ADAPTIVE AND COMPERATIVE MORPHO-ANATOMICAL, BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES OF LEAF OF TWO CUCURBIT WEEDS FROM TWO ECOTYPES UNDER SALT (NACL) STRESS

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Abstract: Salinity causing hazard to crops, menace to agricultural practices affecting adversely to the growth and metabolism of plants. To explore salt resistant plant lines as phytoremediants is a need of time. Leaf being chief photosynthetic structure have central significance to plants on which all attributes depend. Morphological, Physiological and anatomical responses are main adaptations towards stresses which vary with species and ecotype as well as type and level of stress. Two cucurbit weeds from two ecotypes were selected to evaluate their leaf in terms of morphological, physiological and anatomical adaptations against various levels of salt (100, 200 and 400 mMNaCl). Number of leaves, leaf area index, number of tendrils, number of branches, internodal length, water potential, solute potential and turgor potential, chlorophyll, net assimilation rate of CO_2, rate of transpiration and stomatal conductance, total soluble sugars, total soluble proteins, total free amino acids, proline superoxide dismutase, catalase, midrib area, lamina thickness, upper epidermal thickness, lower epidermal thickness of leaf were studied. *Citrullus colocynthis* showed more adaptive response than *Cucumis melo agrestis* and desert ecotype was more successful than agricultural ecotype against stresses in morphological, physiological and anatomical adaptations.

Index-term; physiology, stress, citrullus, cucumis, salt, anatomy

1. INTRODUCTION

Salt stress, continuously or occasionally may be catastrophic or severe gradually during plant development (Hasnuzzaman et al., 2013), by disturbing crop yield, biodiversity, and the overall atmosphere (Liu et al., 2014). Maintenance of the agronomic productivity in saline areas, and selection of tolerant plant genotypes with healthier osmotic regulation is a vital to approach (Chaurasia et al., 2022). Exploration of tolerant species from halophytic lines and commercial improvement of novel salt-tolerant varieties turn into the leading objective of experts to retrieve the saline soils as well as rise the product (Nikaljie et al., 2018).

Plants adapt modifications in morpho-physiological, biochemical, and anatomical characteristics to cope with salt stress, e.g. stimulate the generation of reactive enzymes, that deteriorate biomolecules (like proteins, lipids, and carbohydrates) and modify redox homeostasis (Chaudhery and Sidhu, 2021). The salt stress cause decline in the osmotic potential of the growth medium resulting in physiological drought (Borde et al., 2017). The tolerance to stress levels by the plant may be measured by survival rate and maintenance which is an adjustable character controlled by many features. This variability in plant salinity tolerance generates the glycophytes to halophytes (salt-sensitive to salt-loving) (Ahmed et al., 2021).

Soil salinity may affect seed germination by reducing moisture absorption of seeds and facilitating the entry of ions in excess. The salinity stress level reducing seed germination varies with genotype and environmental conditions (Munawar et al., 2021). However, barley (a salt-tolerant) varieties showed a much higher germination proportion than the sensitive ones (Singh et al., 2018). Salinity persuaded morphophysiological alterations in plant roots were studied and reported in the literature. Excess salinity impedes both cell division as well as expansion (Hasanuzzaman et al., 2021). Tolerance against salt stress is also increased in plants by enhanced translocation of sucrose from source to sink e.g. in *Brassica juncea* by using thiourea (Pandey et al., 2021).

Among various plants of the family Cucurbitaceae, *C.colocynthis* used as traditional folk medicines as a purgative, anti-neoplastic, anti-diabetic, anti-rheumatic and anti-allergic(Shafaei et al., 2012). *Cucumis melo agrestis* L. (Family Cucurbitaceae) is used for easy soy vinegar and sauce, grapevine, and as a raw material of paper and artificial cotton. *C. melo* is a branched, prostrate annual herb mostly permeating cotton, maize, pearl millet, and sorghum. One of the most critical phases in the life of this weed is its germination, emergence, and seedling development (Shatpathy et al., 2018) and is settled by several ecological factors like osmotic and salt stress (Yang and Guo, 2018).

salinity stress has the most obvious effect on photosynthesis reducing the biosynthesis of plant pigments (Abdel et al., 2017). A drop in Chlorophyll content under salinity stress is a generally known phenomenon. Photosynthesis is also congested when excessive levels of Na^+ and/or Cl^- are combined in chloroplasts (Szechyńska et al. 2017). Oxidative stress is also invited by salinity stress through a sequence of actions such as stomatal closure which leads to decreased CO_2 obtainability for fixation of carbon in the leaves.

Proline biosynthesis and/or gathering are a recognized phenomena for diminishing salinity stress (Farouk and Huqail, 2022). Proline improves salt stress tolerance in the plants by flexible antioxidant enzymatic activities, improving the photosynthetic activity, and thus protecting plant growth (Isfahani et al., 2019). Carbohydrates such as sugars (e.g. glucose, fructose, fructans, etc) and starch accumulation occur under salinity stress (Van et al., 2014). Biosynthesis as well as accumulation of antioxidant enzymes, such as catalase (CAT), and superoxide dismutase (SOD), are considered to positively minimize the salinity stress (Shah et al., 2021). SOD recruits the process of ROS cleansing by converting superoxide to hydrogen peroxide (Di et al., 2018).

As it is clear that salinity is crucial problem of the day causing severe loss to the crops yield by polluting soil. Various plant lines with phytoremedial characteristics are inevitable to be explored. *C.colocynthis* and *C.melo agrestis* are important medicinal cucurbit weeds. Leaf has central value among all attributes of plant life. So present study focused on the objectives to explore morphological, physiological and anatomical adaptations of leaf in *C.colocynthis* L.*and C. melo agrestis* L. under various levels of salt (NaCl) stresses.

II. Methodology

Twenty seeds of each ecotype of *C. melo agrests & Citrullus colocynthis* L. were placed in each pot with soil (30% clay, 30% silt, and 40% sand) to a depth of 1- 2 cm in 15 cm diameter plastic pots. Variable combined levels of NaCl and $NiCl_2$ were selected and added to the corresponding pots.

Test	Salt level (mM NaCl)
Tc	0 mM NaCl
T1	100 mM NaCl
T2	200 mM NaCl
T3	400 mM NaCl

2.1 Morphology

1. The number of leaves were counted manually
2. The number of branches were counted manually
3. The number of tendrils were counted manually

4. Internodal length (cm) with the help of a scale
5. Leaf Area Index (cm^2) was measured by using cm scale-

2.2 Physiology

Measurements of the water potential, solute potential and turgor potential were done on fully expanded leaf of four plants of each replicate were done by scholander type pressure chamber and measurement was recorded at 7:00 AM to 10:00 AM. Osmotic potential was recoreded on frozen leaf extract using vapour pressure Osmometer (Wescor 5500). Tugor pressure was calculated by using formula $\Psi_p = \psi_w - \psi_s$.

Photosynthestic parameters such as net assimilation rate of CO_2, rate of transpiration and stomatal conductance (gs) were made using an LCA-4 ADC portable infrared gas analyzer (IRGA). Measurements on fully expanded leaf were done during 10:00AM to 12:00PM recorded at PAR of 1300 µmol m^{-2} s^{-1}, 30± 2 Ċ temperature, 350µmol CO2 concentration, 99 Kpa Atmospheric pressure and 6±2m Bar of water vapor pressure. For the determination of organic solutes/ osmotica, total amino acids were estimated by Moor and Stein method(1948).

2.3 Biochemistry

The chlorophyll a and b were determined according to the method of Arnon (1949). Total soluble sugars were determined according to the method of Yemm and Willis (1954). Total soluble proteins are determined using the method of Lowry et al. (1951). Free amino acids in the sample were evaluated by Hamilton and Van Styke (1943). Proline was estimated according to the method of Bates et al. (1973). CAT actions were measured by using the method of Chance (1955). The activity of SOD was analyzed by the method used by Giannopolitis and Ries (1977) by measuring the capacity of the enzyme to inhibit the photochemical reduction of nitrobluetetazolium (NBT).

2.4 Anatomy

Anatomy of leaf was studied by thin sections, slides were stained with safranin and fast green and observed under microscope. Photographs were taken with the help of camera. Upper epidermis, lower epidermis, midrib and lamina were studied.

Analysis of Data was done by using Microsoft excel and ANOVA by using STATISTIX

III. RESULTS

Results are being described as S1 (*Cucumis melo agrestis*)and S2 (*Citrullus colocynthis* L.)

3.1. Morphology

leaf area in desert ecotypes of S1 (*C. melo agrestis*) was increased at 100 and 200mM of NaCl concentration while a slight decrease was noted was noted at 400mM of NaCl.In agricultural ecotype increase in 100 while gradual decrease at 200 and 400mM of NaCl was observed. Leaf area index of S2 (*C. colocynthis* L.) desert ecotype under single stress of salt showed significant increase at lower salt level (100 mM NaCl) while gradual decrease at moderate salt level (200 mM NaCl) and high salt level (400mM NaCl).

Internodal lengt in desert ecotypes of S1 was increased at 100 and 200mM of NaCl concentration while a slight decrease was noted was noted at 400mM of NaCl. In agricultural ecotype increase in 100 while gradual decrease at 200 and 400mM of NaCl was observed. Internodal length of S2 desert ecotype under single stress of salt showed significant increase at lower salt level (100 mM NaCl) while gradual decrease at moderate salt level (200 mM NaCl) and high salt level (400mM NaCl). While agricultural ecotype showed gradual decrease in internodal length with increasing salt levels.

Number of leaves in desert ecotypes of S1 was increased at 100 and 200mM of NaCl concentration while a slight decrease was noted was noted at 400mM of NaCl. In agricultural ecotype increase in 100 while gradual decrease at 200 and 400mM of NaCl was observed. Number of leaves of S2 desert ecotype under single stress of salt showed significant increase at lower salt level (100 mM NaCl) while gradual decrease at moderate salt level (200 mM NaCl) and high salt level (400mM NaCl).Agricultural ecotype showed gradual decrease in number of leaves with increasing salt.

Number of branches in desert ecotypes of S1 was increased at 100 and 200mM of NaCl concentration while a slight decrease was noted was noted at 400mM of NaCl. In agricultural ecotype increase in 100 while gradual decrease at 200 and 400mM of NaCl was observed. Number of branches of S2 desert ecotype under single stress of salt showed significant increase at lower salt level (100 mM NaCl) while gradual decrease at moderate salt level (200 mM NaCl) and high salt level (400mM NaCl). Agricultural ecotype showed gradual decrease in number of leaves with increasing salt.

Number of tendrils in desert ecotypes of S1 was increased at 100 and 200mM of NaCl concentration while a slight decrease was noted was noted at 400mM of NaCl. In agricultural ecotype increase in 100 while gradual decrease at 200 and 400mM of NaCl was observed. Number of tendrils of S2 desert ecotype under single stress of salt showed significant increase at lower salt level (100 mM NaCl) while gradual decrease at moderate salt level (200 mM NaCl) and high salt level (400mM NaCl). Agricultural ecotype showed gradual decrease in number of leaves with increasing salt.

3.2. Bochemistry and Physiology

In S1 Chlorophyll a level increased at lower and moderate salt levels (100 and 200 mM) while a slight decrease at high level 400mM of salt while in agricultural ecotype a significant increase in chlorophyll a at low salt 100mM and slight increase at moderate level while decrease at high salt level. S2 desert ecotype plants under single stress of salt showed increased level of chlorophyll a at low salt level (100 mM NaCl) and gradual decrease at moderate and high salt levels (200 and 400 mM NaCl). Agricultural ecotype plants showed increased level of chlorophyll a at low salt level (100 mM NaCl) and gradual decrease at moderate and high salt levels (200 and 400 mM NaCl) better as compared to desert ecoype.

In S1 amount of chlorophyll b under single stress of salt showed increase at low salt level (100 mM NaCl) and gradual decrease at moderate and high salt levels (200 and 400 mM NaCl). Maximum incease was observed in agricultural ecotype at 100mM while maximum decrease of chlorophyll b in desert ecotype at 400mM of salt. S2 In comparison with control, both ecotypes amount of chlorophyll b under single stress of salt showed increase at low salt level (100 mM NaCl) and gradual decrease at moderate and high salt levels (200 and 400 mM NaCl).

In S1 total soluble proteins were increased under salt stress. Maximum soluble proteiins were observed in desert ecotype at 100mM of salt. However 400Mm of salt level has no effect on total soluble proteins. S2 desert ecotype amount of soluble proteins under single stress of salt showed increase at high salt level (400 mM NaCl) while decreased at low salt level. Agricultural ecotype showed significant increase in soluble proteins at 100mM, slight increase at 200mM and decrease at high salt levels (400 mM NaCl).

In S1 total soluble sugars significantly increased at high level of salt in both ecotypes while decreased at low level of salt in both ecotypes. Maximum increased soluble sugars were shown in 400 Mm of salt. S2 desert ecotype amount of soluble sugars under single stress of salt showed increase at low salt level (100 mM NaCl) and and high salt levels (400 mM NaCl). Agricultural ecotype showed increased soluble sugars at hig level of salt.

S1 Proline level was increased with increasing salt level. Maximum proline level was observed at high salt level 400mM of NaCl in both ecotypes. S2 In comparison with control, Proline under single stress of salt showed slight increase at low, moderate and high salt level (100, 200 and 400 mM NaCl).

S1 Catalase under single stress of salt showed significant increase at high level (400 mM NaCl). In desert ecotype. Slight increase at low and moderate salts. Maximum increase in desert ecotype under high salt level (400 mM NaCl) was observed. S2 desert ecotype catalase enzyme was increased by increasing stress levels. Catalase under single stress of salt showed slight increase at low level (100 mM NaCl). Catalase under single stress of salt showed significant increase at moderate and high salt level (200 and 400 mM NaCl).

In S1 a gradual increase in superoxide dismutase was observed with increasing salt level in both ecotypes. Maximum amount of superoxide dismutase was observed at high salt level 400mM of NaCl.In S2 In comparison with control, super oxide dismutase enzymes (SOD) was increased by increasing stress levels in both ecotypes. Super oxide dismutase enzymes (SOD) under single stress of salt showed significant increase at low, moderate and high salt level (100, 200 and 400 mM NaCl).

In S1 At low and moderate salt stress level, an increase in CO_2 assimilation was observed while no effect on high salt level. In agricultural ecotype a significant increase in assimilation at low and moderate while decrease at high salt level was observed. In S2 desert ecotype a slight increase at low and moderate salt level while decrease at high salt level while in agricultural ecotype a significant increase at low salt was observed.

S1 both ecotypes, an increase in stomatal conductance at low salt was observed while it gradually decreased by increasing salt level. Stomatal conductance in S2 desert ecotype a slight increase at low salt, no change at moderate salt level while decrease at high salt level while in agricultural ecotype a significant increase at low salt and gradual decrease at moderate and high salt was observed.

Rate of transpiration (u mol m^{-2} s^{-1}) S1 desert ecotype a slight increase at low and moderate salt, no change at high salt level while in agricultural ecotype a significant increase at low and moderate salt and no change at high salt was observed. In S2 desert ecotype a slight increase at low salt and moderate salt level while decrease at high salt level while in agricultural ecotype a significant increase at low salt and slight increase at moderate and no change at high salt was observed.

Water potential (Mpa) of both S1 ecotypes a gradual decrease at low, moderate and high salt level was observed as compared to control. In both S2 ecotypes a gradual decrease in water potential at low, moderate and high salt level was observed as compared to control. Solute potential (Mpa) of both S1 ecotypes a gradual decrease at low, moderate and high salt level was observed as compared to control. In both S2 ecotypes a gradual decrease in solute potential at low, moderate and high salt level was observed as compared to control.

3.3. Anatomy

Midrib area (um^2) of S1desert ecotype, showed no change at low salt while a slight change in moderate and high level of salts. In agricultural ecotype a gradual reduction with increasing salt level was observed. Midrib area of both ecotypes of S2 decreased slightly gradually by increasing levels of salt as compared to control. Slight decrease at low salt (100 mM NaCl) and maximum decrease at high salt (400 mM NaCl) as compared to control. In both ecotypes of S1, a slight increase in upper epidermal thickness (um^2) at low salt level while decrease was observed at moderate and high salt levels. Minimum thickness was observed at high salt level. In desert ecotype of S2 there was decreased upper epidermal thickness at low salt level (100 mM NaCl) and slightly gradual decrease at moderate and high salt level (200 and 400mM NaCl) as compared to control. In agricultural ecotype low salt has no effect while moderate and high salt levels gradually decreased upper epidermal thickness**.**

A slight gradual reduction in lower epidermal thickness was observed with increasing salt levels in both ecotypes of S1. Lower epidermal thickness of S2 was reduced at lower salt level (100 mM NaC1) in comparison to control. After that, as the concentration of NaCl was increased, lower epidermal thickness decreased significantly at moderate and high level (200 and 400 mM NaCl) in both ecotypes. At low concentration of salt, lamina thickness of S1 was increased while a slight decrease at moderate salt and considerable decrease at high salt level was observed in both ecotypes as compared to control.Lamina thickness of of S2 desert ecotype was considerably increased at low salt stress (100 mM NaCI) as compared to control. After that, lamina thickness showed a reduction at moderate and high salt level (200 and 400mM NaC1). Agricultural ecotype showed a slight increase at low salt and then decrease at moderate and high salt levels. In desert ecotype of S1, a slight increase in Spongy mesophyll thickness (um) at low salt and no change at moderate salt while decrease at high salt was observed.maximum reduction in high salt was observed in both ecotypes. Spongy mesophyll thickness of S2 desert ecotype was slightly increased at low salt (100 mM NaCl) while there was no change at moderate (200 mM NaCl) as compared to control. As the concentration of NaC1 increased, a slight decrease in spongy mesophyll thickness was observed and high (400 mM NaCl) salt levels.A slight increase at low salt level, no change in at moderate salt while slight reduction at high salt level in Palisade mesophyll thickness (um) of S1was observed in both ecotypes.In both ecotypes of S2 palisade mesophyll thickness (um) was slightly increased at low salt (100 mM NaC1), no change at moderate level (200 mM NaCl) and slight decrease at high salt level (400 mM NaCl) as compared to control.

Table 1- Analysis of variance for Biochemical and Physiological parameters of *Cucumis melo agrestis* and *Citrullus colocynthis* from two habitats under different levels of Salt (NaCl) Stress in pot experiment.

Source of Variance	d.f.	Net Assimilation of CO2 (μmol $m^{-2}s^{-1}$)	Catalase	Proline Contents (μg/g f.wt.)	Rate of Transpiration (mmol $m^{-2}s^{-1}$)	Stomatal Conductance (mmol $m^{-2}s^{-1}$)	Superoxide Dismutase	Total Soluble Sugar (μg/g f.wt.)	Chlorophyll a (mg/g f.wt.)	Solute Potential (MPa)	Water Potential (MPa)	Total Soluble Protein (μg/g f.wt.)	Chlorophyll (mg/g f.wt.)
Species	1	409.022	12.480***	0.13490**	3.38035*	4.6875**	4.02521**	38116**	0.00366**	0.02200***	0.03052**	4.97083	0.00144**
Habitat	1	24.350**	77.894	0.00016	0.20124**	2.5208	3.25521	6473**	2.221E-04	0.00353	0.00622***	6.54835	0.00152**
Species × Habitat	1	44.467**	0.350***	0.00104**	0.36750**	0.5208**	2.85187***	4650	4.028E-04***	0.00132***	3.991E-04	8.81770	2.517E-04
Salt	3	299.315	342.811	0.06567	2.47367	97.6875	456.098	193792**	0.00273**	1.84000**	0.00200***	7.72574	0.00889***
Species × Salt	3	36.133**	5.092**	0.00982**	0.15995**	1.6319**	2.083E-04**	44556**	1.683E-04**	3.080E-31	5.296E-34***	4.39290	1.801E-04
Habitat × Salt	3	19.354**	12.898**	0.00227**	0.29862	0.5764**	0.00132	16519***	1.083E-04	8.932E-33**	6.821E-35**	1.74839	5.660E-05**
Species ×Habitat × Salt	3	11.785***	0.079***	0.00196***	0.09739**	0.1319***	2.083E-04**	16301**	4.238E-06**	4.303E-32***	7.448E-35***	3.08229	2.144E-04**
Errors	30	0.450	0.061	0.00010	0.00372	0.9875	0.22718	37	3.295	8.239E-05	6.857E-05	0.02584	2.704E-07

Total	47												0.00144**

Key Points: *, **, *** = significance levels at 0.05, 0.01and 0.001, respectively, ns = non-significant.

Table 2- Analysis of variance for morpho-anatomical parameters of *Cucumis melo agrestis* and *Citrullus colocynthis* from two habitats under different levels of Salt (NaCl) Stress in pot experiment.

Source of Variance	d.f.	Lamina Thickness (µm)	Mid Rib Thickness (µm)	Palisade Mesophyll Thickness (µm)	Spongy Mesophyll Thickness (µm)	Lower Epidermal Thickness (µm)	Upper Epidermis Thickness (µm)	Leaf Area Index (cm2)	Internodal Length (cm)	Number of Branches	Number of Leaves	Number of Tendrils
Species	1	22.688**	0.08**	2.5208	14.0833***	0.09810**	0.00021**	1.5696*	0.60750	1.3333	690.083**	102.083
Habitat	1	1.687**	75.00*	2.5208*	8.3333	0.01505**	0.08003***	23.4640*	0.85333*	16.3333*	800.333**	602.083**
Species × Habitat	1	22.688	14.08**	2.5208	0.0833**	0.04025	0.00021	1.2675	0.48000*	8.3333**	884.083*	184.083**
Salt	3	323.188	3990.17	39.1875**	40.3333**	0.85826**	3.25530*	17.7508	2.85250	34.0556**	310.61**	270.583
Species × Salt	3	10.688***	10.92	0.0208**	0.0833	0.04607**	0.03354	2.3177**	0.02250**	0.0556^{8}	67.139*	11.806**
Habitat × Salt	3	22.688*	7.06**	0.0208**	0.3333**	0.02252**	0.04370***	9.0350**	0.03167	2.0556	104.722**	34.694**
Species × Habitat × Salt	3	10.688	2.92***	0.0208	0.0833*	0.02934****	0.03687	12.2899	0.01500**	0.0556*	60.583**	25.694
Errors	30	0.850	3.35	0.5542	0.9708	0.00550	0.00872	1.9876	0.09460	2.1903	29.890	13.635
Total	47											

Key Points: *, **, *** = significance levels at 0.05, 0.01and 0.001, respectively, ns = non-significant.

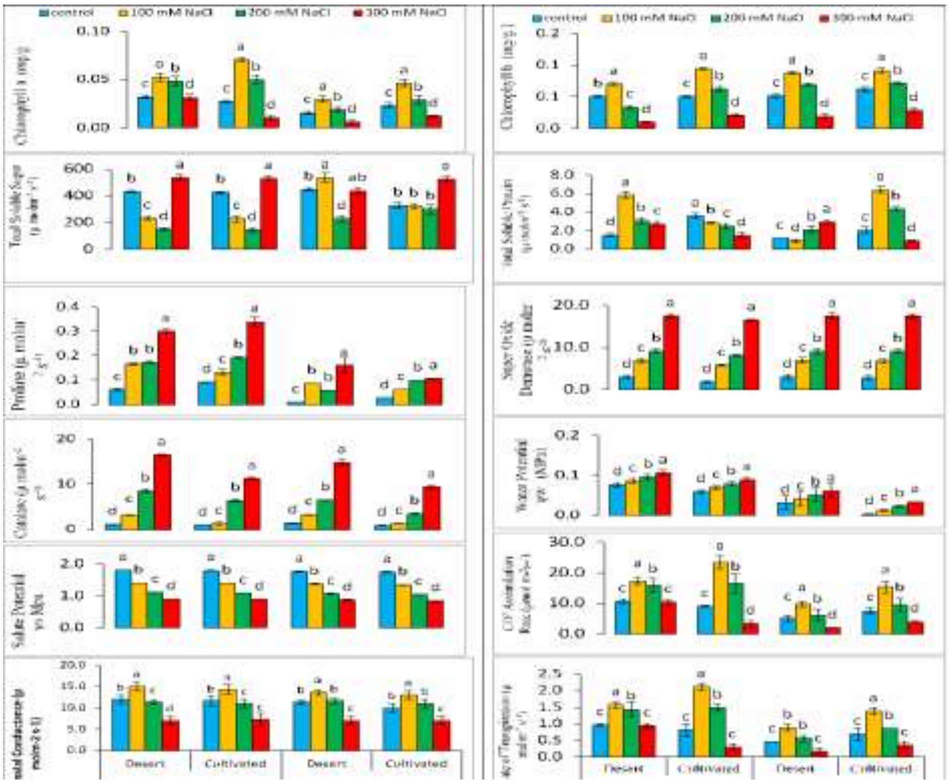


Fig.1. Biochemical and physiological response of leaf of C.colocynthis and C.melo agrestis under various levels of salt (NaCl) stresses.

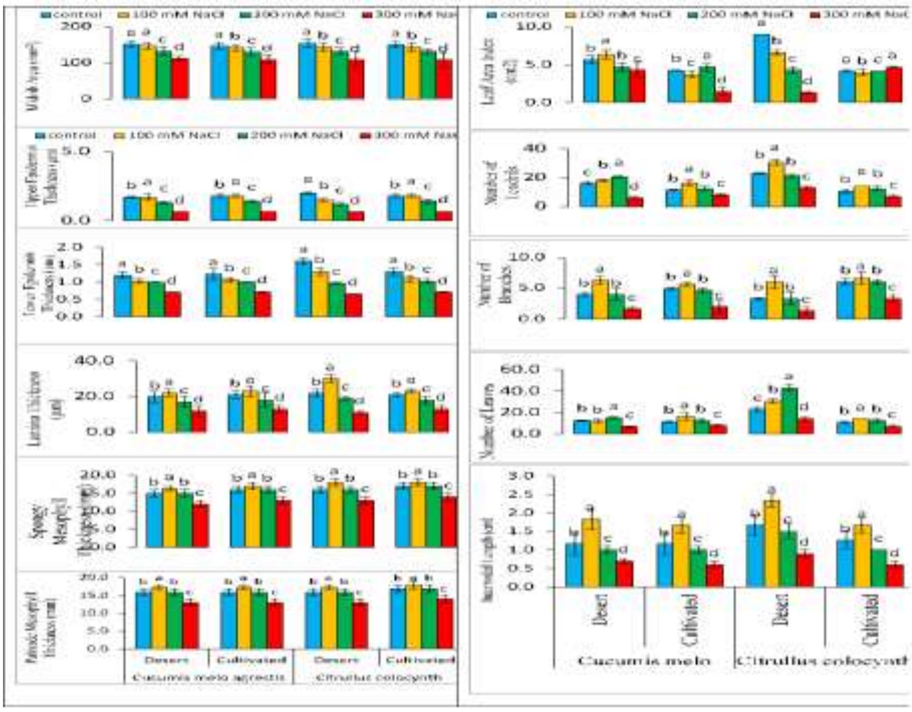
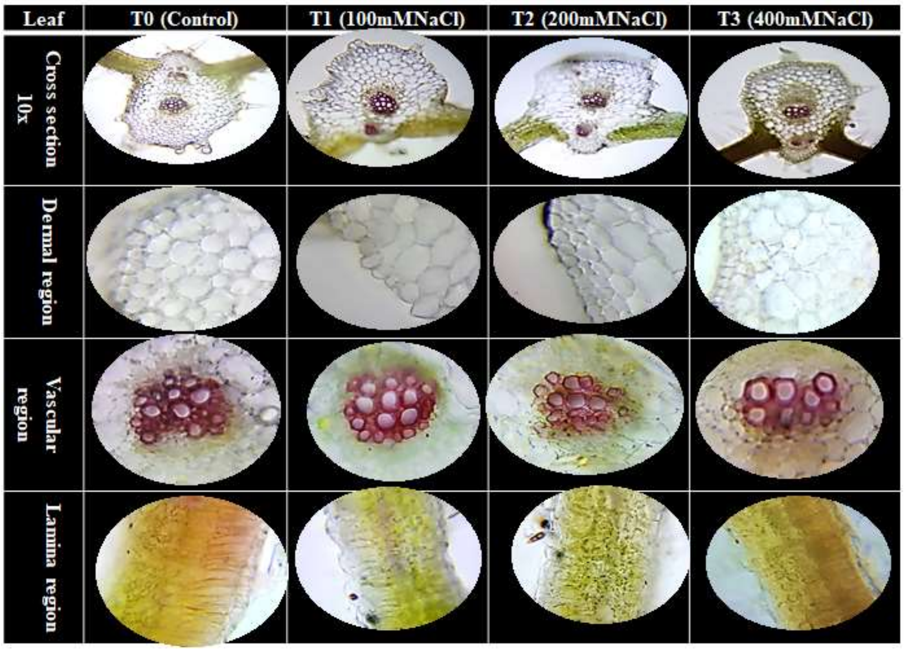
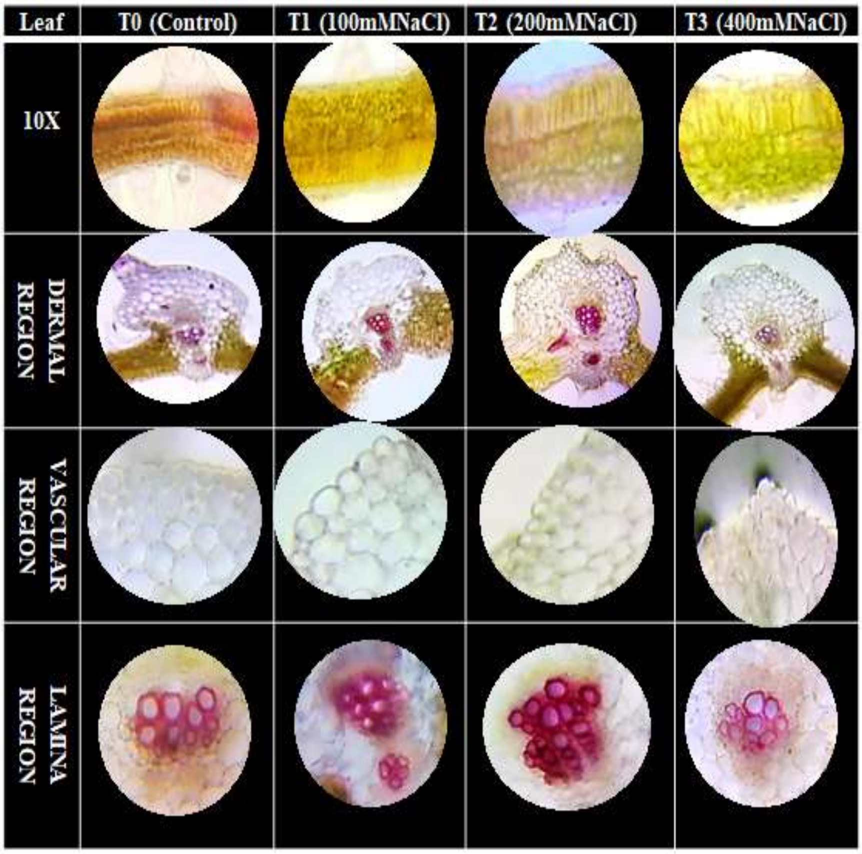


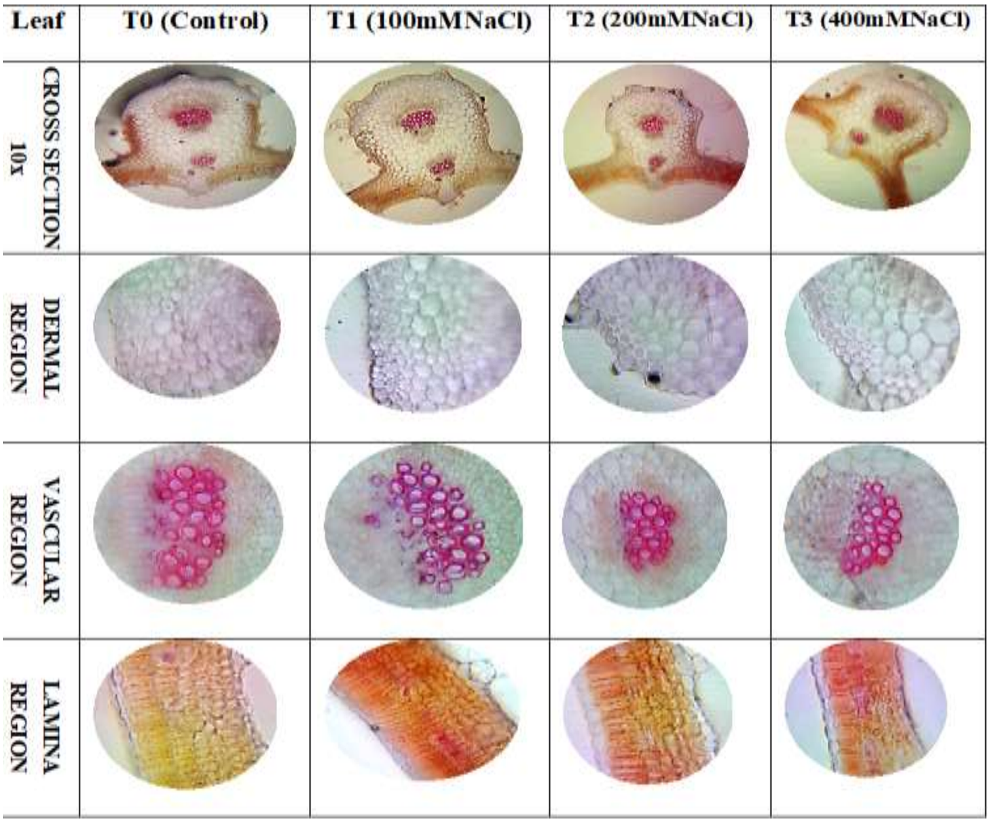
Fig.2 Morphological and anatomical response of leaf of C.colocynthis and C.melo agrestis under various levels of salt (NaCl) stress.



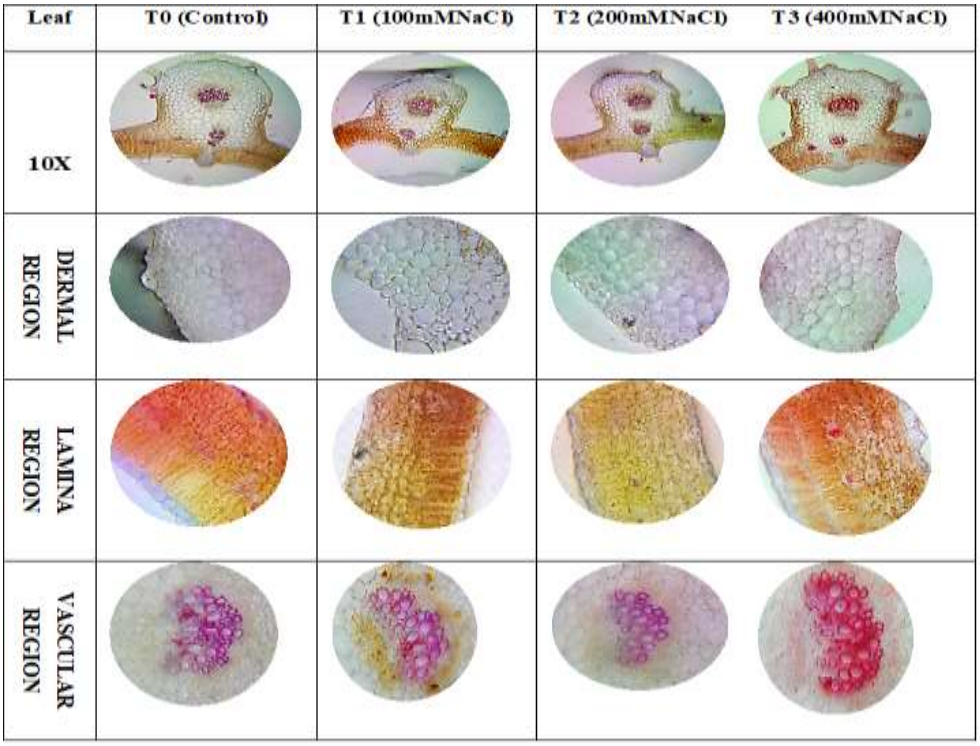
Plate#4.2.1.3.3(a) ; Leaf anatomy of *Cucumis melo agrestis* (Desert Ecotype) under single stress of salt



Plate#4.2.1.3.3(b) ; Leaf anatomy of *Cucumis melo agrestis* (Agricultural Ecotype) under single stress of salt



Plate#4.2.1.3.3(c). ; Leaf anatomy of *Citrullus colocynth* (Desert Ecotype) under single stress of salt



Plate#4.2.1.3.3(d). ;Leaf anatomy of *Citrullus colocynth* (Agricultural Ecotype) under single stress of salt.

IV. DISCUSSION

C. colocynthis L. resulted in adaptable outcomes in variable stresses. In association with control, the extent of chlorophyll was decreased by increasing stress levels of salinity. Plants treated with higher levels of salt resulted in decreased chlorophyll levels and vice versa. The quantity of chlorophyll b was also decreased by rising levels of salinity. The plants retort to hassles at the biomolecular level can be linked with the sensitivity of environmental indicators and their diffusion to the adjusting machinery of the cell to stimulate definite adaptive mechanisms (Xian et al., 2015).

It has been observed that amount of sugars decreased at high levels of salts. Stress signal adversly affect the mechanisms in cells and biochemical reactions (Xian et al., 2015). It has been observed that amount of proteins decreased at low levels of salt. Free amino acids were increased by increasing level of stress in *C. colocynthis* and *C.melo agrestis.* It has also been reported earlier that free amino acids are increased instead of proteins under stress conditions (Amraee et al., 2020). According to Dong et al., 2017, free amino acids are increased in the plants under stress due to the biosynthesis of amino acids and the absence of translational factors. Proline amplified with the escalating level of salinity. The accumulation of proline is also an adaptive response to salinity (Hayat et al., 2022).

It has been detected that antioxidant enzyme accomplishments augmented with the increasing level of salinity in both of our plants. The maximum amount of superoxidase dismutase (SOD) 21.55 u/mg of protein was recorded in high NaCl in comparison to 2.18 u/mg in control. Similar results were observed for catalase (CAT) antioxidant enzyme activities. The maximum amount of catalase (CAT) 18.45 u/mg of protein was recorded in comparison to 0.46 u/mg of control under high NaCI. Oxidative stress becomes an important tool to categorize the salt tolerance level of the plants. Oxidative stress in salt-tolerant plants was directly related to characteristics such as catalase (CAT) and superoxide dismutase (SOD) activity (Wu et al., 2012). Salinity tolerance is controlled by the coordinated action of variable gene involved in the initiation of a diversity of contraptions such as the confiscation of toxic ions, regulation of toxic metabolites, and antioxidative defense (Yasmeen et al., 2020). The augmented level of the enzymes like CAT and SOD at higher salinities showed a significant correlation between plant tolerance levels and these antioxidant systems (Ashraf and Haris, 2004; Rizvi et al., 2018).

C. melo agrestis L. has variable results in variable stresses. Plants treated with higher levels of salt resulted in decreased chlorophyll levels. Chlorophyll a level notably decreased at high NaCl. In comparison with control, the amount of chlorophyll b was significantly decreased by increasing salinity levels. Photosynthetic pigments of *Vigna mungo* were seriously decreased under various levels of salts (Aqeel et al., 2021). It has been observed that amount of sugars in present studies decreased in high levels of salts. Jan et al., 2019, demonstrated that the sugar levels of *Oryza sativa* decreased in higher levels of stress. It has been observed in this study that the amount of proteins increased at moderate and high levels of salt significantly. The reason behind this is that the plant retort to these stresses at the cellular and molecular level can be correlated with the perception of environmental signals and their transmission towards the regulatory machinery of the cell to activate specific adaptive mechanisms (Mantry et al., 2012). A decrease in proteins was also recorded in this study at high levels of salts. Victor et al., 2016 observed a reduction in the protein content of Lettuce and Hyacinth plants under abiotic chemical stress.

Free amino acids were gradually increased with increasing levels of stresses in this study. According to Amraee et al, (2020), free amino acids are increased in the plants under stress due to the biosynthesis of amino acids and the absence of translational factors. and free amino acids are increased instead of proteins under stress conditions (Zhang et al., 2014). Proline gradually amplified with the escalating level of salinity level. The maximum amount of proline was recorded in high NaCI. The accumulation of proline is also an adaptive response to salinity (Hayat et al., 2022).

It has been observed in present study that antioxidant enzyme activities increased with the increasing level of salinity. The maximum amount of superoxidase dismutase (SOD) was recorded in combined high salt (400 mM NaCl). Salt tolerance is regulated by the synchronized action of variable gene involved in the initiation of a variety of mechanisms such as antioxidative defense (Yang et al., 2022). Similar results were observed for catalase (CAT) antioxidant enzyme activities. Oxidative stress becomes an important tool to categorize the salt tolerance level of the plants. Oxidative stress in salt-tolerant plants was directly related to characteristics such as catalase (CAT) and superoxide dismutase (SOD) activity (Wu et al., 2012). The augmented commotion of the enzymes like CAT and SOD at higher salinities showed a significant correlation between plant tolerance levels and these antioxidant systems (Hasanuzzaman et al., 2022; Kibria et al., 2017). These scenarios change physiological processes and consequences decreasing crop quality and yield (Bisbis et al., 2018).

Results of NaC1 stress showed that the midrib area decreased gradually at lower moderate and higher levels in both ecotypes as compared to control. Digioia et al. (2020) revealed leaf anatomy of the plant growing under salinity stress indicated injury in various tissues especially the cells that have thin epidermal and mesophyll cell walls. $NiCl_2$, stress showed that the midrib area was decreased gradually at a lower, moderate and higher level in both ecotypes as compared to control. salt treatment causes a decrease in the size of blade thickness, number of conducting elements, and lowered cell size of the epidermis and aerenchyma tissue (Al-Saadi et al., 2013).

Upper epidermal thickness of leaf in *C. colocynth* decreased gradually with increasing stress levels under salt in comparison with control. The thickness of the upper and lower epidermis of leaves of almost all varieties revealed a trend of decline under abiotic chemical stress (Akhter et al., 2021; Sharma et al., 2022). Cell wall thickening starts in the epidermis and endodermis of rhizomes and also in the cuticle and epidermis of leaves (Tupan and Azrianingsih, 2016). Lower epidermal thickness was not affected at a lower and moderate level in both ecotypes but decreases greatly with increasing stress levels in both ecotypes as compared to control. Salt stress induced a decline in the thickness of the lower epidermis at different levels and a decrease in the size of vascular bundles of wheat leaves (Atabayeva et al., 2016).

Lamina thickness of *C. colocynthis* L. was gradually reduced in both ecotypes with increasing stress levels of salt as compared to control. Tolerating mechanism of plants to salt stresses also includes increasing diameter along with the thickness of these lamina (Longstreth and Nobel, 1979). Al-Saadi et al. (2013) reported that being subjected to salts brought a decrease in the blade thickness in some Potomageton plants. This decline in leaf thickness was due to the consequences of salt-induced water stress or salts accumulation in leaves or a combination of both (Sridhar et al., 2011). The leaf blade showed smaller thickness in the treatments subjected to toxic contamination (Gomes et al., 2011).

Spongy mesophyll thickness was decreased with increasing salt levels in single stresses and maximum decrease at the highest of NaC1 was observed in both ecotypes as compared to control. Sridhar et al. (2011) found that chemical salt accumulation leads to reducing in leaf thickness and a decrease in spongy parenchyma cells in brake fern (*Pteris vittata* L.). subjected to higher salt levels was resulting in a decrease in mesophyll thickness (Akcin et al., 2018). Palisade mesophyll thickness was constant at a lower and moderate level of NaCl in both ecotypes compared to Control. Palisade cell length enhanced and size remained comparatively constant with saline stress for bean and cotton (Longstreth and Nobel, 1979). Palisade mesophyll thickness decreased at a lower and moderate level of $NiCl_2$ while more decrease in highest level as compared to control. Sridhar et al. (2011) found that salt accumulation leads to reduction in leaf thickness and a decrease in palisade parenchyma cells in brake fern (*Pteris vittata* L.).

V. CONCLUSION

Phytoremediation of salinity and heavy metal polluted soils is a reliable technique. For this purpose screening of plant lines is a preliminary requirement. Those plant lines having economic importance especially medicinal importance are very useful for the purpose. Those plants which require low water can be prefferred. The plants adapt various physiological adaptations to tolerate and resist the stress produced due to salt and heavy metals. *C. colocynth* is more resistant against steesses than the *C. melo agrestis* and desert ecotype is more resistant as compared to agricultural ecotype. These plants have minor effect on their physiology but both of these produced resisting chemicals against stresses like SOD and catalase.

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REFRENCES

Abdel Latef, A. A. H., Abu Alhmad, M. F., & Abdelfattah, K. E. (2017). The possible roles of priming with ZnO nanoparticles in mitigation of salinity stress in lupine (Lupinus termis) plants. *J. plant growth regul*, *36*(1), 60-70.

Ahmed, H. A. I., Shabala, L., & Shabala, S. (2021). Understanding the mechanistic basis of adaptation of perennial Sarcocornia quinqueflora species to soil salinity. *Physiol Plant*, *172*(4), 1997-2010.

Akbar, S. (2020). Citrullus colocynthis (L.) schrad.(cucurbitaceae). In *Handbook of 200 Medicinal Plants* (pp. 663-672). Springer, Cham.

Alfaraas, A. J., Khairiah, J., Ismail, B. S., & Noraini, T. (2016). Effects of heavy metal exposure on the morphological and microscopical characteristics of the paddy plant. *J.environ.biol*, *37*(5), 955.

Al Hassan, M., Chaura, J., Donat-Torres, M. P., Boscaiu, M., & Vicente, O. (2017). Antioxidant responses under salinity and drought in three closely related wild monocots with different ecological optima. *AoB Plants*, *9*(2), plx009.

Alloway, B. J. (2013). Heavy metals and metalloids as micronutrients for plants and animals. In *Heavy metals in soils* (pp. 195-209). Springer, Dordrecht.

Amjad, M., Akhtar, S. S., Yang, A., Akhtar, J., & Jacobsen, S. E. (2015). Antioxidative response of quinoa exposed to iso-osmotic, ionic and non-ionic salt stress. *J.Agron. Crop Sci*, *201*(6), 452-460.

Amraee, L., Rahmani, F. and Mandoulakani, B.A., (2020). Exogenous application of 24-epibrassinosteroid mitigates NaCl toxicity in flax by modifying free amino acids profile and antioxidant defence system. *Func. Plant Biol*, *47*(6), pp.565-575.

Aqeel, M., Khalid, N., Tufail, A., Ahmad, R.Z., Akhter, M.S., Luqman, M., Javed, M.T., Irshad, M.K., Alamri, S., Hashem, M. and Noman, A., (2021). Elucidating the distinct interactive impact of cadmium and nickel on growth, photosynthesis, metal-homeostasis, and yield responses of mung bean (Vigna radiata L.) varieties. *ES P R*, *28*(21), pp.27376-27390.

Arora, N. K., Fatima, T., Mishra, I., Verma, M., Mishra, J., & Mishra, V. (2018). Environmental sustainability: challenges and viable solutions. *Env. Sust.1*(4), 309-340.

Ashraf, M. P. J. C., & Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant science*, *166*(1), 3-16.

Asid, A. N., Al-Ghamdi, K. M., Nassar, M. I., & Mangoud, A. (2015). Assessment of bitter apple, Citrullus colocynthis extracts on the housefly, Musca domestica L. *Advances in Environmental Biology*, *9*(8), 72-79.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*(1), 205-207.

Bazihizina, N., Colmer, T. D., Cuin, T. A., Mancuso, S., & Shabala, S. (2019). Friend or foe? Chloride patterning in halophytes. *Trends in plant science*, *24*(2), 142-151.

Bengyella, L., Kuddus, M., Mukherjee, P., Fonmboh, D. J., & Kaminski, J. E. (2021). Global impact of trace non-essential heavy metal contaminants in industrial cannabis bioeconomy. *Toxin Reviews*, 1-11.

Bhattacharjee, S. (2010). Sites of generation and physicochemical basis of formation of reactive oxygen species in plant cell. *Reactive oxygen species and antioxidants in higher plants*, 1-30.

Bisbis, M.B., Gruda, N. and Blanke, M., (2018). Potential impacts of climate change on vegetable production and product quality–A review. *J.Clean.Prod*, *170*, pp.1602-1620.

Borde, M., Dudhane, M., & Kulkarni, M. (2017). Role of arbuscular mycorrhizal fungi (AMF) in salinity tolerance and growth response in plants under salt stress conditions. In *Mycorrhiza-eco-physiology, secondary metabolites, nanomaterials* (pp. 71-86). Springer, Cham.

Chance B, M. A. (1955). Assay catalase and peroxidase. *Methods in Enzymology. Academic Press, New York*, 764-775.

Chaudhry, S., & Sidhu, G. P. S. (2021). Climate change regulated abiotic stress mechanisms in plants: a comprehensive review. *Plant Cell Reports*, 1-31.

Chaurasia, K. N., More, S. J., Kumar, A., Kumar, D., Singh, B., Bhardwaj, V., ... & Lal, M. K. (2022). Salinity responses and tolerance mechanisms in underground vegetable crops: An integrative review. *Planta*, *255*(3), 1-25.

Di Marzo, N., Chisci, E., & Giovannoni, R. (2018). The role of hydrogen peroxide in redox-dependent signaling: homeostatic and pathological responses in mammalian cells. *Cells*, *7*(10), 156.

Dong, Y., Silbermann, M., Speiser, A., Forieri, I., Linster, E., Poschet, G., Allboje Samami, A., Wanatabe, M., Sticht, C., Teleman, A.A. and Deragon, J.M., (2017). Sulfur availability regulates plant growth via glucose-TOR signaling. *Nat. communi*, *8*(1), pp.1-10.

Dreyer, I., & Uozumi, N. (2011). Potassium channels in plant cells. *The FEBS Journal*, *278*(22), 4293-4303.

Elhindi, K. M., El-Din, A. S., & Elgorban, A. M. (2017). The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (Ocimum basilicum L.). *Saudi J. Biol. Sci*, *24*(1), 170-179.

Farouk, S., & Al-Huqail, A. A. (2022). Sustainable Biochar and/or Melatonin Improve Salinity Tolerance in Borage Plants by Modulating Osmotic Adjustment, Antioxidants, and Ion Homeostasis. *Plants*, *11*(6), 765.

Fatima, S., Hameed, M., Naz, N., Shah, S. M. R., Naseer, M., Ahmad, M. S. A., . & Ahmad, I. (2021). Survival strategies in khavi grass [Cymbopogon jwarancusa (Jones) Schult.] colonizing hot hypersaline &arid environments. *Water Air Soil Pollut*, *232*(2),1-17.

Feng, W., Lindner, H., Robbins, N. E., & Dinneny, J. R. (2016). Growing out of stress: the role of cell-and organ-scale growth control in plant water-stress responses. *The Plant Cell*, *28*(8), 1769-1782.

Feghhenabi, F., Hadi, H., Khodaverdiloo, H., & Van Genuchten, M. T. (2020). Seed priming alleviated salinity stress during germination and emergence of wheat (Triticum aestivum L.). *Agric. Water Manag.*, *231*, 106022.

Flowers, T. J., & Colmer, T. D. (2015). Plant salt tolerance: adaptations in halophytes. *Annals of botany*, *115*(3), 327-331.

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant physiol*, *59*(2), 309-314.

Hamilton, P. B., & Van Slyke, D. D. (1943). The gasometric determination of free amino acids in blood filtrates by the ninhydrin-carbon dioxide method. *J.Biol.Chem. 150*(1), 231-250.

Hamim, H., Miftahudin, M., & Setyaningsih, L. (2018). Cellular and ultrastructure alteration of plant roots in response to metal stress. In *Plant growth and regulation-Alterations to sustain unfavorable conditions*. IntechOpen.

Hanin, M., Ebel, C., Ngom, M., Laplaze, L., & Masmoudi, K. (2016). New insights on plant salt tolerance mechanisms and their potential use for breeding. *Front.plant sci*, *7*, 1787.

Hasanuzzaman, M., Parvin, K., Anee, T. I., Masud, A. A. C., & Nowroz, F. (2022). Salt Stress Responses and Tolerance in Soybean. In *Plant Stress Physiology-Perspectives in Agriculture*. IntechOpen.

Hasanuzzaman, M., Raihan, M., Hossain, R., Masud, A. A. C., Rahman, K., Nowroz, F., ... & Fujita, M. (2021). Regulation of Reactive Oxygen Species and Antioxidant Defense in Plants under Salinity. *Int.J.Mol.Sci. 22*(17), 9326.

Hasanuzzaman, M., Nahar, K., & Fujita, M. (2013). Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In *Ecophysiology and responses of plants under salt stress* (pp. 25-87). Springer, New York, NY.

Hayat, K., Zhou, Y., Menhas, S., Hayat, S., Aftab, T., Bundschuh, J., & Zhou, P. (2022). Salicylic Acid Confers Salt Tolerance in Giant Juncao through Modulation of Redox Homeostasis, Ionic Flux, and Bioactive Compounds: An Ionomics and Metabolomic Perspective of Induced Tolerance Responses. *J.Plant Growth Regul.* 1-21.

He, Y., Chen, Y., Zhang, Y., Qin, X., Wei, X., Zheng, D., ... & Yuan, G. (2021). Genetic diversity of Ralstonia solanacearum species complex strains obtained from Guangxi, China and their pathogenicity on plants in the Cucurbitaceae family and other botanical families. *Plant Pathology*, *70*(6), 1445-1454.

Ines, S., Talbi, O., Nasreddine, Y., Rouached, A., Gharred, J., Jdey, A., ... & Abdelly, C. (2022). Drought tolerance traits in Medicago species: A review. *Arid Land Res. Manag.*, *36*(1), 67-83.

Isfahani, F. M., Tahmourespour, A., Hoodaji, M., Ataabadi, M., & Mohammadi, A. (2019). Influence of Exopolysaccharide-Producing Bacteria and SiO 2 Nanoparticles on Proline Content and Antioxidant Enzyme Activities of Tomato Seedlings (Solanum lycopersicum L.) under Salinity Stress. *PJES*, *28*(1).

Islam, A. T., Koedsuk, T., Ullah, H., Tisarum, R., Jenweerawat, S., Cha-um, S., & Datta, A. (2022). Salt tolerance of hybrid baby corn genotypes in relation to growth, yield, physiological, and biochemical characters. *SAJ Bot.*, *147*, 808-819.

Jan, R., Khan, M.A., Asaf, S., Lee, I.J. and Kim, K.M., (2019). Metal resistant endophytic bacteria reduces cadmium, nickel toxicity, and enhances expression of metal stress related genes with improved growth of Oryza sativa, via regulating its antioxidant machinery and endogenous hormones. *Plants*, *8*(10), p.363.

Jiang, X., Gu, S., Liu, D., Zhao, L., Xia, S., He, X., Chen, H. and Ge, J., (2018). Lactobacillus brevis 23017 relieves mercury toxicity in the colon by modulation of oxidative stress and inflammation through the interplay of MAPK and NF-κB signaling cascades. *Front.microbiol. 9*, p.2425.

Joshi, R., Mis;hra, P., Meena, R. K., & Patni, V. (2021). 4. Citrullus colocynthis: A Potential Source of Alternative Medicine.

Karrar, E., Sheth, S., Navicha, W. B., Wei, W., Hassanin, H., Abdalla, M., & Wang, X. (2019). A potential source: Nutritional and antioxidant properties of edible oils from cucurbit seeds and their impact on human health. *J.food biochem. 43*(2), e12733.

Kataria, S., & Verma, S. K. (2018). Salinity stress responses and adaptive mechanisms in major glycophytic crops: the story so far. In *Salinity Responses and Tolerance in Plants, Volume 1* (pp. 1-39). Springer, Cham.

Kibria, M.G., Hossain, M., Murata, Y. and Hoque, M.A., (2017). Antioxidant defense mechanisms of salinity tolerance in rice genotypes. *Rice Science*, *24*(3), pp.155-162.

Kotecha, M., Chaudhary, S., Marwa, N., Deeba, F., Pandey, V., & Prasad, V. (2019). Metals, crops and agricultural productivity: Impact of metals on crop loss. In *Plant-Metal Interactions* (pp. 191-216). Springer, Cham.

Kumar, A., Choudhary, A. K., Pooniya, V., Suri, V. K., & Singh, U. (2016). Soil factors associated with micronutrient acquisition in crops-biofortification perspective. In *Biofortification of food crops* (pp. 159-176). Springer, New Delhi.

Kumar, V., Rathee, P., Kohli, K., Chaudhary, H., & Rathee, S. (2009). Phytochemical and biological potential of indrayan: an overview. *Pharmacog. Rev.3*(5), 193.

Latif, A., Bilal, M., Asghar, W., Azeem, M., Ahmad, M. I., Abbas, A., ... & Shahzad, T. (2018). Heavy metal accumulation in vegetables and assessment of their potential health risk. *J.Environ. Anal.Chem.5*(234), 2380-2391.

Liu, M., Yu, H., Ouyang, B., Shi, C., Demidchik, V., Hao, Z., ... & Shabala, S. (2020). NADPH oxidases and the evolution of plant salinity tolerance. *Plant Cell Enviro*, *43*(12), 2957-2968.

Liu, C., Lu, M., Cui, J., Li, B., & Fang, C. (2014). Effects of straw carbon input on carbon dynamics in agricultural soils: a meta-analysis. *Global change biol*, *20*(5), 1366-1381.

Lowry, O., Rosebrough, N., Farr, A. L., & Randall, R. (1951). Protein measurement with the Folin phenol reagent. *J.biol.chem.*, *193*(1), 265-275.

Manipol, M. M., Tinio, C. E., Maldia, L. S., & Combalicer, M. S. (2020). Salinity-induced changes in the morphology, physiology, and anatomy of seeds and seedlings of smooth narra (Pterocarpus indicus Willd. f. indicus). *Biodivers. J. Biol. 21*(11).

Mantri, N., Patade, V., Penna, S., Ford, R., & Pang, E. (2012). Abiotic stress responses in plants: present and future. In *Abiotic stress responses in plants* (pp. 1-19). Springer, New York, NY.

Matsumoto, K. I., Nyui, M., Ueno, M., Ogawa, Y., & Nakanishi, I. (2019). A quantitative analysis of carbon-ion beam-induced reactive oxygen species and redox reactions. *J.Clinic.Biochem.Nutri.*, 18-34.

Meddich, A., Ait El Mokhtar, M., Bourzik, W., Mitsui, T., Baslam, M., & Hafidi, M. (2018). Optimizing growth and tolerance of date palm (Phoenix dactylifera L.) to drought, salinity, and vascular fusarium-induced wilt

(Fusarium oxysporum) by application of arbuscular mycorrhizal fungi (AMF). In *Root biology* (pp. 239-258). Springer, Cham.

Mohamed, I. A., Shalby, N., Bai, C., Qin, M., Agami, R. A., Jie, K., ... & Zhou, G. (2020). Stomatal and photosynthetic traits are associated with investigating sodium chloride tolerance of Brassica napus L. cultivars. *Plants*, *9*(1), 62.

Mumtaz, S., Saleem, M. H., Hameed, M., Batool, F., Parveen, A., Amjad, S. F., ... & Alyemeni, M. N. (2021). Anatomical adaptations and ionic homeostasis in aquatic halophyte Cyperus laevigatus L. under high salinities. *Saudi J.Biol. Sci.28*(5), 2655-2666.

Munawar, W., Hameed, A., & Khan, M. K. R. (2021). Differential morphophysiological and biochemical responses of cotton genotypes under various salinity stress levels during Early Growth Stage. *Front.Plant Sci.12*, 335.

Munns, R., Day, D. A., Fricke, W., Watt, M., Arsova, B., Barkla, B. J., ... & Tyerman, S. D. (2020). Energy costs of salt tolerance in crop plants. *New Phytol*, *225*(3), 1072-1090.

Nair, R. M., Pandey, A. K., War, A. R., Hanumantharao, B., Shwe, T., Alam, A. K. M. M., ... & Schafleitner, R. (2019). Biotic and abiotic constraints in mungbean production—progress in genetic improvement. *Front.plant sci. 10*, 1340.

Nikalje, G. C., Srivastava, A. K., Pandey, G. K., & Suprasanna, P. (2018). Halophytes in biosaline agriculture: Mechanism, utilization, and value addition. *Land Degrad.Dev.29*(4), 1081-1095.

Pandey, G. K. (Ed.). (2017). *Mechanism of Plant Hormone Signaling Under Stress, 2 Volume Set* (Vol. 1). John Wiley & Sons.

Pandey, M., Paladi, R. K., Srivastava, A. K., & Suprasanna, P. (2021). Thiourea and hydrogen peroxide priming improved K+ retention and source-sink relationship for mitigating salt stress in rice. *Sci. reports*, *11*(1), 1-15.

Pandolfi, C., Mancuso, S., & Shabala, S. (2012). Physiology of acclimation to salinity stress in pea (Pisum sativum). *Environ.Exp. Bot.84*, 44-51.

Panjekobi, M., & Einali, A. (2021). Trehalose treatment alters carbon partitioning and reduces the accumulation of individual metabolites but does not affect salt tolerance in the green microalga Dunaliella bardawil. *Physiol.Mole. Biol.Plants*, *27*(10), 2333-2344.

Parida, A. K., Panda, A., & Rangani, J. (2018). Metabolomics-guided elucidation of abiotic stress tolerance mechanisms in plants. In *Plant metabolites and regulation under environmental stress* (pp. 89-131). Academic Press.

Polash, M. A. S., Sakil, M. A., & Hossain, M. A. (2019). Plants responses and their physiological and biochemical defense mechanisms against salinity: a review. *Trop. Plant Res*, *6*, 250-274.

Polash, M. A. S., Sakil, M. A., Tahjib-Ul-Arif, M., & Hossain, M. A. (2018). Effect of salinity on osmolytes and relative water content of selected rice genotypes. *Trop Plant Res*, *5*(2), 227.

Prajogo, D., & McDermott, C. M. (2014). Antecedents of service innovation in SMEs: Comparing the effects of external and internal factors. *J.Small Busi.Manag.*, *52*(3), 521-540.

Rashid, J. and Al-Marzoqi, A., (2019). Study of Vegetative structure of Middle Eastern Raspberry. *Medbiotech Journal*, *3*(03), pp.111-116.

Ratnakar, A., & Rai, A. (2013). Effect of sodium chloride salinity on seed germination and early seedling growth of Trigonella foenum-graecum L. Var. Peb. *Octa J. Environ. Res*. *1*(4).

Rehman, K., Ashraf, S., Rashid, U., Ibrahim, M., Hina, S., Iftikhar, T., & Ramzan, S. (2013). Comparison of proximate and heavy metal contents of vegetables grown with fresh and wastewater. *Pak. J. Bot*, *45*(2), 391-400.

Rengasamy, P. (2016). Soil salinization. In *Oxford Research Encyclopedia of Environmental Science*.

Rizvi, T. S., Khan, A. L., Ali, L., Al-Mawali, N., Mabood, F., Hussain, J., ... & Al-Harrasi, A. (2018). In vitro oxidative stress regulatory potential of Citrullus colocynthis and Tephrosia apollinea. *Acta Pharmaceutica*, *68*(2), 235-242.

Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A., ... & Sarwar, M. I. (2019). A review: Impact of salinity on plant growth. *Nat. Sci*, *17*(1), 34-40.

Salentijn, E. M., Esselink, D. G., Goryunova, S. V., van der Meer, I. M., Gilissen, L. J., & Smulders, M. J. (2013). Quantitative and qualitative differences in celiac disease epitopes among durum wheat varieties identified through deep RNA-amplicon sequencing. *BMC genomics*, *14*(1), 1-16.

Sarraf, M., Vishwakarma, K., Kumar, V., Arif, N., Das, S., Johnson, R., ... & Hasanuzzaman, M. (2022). Metal/Metalloid-Based Nanomaterials for Plant Abiotic Stress Tolerance: An Overview of the Mechanisms. *Plants*, *11*(3), 316.

Saxena, A. (2021). Application of Siderophore in Crop Productivity and Remediation of Heavy Metal-Contaminated Soil. In *Fungal Siderophores* (pp. 69-77). Springer, Cham.

Shafaei, H., Esmaeili, A., Rad, J. S., Delazar, A., & Behjati, M. (2012). Citrullus colocynthis as a medicinal or poisonous plant: a revised fact. *J.Med. plants Res. 6*(35), 4922-4927.

Shah, T., Latif, S., Saeed, F., Ali, I., Ullah, S., Alsahli, A. A., ... & Ahmad, P. (2021). Seed priming with titanium dioxide nanoparticles enhances seed vigor, leaf water status, and antioxidant enzyme activities in maize (Zea mays L.) under salinity stress. *J.K. Saud Univ.Sci.33*(1), 101207.

Shaheen-Kaleybar, B., Niazi, A., Afsharifar, A., Nematzadeh, G., Yousefi, R., Retzl, B., ... & Gruber, C. W. (2020). Isolation of cysteine-rich peptides from Citrullus colocynthis. *Biomolecules*, *10*(9), 1326.

Sharma, D. K., & Singh, A. (2015). Salinity research in India-achievements, challenges and future prospects.

Shatpathy, P., Kar, M., Dwibedi, S. K., & Dash, A. (2018). Seed priming with salicylic acid improves germination and seedling growth of rice (Oryza sativa L.) under PEG-6000 induced water stress. *IJCMAS*, *7*(10), 907-24.

Sheng, J., Wang, L., Han, Y., Chen, W., Liu, H., Zhang, M., ... & Liu, Y. N. (2018). Dual roles of protein as a template and a sulfur provider: a general approach to metal sulfides for efficient photothermal therapy of cancer. *Small*, *14*(1), 1702529.

Singh, V., Singh, A. P., Bhadoria, J., Giri, J., Singh, J., TV, V., & Sharma, P. C. (2018). Differential expression of salt-responsive genes to salinity stress in salt-tolerant and salt-sensitive rice (*Oryza sativa* L.) at seedling stage. *Protoplasma*, *255*(6), 1667-1681.

Sun, Y., Wen, C., Liang, X., & He, C. (2018). Determination of the phytoremediation efficiency of Ricinus communis L. and methane uptake from cadmium and nickel-contaminated soil using spent mushroom substrate. *ESPR*, *25*(32), 32603-32616.

Szechyńska-Hebda, M., Lewandowska, M., & Karpiński, S. (2017). Electrical signaling, photosynthesis and systemic acquired acclimation. *Front.Physiol*. *8*, 684.

Teets, N. M., & Denlinger, D. L. (2013). Physiological mechanisms of seasonal and rapid cold-hardening in insects. *Physiol.Entomol*. *38*(2), 105-116.l

Ungureanu, N., Vlăduț, V., & Voicu, G. (2020). Water scarcity and wastewater reuse in crop irrigation. *Sustainability*, *12*(21), 9055.

Van den Ende, W., & El-Esawe, S. K. (2014). Sucrose signaling pathways leading to fructan and anthocyanin accumulation: a dual function in abiotic and biotic stress responses?. *Env and Exp.Bot*, *108*, 4-13.

Vicente, V. A., Pratas, J. A., Santos, F. C., Silva, M. M., Favas, P. J., & Conde, L. E. (2021). Geochemical anomalies from a survey of stream sediments in the Maquelab area (Oecusse, Timor-Leste) and their bearing on the identification of mafic-ultramafic chromite rich complex. *Appl.Geochem.126*, 104868.

Victor, K.K., Séka, Y., Norbert, K.K., Sanogo, T.A. and Celestin, A.B., (2016). Phytoremediation of wastewater toxicity using water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes). *I. J.phytorem*, *18*(10), pp.949-955.

Vodyanitskii, Y. N. (2012). Standards for the contents of heavy metals and metalloids in soils. *Eurasian soil science*, *45*(3), 321-328.

Waqas, M. A., Kaya, C., Riaz, A., Farooq, M., Nawaz, I., Wilkes, A., & Li, Y. (2019). Potential mechanisms of abiotic stress tolerance in crop plants induced by thiourea. *Front. Plant Sci*. 1336.

Wu, H., Wu, X., Li, Z., Duan, L. and Zhang, M., (2012). Physiological evaluation of drought stress tolerance and recovery in cauliflower (Brassica oleracea L.) seedlings treated with methyl jasmonate and coronatine. *J.Pt. Growth Regul*, *31*(1), pp.113-123.

Xian, Y., Wang, M., & Chen, W. (2015). Quantitative assessment on soil enzyme activities of heavy metal contaminated soils with various soil properties. *Chemosphere*, *139*, 604-608.

Xie, X., He, Z., Chen, N., Tang, Z., Wang, Q., & Cai, Y. (2019). The roles of environmental factors in regulation of oxidative stress in plant. *BioMed res intl*, *2019*.

Yang, H., Zhang, Q., Dai, Y., Liu, Q., Tang, J., Bian, X., & Chen, X. (2015). Effects of arbuscular mycorrhizal fungi on plant growth depend on root system: a meta-analysis. *Plant and Soil*, *389*(1), 361-374.

Yang, L., Li, N., Kang, Y., Liu, J., Wang, Y., Sun, H., Ao, T. and Chen, W., (2022). Selenium alleviates toxicity in Amaranthus hypochondriacus by modulating the synthesis of thiol compounds and the subcellular distribution of cadmium. *Chemosphere*, *291*, p.133108.

Yang, Y., & Guo, Y. (2018). Unraveling salt stress signaling in plants. *J.Integr. Plant Biol.60*(9), 796-804.

Yasmeen, T., Ahmad, A., Arif, M. S., Mubin, M., Rehman, K., Shahzad, S. M., ... & Wijaya, L. (2020). Biofilm forming rhizobacteria enhance growth and salt tolerance in sunflower plants by stimulating antioxidant enzymes activity. *Plant Physiol. Biochem*. *156*, 242-256.

Yemm, E. W., & Willis, A. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J. 57*(3), 508.

Zhang, X.Q., Xu, C.F., Yu, C.H., Chen, W.X. and Li, Y.M., (2014). Role of endoplasmic reticulum stress in the pathogenesis of nonalcoholic fatty liver disease. *W j gastroent: WJG*, *20*(7), p.1768.

Zulfiqar, F., Akram, N. A., & Ashraf, M. (2020). Osmoprotection in plants under abiotic stresses: New insights into a classical phenomenon. *Planta*, *251*(1), 1-17.

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