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GRAFTING: A TOOL TO AMELIORATE GROWTH- INHIBITION CAUSED BY SALINITY-INDUCED OXIDATIVE DAMAGE IN WATERMELON

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Abstract

Salinity is a significant limitation to agricultural crop productivity worldwide, with varying responses observed among different plant species and cultivars. To address this issue, a study was conducted to identify salt-tolerant rootstocks for watermelon grafting. Several experiments were carried out to select the suitable grafting technique used for grafting of watermelon. In the experiment, effect of 2 salinity levels (control and 9 dS m^{-1} of NaCl) was investigated on the basis of growth, physio-morphological and biochemical attributes of salt tolerant rootstocks (Kalash, Crystal Long, Green Round and Desi Bittergourd) and two grafting techniques (hole insertion and splice grafting) were used for improvement of watermelon cultivar (Augusta). Rootstock and scion cultivars seeds were grown in plastic pots, using fine sand as growth medium. Half strength of Hoagland solution was applied to plants as nutrition. The grafted plants were treated with two different concentrations of NaCl. Symptom scoring was conducted, and subsequent measurements included number of leaves, root length, stem length, and diameter per plant, dry matter production was considered. Data of various morphological, physiological and biochemical parameters were collected and analyzed statistically using standard statistical procedures. The study revealed that Crystal Long variety of bottle gourd was salt tolerant and performed better when watermelon is grafted on it.

1. INTRODUCTION

Abiotic stresses cause more than 50% yield reduction in crops worldwide ((Acquah-Lamptey & Brandl, 2018). Abiotic stresses especially cold, drought, salinity, and heat affect plant survival, lowers biomass and yield up to 70% (Ahmad et al., 2012). Salinity and drought restrict the growth and production of plants leading to food security (Chaves

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et al., 2009). In plants the salt stress ultimately causes the oxidative stress with increased production of reactive oxygen species (Oukarroum et al., 2015). The soil salinity causes both ionic toxicity and osmotic stress, which can be lethal for plants when they are exposed to saline conditions for a long time (Chen & Jiang, 2010). Depending on the genotype and the stage of development plant undergoes different morphological, physiological and biochemical responses which causes severe crop loss (Nabati et al., 2011). Plants growth is retarded due to alteration in physiological process such as photosynthesis, stomatal conductance, osmotic adjustment, ion uptake, protein synthesis, nucleic acid synthesis, enzymatic activity and hormonal balance. Severely affected vegetative growth variables are leaf area, dry mass and plant height because it affects the movement of water and ions resulting in ion toxicity and nutritional imbalance (Evelin et al., 2009).

Grafting is the asexual type of propagation, which may be natural or artificial combination of plant parts to create the continuity between vascular bundles of rootstock and scion (Pina & Errea, 2005). This technique has been used in the in East Asia to preserve inadequate arable land for vegetable production (Kubota et al., 2008). Grafting is affected by type of graft, plant species, incompatibility, environmental circumstances, selection of rootstock and craftsmanship of grafting (Yassin & Hussen, 2015). The common methods for grafting vegetables are the tube, tongue, hole insertion and cleft grafting (Lee et al., 1994). Soil-borne problems can be reduced and tolerance against biotic and abiotic stresses can be improved through grafting method (Keatinge et al., 2014). Grafting of elite and commercial cultivars on vigorous rootstocks is one of adaptive methods for plants to cope with various environmental stresses faced by plants (Yetisir & Sari, 2003). Lowering the amount of salts moved up by the plant and their accumulation in the tissues of rootstocks are the two tolerance mechanisms of the salt resistant rootstocks, which help in preventing the salts from moving into the scion and causing fatal effects (Munns, 1993).

Grafting has been proposed as a substitute technique to improve the tolerance in vegetables against abiotic stresses, especially to overcome salinity (Yassin & Hussen, 2015). In vegetable grafting, the solutes are translocated in scion through xylem, whereas quality traits are influenced by rootstock (Nicoletto et al., 2012). This can incorporate over and under growth of the scion which leads to vital alterations in nutrients, minerals and water uptake (Yassin & Hussen, 2015). Although it is a slow process but it is eco-friendly (Lee et al., 2010). Grafting technique is getting popularity due to the development of well-organized commercial production methods and addition of new rootstocks with adorable attributes that are adaptable to locally selected scion varieties (Kubota et al., 2008).

Watermelon, among other cucurbits has the largest area under cultivation; this is because of its high economic efficiency, large ecological range, ease of cultivation, good ability to transport and storage. It is salt sensitive crop that contains high water and sugar contents (93% water, 7.5-7% sugar) and contains vitamins A, B and C (D. Schwarz et al., 2010). This crop is affected by many diseases like anthracnose, scab, gummy stem blight, alternaria leaf blight and powdery mildew, insect attack, abiotic stresses and flower

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production which ultimately affect the yield. In Pakistan watermelon cultivation occupies an average area of 149,887 ha with the annual production of 565,282 tons (Ekhtiari et al., 2019). Punjab produces more watermelon as compared to other provinces of Pakistan. The causes of low production in Pakistan are scarcity of water and soil salinity. The performance of crops under saline conditions might be enhanced through genetic-engineering approaches, conventional plant breeding or selection (Ashraf & Akram, 2009). Grafting with resistant rootstocks may enhance the ability of watermelon to tolerate salt stress.

2. REVIEW OF LITERATURE

Stress is the state which implements negative effect on an organism resulting in an injury, disease or abnormal physiology (Jaleel et al., 2009). Crop production is reduced by different biotic and abiotic stresses (Sud et al., 2008). The stress occurs in the plants due to living organisms like bacteria, fungi, viruses, insects, cultivated plants and weeds is called biotic stress (Atkinson & Urwin, 2012). Abiotic stress is the stress induced by non-living factors such as drought, irradiations, heavy metals, salinity and temperature (Alexieva et al., 2003). As a result of these stresses the physical and chemical variations take place in the individual and ultimately reduces yield up to 70% (Thakur et al., 2010).

Abiotic stresses like salinity can be controlled by various ways like breeding that may be conventional method or biotechnology, seed priming and exogenous application of growth promoters. The conventional breeding method is most common. It includes emasculation, selective breeding, budding, grafting and many more. In vegetables, grafting technique is getting popular. Grafting is the technique of uniting the branches of one plant with the roots or base of other plant (Youquan et al., 2011). Grafting vegetables onto compatible rootstocks offers several advantages like resistance to soil pathogens mainly Verticillium and Fusarium, improved yield particularly in infested soils and greater tolerance to salt stresses (Bletsos et al., 2003) (Rivero et al., 2003).

Several studies have demonstrated that stress-tolerant rootstocks could improve the growth and early yield of cucumber by reducing the degree of lipid peroxidation and electrolyte leakage under stress conditions (J. Schwarz et al., 2010). Breeding programs to select or improved rootstocks seem to have started a trend toward higher-quality product from grafted plants. (DeSantis et al., 2006) suggested that improved nutrient uptake in grafted seedlings increases photosynthesis. (Itoh et al., 2007) stated that compared with other rootstocks, watermelon grafted onto bottle gourd causes early formation of female flowers. Positive effects of grafting of watermelon, including an increase in fruit firmness, brix, and lycopene content (Davis et al., 2008). The grafted watermelon could increase lycopene and total carotenoids by 20% and amino acids, especially citrulline, by 35% (Davis & Maerz, 2008). Most reports on grafting suggest that changes in the scion are controlled by the rootstock through controlled uptake, synthesis and translocation of water, minerals and plant hormones (Lee et al., 2003). (Castillo et al., 2005) demonstrated that phloem proteins from the rootstock can migrate from the rootstock through the phloem to the scion and accumulate in the phloem and apical

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tissues. Cucurbit plant stems usually secrete xylem sap when decapitated, which is greatly influenced by the rootstock and contains high concentrations of minerals and plant hormones. It was also observed that growth promotion occurs in the cucumbers when Cucurbita rootstocks were used (Farhadi et al., 2016). Watermelon cultivar "Esmeralda" was non-grafted, self-grafted and grafted on two different squash cultivars under three different saline conditions (control, 100 mM, 150 mM NaCl). It was observed that the grafted plants showed significantly better growth on all treatments as compared to non-grafted and self-grafted plants (He et al., 2009). Grafted watermelons with salt-tolerant rootstocks showed increase in yield (Yanyan et al., 2018).

3. MATERIALS AND METHODS

Seeds of rootstocks were collected from National Agricultural Research Centre (NARC), Islamabad. The names of crops and varieties are given in Table 3.1. Seeds were sown by using sand as a media in black plastic pots in growth chamber of Hort. Lab U.C.A., Sargodha at temperature 28 ± 2 ˚C and fluorescent lamps were used to produce a photosynthetic photo flux density of 88 µM m^{-2} s^{-1}. Seeds were sown in black plastic pots of 4 inches size. Salinity levels (control, 3, 6, 9 and 12 dS m^{-1} of NaCl) were induced before sowing of seeds and at seedling stage. The seeds were watered according to their need by observing the moisture of sand. Half strength (0.5) Hoagland nutrient solution (Table 3.2) was applied to plants to fulfill their nutritional needs. Data was collected at seedling stage.

3.1 Morphological Attributes

The morphological attributes of shoot length (cm), root length (cm), shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), root dry weight (g), number of leaves per plant and salt tolerance index were calculated by same procedures as described earlier in 3.1.2.2. to 3.1.2.9 respectively.

3.1.1 Leaf Area (cm^2)

The leaf area of randomly selected leaves from each plant was measured in cm^2 through electric leaf area meter (LI-3100; LI-COR, Inc., Lincoln, Nebr) as done by Amira and Qados, 2011.

3.1.2 Internodal Distance (cm) per Plant

The internodal distance was measured from one node to other node of watermelon plants with the help of meter rule in cm (Yuge et al., 2016).

3.2 Physiological Attributes

3.2.1 Gaseous Exchange Characters

The fully expanded young leaf of each plant (the third leaf from the top) was used to measure the instantaneous net transpiration rate (E) in µmol CO^2 m^{-2} s^{-1} and photosynthetic rate (Pn) in mmol H_2O m^{-2} s^{-1}, by using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England).

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The readings were taken during day time from 10:00 a.m. to 12:00 p.m. with molar flow of air per unit leaf area 403.3 mmol m^{-2} s^{-1}, atmospheric pressure of 99.9 kPa, pressure of water vapors into chamber ranged from 6.0 to 8.9 mbar, the temperature of leaf within the chamber was 30 ± 2 ˚C and the ambient CO_2 concentration was 352 µmol mol^{-1} (Moya, 2003; Colla et al., 2006).

3.2.2 Water Use Efficiency (Pn/E)

Water use efficiency was calculated by formula used by Hatfield and Dold, (2019), that is as following.

WUE = the net photosynthetic rate (Pn) / Transpiration rate (E)

3.2.3 Relative Water Contents (RWC %)

Relative water content of leaves was concluded by the method described by Smart and Bingham (1974) and Bikdeloo et al. (2021). Three youngest fully expanded leaves from randomly selected plants were selected, removed and sealed in clean plastic bags. They were then washed with tap water and dried by tissue paper. After fresh weight determination, the fully hydrating fresh leaves in darkness at 4 °C for 24 h. Following surface drying with the aid of paper towels the turgid weight determined, the leaves were oven-dried at 85 C overnight and then they were reweighed to check the dry weight per leaf. Results were stated as percentage. The formula used for calculation of relative water content was as following.

RWC = [(fresh weight -dry weight) / (turgid weight -dry weight)] × 100

3.2.4 Leaf Water Potential

The third fully expanded young leaf from three randomly selected plants of each treatment were cutted with the surgical blade along petiole and then the leaf water potential was measured with Scholander type pressure chamber (ARIMAD-2, ELE Israel). The measurements were taken during day time from 10:00 a.m. to 12:00 p.m.

3.2.5 Leaf Osmotic Potential

The same leaves which were used for taking leaf water potential were sealed in plastic bags and frozen in refrigerator below -4 ˚C. Then the frozen leaves were thawed and extraction of cell sap was done through surgical syringe. An osmometer (Vapro-5520, USA) was used and 10 µl extracted sample was used to determine the osmotic potential.

3.2.6 Leaf Turgor Potential

The leaf turgor potential was determined by taking difference between osmotic potential and water potential (Barrs, 1968). The formula is as following:

Turgor potential = osmotic potential – water potential

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3.3 Biochemcial Attributes

3.3.1 Chlorophyll Contents

The soil plant analysis development (SPAD) meter was used to check the chlorophyll contents of leaves. It used red and infra-red light of wavelength 660 nm and 940 nm respectively. The values were taken during day time.

3.3.2 Malondialdehyde (MDA)

The malondialdehyde (MDA) which is produced because of lipid peroxidation was obtained by using the thiobarbituric acid (TBA) as done by Wang et al. (2009). The fresh leaves of weight 1 gram were placed in the mortar containing 5 ml 0.6% TBA in 10% trichloroacetic acid (TCA) and were ground with a pestle. The mixture was then heated for 15 min at 100 °C. Later on these samples were placed on ice for 5 min to let them cool down, then the centrifugation of mixtures at 5000 $r \cdot min^{-1}$ was done for 10 min. The absorbance of the supernatant at 450, 532 and 600 nm wavelengths was recorded and MDA contents were calculated on the basis of fresh weight by using following formula:

$$MDA\ (mmol \cdot g^{-1}\ FW) = [6.45 \times (OD532 - OD600) - 0.56 \times OD450] \times Vt / (Vs \times F.W)$$

Here: OD is optical density, Vt is the volume of extracted liquid (ml), Vs is the volume of extraction solution (ml), F.W is the fresh weight of samples (g)

3.3.3 Glycine Betaine (μmol/g f.wt)

The glycine betaine in leaf and root tissues was determined following Grieve and Grattan (1983). From each replicate, the fresh leaf and root material (1.0 g) was shaken for time period of 5 min in 10 ml of 0.5% toluene solution and then was filtered. After filtration, the extract of volume 1 ml was mixed with 2N 39 H_2SO_4 of volume 1 ml. Then 0.5 ml from this mixture was put in a glass tube and 0.2 ml of potassium tri-iodide (KI_3) solution was added in it. The ice cooled distilled water of volume 2 ml was used and 6 ml of 1-2 dichloroethane (cooled at 48 ˚C) were added to the mixture. The upper aqueous layer was discarded. The organic layer was measured at 365 nm by using Spectrophotometer (Hitachi-120, Japan). Blank was developed by using distilled water and the concentrations of glycine betaine in leaves and roots were calculated against standard curve, which was determined as μmol g^{-1} fresh weight.

3.3.4 Proline (μmol/g f.wt)

To evaluate the proline level content, 0.5 g of leaves and roots samples in 3% (w/v) sulfosalicylic acid was homogenized and then filtered homogeneously through filter paper, as described by Bates et al. (1973). Two ml of the filtrate was put in a 25 ml test tube and then reacted with 2 ml acid ninhydrin solution (acid ninhydrin solution was prepared by dissolving 1.25 g ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M orthophosphoric acid) and glacial acetic acid of volume 2 ml and test tubes were heated for 60 minutes at temperature of 100 ˚C. Reaction was abolished in an ice bath; the mixture was extracted by using 10 ml toluene which formed a chromophore. The air stream was passed continuously for about 1-2 minutes in the reaction mixture for the sake

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of separation of aqueous phase from the chromophore having toluene. Isolated colored phase was allowed to settle for about 2-3 minutes at room temperature and its absorbance was noted at 520 nm by using spectrophotometer. Blank was developed by using toluene. The proline concentration was calculated by using a standard curve, which was determined as μmol g-1 fresh weight.

3.4 Enzymatic Attributes

The fresh leaf material of weight 0.5 g from each replicate was grinded well by using an electrical grinder. Then 5 ml of cooled 50 mM phosphate buffer having pH 7.8 was added to the grounded sample. Then the homogenate was vortex and centrifuged at 15,000 rpm for about 15 min at temperature of 48 ◦C. The supernatant was removed and used for the assays of enzymes.

3.4.1 Superoxide Dismutase (SOD) (units/mg protein)

The SOD activity was determined following Giannopolitis and Ries (1977) by appraising the photoreduction of nitroblue tetrazolium (NBT) by the enzyme. The reaction mixture contained methionine (13 mM), NBT (50 mM), riboflavin (1.3 mM), phosphate buffer (20 mM), EDTA (75 nM) and enzyme extract which were homogenized in a test tube. This solution was irradiated for 15 minutes under white fluorescent light (15W lamp) at 80 mmol m^{-2} s^{-1}. Then the optical density of the solutions was read through a spectrophotometer at 560 nm wavelength. The amount of enzymes required to inhibit half of NBT photoreduction was considered equal to one unit of SOD activity.

3.4.2 Peroxidase (POD) (units/mg protein)

The protocol of Chance and Maehly (1955) was followed for the determination of the activities of POD. The changes in absorbance were recorded at 470 nm after every 30 second. A change in the absorbance per minute was considered equal to one unit of POD activity.

3.4.3 Catalase (CAT) (units/mg protein)

The activities of CAT were determined by following procedure stated by Chance and Maehly (1955). Three ml of reaction solution was used for the determination of CAT activity contained 0.1 ml enzyme extract and 50 mM phosphate buffer having pH 7.8. The reaction was started by the addition of enzyme extract. The changes in absorbance of the reaction solution after every 20 second were measured at 240 nm. A change of 0.01 units per minute in absorbance was considered equal to one unit CAT 40 activity. The activity of each enzyme was calculated and expressed on the basis of total protein. Protein concentration of the extract was measured following Bradford (1976).

3.5 Ionic Attributes

3.5.1 Na+ and K+ Contents (mg g^{-1} d.wt)

Firstly the digestion of plant material was done in the way that the dried and grounded plant samples of leaves and roots (0.5 g) were put in digestion tubes and then concentrated H_2SO_4 of (5 ml) was added as described by Wolf, (1982). Then the digestion

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tubes were incubated whole night at room temperature. Then H_2O_2 (0.5 ml) was added and tubes were transferred in digestion block. At that time the tubes were continuously heated for 30 minutes at temperature 350 ˚C unless fumes started to produce. Then they were allowed to cool down for 5 minutes. Then 0.5 ml of H_2O_2 was slowly added again and tubes were put back to digestion block. The above mentioned step was repeated until the colorless digested material was attained. The digest was filtered and put in volumetric flasks to make the volume up to 50 ml. Then the digested material was analyzed for Na+ and K+ ions contents through Flame photometer (Jenway PFP-7, UK). The standard curves were drawn and then the values were computed in mg g-1 D.Wt.

3.5.2 Cl- Contents (mg g^{-1} d.wt)

Chloride ions in the plant samples of leaves and roots were extracted by heating the material in water. Firstly the samples were oven dried and then grounded with the help of mortar and pestle. The grounded leaf and root samples (0.1 g) were taken in test tubes and 10 ml of distilled water was added to them. Then they were incubated overnight at temperature of 25 ˚C. The tubes were then heated at 80 ˚C in a digestion block until the volume in the test tubes remained half of the original volume. After cooling them, distilled water was added to each test tube to maintain the volume up to 10 ml again and Cl^- concentration in the leaf and root extracts was determined by using a chloride analyzer (Corning-920, Germany).

3.6 Statistical Analysis

The experimental units were arranged in a Complete Randomize Design (CRD) two factorial (Steel et al., 1997) with 4 replications. Each replication was consisted of 30 replicates. The collected data was analyzed statistically by using Statistix 8.1 and analysis of variance (ANOVA) and multiple comparisons were done by using Tukeys' test. Significance was considered at $p \leq 0.05$ after statistic analysis.

4. RESULTS

4.1 Effect of salinity stress on morphological attributes of ungrafted and grafted watermelon plants

4.1.1 Effect of salinity stress on number of leaves per plant

Grafted plants have more number of leaves than non-grafted watermelon plants. The plants grafted on bottle gourd rootstock showed maximum number of leaves having average of 11.75 leaves per plant followed by Watermelon/Squash combination which had 10.25 leaves per plant on an average in control whereas, non-grafted watermelon plants had minimum number of leaves with average 5.75. However, the best performance regarding number of leaves per plant was shown by watermelon plants grafted on bottle gourd rootstock at both conditions followed by squash. The worst performance was shown by non-grafted plants. The minimum reduction was at Watermelon/Bottle gourd combination (25.53%), although maximum was noted in ungrafted watermelon plants (56.52%). So, it can be concluded that grafted plants can withstand salinity stress.

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4.1.2 Effect of salinity stress on shoot length per plant

Non-grafted watermelon plants showed poor performance than grafted ones. Shoot length per plant was significantly affected by salinity and rootstock genotype. With the increase of salt level from control to 9 dS m^{-1} of NaCl, the decreased shoot length was noted in ungrafted, self-grafted and other grafted watermelon plants. The self-grafted plants showed good performance as compare to ungrafted plants those produced on Watermelon/Muskmelon combination. This is the clear evidence of negative effect of salinity on plant shoot length. Grafting reduced the salinity effects significantly on shoot length per plant. Among all rootstocks studied under this experiment, bottle gourd rootstock performed better followed by squash. It can be concluded that bottle gourd (Crystal Long) is salt tolerant rootstock as compare to squash (Green Round) and muskmelon (Kalash) rootstocks.

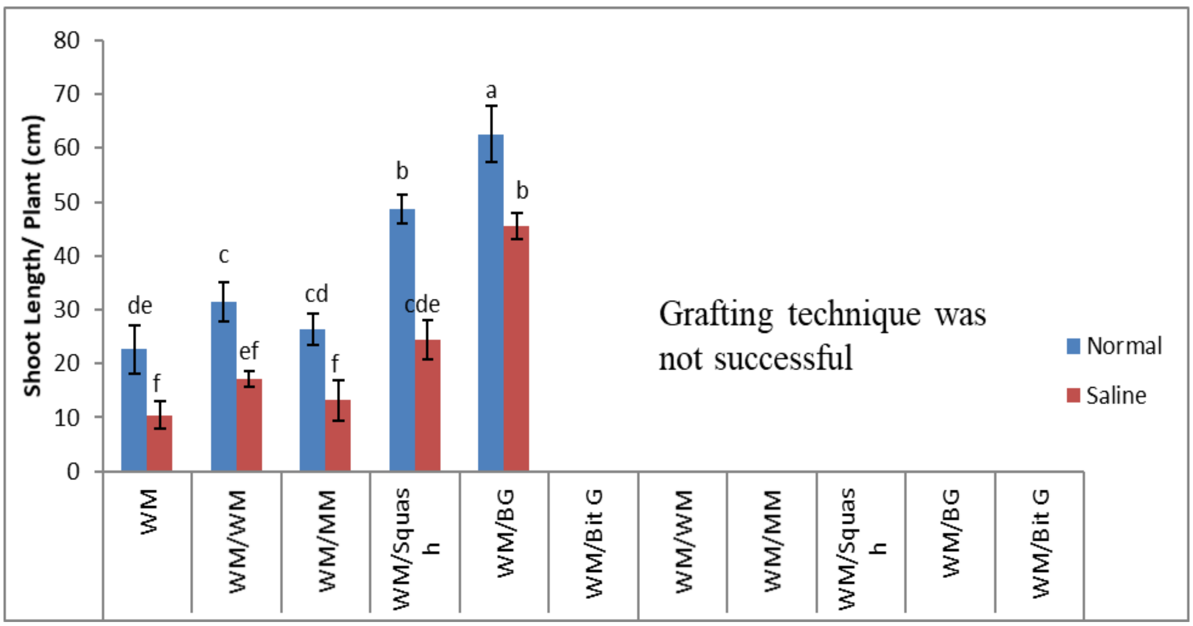

Fig 4.1.2: Effect of grafting combinations and salinity levels on shoot length/plant of watermelon plants

4.1.3 Effect of salinity stress on root length per plant

The significant reduction in root length was observed as the salinity conditions altered from control to 9 dS m^{-1} of NaCl. It decreased in all ungrafted, self-grafted and grafted plants on muskmelon, squash and bottle gourd rootstocks. In control, the maximum root length was obtained by watermelon plants grafted on bottle gourd rootstock with average 28.575 cm per plant although 9.825 cm per plant at average was recorded by the roots of ungrafted watermelon plants, which is the minimum one. Root length of self-grafted, watermelon plants grafted on muskmelon and squash rootstock was 10.2 cm, 11.2 cm and 24.35 cm per plant, respectively. Under salinity level of 9 dS m^{-1} NaCl the root length of plants reduced. Minimum reduction was obtained by plants grafted on bottle gourd and

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squash rootstock with 37.71% and 37.06% reduction respectively. The ungrafted watermelon plants showed maximum decline that was 65.90%.

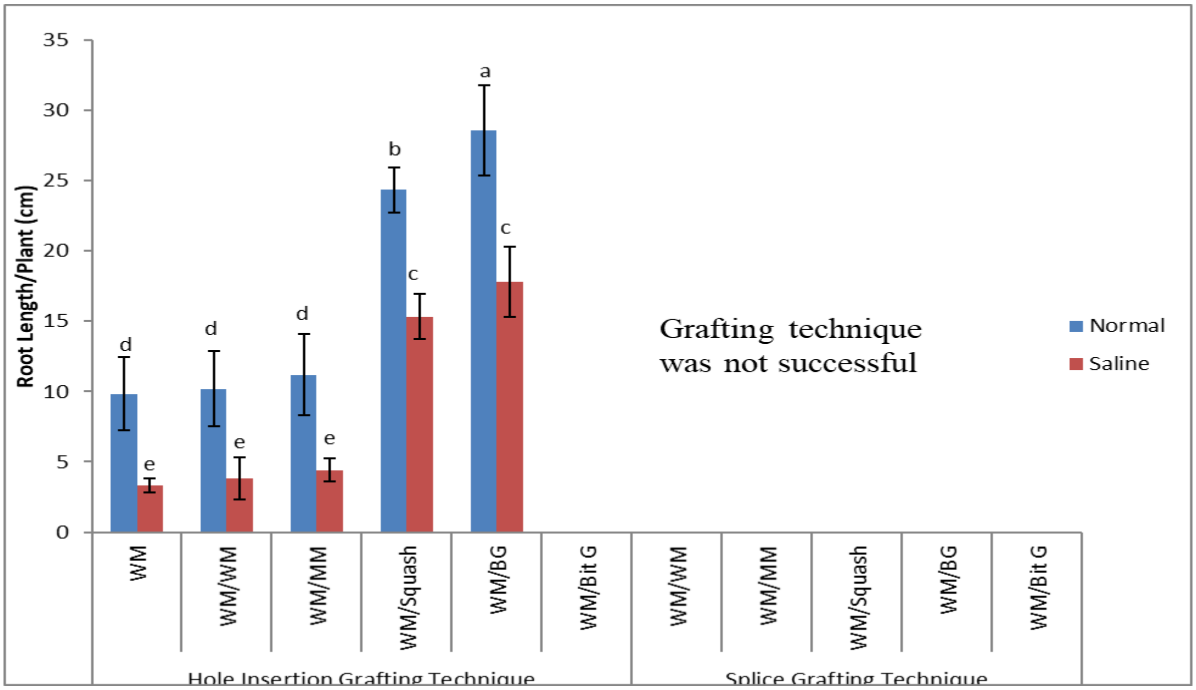

Fig 4.1.3: Effect of grafting combinations and salinity levels on root length/plant of watermelon plants

4.1.4 Effect of salinity stress on shoot dry weight per plant

Salinity imposed negative effects on the shoot dry weight per plant. As the salinity level changed from control to 9 dS m^{-1} of NaCl, the shoot dry weight decreased in ungrafted and grafted watermelon plants (Fig. 4.13). Maximum increase at control level was observed by plants grafted on bottle gourd rootstock followed by squash rootstock while its minimum value was noted in those grafted on muskmelon. In the same time at 9 dS m^{-1} of NaCl the grafted plants on bottle gourd rootstock showed more increase whereas the least was by plants grafted on muskmelon rootstock. Among all rootstocks, the plants grafted on squash rootstock performed good by having lowest reduction percentage (20.69%) while its minimum value was observed in ungrafted plants (87.17%). The self-grafted and watermelon plants grafted on muskmelon and bottle gourd rootstock showed 65.15%, 70.78% and 30% reduction from non-saline to saline levels.

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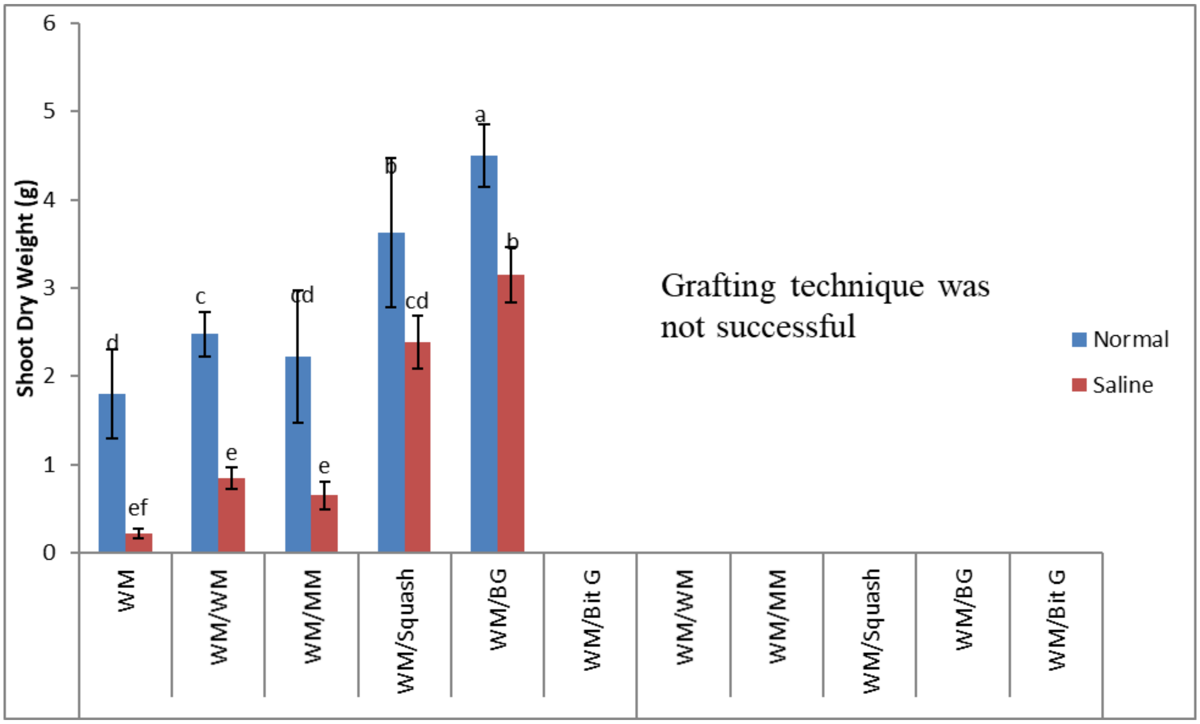

Fig. 4.1.4: Effect of grafting combinations and salinity levels on shoot dry weight/plant of watermelon plants

4.1.5 Effect of salinity stress on root dry weight per plant

Results indicated that root dry weight also decreased because of increase in salinity level (Fig. 4.14). The ungrafted, self-grafted and those which were grafted on muskmelon, squash and bottle gourd rootstock showed 82.22%, 78.86%, 78.33%, 24.32% and 25.49% reduction respectively. It is clear that squash rootstock performed good by producing less reduction in root dry weight at saline conditions. Grafted plants on bottle gourd rootstock performed better followed by squash rootstock. Lowest increase (6%) was observed by grafted plants on muskmelon rootstock while self-grafted showed 17.77% increase in root dry weight at saline level. Similar results were obtained at control level. From the results it is concluded that grafting improved the effects on the roots of grafted watermelon plants by increasing root dry weight.

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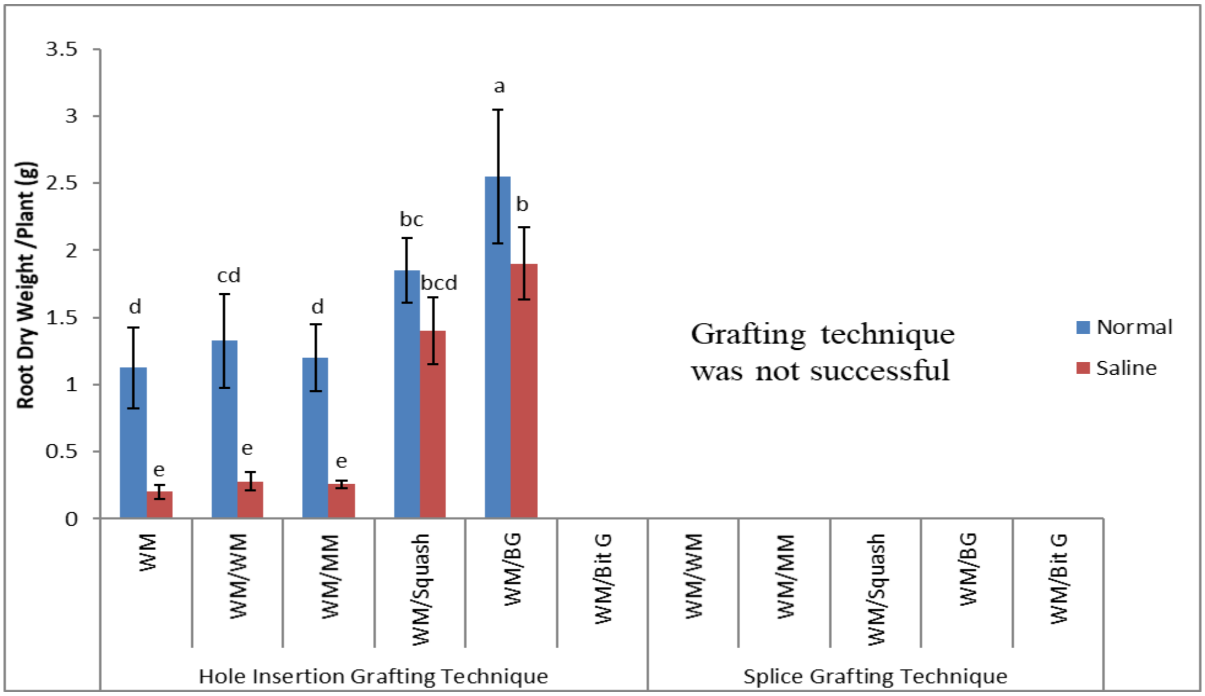

fig. 4.1.5: Effect of grafting combinations and salinity levels on root dry weight/plant of watermelon plants

4.1.6 Effect of salinity stress on stem diameter

The significant decrease of stem diameter was obtained with the change of salinity level from control to 9 dS m^{-1} of NaCl (Fig. 4.15). Among watermelon plants grown at control and saline levels the stem diameter increased in grafted plants as compared to ungrafted ones. Maximum increase was observed in watermelon plants grafted on bottle gourd rootstock (86.08%). Although minimum increase was noted by self-grafted watermelon plants i.e., 9.56% here the ungrafted plants were considered as control. At saline conditions, the stem diameter of grafted and ungrafted plants decreased as compared to plants grown under control level. Minimum reduction was obtained by the plants grown on bottle gourd rootstock while maximum reduction in stem diameter was faced by ungrafted and self-grafted watermelon plants. As compare to ungrafted watermelon plants the grafted plants showed increase in stem diameter at salinity levels also. From results it is obvious that grafting imparted positive effects on the stem diameter of all grafted plants grown under saline or non-saline environment.

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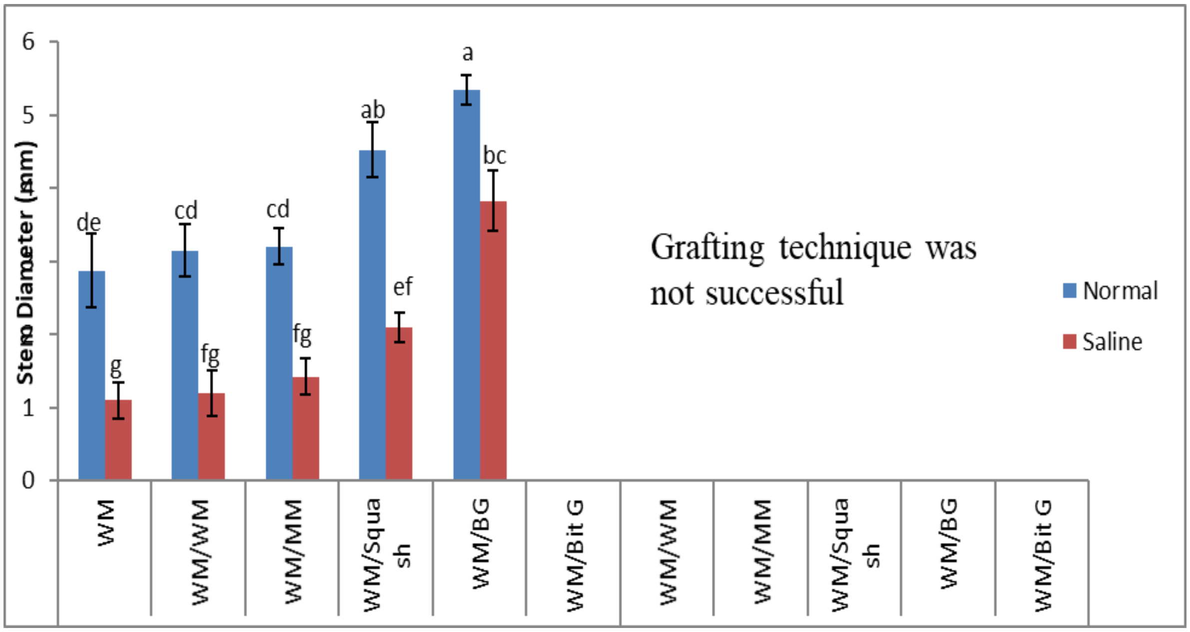

Fig 4.1.6 Effect of grafting combinations and salinity levels on stem diameter/plant of watermelon plants

4.2 Effect of salinity stress on ionic attributes of ungrafted and grafted watermelon plants

4.2.1 Effect of salinity stress on sodium (Na^+) ions contents in leaves.

The influence of rootstocks was significant on the sodium ion concentrations in leaves. Sodium ion contents in leaves were increased by increasing salinity stress. The Na^+ ion contents varied among all rootstocks (Fig. 4.16). In leaves of ungrafted watermelon plants the increase in Na^+ ions concentration was maximum (35.55%) as compared to control plants by sodium chloride treatment. The leaves of plants grafted on bottle ground rootstock showed minimum absorption of Na^+ ions at control as well as 9 dSm^{-1} of NaCl treatment followed by squash and muskmelon root stock. The maximum absorption was by ungrafted and self-grafted plants by having 5.64 and 4.8975 mg g^{-1} D.Wt .Positive response in terms of absorbance of Na+ in leaves by ungrafted and grafted plants was obtained with the increase of salinity level from control to 9 dS m^{-1} of NaCl.

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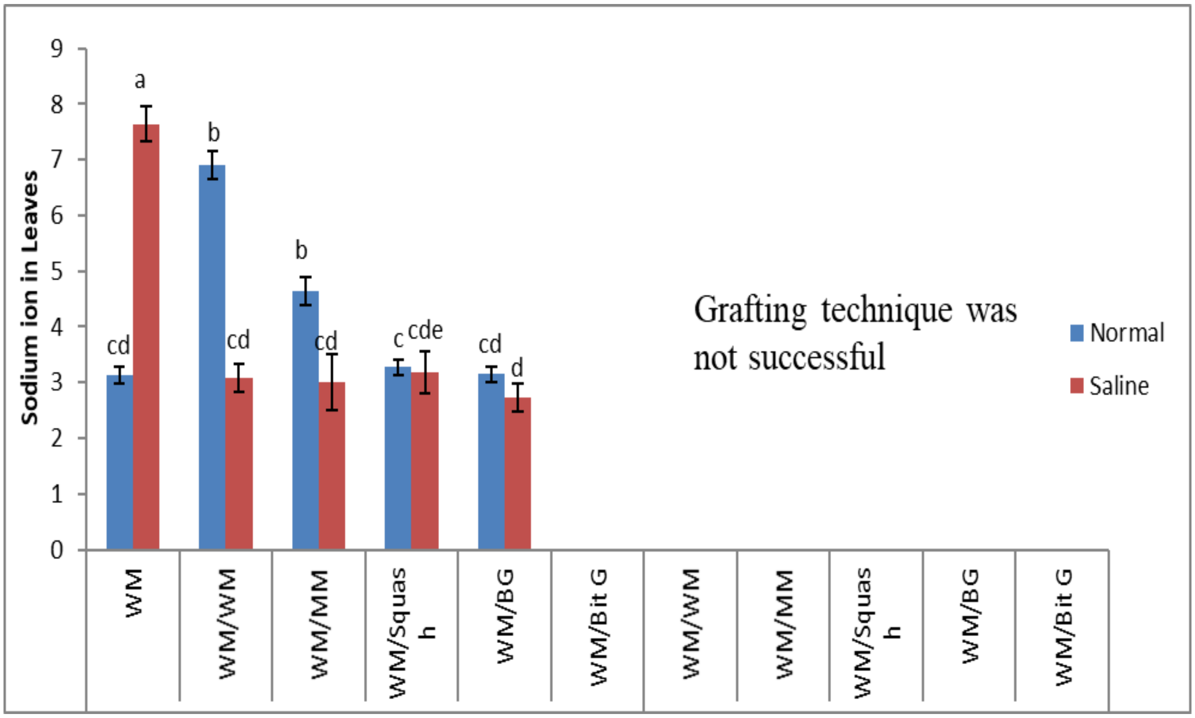

Fig. 4.2.1 Effect of grafting combinations and salinity levels on sodium ions in leaves of watermelon plants

4.2.2 Effect of salinity stress on sodium (Na+) ions contents in roots.

Among ungrafted and grafted plants the Na+ ions absorbance decreased such as in ungrafted plants grown at control level the absorbance of Na+ ions recorded in roots was more than the grafted plants of bottle gourd, squash and muskmelon rootstock. In the same way, at 9 dS m^{-1} of NaCl the minimum quantity of sodium ions was obtained by grafted plants on bottle gourd rootstock followed by squash rootstock while maximum was by ungrafted watermelon plants. The increase of 77.28% was observed in ungrafted plants grown in saline conditions as compare to control level of NaCl. The minimum increase (3.70%) was noted by grafted plants on bottle gourd rootstock. Hence proved that bottle gourd rootstock is salt tolerant one.

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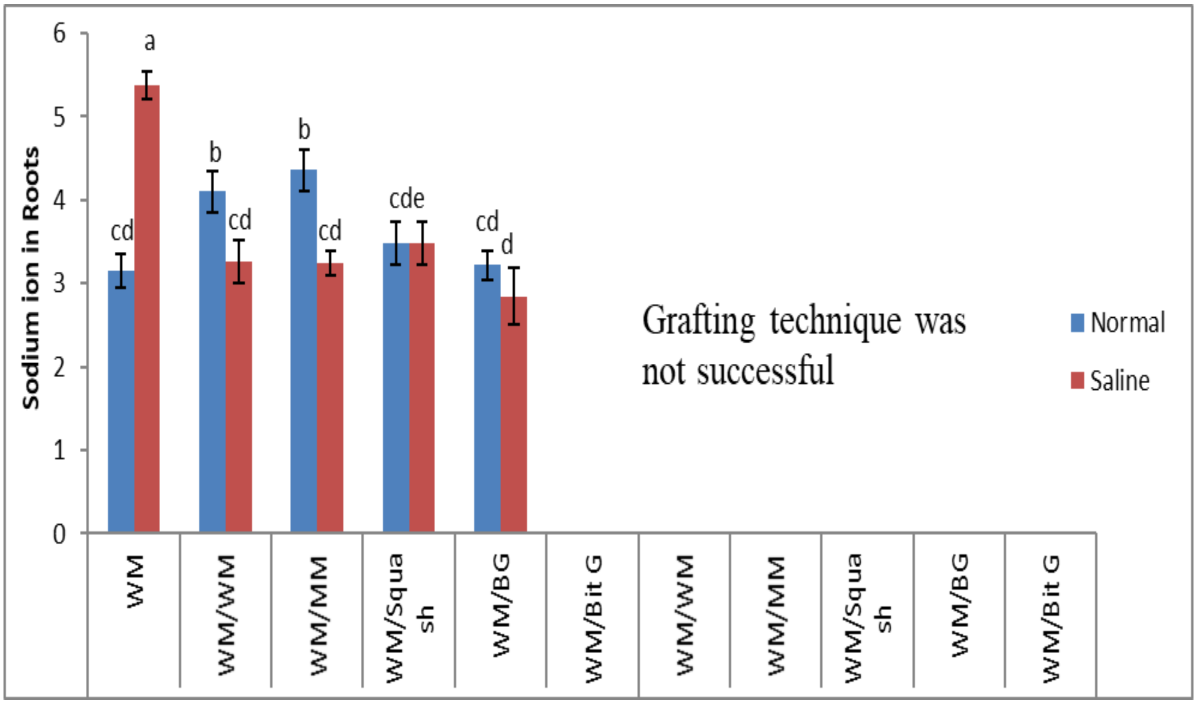

Fig 4.2.2: Effect of grafting combinations and salinity levels on sodium ions in roots of watermelon plants

4.2.3 Effect of salinity stress on chloride (Cl-) ions contents in leaves.

The rootstocks imparted positive influence on the chloride ions accumulation in leaves of grafted watermelon plants whereas it increased with the increase of salt stress (Fig. 4.18). In leaves of ungrafted plants grown at control as compared to 9 dS m^{-1} of NaCl salinity treatment showed maximum increase (40.73%) whereas self-grafted, grafted plants on muskmelon, squash and bottle gourd rootstock produced 9.2%, 12.58%, 2.4% and 1.6% increase. Overall Cl^- ions accumulation in leaves was maximum in ungrafted plants at control and saline level i.e., 4.9225 and 6.9275 mg g^{-1} D.Wt respectively. While minimum was in grafted plants on bottle gourd rootstock at both levels of salt treatment that are 3.135 and 3.19 mg g^{-1} D.Wt Hence, results demonstrates that minimum accumulation of Cl-1 ions was observed by grafted plants on bottle gourd rootstock and is considered as salt tolerant rootstock; while ungrafted plants are sensitive to salinity.

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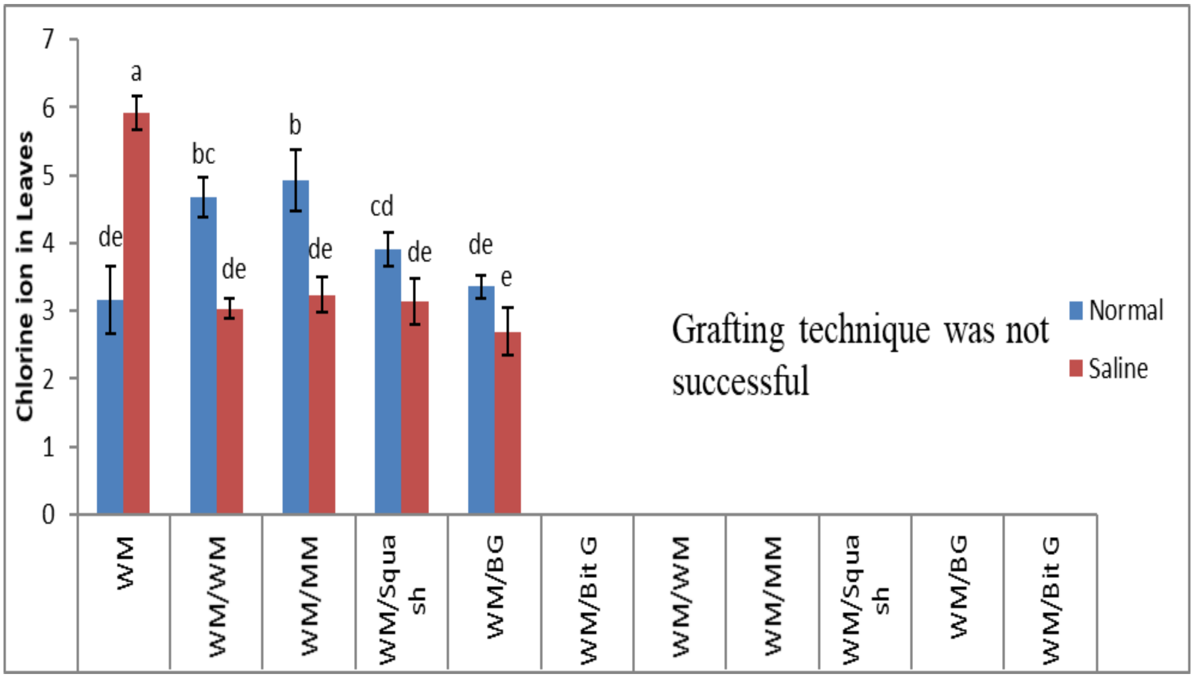

Fig 4.2.3: Effect of grafting combinations and salinity levels on chlorine ions in leaves of watermelon plants

4.2.4 Effect of salinity stress on chloride (Cl^-) ions contents in roots.

The chloride ions concentration in roots is significantly different among ungrafted and grafted watermelon plants. Its accumulation increased with the increase of salinity level (Fig. 4.19). The maximum chloride ions accumulation was observed at control and salinity level by ungrafted plants. The grafted plants on bottle gourd rootstock performed better by minimum accumulation (2.16 and 2.24 mg g^{-1} D.Wt on control and 9 dS m^{-1} of NaCl respectively) of Cl^- ions in roots. The percentage increase of Cl^- ions contents in roots of ungrafted plants was 99.42% and 3.70% increase was observed by the roots of bottle gourd rootstock grown on control and salinity level. According to performance, watermelon plants grafted on bottle gourd rootstock ranked as salt tolerant while ungrafted plants are salt sensitive.

4.2.5 Effect of salinity stress on potassium (K^+) ions contents in leaves.

The potassium ions contents decreased significantly with the increase of salinity from control to 9 dS m^{-1} of NaCl. The maximum concentration of K^+ in leaves was recorded by grafted watermelon plants on bottle gourd rootstock i.e., 22.7175 and 16.925 mg g^{-1} D. Wt at normal and saline level respectively, followed by squash and muskmelon rootstock. The minimum concentration of K^+ ions in leaves was noted by ungrafted watermelon plants at both levels of salt. As the salt level increased from control to 9 dS m^{-1} of NaCl the accumulation of K^+ ions decrease was observed in ungrafted, self grafted, plants grafted on muskmelon, squash and bottle gourd rootstock having 52.06%, 41.18%, 28.83%, 26.68% and 25.49% reduction. Minimum K^+ ions accumulation in leaves of

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ungrafted watermelon plants categorized them as salt sensitive and bottle gourd rootstock having maximum K^+ ions accumulation categorized it as salt tolerant rootstock.

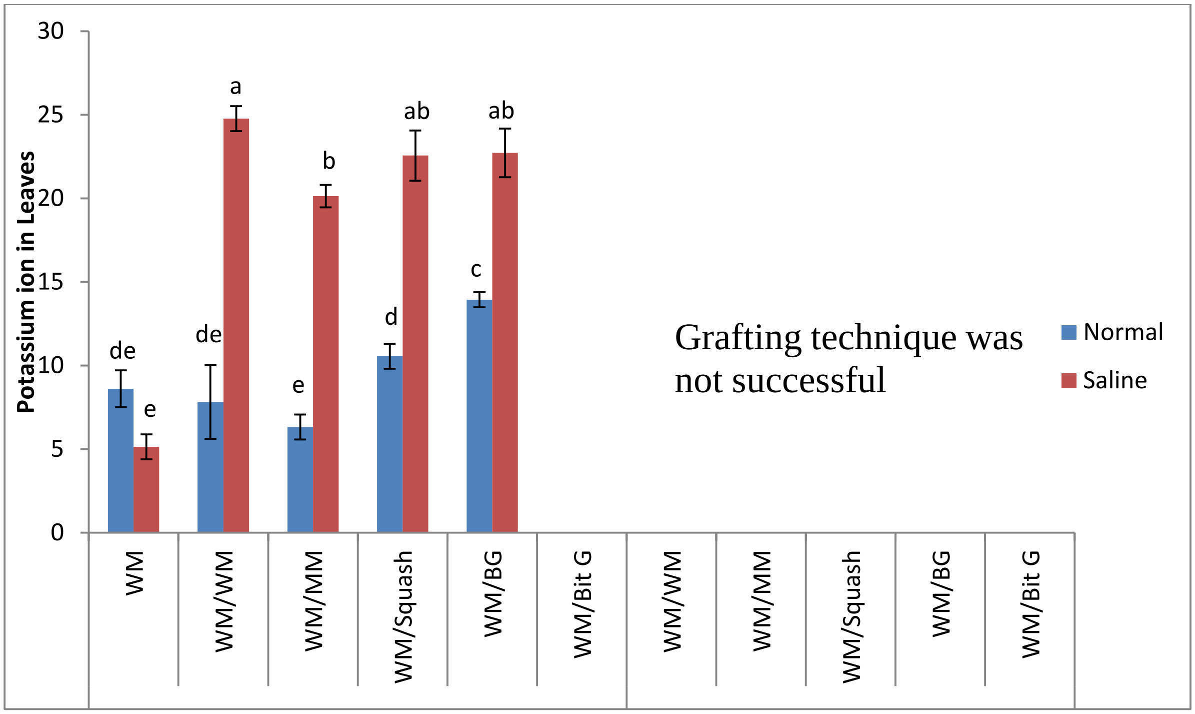

Fig. 4.2.5: Effect of grafting combinations and salinity levels on potassium ions in leaves of watermelon plants

4.2.6 Effect of salinity stress on potassium (K^+) ions contents in roots.

With the increase of salinity level K^+ in roots decreased in ungrafted and grafted watermelon plants. Maximum decrease was obtained by ungrafted plants followed by grafted plants on muskmelon rootstock i.e., 62.43% and 61.08% respectively. Whereas, plants grafted on bottle gourd rootstock showed minimum reduction of 14.84% followed by squash which showed 23.54% and self-grafted plants which showed 55.43% reduction of K^+ ions in roots. Significant change in accumulation of K^+ ions in root organ was observed in all ungrafted, self-grafted and the plants grafted on muskmelon, squash and bottle gourd rootstock at both salinity levels (Fig. 4.21).

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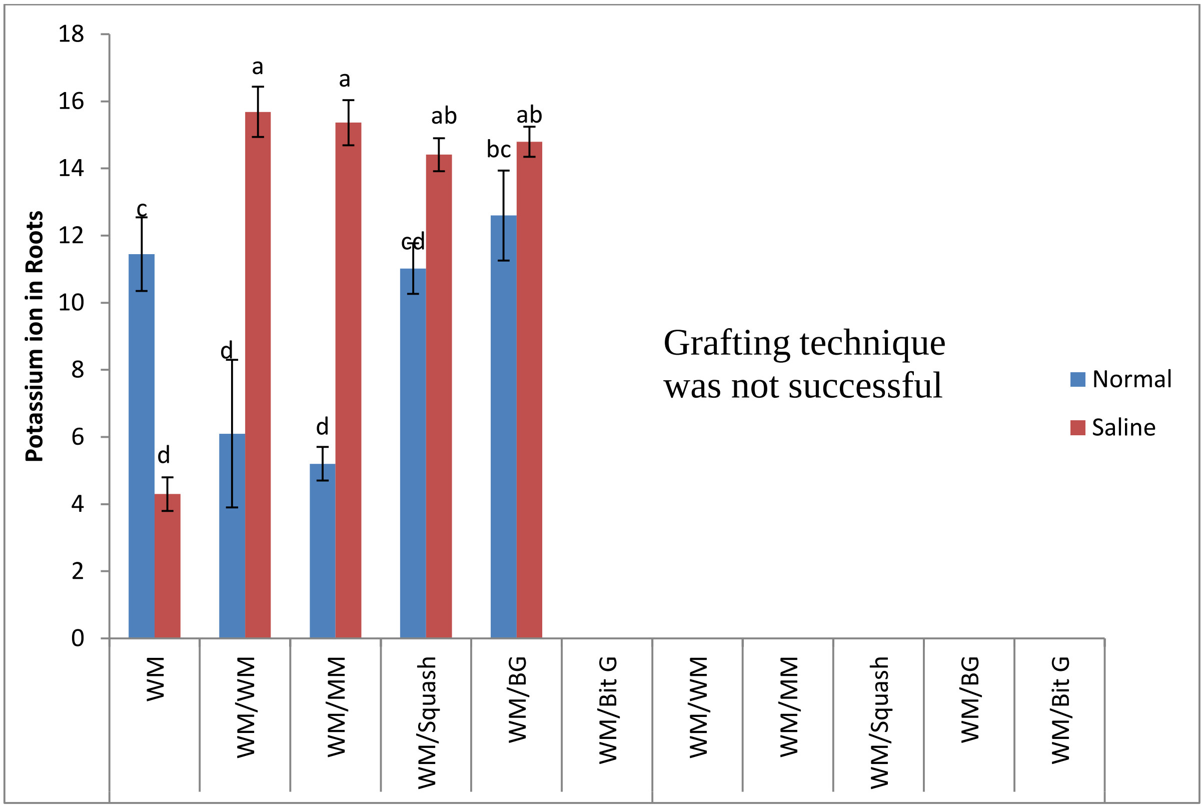

Fig. 4.2.6: Effect of grafting combinations and salinity levels on potassium ions in roots of watermelon plants

4.3 Effect of salinity stress on enzymatic attributes of ungrafted and grafted watermelon plants

4.3.1 Effect of salinity stress on activity of superoxide dismutase (SOD)

From the results it is obvious that SOD values increased with the increase of salt stress. The salt sensitive ungrafted plants showed maximum values of SOD at control and saline level i.e., 513.875 and 561.23 units/mg protein respectively. The plants grafted on salt tolerant bottle gourd rootstock showed minimum values of SOD at control and saline level i.e., 305.305 and 384.02 units/mg protein respectively. The response of watermelon plants is significant in ungrafted and grafted ones (Fig. 4.22). The maximum increase of SOD was obtained by plants grafted on squash and bottle gourd rootstock having 25.84% and 25.78% increased from normal to saline level. While self-grafted, plants grafted on muskmelon and ungrafted plants showed less increase by 1.27%, 4.75% and 9.2% respectively.

4.3.2 Effect of salinity stress on activity of catalase (CAT)

The catalase activity showed an increasing trend by increasing salt level from control to 9 dS m^{-1} of NaCl. Maximum CAT activity was observed by grafted plants grown at 9 dS m^{-1} of NaCl level. Among grafted plants the watermelon plants grafted on bottle gourd

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showed maximum (1.24 units/mg protein) followed by squash rootstock plants having 1.025 units/mg protein value. The ungrafted plants showed minimum values of CAT activity at control and 9 dS m^{-1} of NaCl level by obtaining 0.5225 and 0.855 units/mg protein respectively. Maximum increase was observed by ungrafted plants (63.63%) followed by watermelon plants grafted on bottle gourd rootstock (58.97%). The self-grafted and plants grafted on squash and muskmelon rootstock showed 41.80%, 44.88% and 45.90% increase of CAT values.

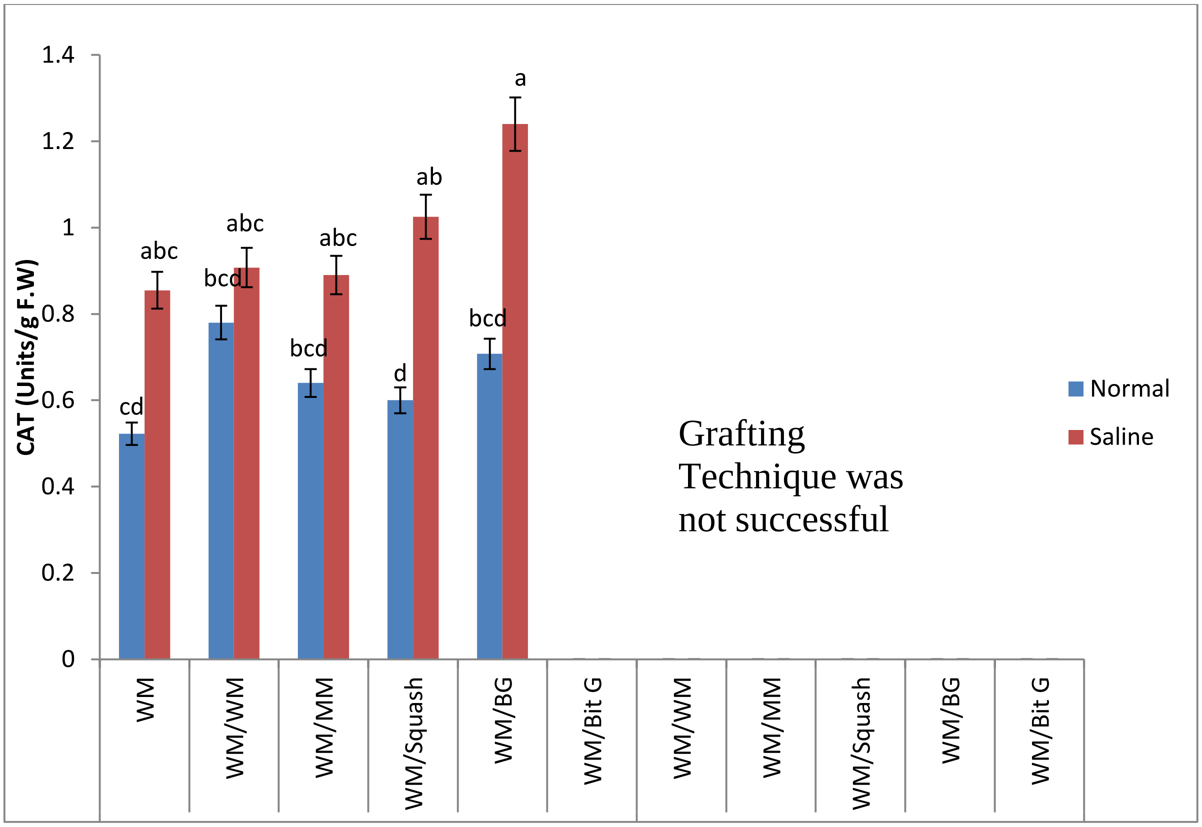

Fig. 4.3.2: Effect of grafting combinations and salinity levels on antibiotic enzyme activity (Catalase) of watermelon plants

4.3.3 Effect of salinity stress on activity of peroxidase (POD)

The POD activity increased as the exposure of plants to salinity levels increased while the salt sensitive plants showed lowest values than salt tolerant plants (Fig. 4.23). The increase in POD values observed in ungrafted plants was 20.81% followed by grafted plants on squash rootstock i.e., 20.80%. Although the increase in POD values obtained by self grafted, plants grafted on muskmelon and bottle gourd rootstock is 7.66%, 6.17% and 36.50% respectively.

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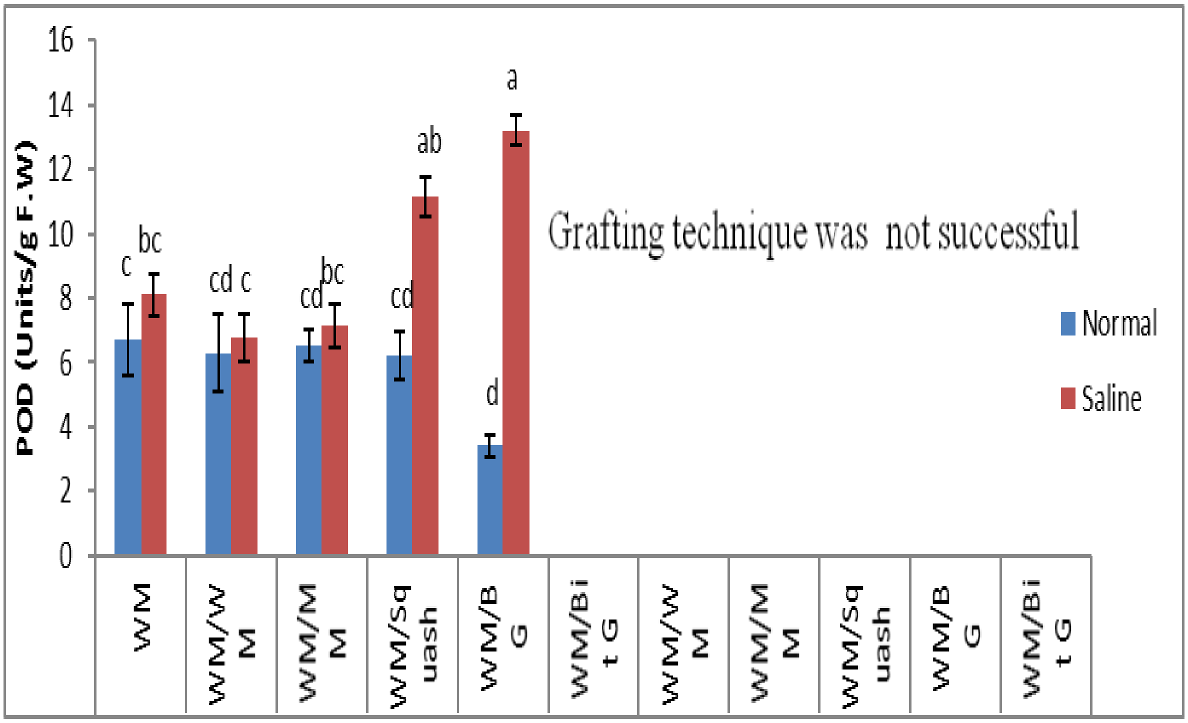

Fig4.3.3: Effect of grafting combinations and salinity levels on antibiotic enzyme activity (Peroxidase)

5. DISCUSSION

Watermelon is a very important cucurbit crop in most regions of the world, but its productivity is severely affected by many abiotic stresses especially salinity stress. All horticultural crops and especially vegetable crops are at serious risk because of rising salts levels of irrigated farmlands. The findings of current research may facilitate watermelon growers of Pakistan by reducing salinity related growth and production losses of watermelon in fairly saline farming. Salt tolerant rootstocks of cucurbit crops can be cultivated in salt affected fields and grafting watermelon on them lessens the undesirable effects of salinity stress on its yield.

The experiment was conducted to understand the effects on the growth of watermelon by grafting it on the selected tolerant rootstocks from each crop. For this purpose, two grafting techniques were used. Unfortunately splice grafting technique was not successful and also bittergourd rootstock did not work because of hollow stem. Here self-grafting was also done to examine whether it can improve watermelon growth or not. The morphological, physiological, biochemical, enzymatic and ionic attributes were studied in ungrafted and grafted plants at two salinity levels (control and 9dS m^{-1} of NaCl). The results clearly conveyed that grafting watermelon onto resistant rootstocks is an effective technique to manage the effects of salinity on the growth. The grafted plants responded different with the change of rootstock genotype while plants grafted on bottle gourd rootstock “Crystal Long” performed better than other rootstocks. The ungrafted plants

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showed the stunted shoot and root length at saline conditions (9dS m^{-1} of NaCl) it indicates their sensitivity to salt stress.

According to number of studies the shoot and root growth are inhibited by elevation of NaCl concentrations in the growth medium, but the inhibition level was varied significantly in ungrafted, self-grafted and those grafted on bottle gourd and squash rootstock (Shah et al., 2001). The minimum reduction was observed by bottle gourd rootstock, and it also promoted growth. The highest value of stem diameter was obtained by watermelon plants grafted on salt tolerant bottle gourd rootstock. A significant decrease in plant growth was observed in NaCl treated watermelon plants either ungrafted or grafted plants and the effect varied with the rootstock used. This may be due to nutritional damage, ionic imbalance and disorders of photosynthetic activities caused as the result of salinity stress (Savvas et al., 2010). Salinity stress resulted in the decreasing of leaf area but the grafted plants had more leaf area as compared to the ungrafted plants. (Neumann et al., 1988) . (Bantis et al., 2020) reported that decrease in leaf area was faced by grafting watermelon on inter-specific and squash rootstock.

Moreover, leaf and root sodium (Na^+), chloride(Cl^-) and K^+ ions contents varies significantly under salinity stress in all ungrafted and grafted watermelon plants (Rouphael et al., 2012). Differences in leaf and root Na^+, Cl^- and K^+ of rootstocks used might be because of their genetic variability and permeability of roots for these ions (Albacete et al., 2009). The results showed that plants that were poor in growth performance accumulated more Na+ as well as Cl^- contents and low K^+ concentrations in leaves and roots under saline regimes in contrast to best performing plants in terms of growth traits. The high accumulation of K^+ and low Na^+ as well as Cl^- in leaves and roots of plants grafted on bottle gourd rootstock increases the salt tolerance potential so these can be used as efficient characterizing mean (Atabayeva et al., 2013). The conclusions of the study revealed that the bottle gourd rootstock is the most tolerant rootstock. The improvement of salt tolerance in plants is related to the activation of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). Superoxide dismutase removes O_2^- which is reduced to H_2O_2 and oxidized to O_2 was reported (Yang et al., 2012). Thus, it plays an important role in the enzymatic defense system as accounted by Zhang et al. (2010). This study clearly demonstrated that rootstocks influenced the ability of watermelon at salt stress by influencing SOD enzyme activity. We determined that the total SOD activity was greatly increased in plants of Watermelon/Bottle. As the most important antioxidative enzymes, CAT, POD and APX catalyze H_2O_2 into H_2O and O_2 under stress conditions (Gong et al., 2014a, Gong et al., 2014b. This study suggested that those grafted onto bottle gourd had higher H_2O_2 scavenging capacity than those grafted onto the other rootstocks.

As a result of salt stress, the photosynthetic activity reduced (García Morales et al., 2012). Maximum reduction was observed by ungrafted and self-grafted plants while less reduction was obtained by plants grafted on bottle gourd rootstock. Grafting enhances the photosynthetic activity which ultimately increases the chlorophyll contents (Zhang et al., 2019). The non-stomatic limitation of photosynthesis is Na^+ and Cl^- contents increase

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in plant tissues, number of studies which reveal that grafting onto the salt tolerant rootstocks can improve the photosynthetic activity of plant at salt stress conditions. (Lemos Neto et al., 2021).The decrease observed in chlorophyll contents by plants at salinity stress is a common process due to many reasons; one of which is deterioration of cell membrane (Ashraf & Bhatti, 2000).

The reduction of osmotic potential is supposed to be an osmotic adoption and also as the defense strategy against salinity (Hajlaoui et al., 2010). The osmotic adjustment includes the overall accumulation of solutes in cells because of salinity (Hnilickova et al., 2017). The reduction in Relative Water Contents (RWC) was less by grafted plants on bottle gourd salt resistant rootstock as compared to non-grafted plants at saline level (Sharf-Eldin et al., 2018). In the present study the RWC was significantly affected by salinity stress. Although RWC decreased under salinity conditions but the grafted plants had higher RWC as compared to ungrafted plants. This indicates that the salt tolerant rootstock have more capability for maintaining water uptake and salinity stress (Rahneshan et al., 2018). In our study the accumulation of proline contents increased with the increase of salinity stress (Lutts et al., 1996). The proline accumulation in our study was affected significantly in all ungrafted and grafted plants. The proline contents increased by exposing grafted watermelon seedlings to saline environment (Rivero et al., 2003).

The MDA significantly increased as the response of salt stress in grafted and non-grafted watermelon plants. Our results are also supported by findings of (Sarkar et al., 2008). In our study the plants grafted on salt tolerant rootstock showed minimum MDA contents as compare to ungrafted plants. The increase of salt stress increased MDA contents was obtained and watermelon plants grafted on salt resistant rootstocks showed less accumulation than those of salt sensitive rootstocks (Yanyan et al., 2018). The yield was enhanced in grafted watermelon plants on salt tolerant bottle gourd rootstock as compared to ungrafted plants. Our study is agreed with (Garnett, 1979). The response of grafting combinations on the fruit yield of watermelon also depends on the genotype of rootstock. Similar results are observed in the yield of tomato which was dependent on the genotype of rootstock (Santa-Cruz et al., 2002).

The pH of fruit is insignificantly affected by salinity but not by grafting combination. The pH of fruit in this study ranged from 5.2 to 5.97. This is similar to that the pH of watermelon ranged from 5.4 to 6.2, as it is dependent on production area (Devi et al., 2020). The pH of fruit in plants grafted on squash rootstock was slightly higher than self-grafted plants (Edelstein et al., 2011). The quality of watermelon fruit on grafted plants enhanced due to increase in endogenous hormones synthesis, changing in secondary metabolites and nutrients (Soteriou et al., 2017). While fruit attributes as seed production in fruit, TSS etc. have been enhanced in grafted plants, these may be due to rootstock genotype used at stress conditions (Al-Harbi et al., 2018). Our results regarding fruit quality on bottle gourd rootstock are contrary to (Rouphael et al., 2010), (Davis et al., 2008), because according to their findings fruit quality was negatively affected by grafting watermelon on bottle

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gourd rootstock. TAA was very low in watermelon fruit which is similar to the findings of (Tlili et al., 2011) and (Bruno et al., 2018).

1. Future Prospects

With the passage of time salinity is getting more common and popular not only in underdeveloped countries like Pakistan but also in developed countries. Due to salinity problems all agricultural including horticultural commodities are also affected which is resulting in shortage of food for ever increasing population. The watermelon grown in lands of Pakistan is of good taste but due to its sensitivity to salinity the production is affected negatively. To overcome these problems there is a dire need to screen out salt tolerant cucurbit crops varieties and also select suitable strategy for improving the crop productivity under saline environment. In current study the varieties of muskmelon, squash, bottle gourd and bittergourd crops were screened out for salt tolerance. The conventional breeding program like grafting on salt tolerant can be used for improving the production of watermelon on useable lands as well as on marginal saline lands. The morphological, physiological, biochemical, enzymatic, ionic and yield attributes of ungrafted and grafted watermelon plants were demonstrated under normal and saline regimes. These markers will be helpful for watermelon breeders with the purpose of breeding and improving it for salinity and other abiotic stress resistance.

The present work has suggested that for the investigations of lethal effects of salt stress can be elaborated at cellular as well as molecular level and also gene extraction stage. Beside the morpho-physiological and biochemical traits examination the gene extraction or molecular study may enhance the screening program. In conventional breeding programs the grafting technique is much beneficial to improve the salt sensitive varieties by using salt tolerant varieties because it is cost effective one. The grafting of vegetables especially watermelon is the oldest method and is used to not only improve the salt tolerance but also help to reduce diseases and pests in less time as compare to other breeding techniques. The present study also suggested that it should be extended to other crops of cucurbit like pumpkin, gourd etc.

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