highest relative water content and osmotic potential was maintained by HJ 541 followed by HJ 513 while lowest was observed in HC 260 at 8 dS $m^{-1}$ of salt stress. This decline in these parameters may be due to reduced or no uptake of water by the plant roots because of ionic stress thereby reducing relative water content in the plant while osmotic withdrawal of water from the cells can result in reduced pressure potential hence reduced water potential creates physiological drought conditions (Rodriguez *et al.*, 1991; Manish 2017). Plants try to adapt the adverse conditions through osmotic adjustment.

These results were confirmed by Duarte and Souza (2016) according to which osmotic potential of leaf sap of *Capsicum annuum* L. decreased with the increasing salt levels. Kukreja *et al.* (2006) and Nandwal *et al.* (2007) also proposed that the low availability of water to root under salt stress reduced the osmotic potential values in chickpea. Stepien and Klobus (2006) also reported decrease in relative water content in cucumber leaves when exposed to salt stress.

### 5.4 Salt affects other physiological and biochemical traits associated with stress tolerance:

Results presented in Fig. 3 indicated that relative stress injury showed increasing trend under salt stress in all the varieties. Minimum injury was observed in HJ 541 while maximum relative stress injury was observed in HC 260. Relative stress injury increased consistently with the imposition of salt stress due to accumulation of $H_2O_2$ content (Kukreja *et al.,* 2006). It was reported that stress may modify the chemical composition, physical structure of biological membranes and finally plasma membranes leading to oxidative damage (Mahlooji *et al.,* 2018). The results are in accordance with Latrach *et al.* (2014) in alfalfa which observed increased relative stress injury with increasing salinity levels. Agami, (2013) also noticed increase in relative stress injury in maize under salt stress.

It is evident from present research findings that osmolytes i.e. proline and glycine betaine content in leaf increased with increasing salt levels. On the other hand, total soluble solids decreased with increasing salt stress (Figs. 4, 5 and 6). The total soluble solids decrease because during summer season, plants have faced high temperature stress during vegetative stage. So to cope up with the high temperature stress plants utilized reserved carbohydrates and also shortens their life cycle. The decrease in both soluble sugar and chlorophyll content under stress lends credence to the idea that low chlorophyll content reduces light absorption by leaves, which in turn reduces soluble sugar content (Hamdia and Shaddad, 2010). Mohammadkhani and Heidari (2008) discovered that the decrease in total soluble proteins was caused by a significant reduction in photosynthesis. Maximum proline, glycine betaine and total soluble solids were observed in HJ 541. These osmolytes plays an important role in plant tolerance to stress (Liang *et al.,* 2013) and their accumulation under stress conditions strengthens the cell's ability to make ionic adjustments (Jaarsma *et al.,* 2013). Proline and glycine-betaine (GB) are involved in turgor maintenance via osmoregulation, as well as maintaining and regulating the performance of PSII protein complexes. (Orlovsky *et al.,* 2016]. Increased carbohydrate accumulation in leaves under salt stress aids in osmotic adjustment (Ali *et al.,* 2018). Accumulated glycine betaine might serve as an intercellular osmotic balance and it can be closely correlated with the elevation of osmotic pressure (Manish 2018).

Results are in favour with Omari *et al.* (2015), Chaparzadeh and Hosseinzad-Behboud (2015) and Moharramnejad *et al.* (2015) who also observed increase in proline and glycine betaine content with the imposition of salt stress in different crops. Massaretto *et al.* (2018) also observed increase in total soluble solids in tomato varieties under salt stress.

Total chlorophyll content, chlorophyll stability index and Fv/Fm were declined with the augmentation of salt from control to 8 dS $m^{-1}$ under salt stress at 50% flowering. (Figs. 7, 8 and 9). Minimum decrease in these parameters was observed in HJ 541 followed by HJ 513 while maximum decrease was seen in HC 260. Under salt stress, total chlorophyll, chlorophyll stability index, and quantum yield may decrease due to oxidative damage of chlorophyll and other pigments in chloroplast that are associated with pigment protein complex volatility (Tariq *et al.,* 2011).

Decreased total chlorophyll content was also observed by Mbinda *et al.* (2019) in sorghum varieties under salt stress. Reduced Fv/Fm with the onset of salt stress was also reported by Ghassemi-Golezani and Lotfi (2015). Reduction in chlorophyll stability index in *Pisum sativum* with increasing salt stress was observed by Ahmad *et al.* (2008).

It is seen in all the varrieties that assimilation rate, transpiration rate and stomatal conductance decreased with increase in salt levels from control to 8 dS $m^{-1}$ (Figs. 10, 11 and 12) at the time of 50% flowering. Yan *et al*. (2012) showed that under salt stress photosynthetic activity declined mainly due to decrease in stomatal conductance. Decrease in assimilation rate was due to decrease in leaf water potential, stomatal conductance and RWC in the plant. Similar observations can be explained in the light of contention of several workers like Rodrigues *et al.* (2014) according to which plants exposed to 150 mM NaCl experienced greater reductions in photosynthetic rate (78%) on comparison to control plants. Rate of photosynthesis reduced under salt stress due to the depression in activity of oxygenase enzymes and photosystem II. Stomatal closure and reduced transpiration rate takes place under salinity by affecting the cytosolic $Ca^{2+}$ ion concentration which affect the stomatal conductance. Similarly decreased transpiration rate under salt stress was also observed by Radi *et al.* (2013) in groundnut. When *Ricinus communis* seedlings were subjected to salt stress, the leaf transpiration (E) was significantly reduced by increasing NaCl levels (Rodrigues *et al.,* 2014). Cornish and Radin (1990) observed that, ABA is the major inhibitory compound, which is released from roots experiencing salt stress and translocated to leaves. Salinity reduced the stomatal conductance value of two rice varieties, Basmati-370 and Basmati-4048, according to Khan and Abdullah (2003).

### 5.5 Enhanced activity of antioxidative enzymes:

Plants defend themselves against ROS using both enzymatic and non-enzymatic mechanisms. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione-synthesizing enzymes are among the enzymes used to scavenge ROS (Maleka *et al.,* 1999; Shanker *et al.* (2011); Xia *et al.,* 2014). Specific activity of SOD, CAT and POX increased significantly under salt stress from control to 8 dS $m^{-1}$ at the time of 50% flowering in all the varieties (Figs. 13, 14 and 15). Maximum increase was observed in HJ 541 followed by HJ 513 while the minimum enzymatic activity was observed in HC 260. SOD causes singlet oxygen to disproportionate to molecular oxygen and hydrogen peroxide (Shahid *et al.,* 2020). When plants are exposed to salt stress, ROS are formed inside the cell, which disrupts metabolic activity and has a negative impact on the plants. To reduce the harmful role of ROS, increasing salt levels activate the specific activity of SOD, CAT, and POX enzymes. Catalases (CATs) are enzymes that are synthesised in peroxisomes and glyoxysomes and are responsible for the conversion of hydrogen peroxide into water and oxygen (Shahid *et al.,* 2020).

The present results are also in concomittent with earlier findings in wheat (Sairam *et al.,* 2002) and chickpea (Singh *et al.,* 2001 and Kukreja *et al.,* 2006, Manish, 2018). Peroxidase is another antioxidative enzyme that scavenges $H_2O_2$. The specific activity of peroxidase increases with $H_2O_2$ content under salt stress. Kukreja *et al.* (2006) also reported increase in peroxidase activity under salt stress in chickpea plant. Das *et al.* (2016) and Sairam *et al.* (2004) reported in wheat, that during abiotic stress conditions activities of SOD, APX, CAT, and GR increases significantly. Ebrahimian and ByBybordi (2012) investigated the specific activity of SOD in barley under salt stress, which was found to be approximately 52% higher than in unstressed barley.

## 5.7 Soil status before sowing and at the time of 50% flowering:

The electrical conductivity (ECe) of soil is a measurement of the amount of salts in the soil (salinity of soil). It is a key indicator of soil health because it influences crop yields, crop suitability, plant nutrient availability and the activity of soil microorganisms, all of which influence key soil processes. Excess salts impede plant growth by interfering with the soil-water balance.

Values of ECe varied with the treatments and varieties. On comparison with before sowing ECe declined at 50% flowering stage. It was observed that pH value remains almost same on comparison with before sowing values (Table 8). Similar observations for ECe and pH were also reported by Kumari *et al.* (2015) and Manish (2018) under salt stress.

## CHAPTER-VI

## SUMMARY AND CONCLUSIONS

The present study entitled “Evaluation of physiological and biochemical traits in sorghum (*Sorghum bicolor* L.) varieties during summer season” was conducted under controlled conditions during the summer season of 2021 (February-May). The pots were saturated with desired levels of salt stress (Control, 5, 8 and 10 dS m$^{-1}$). Sampling was made at 30, 60 DAS and at 50% flowering stage to study the plant growth. Physiological and biochemical traits were estimated at 50% flowering stage and soil studies were observed before sowing and at the time of 50% flowering. The significant findings of the experiment are as follows:

- All the varieties survived up to 8 dS m$^{-1}$ and only one variety i.e. HJ 541 that maintains growth up to 10 dS m$^{-1}$ of salt stress.
- Germination percentage declined with the augmentation of different levels of salt stress in all the varieties. Delayed germination and more number of days to 50% flowering were noticed with the onset of salt stress. The effects were more pronounced at 8 dS m$^{-1}$ of salt stress whereas growth parameters were at par at 5 dS m$^{-1}$ with control at all the stages i.e. 30, 60 DAS and at 50% flowering.
- Plant height, fresh and dry weight, no. of leaves and leaf area declined significantly with the imposition of salt stress at 30, 60 DAS and at 50% flowering stage. Least percent decline in dry weight (67.1%) and leaf area (69.2%) was calculated in HJ 541 at 8 dS m$^{-1}$ of salt stress over their respective control.
- Salinity susceptibility index (SSI%) was calculated on dry weight basis to screen the salt tolerant variety. The variety HJ 541 showed the values less than one which indicated the tolerance to salt stress at 30, 60 DAS and at 50% flowering stage.
- Decreasing trend was also observed in osmotic potential and relative water content of leaves with every increment of salt stress in all the varieties at 50% flowering stage. Improved water status was maintained by HJ 541 i.e. osmotic potential (-1.0 MPa) and RWC (71.5%) at 8 dS m$^{-1}$ of salt stress.
- Reversibly, relative stress injury enhanced significantly under salt stress in all the varieties. This increment was on higher side in HC 260 (34.7%) and lower in HJ 541 (19.2%) at 8 dS m$^{-1}$ of salt stress.
- The accumulation of osmolytes *viz.* proline and glycine betaine enhanced with increasing level of salt stress in all the varieties. Better osmotic adjustment was maintained by the variety HJ 541 under salt stress. HJ 541 accumulated maximum proline (73.7µg), glycine betaine (175.6 µmole) at 8 dS m$^{-1}$ of salt stress. Reversibly,

total soluble solids declined with imposition of different levels of salt stress in all the varieties. Maximum soluble solids was maintained by HJ 541(55.4%) at 8 dS $m^{-1}$ of the salt stress.

- Total chlorophyll content and photochemical quantum yield (Fv/Fm) decreased significantly with increment of salt from control to 8 dS $m^{-1}$. Minimum chlorophyll stability index was maintained at higher salt levels i.e. 8 dS $m^{-1}$. The treatment mean CSI values varied from 90.1% to 74.2% in all the varieties.
- Antioxidative enzymes i.e. specific activity of superoxide dismutase, catalase and peroxidase increased gradually upto 5 dS $m^{-1}$ of salt stress and a sharp enhancement was noticed in all the varieties at 8 dS $m^{-1}$ as compared to control at 50% flowering stage. Antioxidative enzyme activity SOD (212.4 $mg^{-1}$ protein $min^{-1}$), CAT (28.6 $mg^{-1}$ protein $min^{-1}$) and POX (60.5 $mg^{-1}$ protein $min^{-1}$) was maintained on higher side in HJ 541 at 8 dS $m^{-1}$ of salt stress.
- Soil analysis was made before sowing and at 50% flowering stage. Electrical conductivity at its saturation percentage (ECe) declined at 50% flowering stage on comparison with the values of before sowing. Whereas, pH remained almost same at 50% flowering stage in comparison to before sowing.

**Conclusions**

- Based on the above studies it can be concluded that salt stress reduced the overall plant growth and physiological parameters. But this decline was on lower side in HJ 541 as compared to other varieties, based upon maximum dry weight, leaf area, RWC, osmotic potential, more accumulation of osmolytes, enhanced antioxidative enzyme activity mainly at higher (8 dS $m^{-1}$) salt stress. At 50% flowering, the fresh weight of HJ 541 was 4.5% higher over HJ 513 being at par with each other at 8 dS $m^{-1}$ of salt stress.
- Single cut forage sorghum variety, HJ 541 performed better upto 8 dS $m^{-1}$, being the only variety that survived at 10 dS $m^{-1}$ during summer season, hence it is a promising cultivar for salt tolerance.

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## ABSTRACT

| | |
|---|---|
| **Title of Thesis** | **Evaluation of physiological and biochemical traits in sorghum (*Sorghum bicolor* L.) varieties during summer season** |
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| **Number of Words in Abstract** | 459 |

***Keywords:*** Salt stress, *Sorghum bicolor,* Summer season, 50% flowering stage, Salt tolerance

The present investigation entitled "Evaluation of physiological and biochemical traits in sorghum (*Sorghum bicolor* L.) varieties during summer season" was evaluated in the screen house during the summer season of 2021. Before sowing, the desired levels of salt stress (control, 5, 8 and 10 dS m$^{-1}$) were maintained in all pots at its saturation percentage. Seeds of *Sorghum bicolor* L. were grown in pots under controlled conditions on 26th February 2021. All the varieties survived well upto 8 dS m$^{-1}$ and only one variety (HJ 541) maintained its growth upto 10 dS m$^{-1}$ of salt stress. Sampling for growth parameters was made at 30, 60 DAS and at 50% flowering stage and physiological and biochemical parameters were estimated at the time of 50% flowering. Germination and growth studies declined with the augmentation of salt stress at all the stages. Highest dry weight (0.7, 7.1 and 9.1 g/plant) and leaf area (211.9, 270.5 and 278.5 cm$^2$/plant) was estimated in HJ 541 respectively, at 30, 60 DAS and at the time of 50% flowering. Salinity susceptibility index (SSI %) was calculated less than one in HJ 541 at 30, 60 DAS and at 50% flowering stage. Similarly, plant water status, total chlorophyll content, and photochemical quantum yield also showed declining trend with the imposition of salt stress from control to 8 dS m$^{-1}$ in all the varieties. At 8 dS m$^{-1}$ of salt stress, less negative values of osmotic potential and higher RWC (%) was maintained by HJ 541 at 50% flowering stage. Percent decline in total chlorophyll content (26.5%), Fv/Fm (15%), TSS (18.2%) and assimilation rate (35.8%) was noticed in HJ 541 at 8 dS m$^{-1}$. The genotypic mean values ranged from 6.5 - 8.0 mmole $H_2O$ m$^{-2}$s$^{-1}$ and 0.04 – 0.06 m mole $H_2O$ m$^{-2}$s$^{-1}$ respectively in transpiration rate and stomatal conductance. Reversibily, RSI (%) increased with every increment of salt stress at 50% flowering stage. Osmolyte accumulation and specific activity of antioxidative enzymes enhanced gradually at 5 dS m$^{-1}$ and after that a sharp increase was noticed in all varieties at 8 dS m$^{-1}$ of salt stress. Per cent increase was on higher side at 8 dS m$^{-1}$ of salt level in HJ 541 at 50% flowering stage *viz.* proline (61.5%), glycine betaine (60.2%), and SOD (31.1%) CAT (69.4%), POX (34.2%) over their respective control. At 50% flowering, the fresh weight of HJ 541 was 4.5% higher over HJ 513 being at par with each other at 8 dS m$^{-1}$ of salt stress. Based on the dry weight, physiological and biochemical parameters it was concluded that HJ 541 performed better upto 8 dS m$^{-1}$, being the only variety that survived at 10 dS m$^{-1}$ during summer season, hence it is a promising cultivar for salt tolerance.

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**(GAYATRI)**

# UNDERTAKING OF THE COPY RIGHT

I, **Gayatri Kumari**, Admission No. **2019BS36M**, undertake that I give copy right to the CCS HAU, Hisar of my thesis entitled "**Evaluation of physiological and biochemical traits in sorghum (*Sorghum bicolor* L.) varieties during summer season**".

I also undertake that, patent, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of CCS HAU, Hisar.

**Signature of student**