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MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION OF *LESPEDEZA BICOLOR* TURCZ, FROM KATLANG REGION DISTRICT MARDAN, PAKISTAN

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Abstract

Lespedeza bicolor Turcz commonly called bicolor, bush clover or bicolor lespedeza of the family *Papilionaceae* has been collected from high saline field of Ditrict Mardan, Pakistan (34° 05' to 34° 32' north latitudes and 71" 48' to 72° 25' east longitudes). Field capacity of bulk soil was reported 14-16.5%, with EC: 4.1dS/m and pH: 8.5-9.2. Present research reveals bioremediation method to be the most environmentally sustainable method in dealing with soil salinity. According to the plant assessment for mineral uptake and heavy metal accumulation in root, stem and leaves of *Lespedeza bicolor*, Na^{+}, Ca^{+2} and Mg^{+2} were much accumulated whereas, K^{+} ion accumulation was reported least whereas, among the micro minerals Fe^{+2}, Cu^{+3} and Mn^{+2} were found higher than Zn^{+2}, Cr^{+3}, Co^{+3}, Pb^{+4}, Ni^{+3} and Cd^{+2}. Chlorophyll a content of *Lespedeza bicolor* leaves was 2-fold higher than chlorophyll b content whereas, high sugar and carotenoid content was reported in *Lespedeza bicolor* leaves at vegetative stages. *Lespedeza bicolor* appears to use proline and sugar as osmolyte for osmotic adjustment. Among the enzymes study Peroxidase (POD) and Catalase (CAT) activities were found higher in leaves of *Lespedeza bicolor* as compared to superoxide dismutase (SOD) and ascorbate peroxidase (APOX). The production of abscisic acid (ABA) was significantly higher as compared to indole acetic acid (IAA) in the selected plant.

Introduction

Halophytes are considered those plants, which have the ability to tolerate low water potential caused by salinity, so these can survive under saline soils (Munns & Tester, 2008). A diverse halophytic vegetation of 410 species from 58 families has been reported by Khan & Qaiser (2006) in Pakistan. *Lespedeza bicolor* has been reported in old fields, savannas, pine forests, woodlands, and along creek banks in the southeast of United States (Isely & Duane, 1998). *Lespedeza bicolor* is generally limited to old fields and prairies in the upper Midwest (Czarapata & Elizabeth 2005 and Small *et al*., 2001). Hoffman (1986), an agricultural scientist, hypothesized that beneficial effects of plants in reclamation are not well understood but appear to be related to the physical action of the plant roots. Phytoremediation of metals is a cost-effective "Green technology" often referred to as bioremediation, botanical-bioremediation, or green remediation based on the use of specially selected metal-accumulating plants to remove the salt ions, heavy metals, including radionuclides, from soils and water. Boyko (1959) was one of the first to suggest that halophytic plants could be used to desalinate soil and water. However, it stands to reason that plants that are able to accumulate sodium salts could be used successfully to remove sodium from the substrates they are grown in (Helalia *et al*., 1990). From 1930s, *Lespedeza bicolor* was widely planted for erosion control, mine reclamation and wildlife conservation (Mitchell & Wilma 1986, Gorsira *et al.*, 1994 and Thompson *et al.*, 1984). In 1930s 3-4 million *Lespedeza bicolor* were planted for stabilization of gully erosion.

The present study was aimed to describe the assessment of biochemical and physiological mechanism of adaptation of *Lespedeza Bicolor* collected from District Mardan.

Materials and Methods

Collection of plant samples: In the present study, *Lespedeza bicolor* along with three replicates were collected from saline field of District Mardan (altitiude of 400-1,700 meters a.s.l). Three samples of the selected plant species at vegetative stage were collected with their rhizospheric soil, at a depth of 6 inches, the physicochemical analysis of the soil were done and the leaves of the plant samples were used to study the biochemical and physiological analysis.

Elemental analysis: The physiochemical nature of the rhizospheric soil was studied; whereas leaves, stem and root of selected plant were used to study the elemental analysis through atomic absorption spectroscopy technique (AAS).

PH and electrical conductivity: The pH of rhizospheric soil was measured by preparing 1:1 (soil:water) suspension (McKeague, 1978 and Mclean, 1982). Air dried soil sample (10g) was mixed in 10ml distilled water and stirred for 1 hour on magnetic stirrer for homogenous mixing. Then the suspension was filtered with Whatman No. 42 filter paper. The pH of filtrate was determined with pH meter while electrical conductivity meter recorded the EC of extract. Readings were measured in microsiemens per centimeter (μS/cm).

Field capacity of rhizospheric soil: The field capacity of rhizospheric soil was determined following the method;

$$\% \text{ Field capacity} = \frac{\text{Weight of wet soil (g) - Weight of dry soil (g)}}{\text{Weight of dry soil (g)}} \times 100$$

Leaf epidermal anatomy: Fresh specimens were used for anatomical studies. For leaf epidermal anatomy Shultz methods of maceration with improved techniques was followed (Subrahmanyam, 1996). For peeling of epidermis fresh leaves were taken in a test tube covered with 4ml of concentrated nitric acid, to which 0.2g of

potassium chloride and 1ml distilled water was added. The mixture was boiled carefully. After a few second the epidermis of leaves was separated in the form of thin pellicle, the contents were emptied into a Petri dish partly filled with water. The methods of Clark, (1960) were used for Mounting of epidermal strips on Glass Slide. In this procedure peeled epidermis was kept in KOH solution for 24hours. KOH solution was prepared by adding 4-6 KOH pellets in 30ml of water. After 24 hours epidermal strips were washed and placed in bleach for 30 seconds. In the next step the epidermal strip was washed and placed in the center of glass slide with the help of forces and needles. Now 1-2 drops of lactic acid were poured on the epidermis. Cover slop was placed with extreme care to avoid air bubbles.

Physiological and biochemical analysis of the selected halophytes: The analysis of the salt stress response of the selected plant was done taking the leaves of the plants. This foliar material was fractionated for the biochemical and physiological analysis. The chlorophylls and carotenoids content were determined by the method of Arnon *et al*., (1949). The protein content was quantified according to the method of Lowry *et al*., (1951). Sugar content was measure by the method of Dubois *et al*., (1956). The proline contents of leaves were also measured according to the method of Bates *et al.,* (1973).

Determination of antioxidant enzymes: Fresh leaves (5g) were homogenized with 15ml of 0.05N phosphate buffer (PH 7.0) containing 10% poly vinyl poly pyrrolidore (PVPP) and 0.1 M Ethylene diamine tetra acetate (EDTA). Homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. Supernatant was used for SOD and POD assay. SOD was determined by measuring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) using method of Beauchamp, C., and Fridovich, 1971. POD was determined by the method of Vetter *et al*., (1958). APOX and CAT activity was determined according to Asada & Takahashi, (1987).

Extraction and purification of phytohormones: Extraction and purification of Abscisic acid and Indole acetic acid was made following the method of Kettner & Doerffling, (1995). The samples were analyzed on HPLC (Agilent 1100) equipped with variable UV detector and C18 column (39×300 mm) [BondaPack Porasil C-18, 37/50 µm, Waters, Eschborn, BRD). Methanol and water in the ratio of 30:70 v/v were used as mobile phase @ of 1,500 µl min^{-1} with a run time of 20 min $sample^{-1}$. The growth hormones were identified on the basis of retention time of phytohormone standards. IAA (indole-3-acetic acid) was studied at 280nm wavelengths while ABA was analyzed at 254nm respectively.

Statistical analysis: Analysis of variance was used for comparison between all the data using Statistica 8.0.

Results

Morphology: Lespedeza bicolor Turcz, commonly called Bush Clover is an erect, robust, deciduous, perennial shrub 1-2.5 m tall with neumerous slender stems and branches. Leaves trifoliate, alternate, elliptic, dark green in color; leaflets 1-1.5 x 0.5cm, oblong lower surface light in color, upper surface dark green. Flower arranged in terminal racemes or in the axils of upper leaves, 1.3cm long, purple in color. Fruit globose-ellipsoid, one-seeded. Flowering Period: August-September.

Anatomy: Epidermal cells were mostly pentagonal to hexagonal in shape, small in size, with about 84 cells /mm^2 were present abaxially and ±98 cells 20/mm^2 adaxially (Fig. 1a and 1b). Length of epidermal cells: 57.21 ± 1.24 (37.1, 76.9) µm, width: 39.62 ± 0.9 (20.6, 51.5) µm, thickness of cell wall: 5.27 ±0.09 (3.6, 6.3) µm with smooth surface. Stomatal length: 18.3 ± 0.37 (13.1, 23.3) µm, width: 11.15 ± 0.36 (5, 18.7) µm, length of guard cells: 36.75 ± 0.24 (33.6, 40) µm, width: 12.68 ± 0.3 (9.8, 16.5) µm, mostly Ranunculaceous or anomocytic type of stomata were found on both surfaces, being more numerous adaxially. About 20/mm^2 stomata were present on abaxial surface and ± 30 /mm^2 on adaxial surface. Uniseriate, multicellular, bulbous at base, glandular trichomes were observed with an average length of about 380 µm.

Macronutrients analysis (µg/g): Table 1 indicated that among the macro minerals Na^+ and Ca^{++} ion concentration of rhizospheric soil was higher in the range of 15µg/g, followed by Mg^{++}, whereas K^+ ion concentration was reduced in the rhizospheric soil. P and NO_3-N ion concentration of the rhizospheric soil was recorded 0.436µg/g and 0.824µg/g respectively. There was hyper accumulation of Na^+, Ca^{++} and Mg^{++} ions in the leaves of *Lespedeza bicolor* as compared to that of stem, root and rhizospheric soil. Among the macro minerals Ca^{++} ion concentration was found higher in *Lespedeza bicolor* with 16298-19502 µg/g followed by Na^+ and Mg^{++} respectively. The dominant cation in the present work was calcium (Ca^{++}) which is not supporting the work of Shirazi *et al*., (2011).

Micronutrients analysis (µg/g): It has been revealed in Table 2 that among the micro minerals Cu^{+3} ion concentration was higher of 1.575 µg/g in the rhizospheric soil followed by similar and considerable amount of Fe^{++}, Co^{+3} and Mn^{+2} whereas $Zn^{+2}$and Cr^{+3} showed the least concentrations of 0.045 µg/g and 0.068 µg/g respectively. Among the different plant parts of *Lespedeza bicolor*, leaves showed the hyper accumulation of Micronutrients Fe^{++}, Cu^{+3}, Zn^{+2}, Cr^{+3}, Co^{+3} and Mn^{+2} ions followed by stem and root. The overall uptake of the Fe^{++} concentration was found higher of 324-409 µg/g followed by Cu^{+3} (143-167 µg/g) and Mn^{+2} (64-72 Pb^{+4}), whereas Cr^{+3}, Zn^{+2} and Co^{+3} showed the least amount of accumulation in plant parts.

Heavy metal analysis (µg/g): Table 3 indicated that there was hyper accumulation of heavy metals Ni^{+3}, Li^{+1}, Pb^{+4} and Cd^{+2} ions in the leaves of *Lespedeza bicolor* in comparison with stem and root. The concentration of Ni^{+3}, Li^{+1}, Pb^{+4} and Cd^{+2} ions in rhizospheric soil was found in the range of 0-1.4µg/g. In the overall result the Pb^{+4} accumulations was found higher of (347-362 Pb^{+4}) followed by similar accumulation of Ni^{+3} and Cd^{+2} in the range of 18.9-27.8µg/g) in all parts of the plant. Li^{+1} showed the least accumulation of 7-10µg/g in the selected plant parts.

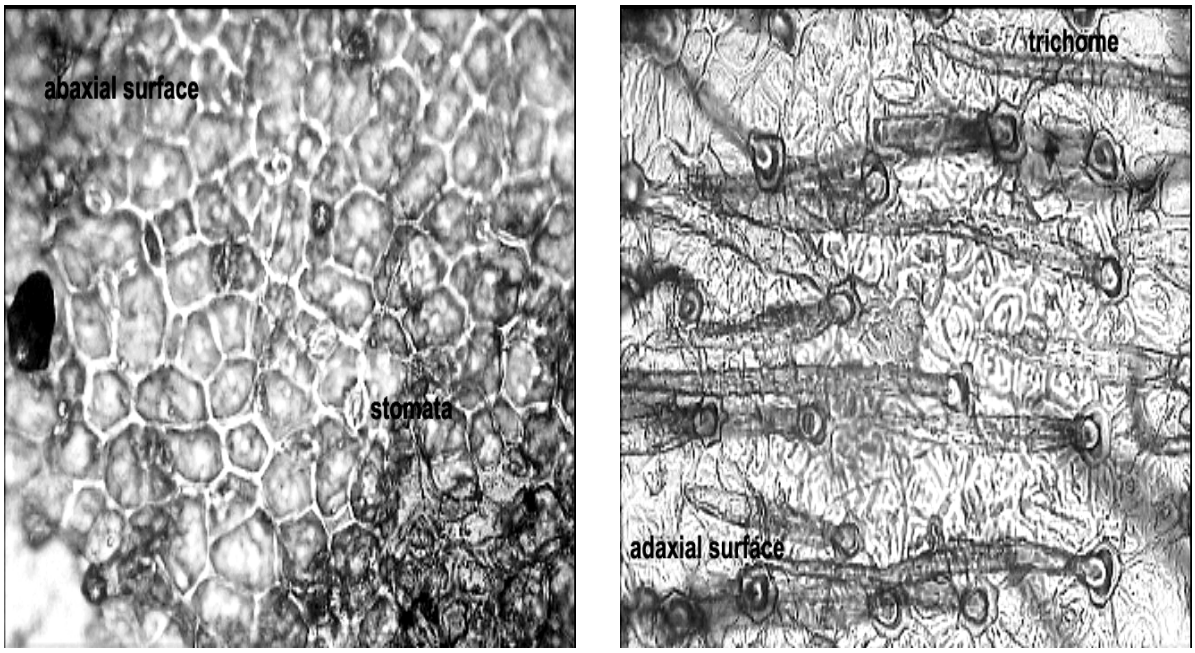

Fig. 1. (a) Showing abaxial surface (b) showing adaxial surface with multicellular trichomes.

Table 1. Macronutrient content (µg/g) of rhizospheric soil and different parts of *Lespedeza bicolor* collected from District Mardan, Pakistan.

Samples	Na^{+}	Ca^{+2}	K^{+}	Mg^{+2}	P	NO_3-N
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Rhizospheric soil	15.4	15.36	0.087	3.68	0.448	0.866
	+/-1.7	+/-1.1	+/-0.006	+/-0.97	+/-0.09	+/-0.06
Lespedeza root	2363	16676	2.6	1651	-	-
	+/-269.1	+/-422.7	+/-0.9	+/-78.5	-	-
Lespedeza shoot	2642	16451	3.3	1852	-	-
	+/-113.8	+/-655.5	+/-1.02	+/-120.3	-	-
Lespedeza leaves	2721	19268	3.6	1920	-	-
	+/-130.5	+/-1131.2	+/-0.79	+/-125.4	-	-

Data are expressed as mean ± SEM (n = 3) of three independent experiments

Table 2. Micronutrient content (µg/g) of rhizospheric soil and different parts of *Lespedeza bicolor* collected from District Mardan, Pakistan.

Samples	Fe^{+2}	Zn^{+2}	Cu^{+3}	Cr^{+3}	Co^{+3}	Mn^{+2}
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Rhizospheric soil	0.43	0.067	1.4	0.044	0.344	0.551
	+/-0.10	+/-0.01	+/-0.57	+/-0.01	+/-0.23	+/-0.12
Lespedeza root	324	15.7	144	25	5.3	64
	+/-15	+/-1.3	+/-5.5	+/-5.1	+/-1.6	+/-6.06
Lespedeza shoot	386	18.3	156	29	5.3	67
	+/-7.21	+/-1.7	+/-4.9	+/-2.8	+/-1.2	+/-7.2
Lespedeza leaves	408	21.6	168	35	6.4	72
	+/-2.08	+/-1.2	+/-2.6	+/-2.2	+/-1.09	+/-4.9

Data are expressed as mean ± SEM (n = 3) of three independent experiments

Table 3. Heavy metal analysis (µg/g) of rhizospheric soil and different parts of *Lespedeza bicolor* collected from District Mardan, Pakistan.

Samples	Ni^{+3}	Li^{+1}	Pb^{+4}	Cd^{+2}
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Rhizospheric soil	0.053	0.052	1.4	0.12
	+/-0.007	+/-0.01	+/-0.40	+/-0.02
Lespedeza root	28.6	7.3	346	20
	+/-3.27	+/-1.19	+/-4.91	+/-1.7
Lespedeza shoot	22	7.4	356	23
	+/-5.6	+/-1.05	+/-5.2	+/-1.7
Lespedeza leaves	27	9.5	362	26
	+/-1.7	+/-1.2	+/-4.9	+/-1.2

Data are expressed as mean ± SEM (n = 3) of three independent experiments

Chlorophylls and carotenoid contents, a growth response to saline condition: Fig. 2 showed that chlorophyll a content was found higher than chlorophyll b content in *Lespedeza bicolor* and a significant level of carotenoids was also recorded in *Lespedeza bicolor* (10.20mg/g). But the total chlorophyll content in the present work reduced under water stress condition is not supporting the work of Azhar *et al.,* (2011).

Osmolite contents: (sugar, proline and protein): Results in Fig. 3 revealed that higher proline content (mg/g) was found in *Lespedeza bicolor* followed by sugar content whereas, protein content was reported in minimum concentration in *Lespedeza bicolor*.

Antioxidant enzymes in response to oxidative stress: Results indicated in Fig. 4 showed that Peroxidase (POD) activity was found higher in *Lespedeza bicolor* followed by Ascorbate Peroxidase (APOX) whereas, Superoxide Dismutase (SOD) and Catalase (CAT) were found minimum in *Lespedeza bicolor.* The present results have been supported by the work of Hongyu *et al*., (2011).

Phytohormones in saline response: abscisic acid and indole acetic acid content: ABA referred as a "stress phytohormone" because its level increases in response to various environmental stresses. Fig. 5 showed that ABA contents were found higher in *Lespedeza bicolor* whereas, considerable amount of IAA was also reported.

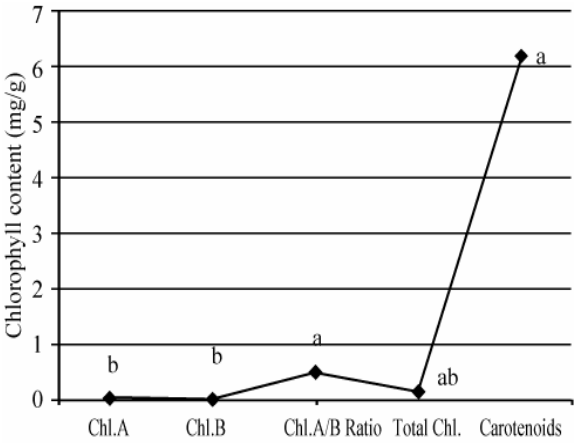

Fig. 2. Comparison of chlorophyll "a", chlorophyll "b", chlorophyll a/b ratio, total chlorophyll and carotenoid content (mg/g) of *Lespedeza bicolor* collected from District Mardan.

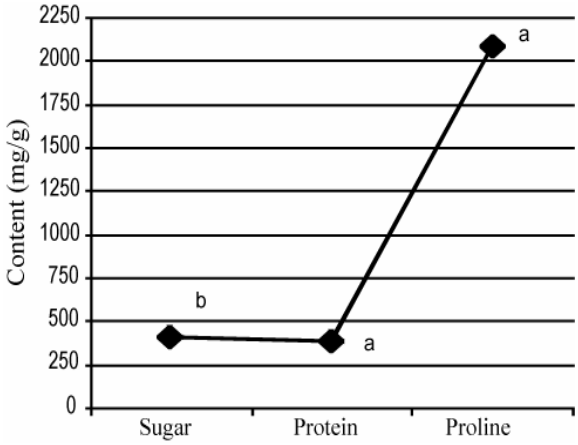

Fig 3. Comparison of sugar, protein and proline content (mg/g) of *Lespedeza bicolor* collected from District Mardan.

Discussion

Halophytes have evolved different mechanisms to deal with excess sodium and other salts in their environments (Gorham *et al.,* 1987). Some vascular halophytes accumulate high levels of sodium and other salts in their above ground tissue which support the present results. Holmes, (2001) reports that content of sodium in the soil was decreased by 65% with passage of time after planting salt accumulating plants species supporting the present experiment. According to Rush & Epstein, (1981) the accumulation of salts is thought to reduce the requirements for increased wall extensibility, leaf thickness, and water permeability that might otherwise be required to maintain positive growth and turgor at low soil water potentials. The previous work of Redman &

Fedec, (1987) described that most of halophytes exhibited relatively high concentrations of K^+, Mg^{2+}, and Ca^{2+}, correlates with the present work. According to Gebauer & Ebert (2003) and Abdel-Kader, (2000) chlorophyll *a, b* and carotenoid content in plant leaves decreases under soil salinity due to the increase in their degradation under stress condition. Higher amount of Proline and sugar was found in *Lespedeza bicolor* supported that a strong correlation is present between osmolites accumulation and the osmotic adjustment (Gilmour *et al*., 2000; Ashraf & Foolad 2007). According to Agastian *et al*., (2000) proteins level decreased under salinity due to low uptake of nitrate ions supporting the present work. Sreenivasulu *et al*., (2000) demonstrated that salt stress leads to a decrease in SOD activity in salt-sensitive plants but to an increase in salt-tolerant one. ABA contents were found higher in *Lespedeza bicolor* which plays a key role in plants under salinity condition (Zhang *et al*., 2006).

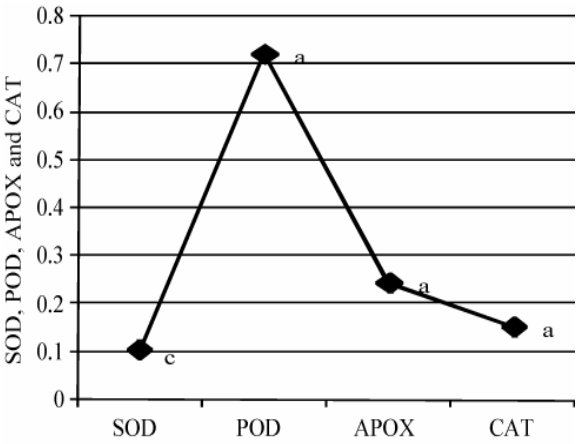

Fig 4. Comparison of SOD (units g^{-1} f.w), POD (OD/min/g f.w), APOX (OD $min^{-1}g^{-1}$f.w), and CAT Activity (OD $min^{-1}g^{-1}$f.w) of *Lespedeza bicolor* collected from District Mardan.

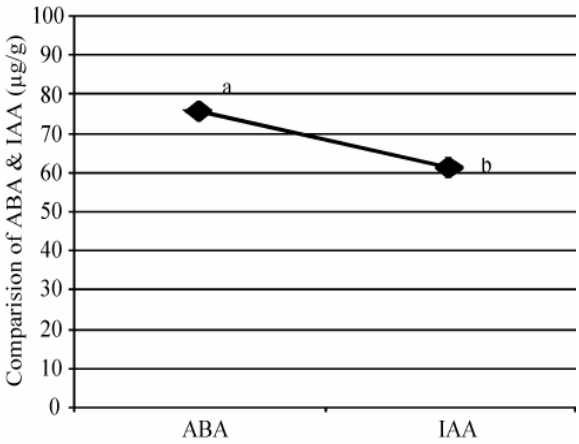

Fig 5. Comparison of the ABA and IAA (µg/g) of *Lespedeza bicolor* collected from District Mardan.

Conclusion

The present experiment showed that ABA and proline production interaction can be treated as a key character for salt tolerance in *Lespedeza bicolor* during hyper accumulation of heavy metal and ions.

References

Agastian, P., S.J. Kingsley and M. Vivekanandan. 2000. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica*, 38: 287-290.

Arnon, D.I. 1949. Copper enzyme in isolated chloroplast. Polyphenol oxidase in beta vulgaris. *Plant Physiol*., 24: 1-15.

Asada, K. and M. Takahashi. 1987. Production and scavenging of active oxygen in photosynthesis. In: (Eds.): C.J. Arntzen, Amsterdam. 227-287.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Env. and Exp. Botany*, 59(2): 206-216.

Azhar, N., B. Hussain, M.Y. Ashraf and K.Y. Abbasi. 2011. Water stress mediated changes in growth, physiology and Secondary metabolites of desi ajwain (*Trachyspermum ammi* L.). *Pak. J. Bot*., 43(1): 15-19.

Bates, I.S., R.P. Waldern and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 205-207.

Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem*., 44: 276-287.

Boyko, H. and E. Boyko. 1959. Seawater irrigation, a new line of research on a bioclimatic plant-soil complex. *Int. J. Bioclim*. 3: 1–17.

Clark, J. 1960. Preparation of leaf epidermis for topographic study. *Stain. Techno*., 35: 35-39.

Czarapata, E.J. 2005. Invasive plants of the Upper Midwest: An illustrated guide to their identification and control. Madison, WI: The University of Wisconsin Press. 215.

Dubois, S.M., K.A. Giles., J.K. Hamilton., P.A. Rebers and F. Smith. 1956. Calorimetric method of sugar and related substances. *Anal. Chem*., 28: 350.

Gebauer, J. and G.D. Ebert. 2003. Tamarinde (*Tamarandus arindus indica* L.). Anabu and Verwendungeiner interessanten Obstart der Tropen and Subtropen. *Erwerbsobstbau*. 45: 181-185.

Gilmour, S.J., A.M. Seblot, M.P. Salazar, J.D. Everard and M.F. Thomashow. 2000. Overexpression of Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. *Plant Physiol*., 124: 1854-1865.

Gorham, J., C. Hardy, J. Wyn, L.R. Joppa and C.N. Law. 1987. Chromosomal location of the K/Na discriminating character in the D genome of wheat. *Theor. Appl.Genet*., 74: 584-588.

Gorsira, B. and K.L. Risenhoover. 1994. An evaluation of woodland reclamation on strip-mined lands in east Texas. *Environmental Management*, 18(5): 787-793.

Helalia, A.M., S.E. Amir., S.T. Abou-Zeid and K.F. Zagholoul. 1990. Bioremediation of saline-sodic soil by amshot grass in northern Egypt. *Soil and Tillage Research*, 22: 109-116.

Hoffman, G. 1986. Guideline for reclamation of salt-affected soils. *Appl. Agric. Res. J*., 1(2): 65-72.

Holmes, P.M. 2001. Mycorrhizal colonization of halophytes in central European salt marshes. Referenced by E.P. Glenn in Scientific Amer. April 2001, 112-114.

Hongyu, M., R. Yang., Z. Wang., T. Yu., Y. Jia., H. Gu., X. Wang and H. Ma. 2011. Screening of salinity tolerant jute

(*corchorus capsularis & c. Olitorius*) genotypes via phenotypic and phsiology-assisted procedures. *Pak. J. Bot*., 43(6): 2655-2660.

Isely, D. 1998. Native and naturalized Leguminosae (Fabaceae) of the United States (exclusive of Alaska and Hawaii). Provo, UT: Brigham Young University, Monte L. Bean Life Science Museum. 1007.

Kader, A.Z. 2000. Salinity and adaptation effects on lipids peroxidation and antioxidant enzymes in Raphanus sativus and Eruca sativa seedling. *J. Union Arab Biol*., Vol. (8 B): 59-71.

Kettner, J. and Doerffling. 1995. Biosynthesis and metabolism of abscisic acid in tomato leaves infected with Botrytis cinerea. *Planta.*, 196: 627-634.

Khan, M.A. and M. Qaiser. 2006. Halophytes of Pakistan: Distribution, Ecology, and Economic Importance In: (Eds.): M.A. Khan, H. Barth. G.C. Kust & B. Boer. Sabkha Ecosystems: Volume II: The South and Central Asian Countries. 129-154. Kluwer Academic Press, Netherlands.

Lowry, H.O., N.J. Poesenbrough, A.L. Fal and R.J. Randall. 1951. Protein measurement with folin phenol reagent, *J. Biol. Chem*., 193: 265-275.

McKeague, J.A. 1978. Manual on soil sampling and methods of analysis. *Canadian Society of Soil Science*, pp. 66-68.

McLean, E.O. 1982. Soil pH and lime requirement. In: *Methods of soil analysis*. (Ed.): A.L. Page. Part 2: chemical and microbiological properties. Am. Soc. Agron, Madison, WI, USA. 199-224.

Mitchell, W.A. 1986. Bicolor *lespedeza* (*Lespedeza bicolor*). Technical Report EL-86-23. Section 7.3.2-US Army Corps of Engineers Wildlife Resources Management Manual. Vicksburg, MS: Department of the Army, Corps of Engineers, Waterways Experiment Station, *Environmental Impact Research Program, Work Unit*., 31631. 12.

Munns, R. and M. Tester. 2008. Mechanism of Salinity Tolerance. *Annual Review of Plant Biology*, 59(1): 651-681.

Redman, R.E. and P. Fedec. 1987. Mineral composition of halophytes and associated soils in Western Canada. In: Field survey of Kocia scoparia inland halophytes of the United States. *Commun. Soil Sci. Plant Anal*., 18: 559.

Rush, D.W. and E. Epstein. 1981. Breeding and selecting for crop tolerance by the incorporation of wild germplast into a domesticated tomato. *J. Amer. Soc. Hort. Sci*., 106: 669-670.

Shirazi, U.M., M. T. Rajput, M. A. Khan, M. A. Shaikh, M. Mujtaba, A. Shereen, S. Mumtaz and M. Ali. 2011. Growth and ions (Na^+, K^+ and Cl^-) accumulating pattern of some *brassica* genotypes under saline–sodic field condition. *Pak. J. Bot*., 43(6): 2661-2664.

Small, C.J., M. Carthy and C. Brian. 2001. Vascular flora of the Waterloo Wildlife Research Station, Athens County, Ohio. *Castanea*. 66(4): 363-382.

Sreenivasulu, N., B. Grimm, U. Wobus and W. Weschke. 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerance and salt-sensitive seedlings of foxtail millet (*Setaria italica*). *Physiol. Plant*., 109: 435-442.

Subrahmanyam, N.S. 1996. *Labortary Manual of Plant Taxonomy*. Vikas Publishing house Pvt. Ltd. New Delhi. 153-156.

Thompson, R.L., G. Vogel, W. Taylor and D. David. 1984. Vegetation and flora of a coal surface-mined area in Laurel County, Kentucky. *Castanea*, 49(3): 111-126.

Vetter, J.L., M.P. Steinberg and A. Nelson. 1958. Quantitative determination of peroxidase in sweet corn. *I. J. Agric. Food Chem*. 6: 39.

Zhang, G., W.G. Zhizheng, W.P. Shi and W.J. Wang. 2006. Study on the impact of different salinity of sea-ice water on seed germination of cotton, wheat and maize. *Journal of Beijing*, 42(2): 209-212.

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