

*Original Research*

# Exogenous Methyl Jasmonate Acclimate Morpho-Physiological, Ionic and Gas Exchange Attributes of Wheat Genotypes (*Triticum aestivum* L.) under Salt Stress

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## Abstract

Salinity is a serious menace that has a significant influence on sustainability of agriculture and threatens food security of the world. Methyl jasmonate appeared to be an important modulator with different specific functions in plants and increase the resistance against different abiotic stresses. A hydroponic study was performed with three salt (control, 100 mM and 200 mM NaCl) and two methyl jasmonate concentrations (50  $\mu$ M and 100  $\mu$ M) applied in sole and integrate form. Methyl jasmonate treated plants exhibits improved growth mass and antioxidant safeguard. The imposition of salt in growth medium drastically reduced the plant growth, physiological, gas exchange, ionic and biochemical attributes. Application of methyl jasmonate significantly alleviated the deleterious effects of salinity by depicting the improvement in plant biomass, RWC, MSI, transpiration rate, photosynthetic rate, stomatal conductance, internal CO<sub>2</sub> concentration, chlorophyll contents, oxidative stress indicators, activity of antioxidant enzymes (POD, CAT, APX and SOD) and K<sup>+</sup>/Na<sup>+</sup> ratio. The results also disclose that response of wheat genotypes against methyl jasmonate application under saline condition show a concentration effect and varietal diversity. Moreover, the application of 100 $\mu$ M methyl jasmonate under low and elevated salt concentration shows prominent response towards wheat genotype Faisalabad-2008 while least response was observed in wheat genotype Victoria.

**Keywords:** antioxidant enzymes; K<sup>+</sup>/Na<sup>+</sup> ratio, methyl jasmonate, photosynthetic rate, wheat, relative water contents

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## Introduction

Soil salinization is a global issue that threatens crop growth and productivity, as well as the long-term viability of modern agriculture. Salinity affects more than one third of the world's irrigated land. Salinization is caused by rising groundwater levels with high salt content, as well as inefficient drainage and irrigation systems [1]. Plant growth and development are hampered by salinity stress, which imposes many primary restrictions. The first restriction is osmotic stress, which harms plants capacity to absorb water by reducing the external water potential [2]. Salt stress has a significant negative impact on crop performance, biochemical alterations and physiology, resulting in decreased seed germination, fresh and dry biomass, photosynthesis and mineral nutrient accumulation [3-6]. Biochemical alterations occur when plants are subjected to the detrimental effects of reactive oxygen species (ROS) in a saline environment which resulting in growth seizure and metabolic damage [7]. Antioxidant enzymes (CAT, SOD and POD) and nonenzymatic antioxidants (tocopherols, ascorbate and phenolic compounds) have boosted their activity, which protecting plants from salt-induced reactive oxygen species [8]. Wheat (*Triticum aestivum* L.) is a key staple food that is cultivated in ninety countries [9]. Wheat is the sole food available in many nutrient-deficient areas across the world [10]. Global food production has increased by several times in comparison to the past, yet the slow pace of agricultural progress is insufficient to meet the needs of the world fast-growing population. Wheat is responsible for 30% of world grain output and 45% of cereal nutrients [11]. It is grown all across the world and accounts for around one-sixth of all arable land. Among all the grains cultivated in Pakistan, it ranks top in terms of both acreage and output. In 2010-11, Pakistan produced 24214 thousand tonnes of wheat and had an area under cultivation of 8805 thousand hectares [12]. Soil characteristics and water availability have an impact on wheat growth [13]. Wheat is a moderately salt-resistant crop with large genotypic differences in salinity tolerance [14]. Wheat is one of the most important crops for provision of daily protein and calorie demands of the people [15]. Wheat productivity in Pakistan is poor in salt-affected regions, with yield losses as high as 65% in moderately saline-soils [16].

Methyl jasmonate (MeJA) is a cellular regulator that plays a role in a variety of developmental processes, including seed sprouting, root growth, fertilization, fruit maturing and senescence [17]. They stimulate plant protection mechanisms in response to insect-caused wounds, pathogens, and environmental challenges such as drought, chilling injuries and salt stress [18]. Under salinity stress, jasmonates restored salt restriction on maize dry mass production [19] and reduced the inhibitory impact of NaCl and improve antioxidant activities in strawberries plants [20]. MeJA

has been shown to reduce the adverse effect of salt and water stress in a variety of agricultural plants, including cowpea, alfalfa, tomato, chilies and broccoli [21-25]. The objective of the current study was to better understand the morpho-physiological responses of salt-resistant and salt-sensitive wheat genotypes to a saline environment, as well as to alleviating the effects of methyl jasmonate on plant growth and physiology, improve nutrition, water relations, and gas exchange characteristics, and enhance antioxidant enzyme activities in wheat exposed to salinity stress. We want to know if these wheat genotypes have similar salinity adaptation mechanisms, and if so, what are the primary susceptible or tolerant features in these genotypes. Does methyl jasmonate help to improve these qualities in wheat genotypes that have been exposed to salt stress?

## Material and Methods

### Experimental Site and Growth Conditions

A Hydroponic experiment was conducted in a rain protected wire house at Soil Salinity Laboratory, The Islamia University of Bahawalpur, Pakistan. In moist sand culture, seed of two salt resistant wheat genotypes (Faisalabad-2008, Anaj-17) and two salt susceptible wheat genotypes (Gandum-09 and Victoria) were sown. Uniform seedlings at two leaf stage were transplanted to thermopole sheet in 50-liter capacity iron tubs having half strength Hoagland solution as a nutrient media [26]. The desired salinity levels (control, 100 and 200 mM) were attained by adding the appropriate quantity of NaCl, while methyl jasmonate was supplied at 50 and 100  $\mu\text{M L}^{-1}$ . The study used a complete randomized design with split plot layouts, with each treatment being repeated four times. The following treatments were used: (T<sub>1</sub>) control, (T<sub>2</sub>) 100 mM NaCl  $\text{L}^{-1}$ , (T<sub>3</sub>) 200 mM NaCl  $\text{L}^{-1}$ , (T<sub>4</sub>) 100 mM NaCl + 50  $\mu\text{M L}^{-1}$  methyl jasmonate, (T<sub>5</sub>) 100 mM NaCl + 100  $\mu\text{M L}^{-1}$  methyl jasmonate, (T<sub>6</sub>) 200 mM NaCl + 50  $\mu\text{M L}^{-1}$  methyl jasmonate, (T<sub>7</sub>) 200 mM NaCl + 100  $\mu\text{M L}^{-1}$  methyl jasmonate. Aeration pumps were used to deliver oxygen to the plants. The pH of the solution was regularly monitored and regulated at  $6.0 \pm 0.5$  every day.

### Plant Growth Measurement

The plants were harvested at vegetative stage and root and shoot lengths, as well as fresh and dry weight were measured according to standard procedures.

### Root Traits

Root attributes (root surface area, root volume, root diameter and number of root tips) were determined by utilizing a root scanner by extract fresh roots from each plant (Win RHIZO Pro, STD, 2017, Netherland).

### *Leaf Area and Chlorophyll Content (SPAD)*

The leaf area was determined by using a leaf area meter. Fully enlarged leaves from all treatments were used for this purpose. The leaf area was measured in cm<sup>2</sup> (Win FOLIA Pro, STD, 2016, Netherland). Using a SPAD meter, the chlorophyll contents (SPAD values) of fresh plant leaves were determined at vegetative stage (Minolta, Japan).

### *Measurements of Gas Exchange Characteristics*

On a fully expanded youngest leaf, a portable LCA-4 ADC open system infrared gas analyzer was used to quantify net photosynthetic rate (A), transpiration rate (E), stomatal conductance (Gs) and sub-stomatal CO<sub>2</sub> concentration (Ci) at vegetative stage of plants. From 10 A.M. until 2 P.M., the following specifications/adjustments were utilized to collect these measurements: The leaf chamber temperature (Tch) ranged from 39.2 to 43.9°C, the leaf chamber volume gas flow rate (v) 396 mL min<sup>-1</sup>, the leaf chamber molar gas flow rate (U) 251 mol S<sup>-1</sup>, the ambient pressure (P) 99.95 kPa, the molar flow of air per unit leaf area (Us) 221.06 mol m<sup>-2</sup> S<sup>-1</sup> and the PAR (Qleaf) at the leaf surface peaked at 918 µmol m<sup>-2</sup>.

### *Relative Water Contents*

After acquiring fresh, dry and turgid weight, RWC was measured using the technique of [27]; after selecting fully expanded youngest leaves of each treatment.

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

### *Membrane Stability Index*

The Membrane stability index was calculated using Sairam's approach [28]. In test tubes containing double distilled water, 0.1g leaf discs of all treatments were placed. They were maintained at 40°C in a water bath. The EC was measured after 30 minutes of incubation (C<sub>1</sub>). The identical leaf sample was subsequently held in a water bath at 100°C for 10 minutes and the EC was measured once more (C<sub>2</sub>). The formula used was:

$$\text{Membrane Stability Index} = [1 - C_1/C_2] \times 100$$

### *Estimation of Proline Content*

To quantify proline from freeze-dried leaves, sulfosalicylic (3%) and extraction buffers ninhydrin [29] were used. 0.04 g dried leaves were extracted with sulfosalicylic acid (3% w/v). The extract was centrifuged

at 3000 g for 10 minutes. A 200 mL supernatant aliquot was combined with 400mL of the reagent combination (1.25 g ninhydrin, 20 Ml phosphoric acid, and 30 mL glacial acetic acid) and heated in sealed test tubes at 100°C for 1 hour. The mixture was then allowed to cool. To each sample's combination, exactly 4 mL of toluene was added. At 520 nm, a Cecil Aquarius CE 7200 Double Beam Spectrophotometer was used to calculate proline content. The results were expressed as millimoles per gramme of dry weight (mmol g<sup>-1</sup> DW).

### *Determination of MDA and H<sub>2</sub>O<sub>2</sub>*

Jambunathan's approach was used to determine the levels of lipid peroxidation (MDA) [30]. The supernatant was removed by centrifuging for fifteen minutes at 10,000 r with 0.25 g fresh leaves and 0.1% trichloroacetic acid, TCA (5mL). Absorbance at 532 nm and 600nm were removed for non-specific turbidity adjustments. The researchers employed a 0.25% blank sample (TBA) and a specific activity coefficient of 155 mM<sup>-1</sup>cm<sup>-1</sup> respectively. The concentrations of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were determined using Velikova's technique [31].

### *Ionic Attributes*

In dried and crushed shoot material (0.01 g), sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) concentrations were determined. Using a Flame Photometer (BWB-XP, UK), the samples were digested with hydrogen peroxide and sulfuric acid, then heated at 350°C until fumes were produced and the compound was colorless, as described by Wolf [32].

### *Biochemical Attributes*

A 10 ml extraction buffer containing 50 mM phosphate, pH 7.8, 1 mM EDTA, 1 g polyvinylpyrrolidone (PVP) and 0.5% Triton X-100 was used to homogenize fresh leaf segments weighing 0.5 g. The homogenate was centrifuged at 12,000 rpm for 20 minutes, and the supernatant was used to test for SOD, POD, CAT and APX. At a temperature of 0-4°C, all procedures were carried out [33].

### *Statistical Analysis*

The statistics package Statistics 8.1 (Statistics, IL, USA) was used to analyze the data in this study and the results are provided in tables and graphs. The graph bars represent the average value of four replicates while the error bars represent the standard deviations. The honest significant difference (HSD) test was used to assess and analyze the source of variance with a 5% probability [34].

## Results

### Plant Biomass

Salt stress has been shown to divide wheat genotypes into two groups. Wheat genotypes Faisalabad-2008 and Anaj-17 (salt resistant) are in the first group, whereas wheat genotypes Gandum-09 and Victoria are in the second (salt susceptible). Wheat genotypes in the first group have longer roots and shoots, as well as fresh and dry biomass than genotypes in the second group (Table 1, 2 and 3). Wheat genotypes Victoria demonstrates the greatest decline in growth (66% and 72% root and shoot length, 64% and 70% root and shoot fresh weight, 65% and 75% root and shoot dry weight) when exposed to extreme salt stress (200 mM NaCl). As indicated in this study, salinity has a substantial impact on all wheat genotypes with wheat genotype Faisalabad-2008 exhibiting the highest growth at both salt levels. Under high salt stress, visual signs such as leaf tip burn were observed in wheat genotype Victoria, which proved to be the most salt sensitive of all wheat genotypes. When a high dose of methyl jasmonate (100  $\mu$ M) was combined with 100 mM salt stress, the growth of both categories of wheat genotypes was significantly altered. A notable effect was observed in wheat genotypes Faisalabad-2008, 13% and 12% improvement in root and shoot length, 20% and 21% increase in root and shoot fresh weight and 18% and 20% increase in root and shoot dry weight.

### Root Traits

Increased salt in the growing environment has a considerable detrimental influence on both types of wheat genotypes root characteristics (Table 4 and 5). Wheat genotypes Victoria had the lowest values in root surface area and root volume (64% and 61%) when exposed to a high level of salinity (200mM NaCl) while root diameter and number of root tips were reduced by 43% and 61% respectively when compared to control. As revealed in the current study, wheat genotypes Faisalabad-2008 respond eminently to low and high doses of exogenously applied methyl jasmonate, manifesting improved plant root surface area and root volume (20% and 21%), root diameter and number of root tips (22% and 21%) when subjected to 100 $\mu$ M methyl jasmonate with low salt stress (100mM NaCl) revealing its better tolerance towards salt stress. Between these two wheat genotypes, other wheat genotypes exhibit a decline.

### Physiological Attributes

The results of the analysis (Fig. 1) of relative water contents, membrane stability index, chlorophyll contents (SPAD) and leaf area show that higher salt levels reduce plant water relations and membrane integrity in both groups of wheat genotypes, with wheat genotype Victoria showing the greatest reduction (45% RWC,

Table 1. Effect of exogenously applied methyl jasmonate on shoot and root length of four wheat genotypes under salt stress. Each value represents the average of four replicates and column not sharing the same letter differ significantly at 0.05 probability level.

Treatments	Shoot Length (cm)				Root Length (cm)			
	FSD-2008	Anaj	Gandum	Victoria	FSD-2008	Anaj	Gandum	Victoria
Control	60.3 $\pm$ 3.7 a	61.2 $\pm$ 3.8 a	49.3 $\pm$ 3.0 ef	57.4 $\pm$ 3.5 d	40.4 $\pm$ 2.5 a	36 $\pm$ 2.1 b	28 $\pm$ 1.7 f	32.1 $\pm$ 2.1 h
S1 = 100 mM NaCl	48 $\pm$ 2.9 g	46.3 $\pm$ 2.7 h	30.1 $\pm$ 1.8 n	32.7 $\pm$ 1.9 m	32.4 $\pm$ 2.0 d	28.1 $\pm$ 1.7 f	21 $\pm$ 1.3 k	17.2 $\pm$ 1.1 m
S2 = 200 mM NaCl	39 $\pm$ 3.3 jk	38.4 $\pm$ 2.3 k	16.2 $\pm$ 1.1 o	15.6 $\pm$ 1.0 o	25.6 $\pm$ 1.5 h	21.9 $\pm$ 1.3 jk	12.1 $\pm$ 0.7 nop	10.7 $\pm$ 0.6 q
S1 + 50 $\mu$ M MeJA	50.4 $\pm$ 3.0 e	48.6 $\pm$ 2.9fg	31.2 $\pm$ 1.8 n	33.6 $\pm$ 2.0 l	34.1 $\pm$ 2.1 c	29.5 $\pm$ 1.8 e	22 $\pm$ 1.4 ijk	17.7 $\pm$ 1.1 lm
S1 + 100 $\mu$ M MeJA	53.7 $\pm$ 3.2 c	52.3 $\pm$ 3.1 d	33.3 $\pm$ 2.0 m	35.9 $\pm$ 2.1 l	36.2 $\pm$ 2.2 b	31.7 $\pm$ 1.9 d	23.1 $\pm$ 1.3 ij	18.9 $\pm$ 1.2 l
S2 + 50 $\mu$ M MeJA	40.1 $\pm$ 2.4 ij	39.9 $\pm$ 3.4 ij	16.4 $\pm$ 1.1 o	15.9 $\pm$ 1.1 o	26.3 $\pm$ 1.6 h	22.7 $\pm$ 1.4 ij	12.3 $\pm$ 0.8 no	10.9 $\pm$ 0.5 pq
S2 + 100 $\mu$ M MeJA	41.3 $\pm$ 2.5 i	40.7 $\pm$ 2.6 i	16.8 $\pm$ 1.2 o	16.2 $\pm$ 1.1 o	27.1 $\pm$ 1.7 fg	23.2 $\pm$ 1.5 i	12.5 $\pm$ 0.9 n	11.1 $\pm$ 0.7 opq

Table 2. Effect of exogenously applied methyl jasmonate on shoot and root fresh weight of four wheat genotypes under salt stress. Each value represents the average of four replicates and column not sharing the same letter differ significantly at 0.05 probability level.

Treatments	Shoot Fresh Weight (g plant <sup>-1</sup> )				Root Fresh Weight (g plant <sup>-1</sup> )			
	FSD-2008	Anaj	Gandum	Victoria	FSD-2008	Anaj	Gandum	Victoria
Control	50±2.9 a	48±2.8 ab	45±2.7 cd	46±2.8 bc	6.6±0.36 a	6.1±0.36 b	5.2±0.31 d	5.8±0.25 bc
S1 = 10 mM NaCl	36±2.1 gh	35.1±2.1 h	23.8±1.5 mn	23.1±1.3 n	4.75±0.28 fg	4.45±0.27 g	3.38±.20 k	3.45±0.21 k
S2 = 200 mM NaCl	29±1.7 ij	28.8±1.7 jk	12.2±0.74 o	13.6±0.8 o	3.82±0.22 hij	3.6±0.22 jk	1.8±0.10 m	2.05±0.13 lm
S1 + 50 $\mu$ M MeJA	39.2±2.3 f	37.9±2.3 fg	25.4±1.5 lmn	24.4±1.4 lmn	5.1±0.30 de	4.8±0.28 ef	3.6±0.21 jk	3.6±0.21 jk
S1 + 100 $\mu$ M MeJA	43.2±2.5 de	42.4±2.5 e	26.6±1.6 kl	26.1±1.5 lm	5.7±0.34 c	5.3±0.31 d	3.7±0.22 ijk	3.8±0.24 hij
S2 + 50 $\mu$ M MeJA	30.4±1.8 ij	30.2±1.8 ij	12.5±0.8 o	14.1±0.8 o	4.0±0.25 hi	3.7±0.22 ijk	1.82±0.11 m	2.1±0.13 lm
S2+100 $\mu$ M MeJA	31.3±1.9 i	31.1±1.9 ij	12.9±0.8 o	14.4±0.9 o	4.1±0.25 h	3.8±0.23 hi	1.9±0.13 lm	2.2±0.15 l

Table 3. Effect of exogenously applied methyl jasmonate on shoot and root dry weight of four wheat genotypes under salt stress. Each value represents the average of four replicates and column not sharing the same letter differ significantly at 0.05 probability level.

Treatments	Shoot Dry Weight (g plant <sup>-1</sup> )				Root Dry Weight (g plant <sup>-1</sup> )			
	FSD-2008	Anaj	Gandum	Victoria	FSD-2008	Anaj	Gandum	Victoria
Control	6.9±0.40 a	6.1±0.35 b	5.3±0.28 d	5.9±0.24 e	1.51±0.11 ab	1.57±0.12 a	1.41±0.09 c	1.44±0.1 bc
S1 = 100 mM NaCl	4.9±0.24 f	4.4±0.24 h	2.7±0.13 o	2.4±0.12 d	1.1±0.06 fgh	1.14±0.07 fg	0.78±0.05 p	0.79±0.05 op
S2 = 200 mM NaCl	4.1±0.21 j	3.6±0.18 l	1.5±0.08 qp	1.4±0.07 r	0.87±0.05 mn	0.94±0.06 klm	0.42±0.02 q	0.47±0.04 q
S1 + 50 $\mu$ M MeJA	5.3±0.27 d	4.7±0.23 g	2.9±0.15 n	2.5±0.12 p	1.19±0.07 ef	1.23±0.07 f	0.83±0.05 nop	1.02±0.06 hij
S1 + 100 $\mu$ M MeJA	5.8±0.29 c	5.3±0.28 d	3.1±0.17 m	2.7±0.13 o	1.32±0.08 d	1.37±0.08 cd	0.87±0.05 mn	1.09±0.07 ghi
S2 + 50 $\mu$ M MeJA	4.2±0.22 ij	3.7±0.19 kl	1.54±0.09 qr	1.44±0.08 qr	0.91±0.06 lmn	0.98±0.05 jkl	0.43±0.03 q	0.48±0.05 q
S2 + 100 $\mu$ M MeJA	4.3±0.24 hi	3.8±0.24 k	1.59±0.10 q	1.48±0.09 qr	0.93±0.06 klm	1.01±0.06 ijk	0.44±0.03 q	0.49±0.05 q

Table 4. Effect of exogenously applied methyl jasmonate on root surface area and root volume of four wheat genotypes under salt stress. Each value represents the average of four replicates and column not sharing the same letter differ significantly at 0.05 probability level.

Treatments	Root Surface Area (cm <sup>2</sup> )			Root Volume (cm <sup>3</sup> )				
	FSD-2008	Anaj	Gandum	Victoria	FSD-2008	Anaj	Gandum	Victoria
Control	245±12.4 a	237±10.4 a	232±11.7 b	230±10.3 b	18.3±1.1 b	19.1±1 a	16.9±0.8	17.1±0.9 c
S1 = 100 mM NaCl	174±8.5 ef	171±8.1 fg	148±7.3 ijk	145±7.4 jk	12.6±0.7 g	13.4±0.6 f	10.6±0.6	10.6±0.5 lm
S2 = 200 mM NaCl	140±7.1 k	141±7.1 k	79±3.9 l	85±4.3 l	10.1±0.6 m	11.1±0.6 jkl	5.5±0.3	6.1±0.3 nop
S1 + 50 µM MeJA	189±9.5 d	184±9.5 de	159±8.2 hi	153±7.8 hij	13.8±0.6 f	14.6±0.7 e	11.4±0.6	11.3±0.6 ijk
S1 + 100 µM MeJA	208±10.5 c	206±10.2 c	163±8.5 gh	164±8.8 fgh	15.2±0.8 e	16.3±0.8 d	11.9±0.6	12.1±0.6 gh
S2 + 50 µM MeJA	146±7.7 jk	142±7.2 k	88±4.7 l	81±4.2 l	10.6±0.5 lm	11.6±0.6 hij	5.6±0.3	6.2±0.3 no
S2 + 100 µM MeJA	150±7.1 ijk	143±7.7 k	90±5.1 l	83±4.3 l	10.9±0.6 kl	11.9±0.6 ghi	5.8±0.4	6.4±0.3 n

Table 5. Effect of exogenously applied methyl jasmonate on root diameter and number of root tips of four wheat genotypes under salt stress. Each value represents the average of four replicates and column not sharing the same letter differ significantly at 0.05 probability level.

Treatments	Root Diameter (mm)				Number of Root Tips			
	FSD-2008	Anaj	Gandum	Victoria	FSD-2008	Anaj	Gandum	Victoria
Control	3.92±0.20 a	3.73±0.17 b	3.45±0.13	3.51±0.18 a	4620±226 a	4570±218 a	4350±217 b	4310±215 b
S1 = 100 mM NaCl	2.74±0.14 f	2.76±0.14 fg	2.27±01.2	2.28±0.12 f	3230±164 e	3199±157 ef	2827±136 ij	2758±138 jk
S2 = 200 mM NaCl	2.23±0.12 k	2.31±0.12 jk	1.29±0.07	1.33±0.07 k	2587±130 mn	2513±126 n	1609±79 q	1680±85 pq
S1 + 50 µM MeJA	2.98±0.15 e	2.99±0.15 e	2.42±0.12	2.41±0.13 e	3488±164 d	3422±167 d	2968±134 gh	2895±140 hi
S1 + 100 µM MeJA	3.34±0.16 cd	3.33±0.16 d	2.54±0.13	2.57±0.14 cd	3908±190 c	3870±185 c	3110±157 f	3006±157 g
S2 + 50 µM MeJA	2.34±0.12 ijk	2.42±0.13 hij	1.32±0.07	1.36±0.08 ijk	2716±135 kl	2638±135 lm	1657±79 pq	1730±86 op
S2 + 100 µM MeJA	2.40±0.13 hij	2.49±0.13 hi	1.36±0.08	1.40±0.07 hij	2793±142 jk	2715±134 kl	1689±85 opq	1780±89 o

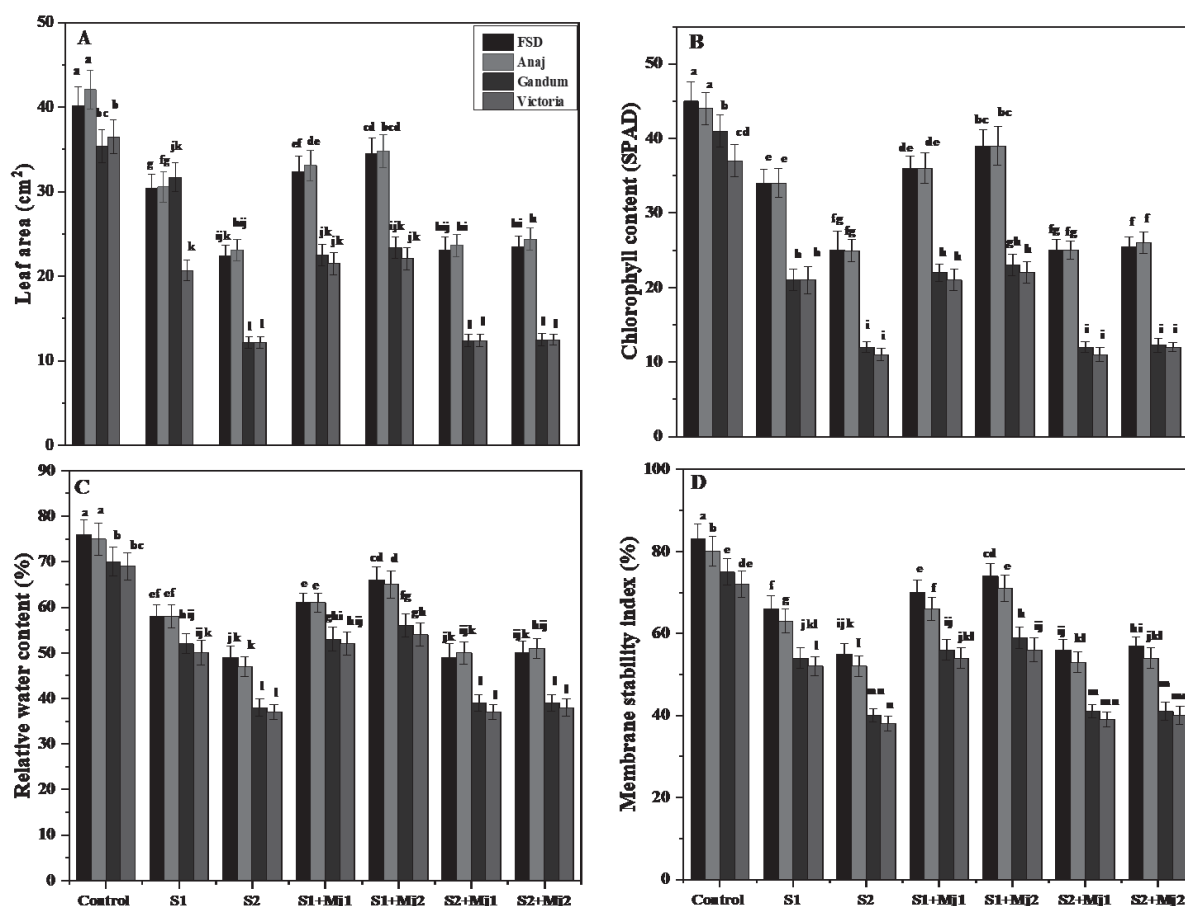


Fig. 1. Effect of exogenously applied methyl jasmonate on physiological attributes such as a) Chlorophyll content (SPDA), b) Leaf Area, c) Relative water content and d) Membrane stability index of four wheat genotypes under salt stress. The bar values depict the average of three replications and the bars not sharing the same lowercase letters differ significantly from each other according to the HSD test at  $P < 0.05$  probability level.

43% MSI, 60% SPAD and 65% leaf area). In terms of chlorophyll concentration and leaf area, all wheat genotypes follow a parallel pattern, with lower values as salt level increases. When compared to other wheat genotypes described in this trial, Faisalabad-2008 reacts admirably to methyl jasmonate and exhibits enhanced RWC, MSI, chlorophyll and leaf area contents.

#### Gas Exchange Attributes

Salt stress in the growth medium has a significant detrimental influence on the gas exchange characteristics of both wheat genotypes (Fig. 2). Wheat genotypes Victoria had the lowest photosynthetic rate and transpiration rate (69% and 67%) when exposed to a high level of salinity (200 mM NaCl), while stomatal conductance and internal  $\text{CO}_2$  concentration were reduced by 56 and 54% respectively when compared to control. Wheat genotypes Faisalabad-2008 respond prominently to low and high doses of exogenously applied methyl jasmonate, as showed in the current study and exhibit enhanced plant photosynthetic and transpiration rate (15 and 17%) and other gas exchange characteristics when exposed to 100  $\mu\text{M}$  methyl

jasmonate with low salt stress (100 mM NaCl), revealing its better tolerance towards salinity stress. Between these two wheat genotypes, other wheat genotypes exhibit a decline.

#### Oxidative Stress Indicators

Effects of NaCl and methyl jasmonate on MDA,  $\text{H}_2\text{O}_2$  and proline levels in four wheat genotypes shown in Fig. 3. When compared to control, salinity treatment resulted in significant increases in MDA,  $\text{H}_2\text{O}_2$  and proline contents in all four wheat genotypes, but this pattern is reduced at low salt stress (100mM NaCl) in both salt resistant wheat genotypes (Faisalabad-2008 and Anaj-17) and more prominent at high salinity stress in both salt susceptible wheat genotypes (Gandum-09 and Victoria). In comparison to plants grown in low salinity level at 100 mM, high level of methyl jasmonate (100  $\mu\text{M}$ ) application reduced the concentration of MDA,  $\text{H}_2\text{O}_2$  and proline in the plants by 11%, 13% and 18% in the wheat genotype Faisalabad-2008 plants and by 7%, 6% and 9% in the wheat genotype Victoria plants when exposed to salinity level at 100 mM.

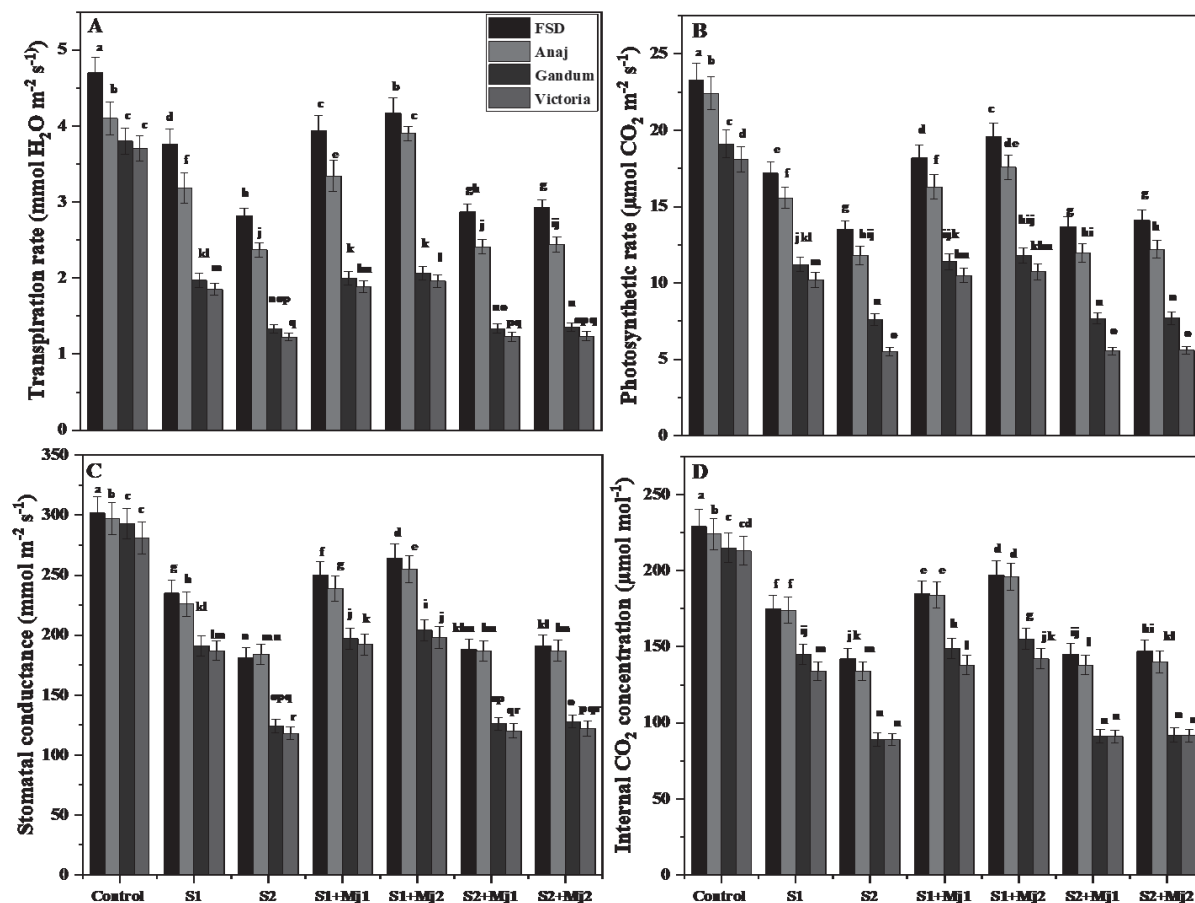


Fig. 2. Effect of exogenously applied methyl jasmonate on gas exchange attributes such as a) Photosynthetic rate, b) Transpiration rate, c) Stomatal conductance and d) Internal  $\text{CO}_2$  concentration of four wheat genotypes under salt stress. The bar values depict the average of three replications and the bars not sharing the same lowercase letters differ significantly from each other according to the HSD test at  $P < 0.05$  probability level

#### $K^+/Na^+$ ratio

The result depicts plant exposure to salt stress and methyl jasmonate as well as their interaction on potassium and sodium concentrations and the  $K^+/Na^+$  ratio as shown in Fig. 4. The  $K^+/Na^+$  ratio of all wheat genotypes was strongly altered by increasing salinity stress, and wheat genotypes Victoria with the greatest  $\text{Na}^+$  content was found. Victoria appears to be the most sensitive wheat genotypes to salinity at both levels of salinity. Wheat genotypes Faisalabad-2008 and Anaj-17 exhibited the highest tendency to retain  $K^+$  in the leaves across all treatments and the highest  $K^+$  levels were seen in these genotypes. Methyl jasmonate supply at  $100 \mu\text{M}$  in the presence of  $100\text{mM}$  salt stress highlighted a critical mechanism governing  $\text{Na}^+$  and  $K^+$  concentrations, resulting in an enhanced  $K^+/Na^+$  ratio in wheat genotypes Faisalabad-2008 compared to all other genotypes.

#### Antioxidant Enzymes

Salinity stress decreased antioxidant enzyme activity compared to the control, notably in salt

susceptible wheat genotypes compared to salt resistant wheat genotypes (Fig. 5). Wheat genotypes at high saline level ( $200\text{mM NaCl}$ ) showed a 58% decrease in POD, which functions as a first barrier for the removal of ROS, which resulted in a 57% drop in APX and 55 and 61% reductions in SOD and CAT respectively which successfully resist hydrogen peroxide buildup in plants. Under both levels of salt stress, exogenously provided methyl jasmonate ( $100\mu\text{M}$ ) enhanced plant growth and physiology while also increasing the biochemical activities and reducing the impairment caused by oxidative stress in wheat. A distinguished increase in these protective enzyme's concentration was found in wheat genotypes Faisalabad-2008 and Anaj-17.

#### Discussion

Agricultural plant productivity is dramatically declined by increased salt stress in the growth medium causes a drop in plant growth attributes such as physiological features, biochemical and gas exchange properties and ion hemostasis [35, 36]. Methyl jasmonate provision considerably reduces the

harmful effects of salt because of its acclimating role in modifying morpho-physiological features, mineral nutrition and antioxidant defense mechanisms, methyl jasmonate provision considerably reduces the harmful effects of salt [37, 38]. The greater concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  in the growth medium affect membrane stability, interrupt photosynthetic action and assemble

toxic ions at the tissue level resulting in an imbalance in nutrient uptake, suspend cell division and produce reactive oxygen species (ROS) all of which significantly reduce plant biomass [39]. Plant physiological properties such as photosynthetic rate, transpiration rate and stomatal opening and shutting are all affected by high levels of  $\text{Na}^+$  ions in the soil [40]. Under some stresses,

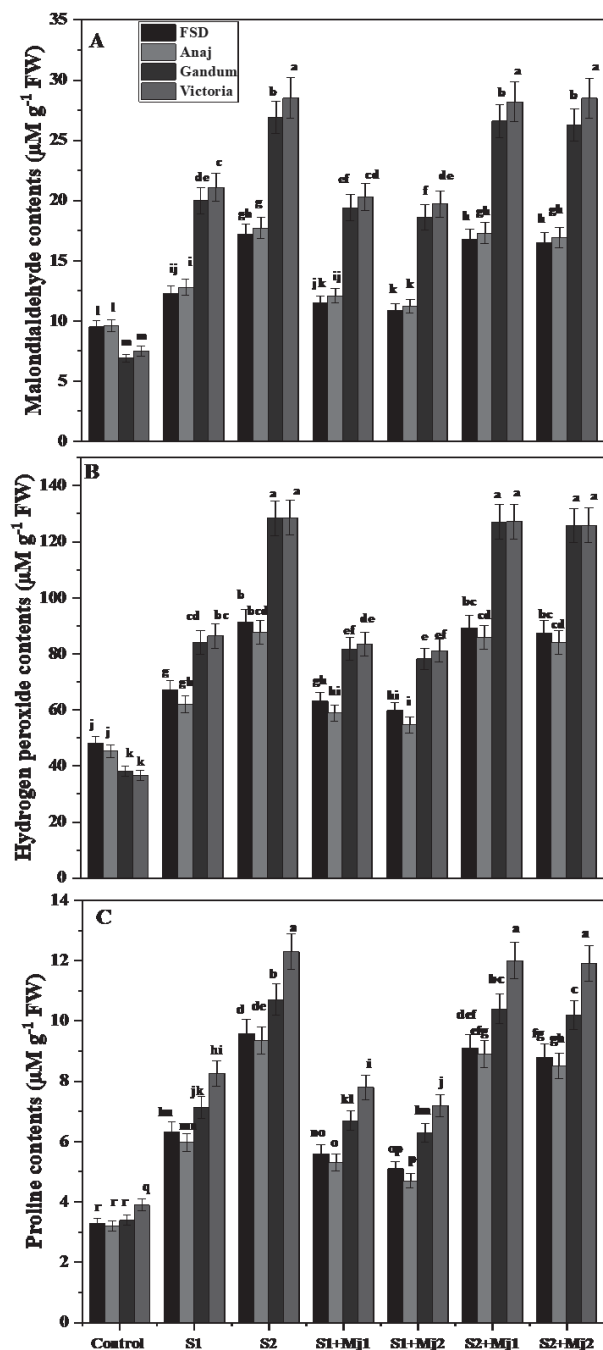


Fig. 3. Effect of exogenously applied methyl jasmonate on oxidative stress indicators such as a) Malonaldehyde, b) Hydrogen peroxide and c) Proline of four wheat genotypes under salt stress. The bar values depict the average of three replications and the bars not sharing the same lowercase letters differ significantly from each other according to the HSD test at  $P < 0.05$  probability level

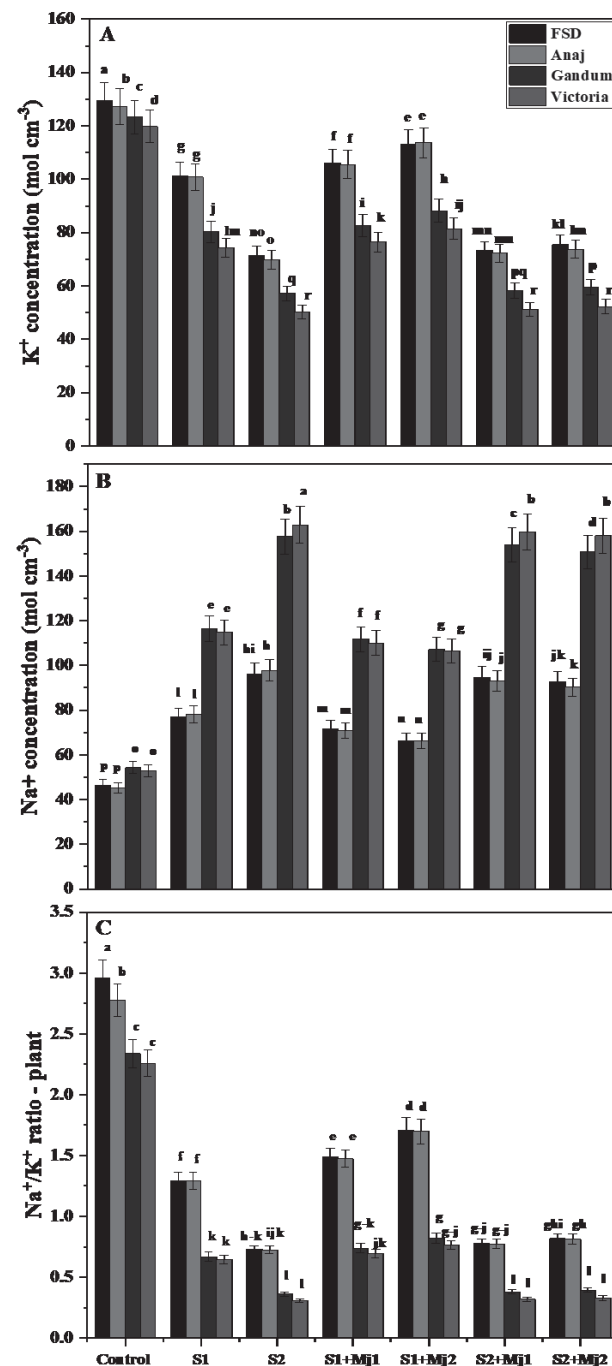


Fig. 4. Effect of exogenously applied methyl jasmonate on ionic attributes such as a)  $\text{K}^+$  concentration, b)  $\text{Na}^+$  concentration and c)  $\text{K}^+/\text{Na}^+$  ratio of four wheat genotypes under salt stress. The bar values depict the average of three replications and the bars not sharing the same lowercase letters differ significantly from each other according to the HSD test at  $P < 0.05$  probability level

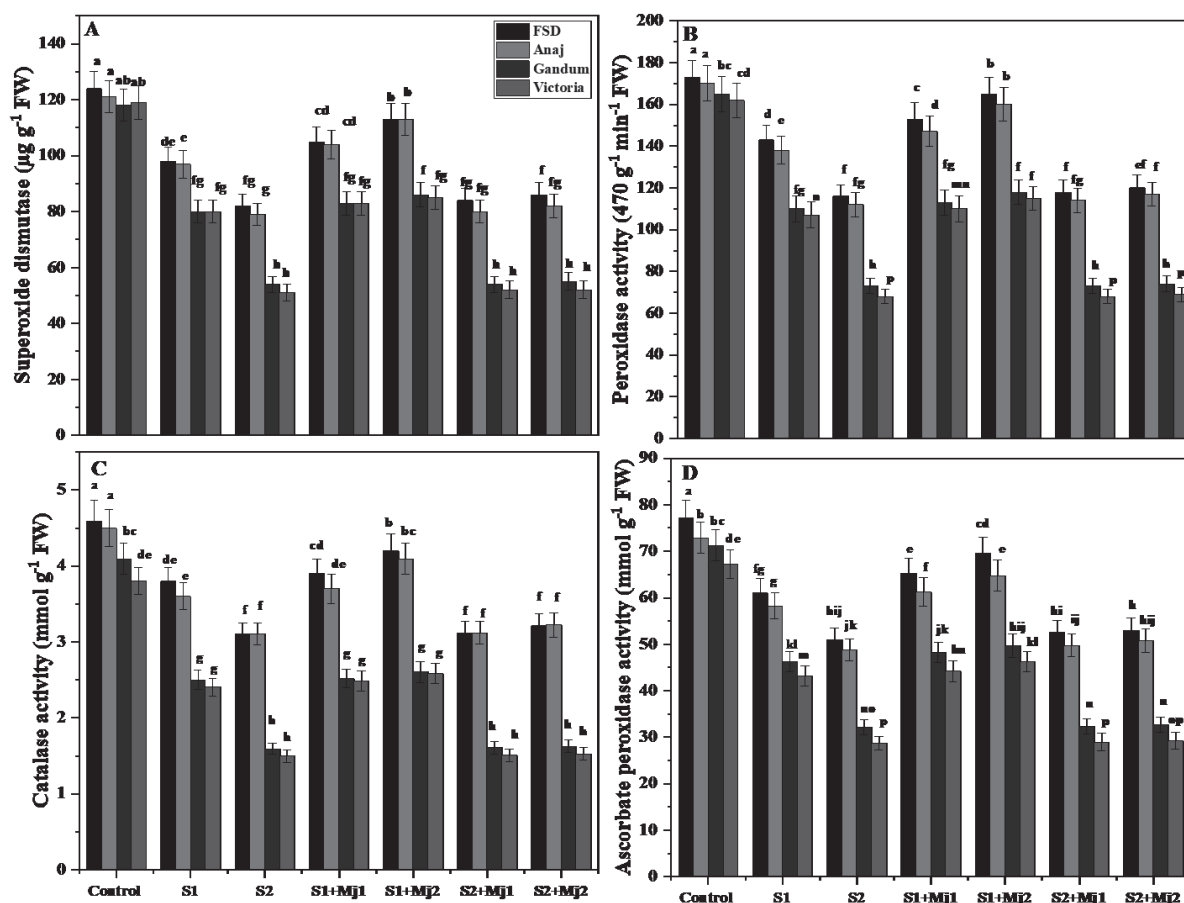


Fig. 5. Effect of exogenously applied methyl jasmonate on biochemical attributes such as a) Superoxide dismutase activity, b) Peroxidase activity, c) Catalase activity and d) Ascorbate peroxidase activity of four wheat genotypes under salt stress. The bar values depict the average of three replications and the bars not sharing the same lowercase letters differ significantly from each other according to the HSD test at  $P < 0.05$  probability level

a decreased in photosynthetic activity and a restriction in the intake of necessary nutrients leads to a shortage of critical metabolites [41]. Provision of methyl jasmonate improves plant physiology in a salt-stressed environment by reducing  $\text{Na}^+$  inflow, up-regulating the antioxidant defense system, enhancing photosynthetic rate-related leaf ultra-structure and increasing ribulose biphosphate carboxylase activity [42]. Furthermore, by modifying the efflux flow of  $\text{Na}^+$  in the roots of salt treated plants, methyl jasmonate leads to improved agricultural plant development by regulating increased salt concentrations [43]. According to a previous study done by Avalbaev methyl jasmonate supplementation can ameliorate the negative effects of salt toxicity on wheat cultivar development [44]. As shown in the current study, salt stress modifies plant cell physiology by disrupting plant water relations, deteriorating membrane stability and lowering chlorophyll concentration. When comparing wheat genotypes Victoria and Faislabad-2008, this decline is more pronounced. Plant water potential and ion toxicity are intimately linked to plant water content and membrane stability. They are the most important indication for evaluating genotype performance under various forms of abiotic stress. The rising trend of NaCl

in the growth medium significantly lowers the RWC and MSI resulting in decreased water absorption by the plant, leaf succulence and cell membrane damage due to scratched membrane-associated fatty acids [45]. Under high salt stress, because of metabolic changes, disruption in hormonal balance at the molecular level, stomata closing and changes in the creation of pigments involved in plant life sustenance such as chlorophyll, leaves develop chlorosis and eventually fall resulting in lower chlorophyll contents [46]. Methyl jasmonate nourishment significantly increased plant water content by improving plant root growth and ability absorb more water from the growth channel and flow from bottom to top, stable osmotic alteration, regulating transpiration rate with stomatal conductance and regulating plant water potential [47]. By functioning as a scavenger against  $\text{H}_2\text{O}_2$  and  $\text{O}_2$ , methyl jasmonate improved antioxidant machinery and maintained ROS balance at the cellular level, promoting membrane integrity and permeability [48]. Provision of methyl jasmonate not only improves the absorption of certain plant nutrients but also promotes the translocation of magnesium (Mg) which is the primary component of chlorophyll synthesis in plant shoots [49].

Salt-induced oxidative damage in wheat (Fig. 3), may be responsible for plasma membrane rupture, electrolyte leakage, lipid peroxidation and nutrient uptake inhibition as seen by higher oxidative stress markers (MDA and  $H_2O_2$ ) under salinity stress [50]. These findings are consistent with those of [51], who found oxidative stress in wheat in a saline environment. Lower  $Na^+$  absorption greater plasma membrane stability and decreased exposure of wheat plant roots to a salty environment might all contribute to a considerable drop in MDA and  $H_2O_2$  following methyl jasmonate treatment. Under the influence of salt stress, a variety of physiological indicators can reveal information on the lowering of gas exchange properties. The decreased in photosynthetic and transpiration rate, as well as stomatal conductance was seen in our experiment with an increasing trend of  $Na^+$ , with wheat genotypes Victoria showing the greatest drop when compared to wheat genotype Faisalabad-2008. It might be owing to the plant response to altered water contents and low water availability in the rhizosphere due to salt which was triggered by changing stomata packing [52]. The administration of methyl jasmonate under saline circumstances had no significant effect on internal  $CO_2$  content, which might be related to differences in stress duration and severity, plant development stage and plant species. Exogenously provision of methyl jasmonate improved chlorophyll activity and water availability by adjusting osmotic pressure, stopping chlorophyll breakdown and lowering  $H_2O_2$  production in leaves [53].

Due to its role in nutrient availability, feasible absorption and transport within the plants, soil salinity causes a nutritional imbalance in wheat [54]. The plasma membrane's existence of  $Na^+$  transport channels allow it to enter the cell [55]. The plant cell's ability to retain  $K^+$  is a key aspect in improving plant stress tolerance. Reduced  $K^+$  uptake in a saline environment might be related to nutritional imbalance and competitive uptake of  $Na^+$  and  $Cl^-$  with specific nutrients like  $K^+$ ,  $Mn^{2+}$  and  $Ca^{2+}$  [56]. In plants, a lower  $Na^+/K^+$  ratio aids salt-tolerant genotypes in performing well in saline environments and sustaining cellular metabolism by boosting protein synthesis, controlling enzyme activation, photosynthetic activity, osmoregulation and enhanced cell turgor [57]. In the current study, wheat plants treated with methyl jasmonate had higher  $K^+$  levels in their shoots than those not treated with methyl jasmonate, as previously reported [58]. This elevated  $K^+$  concentration can be attributable to a rise in root plasma membrane H-ATPase pumps when methyl jasmonate is applied. Under salt stress, methyl jasmonate might reduce  $Na^+$  loading in roots, while increased  $Na^+$  retrieval from shoots resulted in higher  $K^+$  accumulation, osmotic balance and ionic ratio, as well as a decline in  $Na^+/K^+$  ratio [59]. Excessive production of reactive oxygen species (ROS) in plants is toxic and damages subcellular apparatuses and DNA, resulting in cell death [60]. By efficient destruction induced by  $O_2^-$  and  $H_2O_2$  in the chloroplast and cytoplasm of the plant cell,

salinity affected the quantity and activity of antioxidant enzymes in scavenging ROS [61]. Methyl jasmonate has also been shown to operate as an antioxidant, enhancing the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POD) in the leaves of various agricultural plants, which act as the primary line of defense in the photosystem I to decrease ROS generation [62]. Our data imply that the addition of methyl jasmonate to all wheat genotypes influences the facilitation of antioxidant enzyme activities, perhaps protecting plant tissues from oxidative membrane damage and modifiable ROS scavenging enzymes in a salt medium.

## Conclusions

Wheat genotypes that are subjected to salt stress exhibit interrupted growth, poor plant water relations, membrane destabilization, excessive cellular  $Na^+$  levels, decreased photosynthetic activity, as well as oxidative damage. Exogenous treatment of methyl jasmonate performed a multifunctional effect in enhancing salt tolerance in wheat genotypes, according to an increase in plant biomass, increased water contents, higher  $K^+/Na^+$  ratio, gas exchange characteristics, and antioxidant enzyme activity. Wheat genotype Faisalabad-2008 has the greatest  $K^+/Na^+$  ratio and maximum stress-resistance antioxidant enzyme activities, as well as enhanced morpho-physiological properties, allowing for the designation of salt tolerance when subjected to methyl jasmonate at a concentration of 100  $\mu M$ . Contrarily, among all the wheat genotypes tested in saline environment, Victoria responds the least favorably to the treatment of methyl jasmonate. Our findings can aid scientists investigating saline agriculture by illuminating the efficacy of methyl jasmonate in preventing salt stress and presenting fresh opportunities for its use in farming.

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## Conflict of Interest

The authors declare no conflict of interest.

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