

Implication of Jasmonic acid on physiological alterations on salt stressed fodder sorghum (*Sorghum bicolor* L.)

ABSTRACT

One of the main environmental factors that restricts the growth and productivity of sorghum is salt stress, which interferes with physiological processes including relative water content, NBI (nitrogen balance index), photosynthetic rate, etc. Agricultural land affected by soil salinity is growing significantly worldwide, therefore, strategies are needed to improve the salinity tolerance and most efficient one can be opted by studying the response of sorghum against salinity. Salt stress can be reduced by plant stress hormones such as jasmonates, ABA, brassinosteroids, etc. In order to assess the effects of jasmonic acid (JA) (4.5, 6.5 & 8.5 mM) on the physiological properties of sorghum (HJ 541 and CSV 21F) grown in saline soil (4, 6 & 8 dS/m), green house experiment was executed on a complete randomized design (CRD), with three replications. The results show that the salinity adversely affected photosynthetic efficiency, chlorophyll content, antioxidants (anthocyanin and flavonoids), nitrogen balance index, ash content which can be used as the most suitable parameter for determining tolerance to salinity stress. While, foliar application of jasmonic acid significantly improved the relative water content, chlorophyll content, photosynthetic rate, stomatal conductance, etc. Jasmonic acid concentrations of 8.5 mM had more noticeable effects. Percent enhancement in chlorophyll index, transpiration rate, stomatal conductance, was 14.5% and 13.4%, 16.7% and 19.9%, 42.0% and 58.0% in HJ 541 and CSV 21F, respectively at 8 dS/m as compared to control. CSV 21F performed better as compared to HJ 541 at all levels of salt stress after application of jasmonic acid.

Keywords: Antioxidants, Jasmonic acid, Salinity, Sorghum

1. INTRODUCTION

Second most destructive stress among abiotic stresses is salt stress, as it persists throughout the crop life cycle. Changing climatic scenario, usage of low-quality water for irrigation and use of excessive ground water, poor irrigation practices, rising population, and industrial pollution are some of the reasons behind soil salinity (Behera et al., 2022). Due to the salinity stress approximately 12 billion USD are lost worldwide on an average every year which greatly affects the agriculture production. The key challenge of modern agriculture is to fulfill the nutritional and food security of the global growing population. Salt-affected soils are found in arid and semi-arid climates in more than one hundred countries of the world, where many regions are also affected by irrigation-induced salinization. Presently around, 830 million hectares global area is estimated to be affected by salinity and alkalinity and increasing continuously (Abdi et al., 2022). In India, nearly 6.73 million ha of land is salt affected, which is supposed to be further increased at the rate of almost 10% per year if precautionary measures are not adopted (Sanwal et al., 2022). The saline area in Haryana has grown by 35% to 80,000 ha in 20 years, as per a 2016 study by the ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal. Short-term effects of salinity include reduction of growth by salt due to osmotic effects. (Jangra et al., 2022). Amelioration of salt stress can be achieved by overcoming the irregularities in plant physiological mechanisms by some of the plant growth regulators and nutrients (Iqbal and Ashraf, 2007).

Sorghum, botanically known as *Sorghum bicolor* L., is an important agricultural crop of the Gramineae family and known as the fifth most important cereal crop in the world. It is also known as the "King of Millets" and is extensively produced in Africa, China, the United States, Mexico and India (Hossain et al., 2022). Worldwide sorghum is mainly grown in hot and dry regions on large-scale for commercial operations, as it is broadly adapted to temperate, subtropical and tropical drylands, and irrigated environments. India has the largest sorghum area (10.06 mha), while USA is the World's leading producer of sorghum, with a total production of 9.4 million metric tonnes followed by, Nigeria, Ethiopia, Mexico, Sudan, India, China, Argentina, Australia and Brazil during 2020-21. Sorghum is not only one of the most important carbohydrate-rich crops but also a key staple food crop for millions of peoples in semi-arid tropics areas. It is naturally drought and salt-tolerant crop that is grown for food, fodder, forage and biofuel, globally. It is also being considered as a potential model for studying the physiological mechanisms in cereals under salt stress (Punia et al., 2021).

Under salinity stress most plants demonstrate wide interactive and complex adaptive morpho-physiological responses. Salinity-induced oxidative stress in Sorghum could affect the qualitative and quantitative value of sorghum as this oxidative stress could lead to a plethora of biochemical and physiological changes in plants (Prakriti et al., 2022). The most common of them include photosynthetic rate, leaf water content, membrane integrity, chlorophyll. These changes ultimately affect the physiological activities like

respiration, photosynthesis, transpiration, water use efficiency, production of antioxidants, and plasma membrane permeability (Chourasia *et al.*, 2021). Sorghum possesses a diverse range of morphological, anatomical and physiological, characteristics that enables it to survive under stress conditions.

There is an urgent need to further increase crop productivity to fulfill the food demand of increasing global human population and thus, to meet these goals, it is imperative to develop new crops that have improved resistance to salt stress. Plant hormones play an important physiological role in regulating plant growth and development, under stress conditions. For producing abiotic stress-tolerant crop plants, plant hormones may prove to be important metabolic engineering targets and jasmonic acid is one of those hormones. JA reduces the negative impact of salt stress while improving plant growth and yield via reactive oxygen species (ROS) scavenging by antioxidant enzymes and ion absorption (Prakriti *et al.*, 2022). Jasmonic acid (JA) under high salinity conditions improves the level of solutes leading to higher osmotic potential within cells and the ability to absorb more water while maintaining the desired level of relative water content. JA also reduces the inhibitory effect of NaCl on photosynthetic rate by modifying the concentration of endogenous hormones such as ABA and increases anthocyanin content due to stress-induced ROS signalling under salt stress (Yosefi *et al.*, 2018). Therefore, the present study was carried out to study the efficacy of jasmonic acid on physiology of sorghum (*Sorghum bicolor* L.) under salt stress.

2. MATERIALS AND METHODS

2.1 Plant material and Treatments

Healthy seeds of two varieties of *Sorghum bicolor* L. (HJ541 and CSV 21F) were procured from Forage Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana), India. Sorghum genotypes (CSV 21F and HJ 541) were selected for analysis based on salt tolerance as HJ 541 is salt tolerant cultivar and CSV 21F is a new variety with salt tolerant characters. Under screen house conditions in screen house of the Department of Botany and Plant Physiology on July 16, 2021, seeds of both the genotypes were sown in pots containing 10 kg of dune sand. Before sowing pots were saturated with desired levels of salt i.e., Control (0), 4, 6 and 8 dS m⁻¹. The control pots were irrigated with canal water. Initially, each pot contained five seeds, which were sown. However, on the emergence of seedlings, thinning was conducted, and three seedlings were left in each pot. At different time intervals nutrient solution was given (Hoagland and Arnon, 1950). Jasmonic acid (0, 4.5, 6.5 and 8.5 mM) was applied exogenously with the help of manual sprayer at 30 days after sowing (DAS). In the experiment, treatments were arranged in complete randomized design (CRD) and plants were sampled for physiological estimations at 40 days after sowing (DAS).

2.2 Climate and weather conditions

The mean weekly meteorological data during the crop season of *Kharif* 2021 recorded at meteorological observatory located at Research Farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar are given in Table. 1. The total rainfall received during the crop growing period was 394.1 mm. Weekly maximum and minimum temperature remained under a suitable range for different crop growth stages. Average temperature on sowing date for crop season was 30.7°C while average temperature at harvesting was 29.4°C. On the other hand, mean weekly maximum and minimum temperature ranged between 31.8-37.8°C and 25.2-28.1°C, respectively during crop season. The mean weekly values for morning and evening relative humidity (%) ranged between 79 to 95 and 54 to 79, respectively during crop season. The bright sunshine during crop growth period ranged between 2.6 and 9.6 hours. Availability of favorable temperature and solar radiation proved conducive for growth and yield attributing characters and eventually to the higher yield.

Table 1: Mean weekly weather data recorded at agricultural meteorology observatory, CCS HAU, Hisar during *Kharif* 2021

Year	Date and Month	Week No.	Temperature (°C)		Relative Humidity %		Bright Sun-Shine Hours	Rainfall (mm)
			Maximum	Minimum	Morning	Evening		
2021	12-18 Jul	28	37.8	28.1	79	61	5.2	18.5
	19-25 Jul	29	35.9	26.9	86	63	4.3	9
	26 Jul-1 Aug	30	33.7	27.4	92	76	3.5	56

	2-8 Aug	31	31.9	26.5	94	79	2.6	149.9
	9-15 Aug	32	35.6	26.6	88	58	7.6	0
	16-22 Aug	33	37.1	26.3	80	54	9.6	0
	23-29 Aug	34	34.7	26.7	86	69	5.2	0.5
	30 Aug-5 Sep	35	35.9	25.9	88	66	6.7	15.7
	6-12 Sep	36	32.7	25.8	95	75	4.3	100.2
	13-19 Sep	37	31.8	25.2	89	72	4.7	44.3

2.3 Measurement of Water potential, Osmotic potential, Electrolytic leakage, Relative water content, Nitrogen balance index, Anthocyanin and Flavanoid

The third leaf from the top was separated from the plant with the help of a sharp edge knife and sealed in the pressure chamber one by one with the cut end protruding outside, and pressure was developed until the sap just appeared at the end. That pressure (bar) (1 Bar = 0.1 MPa) was recorded as water potential and a pressure chamber (Model 3005, Soil Moisture Corporation, Santa Barbara, CA, USA) was used to assess the water potential of leaves. Osmotic potential was measured by psychrometric technique (Model 5199-B vapour Pressure Osmometer, Wescor Inc. Logan, Utah, USA). Nitrogen balance index (NBI), Anthocyanin and Flavanoid content were measured using a Dualex 4 Scientific (Dx4) analyzer. While, relative water content (%) was estimated by using Weatherley's (1950) formula (percent).

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

Method of Sullivan and Ross (1979) was used to analyse membrane injury. The electrolyte leakage was expressed by the following formula:

$$\text{Electrolyte leakage (\%)} = \frac{EC_a}{EC_b} \times 100$$

EC_a - Before boiling

EC_b - After boiling

2.4 Determination of Photosynthetic Gas Exchange Parameters, photochemical quantum yield and Chlorophyll Content

Chlorophyll content was determined by SPAD 502 plus instrument by measuring the absorbance of the leaf in two wavelength regions (Blue 400-500 nm and Red 600-700 nm). The Fv/Fm of the fully expanded third leaf from the top of the plant was determined using a chlorophyll fluorometer (OS-30p, Opti-Science, Inc., Hudson, USA). Infrared gas analyzer (IRGA LCI-SD, ADC Biosciences) was used to determine photosynthetic gas exchange parameters (such as net photosynthetic rate, stomatal conductance and intercellular CO₂ concentration) in fully expanded third leaf from top.

2.5 Statistical Analysis

Data were analyzed statistically by using analysis of variance (ANOVA) by SPSS 17.0 for windows and presented as mean ± SE (n = 4), and the significance level at p < 0.05 was calculated using the least significant difference (LSD) test.

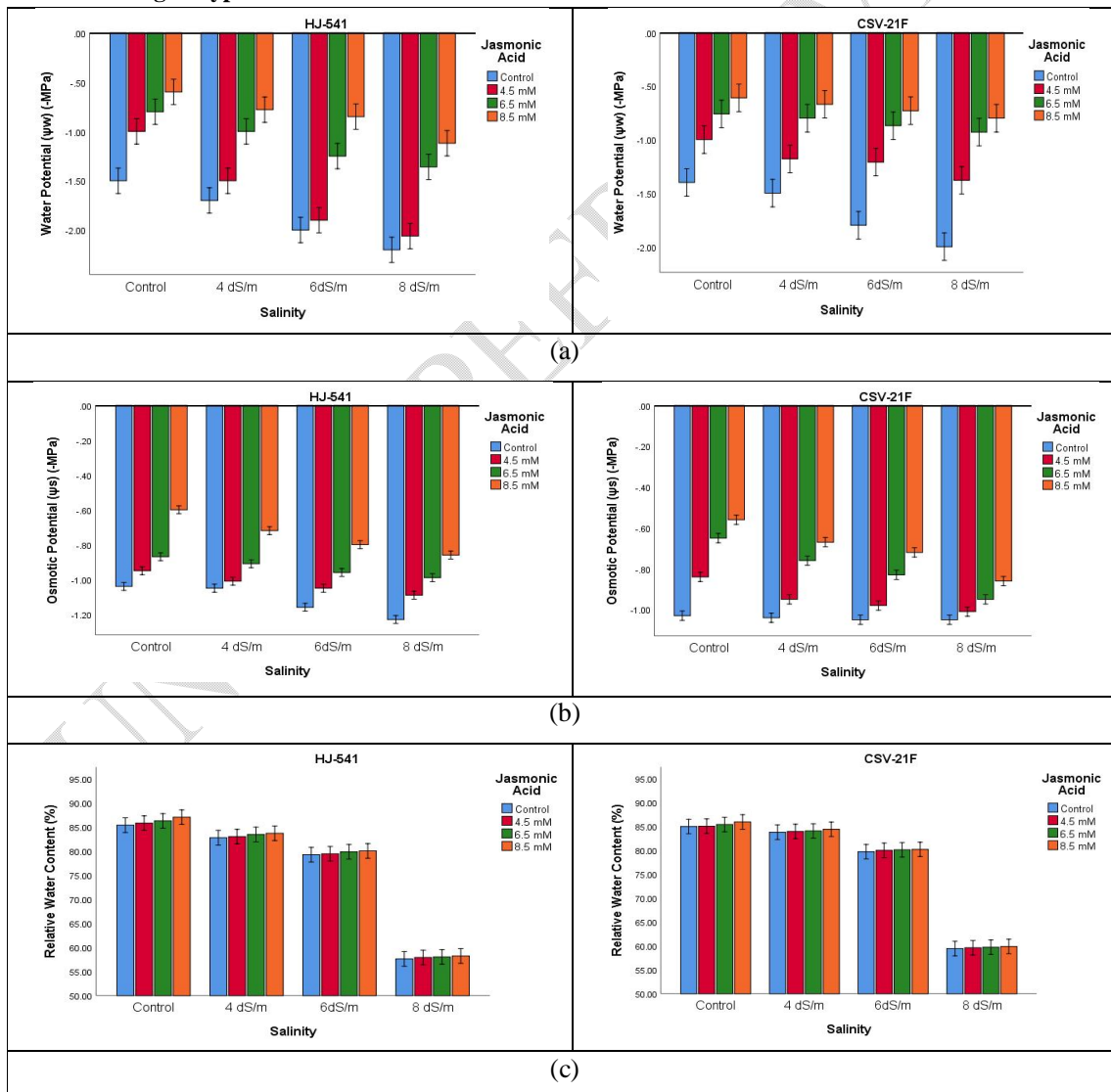
3. RESULTS AND DISCUSSION

Effect of JA on Plant water relations under salinity conditions

Leaf water potential decreases with every increment in salt levels from control to 8 dS m⁻¹, however it increases with the application of jasmonic acid (4.5, 6.5, and 8.5 mM) in both genotypes (Fig. 1). At 8 dS m⁻¹ of salt level, HJ 541 (-2.20 MPa) had a higher negative value than CSV 21F (-2.00 MPa) but after applying 8.5 mM of JA, CSV 21F exhibited a lower negative value (-0.70 MPa) than HJ 541 (-0.84 MPa). Similarly, as the salt content increased from 4 to 8 dS m⁻¹, the osmotic potential of the leaf became more negative (Fig. 1). Values of osmotic potential in CSV 21F (-1.33 MPa) were at par with HJ 541 (-1.31 MPa), at 8 dS m⁻¹ of salt level. However, foliar application of jasmonic acid (4.5, 6.5 and 8.5 mM) enhance the osmotic potential *i.e.* become

less negative, in stressed plant as well as in un-stressed plants. Maximum enhancement in osmotic potential value was observed at 6 dS m⁻¹ with the application of 8.5 mM of JA in both genotypes i.e., CSV 21F (42.4%) and HJ 541 (36.4%), when compared to control. In both genotypes, relative water content (RWC) decreased progressively with the increasing salt stress (Fig. 1). Maximum decrement in relative water content was at 8 dS m⁻¹ in both genotypes i.e., 32.5% in HJ 541 and 30.0% in CSV 21F on comparison to control but the foliar application of 8.5 mM of JA increased the relative water content at each level of salt stress in both genotypes i.e., 82.75 to 83.67% at 4 dS m⁻¹, 79.23 to 80.01% at 6 dS m⁻¹ and 57.61 to 58.23% at 8 dS m⁻¹ in HJ 541, likewise enhancement in relative water content was also observed in CSV 21F. Reduction in water and osmotic potential may be due to accumulation of salt ions inside the cell that disturbs the osmotic balance in the cell, which reduces absorption and translocation of water (Hao *et al.*, 2021) and the hydrolysis of macromolecules into smaller molecules (such as mono and disaccharides, amino acids, particularly proline), may cause high osmotic potential under salt stress (Bruno *et al.*, 2013). Under salt stress, JA increases the water potential in plant cells by stimulating the synthesis of some osmo-regulators (Ollaset *et al.*, 2013). These results were in corroboration with the findings of Soni *et al.*, 2021, Yosefi *et al.* (2018) & Punia *et al.* (2021).

Fig. 1: Effect of salt stress on water potential (ψ_w) (-MPa), osmotic potential (ψ_s) (-MPa), relative water content (%), electrolyte leakage (%) and its alleviation by jasmonic acid in sorghum genotypes.



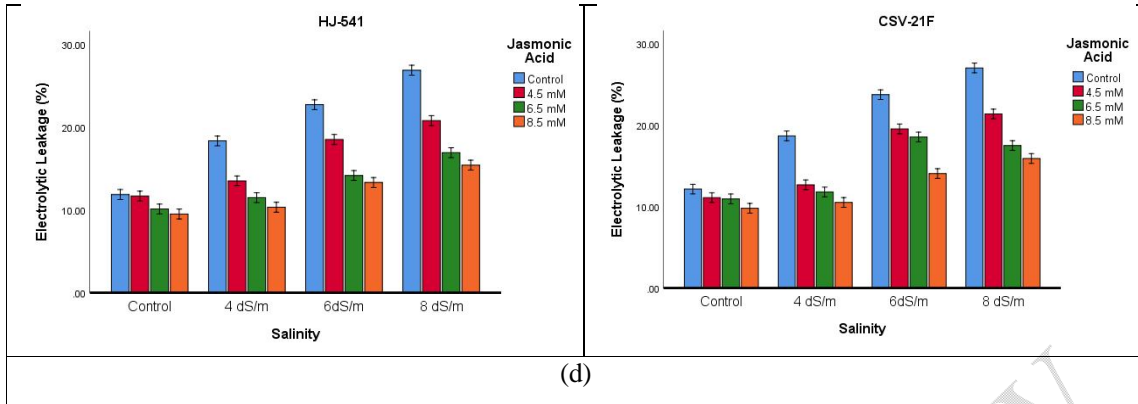


Table. 2: Effect of salt stress on chlorophyll index (mg g^{-1} DW), photochemical quantum yield (Fv/Fm), anthocyanin content, flavonoids content, nitrogen balance index (NBI) and its alleviation by jasmonic acid in sorghum genotypes.

Treatments	Chlorophyll index	Fv/Fm	Anthocyanin content	Flavonoids content	NBI
Genotypes (G)					
G ₁ - HJ-541	22.9 ± 2.54	0.728 ± 0.03	0.269 ± 0.022	0.708 ± 0.106	41.18 ± 1.26
G ₂ - CSV-21F	21.5 ± 1.77	0.726 ± 0.02	0.264 ± 0.018	0.793 ± 0.104	38.56 ± 1.91
SEm	0.24	0.002	0.001	0.017	0.14
CD (0.05)	NS	NS	0.004	0.048	0.39
Salt Stress (S)					
S ₀ - Control	23.48 ± 2.24	0.748 ± 0.021	0.251 ± 0.014	0.716 ± 0.113	41.15 ± 1.69
S ₁ - 4 dS m ⁻¹	22.09 ± 1.58	0.731 ± 0.021	0.265 ± 0.017	0.748 ± 0.113	40.61 ± 1.35
S ₂ - 6 dS m ⁻¹	21.32 ± 1.76	0.723 ± 0.020	0.270 ± 0.018	0.762 ± 0.115	39.03 ± 2.10
S ₃ - 8 dS m ⁻¹	20.13 ± 1.74	0.706 ± 0.025	0.280 ± 0.020	0.776 ± 0.109	38.69 ± 2.07
SEm	0.33	0.003	0.002	0.024	0.20
CD (0.05)	0.94	0.008	0.006	NS	0.55
Jasmonic Acid (JA)					
J ₀ - Control	20.51 ± 1.72	0.707 ± 0.025	0.250 ± 0.014	0.711 ± 0.120	38.92 ± 2.12
J ₁ - 4.5 mM	21.17 ± 1.63	0.720 ± 0.021	0.260 ± 0.012	0.739 ± 0.115	39.44 ± 2.08
J ₂ - 6.5 mM	21.95 ± 2.08	0.732 ± 0.016	0.268 ± 0.015	0.766 ± 0.106	40.21 ± 2.04
J ₃ - 8.5 mM	23.39 ± 2.25	0.749 ± 0.023	0.287 ± 0.019	0.786 ± 0.103	40.90 ± 1.58
SEm	0.33	0.003	0.002	0.024	0.20
CD (0.05)	0.94	0.008	0.006	NS	0.55
Interaction (GxSXJA)					
G ₁ S ₀ J ₀	22.80 ± 1.99	0.733 ± 0.031	0.230 ± 0.010	0.63 ± 0.101	41.81 ± 1.48
G ₁ S ₀ J ₁	23.40 ± 1.06	0.740 ± 0.010	0.250 ± 0.017	0.67 ± 0.098	41.96 ± 1.88
G ₁ S ₀ J ₂	24.70 ± 2.42	0.757 ± 0.006	0.257 ± 0.012	0.71 ± 0.120	42.23 ± 1.96
G ₁ S ₀ J ₃	26.80 ± 2.22	0.790 ± 0.000	0.267 ± 0.006	0.73 ± 0.121	42.46 ± 2.05
G ₁ S ₁ J ₀	21.20 ± 1.82	0.703 ± 0.012	0.250 ± 0.010	0.67 ± 0.102	40.33 ± 0.09
G ₁ S ₁ J ₁	21.80 ± 0.27	0.730 ± 0.010	0.263 ± 0.006	0.69 ± 0.135	41.10 ± 0.18
G ₁ S ₁ J ₂	22.60 ± 1.87	0.740 ± 0.010	0.277 ± 0.006	0.72 ± 0.121	41.98 ± 0.58
G ₁ S ₁ J ₃	24.20 ± 2.15	0.767 ± 0.006	0.297 ± 0.012	0.74 ± 0.112	42.23 ± 0.56
G ₁ S ₂ J ₀	20.18 ± 0.23	0.690 ± 0.010	0.260 ± 0.010	0.68 ± 0.120	40.21 ± 1.07
G ₁ S ₂ J ₁	20.76 ± 0.87	0.707 ± 0.006	0.260 ± 0.010	0.69 ± 0.108	40.37 ± 0.93
G ₁ S ₂ J ₂	21.40 ± 1.99	0.733 ± 0.015	0.277 ± 0.015	0.73 ± 0.129	41.06 ± 1.01
G ₁ S ₂ J ₃	23.10 ± 2.65	0.753 ± 0.006	0.303 ± 0.006	0.76 ± 0.121	41.26 ± 0.12
G ₁ S ₃ J ₀	18.60 ± 0.46	0.673 ± 0.012	0.257 ± 0.006	0.70 ± 0.137	39.98 ± 0.63
G ₁ S ₃ J ₁	19.20 ± 1.22	0.690 ± 0.026	0.273 ± 0.012	0.71 ± 0.102	40.00 ± 0.10
G ₁ S ₃ J ₂	19.90 ± 1.64	0.710 ± 0.017	0.280 ± 0.010	0.73 ± 0.084	40.81 ± 1.67
G ₁ S ₃ J ₃	21.30 ± 2.01	0.727 ± 0.025	0.310 ± 0.000	0.77 ± 0.100	41.05 ± 0.54
G ₂ S ₀ J ₀	21.13 ± 0.42	0.733 ± 0.006	0.240 ± 0.010	0.65 ± 0.121	38.80 ± 0.33
G ₂ S ₀ J ₁	22.02 ± 1.43	0.737 ± 0.006	0.253 ± 0.006	0.74 ± 0.038	40.02 ± 0.92
G ₂ S ₀ J ₂	22.90 ± 1.99	0.737 ± 0.006	0.250 ± 0.010	0.79 ± 0.098	40.48 ± 0.55
G ₂ S ₀ J ₃	24.10 ± 1.96	0.753 ± 0.006	0.260 ± 0.010	0.81 ± 0.026	41.42 ± 0.87

G ₂ S ₁ J ₀	20.83 ± 1.04	0.717 ± 0.025	0.250 ± 0.000	0.76 ± 0.119	38.34 ± 0.42
G ₂ S ₁ J ₁	21.16 ± 1.40	0.727 ± 0.012	0.253 ± 0.006	0.78 ± 0.121	39.23 ± 0.23
G ₂ S ₁ J ₂	21.79 ± 1.71	0.727 ± 0.006	0.260 ± 0.017	0.80 ± 0.111	40.51 ± 0.43
G ₂ S ₁ J ₃	23.12 ± 0.58	0.737 ± 0.012	0.270 ± 0.010	0.82 ± 0.099	41.20 ± 1.19
G ₂ S ₂ J ₀	20.10 ± 2.64	0.713 ± 0.006	0.253 ± 0.015	0.78 ± 0.119	36.01 ± 0.96
G ₂ S ₂ J ₁	20.80 ± 0.42	0.723 ± 0.006	0.257 ± 0.006	0.81 ± 0.120	36.81 ± 0.85
G ₂ S ₂ J ₂	21.40 ± 0.96	0.733 ± 0.006	0.267 ± 0.012	0.82 ± 0.108	37.42 ± 1.23
G ₂ S ₂ J ₃	22.80 ± 1.64	0.730 ± 0.017	0.283 ± 0.006	0.83 ± 0.116	39.12 ± 0.99
G ₂ S ₃ J ₀	19.23 ± 0.80	0.690 ± 0.010	0.263 ± 0.021	0.82 ± 0.128	35.92 ± 0.48
G ₂ S ₃ J ₁	20.20 ± 2.50	0.703 ± 0.021	0.273 ± 0.006	0.82 ± 0.121	36.06 ± 0.09
G ₂ S ₃ J ₂	20.90 ± 2.55	0.723 ± 0.015	0.280 ± 0.010	0.83 ± 0.126	37.20 ± 0.04
G ₂ S ₃ J ₃	21.70 ± 0.36	0.733 ± 0.012	0.303 ± 0.006	0.83 ± 0.121	38.48 ± 0.48
SEm	0.94	0.008	0.006	0.068	0.55
CD (0.05)	NS	NS	NS	NS	NS

Effect of JA on Electrolytic leakage and Photosynthetic Characteristics under salinity

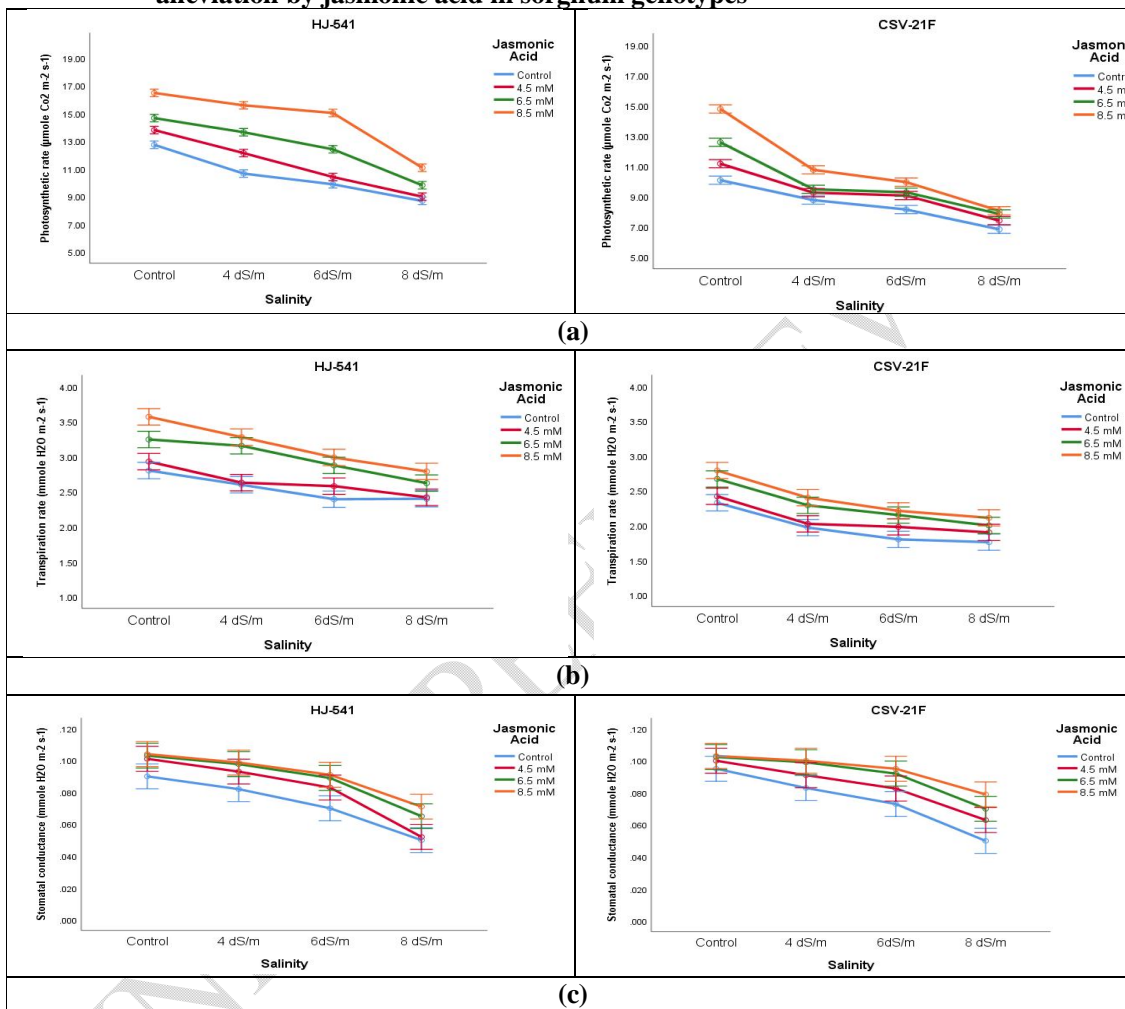
In present study, maximum electrolyte leakage was estimated at 8 dS m⁻¹ in both genotypes *i.e.*, 26.85 in HJ 541 and 27.00 in CSV 21F with respect to control. While jasmonic acid reduced electrolyte leakage to some extent. (Fig. 1). Electrolyte leakage increases with salt stress due to increased lipid peroxidation, which is directly related to ROS accumulation leading to membrane instability, but JA reduces electrolyte leakage under salt stress by increasing anti-oxidative enzyme activity, which can remove reactive oxygen species (ROS) and reduces membrane lipid breakdown (Hnilickova *et al.* (2019)). Dehnavi *et al.* (2022) and Dinler *et al.* 2019 also reported similar findings.

Leaf chlorophyll is a key indicator of leaf greenness and is frequently used to investigate leaf nutrient deficiencies and changes in chlorophyll. According to present study under salt stress total chlorophyll and photochemical quantum yield (Table. 2) were decreased with the increasing levels of salt from control to 8 dS m⁻¹ in both the sorghum genotypes. Percent chlorophyll decrease was maximum at 8 dS m⁻¹ of salt stress *i.e.*, 18.4% in HJ 541 and 9.0% CSV 21F with respect to control. (Table. 2) The loss of chlorophyll under salt stress may be due to photoinhibition, ROS formation or due to the reduction in photosynthesis (Heidari, 2012). These results were in accord with Akhter *et al.* (2021). With application of different concentrations of jasmonic acid progressive increase in total chlorophyll was observed in both genotypes, with maximum at 8.5 mM of jasmonic acid in HJ 541 (14.5%) and CSV 21F (13.4%) at 6 dS m⁻¹ of salt level over control. Photochemical quantum yield (Fv/Fm) decreased from control to 8 dS m⁻¹ *i.e.*, 0.73 to 0.67 and 0.73 to 0.69, in HJ 541 and CSV 21F, respectively while, with the foliar application of 8.5 mM JA progressive increase in quantum yield was reported at each salt level as well as in control, but maximum increase was noticed at 4 dS m⁻¹ of salt level in HJ 541 from 0.70 to 0.77. JA enhanced the activity of enzymes involved in chlorophyll production, such as protochlorophyllide reductase and -aminolevulinic acid dehydratase (Noor *et al.*, 2022) and also improved photochemical quantum yield by decreasing Tfm (time required to reach maximum fluorescence), increasing maximum fluorescence, Ptotal (Total performance index), and area, which boosts photosystem II efficiency (Ghassemi-Golezani *et al.* 2015). Sheyhakinia *et al.* (2020) and Sirhindi *et al.* (2017) also found JA to be beneficial in improving salt tolerance.

Rate of photosynthesis declined with the increasing levels of salt stress in both genotypes. Percent decrease in photosynthetic rate was 31.8% in HJ 541 and 32.2% in CSV 21F at 8 dS m⁻¹ of salt level. Maximum enhancement in the rate of photosynthesis of both genotypes was noticed with 8.5 mM JA. (Fig. 2) With jasmonic acid increase was noticed at each level of salt stress in HJ 541 *i.e.*, 46.4% at 4, 52.1% at 6 and 27.6% at 8 dS m⁻¹ over control, likewise increment was also observed in CSV 21F. Similarly, a progressive decline in transpiration rate and stomatal conductance was noticed with every increment in salt levels (Fig. 2). At 8 dS m⁻¹, 14.6% and 24.4% decline in transpiration was observed in HJ 541 and CSV 21F, respectively with respect to control. However, decline in stomatal conductance at 4, 6 and 8 dS m⁻¹ of salt level 8.9%, 22.2% and 44.4%, respectively in HJ 541 while, 12.6%, 23.2% and 47.4%, in CSV 21F over their respective control. Foliar application of JA (4.5, 6.5 and 8.5 mM) lead to enhancement of stomatal conductance and transpiration rate in both genotypes and maximum increase was observed at 8.5 mM. At 8 dS m⁻¹, application of 8.5 mM JA showed 16.7% in HJ 541 and 19.9% in CSV 21F increase in transpiration rate and 42.0% and 58.0% in stomatal conductance of HJ 541 and CSV 21F, respectively. Under salt stress, the rate of photosynthesis decreased due to

a decrease in the activity of oxygenase enzymes and photosystem II. Under salinity, stomatal closure and reduced transpiration rate occur by affecting the cytosolic Ca^{2+} ion concentration, which affects stomatal conductance Devi *et al.* 2020. JA enhances photosynthetic rate, transpiration rate and stomatal conductance by improving the plant water status and chlorophyll content. These results are in accord with the observations of Hussain *et al.* (2020); Dourado *et al.* (2022), and Orzechowska *et al.* (2021).

Fig. 2: Effect of salt stress on (a) photosynthetic rate ($\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (b) transpiration rate ($\text{mmole H}_2\text{O m}^{-2} \text{ s}^{-1}$), (c) stomatal conductance ($\text{mmole H}_2\text{O m}^{-2} \text{ s}^{-1}$) and its alleviation by jasmonic acid in sorghum genotypes



Application of JA enhanced antioxidants and NBI content under salinity

Anthocyanin and flavonoids act as antioxidants under salt stress and plays an important role in defense reactions of plants under salt stress. (Table.2) Anthocyanin content increased with increasing level of salt stress as well as JA (4.5, 6.5 and 8.5 Mm) under stress and control conditions. Maximum anthocyanin content was noticed at 8 dS m^{-1} of salt with the application of 8.5 mM JA in both genotypes *i.e.*, 0.31 and 0.30 in HJ 541 and CSV 21F, respectively. Similarly, the flavonoids content also exhibited more values as salt levels increases and the maximum flavonoid content was noticed at 8 dS m^{-1} in both the genotypes (HJ 541 and CSV 21F). On the application of jasmonic acid of 4.5, 6.5 and 8.5 mM concentrations a significant increase in flavonoid content was observed. Maximum percent increase was observed in HJ 541 at 6 dS m^{-1} *i.e.*, 11.76% and in CSV 21F at 4 dS m^{-1} that was 7.89% over their respective control. Similar findings were also reported by Kianiet *al.* (2021) and Jeon *et al.* (2020).

A significant decline was noticed in nitrogen balance index with every increment from control to 8 dS m^{-1} (Table.2). Among all the salt treatments, least nitrogen balance index was found minimum at 8 dS m^{-1} in both

genotypes i.e., 4.4% and 7.4% in HJ 541 and CSV 21F, respectively. However, with the application of jasmonic acid NBI content increased noticeably and maximum was noticed at 8.5 mM concentration of JA in all salt levels on comparison to control in both genotypes. Significant decrease in nitrogen uptake with salt levels has been advocated by Kumar (2019) and Kaur *et al.* (2017) also.

Conclusions

Salt stress reduced the morpho-physiological parameters leading to reduction in growth yield and quality in both genotypes. Conclusively, the forage sorghum variety CSV 21F performed better and showed effective response in alleviating salt stress with foliar application of jasmonic acid, however HJ 541 was also at par with CSV 21F. Deleterious effects were more pronounced at higher level of salt (8 dS m⁻¹) stress. Ameliorating effect was seen more effective at 8.5 mM of JA which was at par with 6.5 mM. Hence, this study concluded that sorghum genotype CSV 21F performed better as compared to HJ 541 at all levels of salt stress after application of jasmonic acid.

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