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IMPACT OF PROLINE, GLYCINE BETAINE AND MANNITOL APPLICATION ON *LEPIDIUM SATIVUM* L. PLANTS GROWN UNDER ABIOTIC STRESS CONDITIONS (WATER STRESS)

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Abstract

The effects of osmolyte proline, glycine betaine and mannitol on growth and physiology were investigated in *Lepidium sativum* L. Plants were grown under controlled temperature (25°C) and light conditions (16 hours light and 8 hours dark). Growth parameters (shoot and root length) physiological analysis (total chlorophyll, electrical conductivity, membrane stability index and relative water content) were measured after 35,55,75,95 and 110 days. Exogenous application of each osmolytes (50µg/l, 100µg/l, and 250µg/l) were standardized and applied to different water potential $-0.01q_w$ MPa, $-0.02q_w$ MPa and $-0.03q_w$ MPa. Proline enhanced more growth in stressed plants as compared to glycine betaine and mannitol; it is high enough to play a significant role in cellular osmotic adjustment. Despite the present study indicating that osmolytes play a fundamental role under water stress in *Lepidium sativum* L. Therefore, it appears that the plants can cope with abiotic stress when exogenous osmolytes are applied.

Key words : Glycine betaine, Mannitol, Proline, Relative water content, Water stress.

Introduction

Lepidium sativum (Garden cress) is an annual herb belonging to the Brassicaceae family. In Ayurveda, it is an important medicinal plant; its seeds, leaves, and roots are economically and medicinally important. It is cultivated all over India and is consumed as a leafy vegetable. It is an erect, glabrous, annual, herbaceous plant growing up to 15-60 cm in height. The leaves are used in salads, cooked with other vegetables, and used to garnish food (Wadhwa *et al.,* 2012). Due to Abiotic stress conditions causing cellular dehydration in plants, such as cold, elevated temperatures, water or exposure to heavy metals is based on the synthesis and cytoplasmic accumulation of osmolytes, a well-maintained phenomenon detected in all plants, tolerant as well as sensitive to stress (Parvaiz and Satyawati, 2008). Water stress is the primary environmental stress that limits plant growth as well as development. Plants have complicated developmental mechanisms at cellular and molecular levels to alleviate the damaging effects of water deficiency (Chaves *et al.,* 2009; Shen *et al.,* 2014). Drought stress induces the generation of reactive oxygen species, leading to oxidative stress (Bartels and Sunkar, 2005). When ROS accumulate in plant tissues, it damages lipids, proteins, DNA, and accordingly leading to cell death (Molassiotis *et al.,* 2006). To alleviate the toxic effects of ROS, plants developed an antioxidant defense system including both enzymatic that is superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), glutathione reductase (GR), non-enzymatic antioxidants including ascorbate and glutathione (Foyer and Noctor, 2005; Mittler, 2002; Gill and Tuteja, 2010; Suzuki *et al.,* 2012). It seems that the balance between ROS production and capability of scavenging ROS via antioxidant system effects on drought tolerance to plant (Boaretto *et al.,* 2014). Osmolytes are 'compatible solutes' very soluble, low-molecular-weight organic complexes that do not interfare with normal metabolism even when present at high

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concentrations. While harmful inorganic ions sequestered in vacuoles, organic osmolytes accumulate predominantly in the cytoplasm, avoiding or preventing cellular dehydration (Bussis and Heineke, 1998; Handa *et al.,* 1986; Stewart and Lee, 1974). A reduction of the osmotic potential due to accumulation of osmolytes in response to stress improves the capability of the plant cells to maintain turgor pressure at low water potentials, which is essential for biological processes such as photosynthesis or cell expansion, as well as for maintaining enzymatic activities (Tyree and Jarvis, 1982). Moreover, their role in osmotic adjustment, osmolytes act as osmoprotective substances, directly stabilizing proteins, and cell membranes under dehydration conditions. Osmolytes also protect cells from oxidative stress by inactivating 'reactive oxygen species' (ROS) (Szabados and Savoure, 2010). The amino acid proline (Pro) and glycine betaine (GB), a quaternary amine, are certainly the most common compatible solutes synthesized by plants as a reply to abiotic stress (Ashraf and Foolad, 2007; Chen and Murata, 2008; Verbruggen and Hermans, 2008). As for other osmolytes, and their role in osmoregulation, both compounds can act as 'low-molecular-weight chaperons,' contributing to maintain the active conformation of macromolecules in stressed plants and contribute in detoxification of ROS. Furthermore, Pro and GB appear to be involved, directly or indirectly, in the regulation of gene expression as signaling molecules, also contribution as metabolites for the cellular storage of carbon and nitrogen throughout stress, which would be used by the cell once stress has ceased (Szabados and Savoure, 2010). Compatible solutes similarly comprise soluble carbohydrates, such as sugars (e.g., sucrose, glucose, fructose or trehalose), sugar alcohols (sorbitol, mannitol, thriving as different inositol isomers and derivatives), and the raffinose family of oligosaccharides (Gavaghan *et al.,* 2011; Parida *et al.,* 2002). Although sugars shown to act as functional osmolytes in several species, it is not so easy to assess their specific functions in the responses to stress, which can be masked by their multiple additional roles as direct products of photosynthesis, components of the primary metabolism and regulatory molecules (Gil *et al.,* 2013). A secondary effect of abiotic stresses, including drought and salinity, is the increased generation of 'reactive oxygen species' (ROS), including highly reactive free radicals such as superoxide, singlet oxygen, hydroxyl or per-hydroxyl radicals, as well as hydrogen peroxide, molecular oxygen, ozone and other strong oxidant molecules (Apel and Hirt, 2004). ROS continuously generated by plants as by-products of different metabolic pathways, but under stress their production increases leading to oxidative damage of cellular membranes, proteins, carbohydrates and DNA (Van Breusegem and Dat, 2006). In a comeback to stress, plants activate powerful antioxidant systems, both enzymatic and non-enzymatic (Apel and Hirt, 2004). The aims of the present study were to analyze the effects on the growth, and physiology of *Lepidium sativum* L. plant to water stress tretments, applied under different osmolyte (proline, glycine betaine and mannitol) concentration beyond the tolerance threshold, to allow detection of time-water stress concentration dependent effects. These growth responses were correlated with abiotic stress tolerance: the main osmolytes (proline, glycine betaine and mannitol), responsible for cellular osmotic adjustment. The experiment were carried out in Lepidium sativum L. a plant that were not been extensively studied despite its growing commercial interest.

Materials and Methods

Seed source

The seeds of L. *sativum* L. obtained from Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P). Seeds were grown in polyhouse at Shoolini University of Biotechnology and Management Sciences, Solan (H.P) India.

Plant Growth

The seeds were sown in seed trays having soil placed in a polyhouse through regulated temperatures ranging between 20 to 25 °C, under a long-day photoperiod (16 h light / 8 h dark). 10days old seedling shifted to individual pots. After a sowing of 20 days water stress and osmolytes, treatments started. Water stress imposed by watering while weighing method. Different water potential ($-0.01q_w$ MPa, $-0.02q_w$ MPa and $-0.03q_w$ MPa) was achieved at 20 days of seed sowing. Seedling fertilized by adding Hoagland nutrient solution to each pot after every seven days. Plants parts (Leaves) sampled to determine the morphology and physiology of plants after 35,55,75,95 and 110 days. Osmolytes concentration used for treatment 50µg/l, 100µg/l, 250µg/l, and applied through foliar spray.

Shoot and Root Length

The shoot and root length of L. *sativum* L. in centimeter were measured by using the scale.

Relative water content (RWC)

The fresh weight of leaves from each treatment weighed. The leaves dipped in distilled water in a beakers and left for 24 h. Then, fully turgid leaves weighed again. The leaves desiccated in oven intended for 72 h at 70 ºC, up to the constant weight of leaves obtained. The relative water content of leaves calculated according to

(Wheatherley, 1950).

$$\mathrm{RWC} = \frac{\text{Fresh mass} - \text{Dry mass}}{\text{Saturated mass} - \text{Dry mass}} \times 100$$

Chlorophyll

Chlorophyll extraction is done by using dimethyl sulphoxide (DMSO) chlorophyll extraction technique (Hiscox and Israelstam, 1979). Instead of the extractions, glass centrifuge vessels containing 7 ml DMSO heated to 65°C in a water bath. The spectrophotometer calibrated to zero utilizing an absolute of pure DMSO. The absorbance of individually blank as well as sample measured at 645 and 663 nm.

Electrical Conductivity

Weighed and transferred the 100 mg of leaf sample in a 100 ml beaker additional 40 ml of distilled water and kept for 1 h on a shaker. Allowed to stand until vibrant supernatant liquid obtained. Calibrated the conductivity bridge through the help of a standard KCl solution then determined the cell constant.

Membrane Stability Index

The membrane stability index was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40º and 100°C (Sairam, 1994). Leaf samples (100mg) cut into discs of undeviating size and put in test tubes containing 10 ml of double distilled water in two sets. The single set kept at 40°C for 30 min and another set at 100°C in boiling water bath for 15 min and their relevant electric conductivity's C_1 and C_2 measured by a conductivity meter.

$$\text{Membrane stability index} = \left[1 - \left(\frac{C_1}{C_2}\right)\right] \times 100$$

At the end of the experiment, data subjected to analysis of variance (ANOVA) and mean separation. The statistical analysis was done using Graph Pad Prism® 5.2. The least significant difference (LSD) at 5% level used to compare the means of different test parameters. Data are mean ± SD, of three replicates (n=3) were examined by Two way Anova followed by Bonnferoni multiple comparison post-tests $P<0.05^{*}$, $P<0.01^{**}$, $P<0.001^{***}$ significance level.

Results

Shoot and Root length

The shoot and root length of the *Lepidium sativum* L. increased. When exogenous proline, glycine betaine and mannitol was applied by 4,3% in 50µg/l, 5,7% in 100µg/l and 5,12% in 250µg/l at 35 days. 6,5% in 50µg/l, 7,6% in 100µg/l and 7,10% in 250µg/l at 55 days. By 7,7% in 50µg/l, 8,11% in 100µg/l and 9,16% in 250µg/l at 75 days then by 6,2% in 50µg/l, 7,4% in 100µg/l and 8,9% in 250µg/l at 95 days. By 5,5% in 50µg/l, 5,9% in 100µg/l and 5,14% in proline 250µg/l at 110 days. In case of glycine betaine it is increased by 2,1% in 50µg/l, 4,9% in 100µg/l and 4,14% in 250µg/l at 35 days. 4,1% in 50µg/l, 5,5% in 100µg/l and 5,8% in 250µg/l at 55 days. By 6,3% in 50µg/l, 7,6% in 100µg/l and 8,8% in 250µg/l at 75 days then by 5,2% in 50µg/l, 6,1% in 100µg/l and 7,1% in 250µg/l at 95 days. By 4,3% in 50µg/l, 5,6% in 100µg/l and 5,11% in glycine betaine 250µg/l at 110 days. Moreover in case of mannitol it enhanced 1,1% in 50µg/l, 2,1% in 100µg/l and 3,2% in 250µg/l at 35 days. 3,12% in 50µg/l, 4,18% in 100µg/l and 5,24% in 250µg/l at 55 days. By 5,3% in 50µg/l, 6,5% in 100µg/l and 4,10% in 250µg/l at 75 days then by 5,1% in 50µg/l, 6,1% in 100µg/l and 6,1% in 250µg/l at 95 days. By 4,3% in 50µg/l, 4,12% in 100µg/l and 5,13% in mannitol 250µg/l at 110 days shoot and root length respectively compared to their respective control shown in (Table 1a, b, c & Table 2a, b, c) (Figs. 1a, b, c. Figs. 2a, b, c. respectively). The shoot and root length maximum increased in case of proline as compared to glycine betaine and mannitol.

Relative water content

The relative water content was enhanced in *Lepidium sativum* L. With exogenous application of proline, glycine betaine and mannitol was applied by 13% in 50µg/l, 16% in 100µg/l and 21% in 250µg/l at 35 days. 6% in 50µg/l, 6% in 100µg/l and 7% in 250µg/l at 55 days. By 5% in 50µg/l, 7% in 100µg/l and 9% in 250µg/l at 75 days then by 7% in 50µg/l, 8% in 100µg/l and 9% in 250µg/l at 95 days. By 6% in 50µg/l, 7% in 100µg/l and 8% in proline 250µg/l at 110 days. In case of glycine betaine it is increased by 10% in 50µg/l, 13% in 100µg/l and 18% in 250µg/l at 35 days. 4% in 50µg/l, 5% in 100µg/l and 6% in 250µg/l at 55 days. By 3% in 50µg/l, 6% in 100µg/l and 8% in 250µg/l at 75 days then by 7% in 50µg/l, 5% in 100µg/l and 5% in 250µg/l at 95 days. By 5% in 50µg/l, 6% in 100µg/l and 8% in glycine betaine 250µg/l at 110 days. Then in mannitol it is increased 8% in 50µg/l, 11% in 100µg/l and 18% in 250µg/l at 35 days. 2% in 50µg/l, 4% in 100µg/l and 5% in 250µg/l at 55 days. By 2% in 50µg/l, 6% in 100µg/l and 7% in 250µg/l at 75 days then by 4% in 50µg/l, 4% in 100µg/l and 4% in 250µg/l at 95 days. By 5% in 50µg/l, 6% in 100µg/l and 8% in mannitol 250µg/l at 110 days compared to control shown in (Table 3a, b, c & Figs. 3a, b, c respectively). The relative water content maximum increased was observed in proline.

Total Chlorophyll Content

Table 1: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on shoot length (cm) of *Lepidium sativum* L. Data are mean ± SD of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonnferoni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level . Different lower case letters in a table indicate significant difference between control and treatment.

Treatments	35Days	55Days	75 days	95Days	110Days
Control	13.236±0.247	25.910±0.115	36.866±0.995	40.816±0.343	47.566±0.440
Control Proline (50) µg/l	13.816±0.033	27.500±0.458a	38.966±0.392a	43.290±0.025a	49.866±0.260a
Proline50 µg/l and -0.01q_wMPa	13.570±0.226	27.233±0.338	38.633±0.296	42.953±0.347	49.466±0.218
Proline50 µg/l and -0.02q_wMPa	13.396±0.409	27.066±0.284	38.400±0.305	42.783±0.125	49.366±0.497
Proline50 µg/l and -0.03q_wMPa	12.940±0.446a	26.766±0.260a	38.333±0.166	42.516±0.114a	49.266±0.120
Control Proline (100) µg/l	13.894±0.045	27.666±0.384b	39.200±0.513b	43.726±0.427b	50.033±0.133b
Proline100 µg/l and -0.01q_wMPa	13.678±0.237	27.133±0.176	38.900±0.378	43.393±0.096	49.766±0.352
Proline100 µg/l and -0.02q_wMPa	13.506±0.182	26.966±0.120b	38.800±0.305	43.173±0.360	49.600±0.351
Proline100 µg/l and -0.03q_wMPa	13.133±0.386b	26.633±0.284c	38.600±0.378	42.990±0.337b	49.366±0.218
Control Proline (250) µg/l	13.884±0.035	27.766±0.437d	39.466±0.260c	43.972±0.358c	50.133±0.120c
Proline250 µg/l and- 0.01q_wMPa	13.651±0.197	27.433±0.240	39.333±0.166	43.639±0.480	49.766±0.133
Proline250 µg/l and -0.02q_wMPa	13.640±0.120	27.266±0.202	39.133±0.409	43.516±0.243	49.600±0.251
Proline250 µg/l and -0.03q_wMPa	13.634±0.165	27.133±0.338	38.800±0.152	43.493±0.588	49.566±0.185

(a)

Treatments	35Days	55Days	75 days	95Days	110Days
Control	13.236±0.247	25.910±0.115	36.866±0.995	40.816±0.343	47.566±0.440
Control Glycine Betaine (50) µg/l	13.564±0.232	26.900±0.115a	38.633±0.296a	42.960±0.305a	49.433±0.088a
Glycine Betaine 50 µg/l and -0.01q_wMPa	13.363±0.238	26.700±0.115	38.300±0.298	42.656±0.306	49.300±0.057
Glycine Betaine 50 µg/l and -0.02q_wMPa	13.168±0.380	26.566±0.066	38.233±0.145	42.410±0.577	49.233±0.120
Glycine Betaine 50 µg/l and -0.03q_wMPa	13.032±0.329	26.333±0.088	38.066±0.066	42.266±0.126a	49.133±0.088
Control Glycine Betaine (100) µg/l	13.753±0.095a	27.112±0.657a	38.866±0.523b	43.430±0.620b	49.766±0.133b
Glycine Betaine 100 µg/l and -0.01q_wMPa	13.514±0.202	26.833±0.176	38.533±0.202	43.196±0.692	49.566±0.176
Glycine Betaine 100 µg/l and -0.02q_wMPa	13.387±0.092	26.766±0.240	38.333±0.333	42.984±0.633	49.400±0.251
Glycine Betaine 100 µg/l and -0.03q_wMPa	13.211±0.030	26.566±0.066	38.102±0.288b	42.403±0.514b	49.266±0.120
Control Glycine Betaine (250) µg/l	13.781±0.187	27.166±0.176b	39.133±0.409c	43.636±0.192c	50.033±0.088c
Glycine Betaine 250 µg/l and -0.01q_wMPa	13.635±0.214	26.833±0.176	38.833±0.166	43.440±0.067	49.700±0.251
Glycine Betaine 250 µg/l and -0.02q_wMPa	13.536±0.183	26.566±0.176	38.666±0.440	43.111±0.349	49.566±0.120
Glycine Betaine 250 µg/l and -0.03q_wMPa	13.410±0.215	26.466±0.317b	38.333±0.166c	43.001±0.318	49.466±0.260

(b)

Treatments	35Days	55Days	75 days	95Days	110Days
Control	13.236±0.247	25.900±0.115	36.366±0.995	40.816±0.343	47.566±0.440
Control Mannitol (50) µg/l	13.352±0.201	26.800±0.152a	38.233±0.504a	42.673±0.293a	49.233±0.120a
Mannitol 50 µg/l and -0.01q_wMPa	13.136±0.034	26.633±0.317	38.104±0.288	42.330±0.052	49.066±0.284
Mannitol 50 µg/l and- 0.02q_wMPa	13.110±0.052	26.466±0.033	37.733±0.233	42.143±0.598	48.933±0.233
Mannitol 50 µg/l and -0.03q_wMPa	13.085±0.044	26.266±0.120	37.700±0.351	41.883±0.195a	48.866±0.185
Control Mannitol (100) µg/l	13.566±0.283	26.966±0.290b	38.400±0.493b	43.076±0.322b	49.533±0.202b
Mannitol 100 µg/l and -0.01q_wMPa	13.327±0.259	26.833±0.405	38.233±0.504	42.830±0.345	49.400±0.328
Mannitol 100 µg/l and -0.02q_wMPa	13.423±0.248	26.666±0.284	38.066±0.296	42.453±0.217	49.300±0.368
Mannitol 100 µg/l and -0.03q_wMPa	13.350±0.252	26.633±0.185	37.896±0.208	42.370±0.226b	49.236±0.031
Control Mannitol (250) µg/l	13.607±0.188	27.133±0.185c	38.900±0.208c	43.302±0.166c	49.933±0.033c
Mannitol 250 µg/l and -0.01q_wMPa	13.350±0.068	26.966±0.290	38.733±0.371	42.968±0.490	49.766±0.133
Mannitol 250 µg/l and -0.02q_wMPa	13.279±0.139	26.800±0.152	38.600±0.057	42.643±0.162c	49.633±0.185
Mannitol 250 µg/l and -0.03q_wMPa	13.211±0.112	26.633±0.240	38.566±0.348	42.456±0.272d	49.566±0.202

(c)

Table 2: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on root length (cm) of *Lepidium sativum* L. Data are mean ± SD of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonnferoni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level . Different lower case letters in a table indicate significant difference between control and treatment.

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	4.866±0.202	5.166±0.145	6.033±0.328	6.800±0.243	7.933±0.218
Control Proline (50) µg/l	5±0.200	5.433±0.338	6.466±0.218	6.933±0.025	8.333±0.296
Proline50 µg/l and -0.01q_wMPa	4.866±0.176	5.366±0.497	6.133±0.497	6.600±0.347	8.100±0.132
Proline50 µg/l and -0.02q_wMPa	4.766±0.338	5.200±0.721	5.903±0.347	6.500±0.125	8±0.112
Proline50 µg/l and -0.03q_wMPa	4.600±0.230	4.733±0.688a	5.633±0.352a	5.766±0.114a	7.866±0.145
Control Proline (100) µg/l	5.200±0.173	5.500±0.360	6.700±0.550	7.100±0.427	8.666±0.233a
Proline100 µg/l and -0.01q_wMPa	5.066±0.088	4.800±0.404b	6.333±0.466	6.766±0.096	8.433±0.233
Proline100 µg/l and -0.02q_wMPa	4.933±0.088	4.666±0.296c	6.200±0.152	6.500±0.360	8.366±0.272
Proline100 µg/l and -0.03q_wMPa	4.866±0.185	4.566±0.033d	6.033±0.589	6.333±0.337b	8.200±0.404
Control Proline (250) µg/l	5.466±0.145	5.666±0.272	7±0.251b	7.433±0.358	9.033±0.433b
Proline250 µg/l and- 0.01q_wMPa	5.300±0.102	5.333±0.088	6.666±0.272	6.966±0.480	8.900±0.300
Proline250 µg/l and -0.02q_wMPa	5.100±0.057	5.100±0.057	6.566±0.176	6.633±0.243c	8.800±0.416
Proline250 µg/l and -0.03q_wMPa	4.933±0.133	5±0.251	6.366±0.088	6.300±0.588d	8.733±0.133

(a)

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	4.866±0.202	5.166±0.145	6.033±0.328	6.800±0.243	7.933±0.218
Control Glycine Betaine (50) µg/l	4.900±0.400	5.204±0.057	6.233±0.145	6.910±0.305	8.133±0.120
Glycine Betaine 50 µg/l and -0.01q_wMPa	4.733±0.371	4.766±0.384	6.066±0.066	6.133±0.306a	7.900±0.230
Glycine Betaine 50 µg/l and -0.02q_wMPa	4.633±0.317	4.433±0.233a	5.866±0.133	5.900±0.577b	7.833±0.233
Glycine Betaine 50 µg/l and -0.03q_wMPa	4.533±0.272	4.266±0.176b	5.666±0.166	5.566±0.126c	7.700±0.251
Control Glycine Betaine (100) µg/l	5.300±0.100	5.433±0.284	6.366±0.497	6.866±0.620	8.400±0.251
Glycine Betaine 100 µg/l and -0.01q_wMPa	5.200±0.173	5.100±0.057	6.033±0.218	6.566±0.692	8.200±0.404
Glycine Betaine 100 µg/l and -0.02q_wMPa	5.133±0.033	5.033±0.523	5.966±0.185	6.266±0.633	8.133±0.033
Glycine Betaine 100 µg/l and -0.03q_wMPa	5.066±0.133	4.900±0.208	5.866±0.384	6±0.514d	8.066±0.088
Control Glycine Betaine (250) µg/l	5.533±0.202	5.600±0.450	6.533±0.202	6.900±0.192	8.833±0.560a
Glycine Betaine 250 µg/l and -0.01q_wMPa	5.400±0.102	5.266±0.120	6.200±0.173	6.566±0.067	8.566±0.317
Glycine Betaine 250 µg/l and -0.02q_wMPa	5.300±0.104	5.106±0.057	6.033±0.417	6.133±0.349e	8.400±0.793
Glycine Betaine 250 µg/l and -0.03q_wMPa	5±0.264	5.933±0.218	5.900±0.208	5.700±0.318f	8.266±0.545

(b)

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	4.866±0.202	5.166±0.145	6.033±0.328	6.800±0.328	7.933±0.218
Control Mannitol (50) µg/l	4.883±0.317	5.790±0.218	6.213±0.088	6.833±0.293	8.166±0.384
Mannitol 50 µg/l and -0.01q_wMPa	4.466±0.260	4.766±0.384a	5.866±0.375	6.233±0.052	7.600±0.173
Mannitol 50 µg/l and- 0.02q_wMPa	4.400±0.208	4.566±0.348b	5.700±0.288	5.700±0.598a	7.466±0.233a
Mannitol 50 µg/l and -0.03q_wMPa	4.366±0.233	4.466±0.260c	5.466±0.218a	5.633±0.195b	7.266±0.120b
Control Mannitol (100) µg/l	4.900±0.152	6.106±0.057b	6.334±0.120	6.870±0.322	8.900±0.057c
Mannitol 100 µg/l and -0.01q_wMPa	4.633±0.133	4.866±0.185b	5.900±0.173	6.066±0.345c	7.933±0.218c
Mannitol 100 µg/l and -0.02q_wMPa	4.500±0.208	4.633±0.133c	5.866±0.218	5.600±0.217d	7.866±0.384d
Mannitol 100 µg/l and -0.03q_wMPa	4.400±0.152	4.433±0.384d	5.766±0.088	5.500±0.226e	7.600±0.152e
Control Mannitol (250) µg/l	4.950±0.057	6.400±0.251e	6.620±0.173	6.893±0.166	8.933±0.633f
Mannitol 250 µg/l and -0.01q_wMPa	4.633±0.218	5.333±0.284e	6±0.585	6.233±0.490f	8.300±0.300f
Mannitol 250 µg/l and -0.02q_wMPa	4.566±0.120	5.107±0.057f	5.933±0.033b	6±0.162g	8.200±0.854g
Mannitol 250 µg/l and -0.03q_wMPa	4.433±0.284	5±0.288g	5.866±0.375c	5.800±0.272h	8.133±0.517h

(c)

Table 3: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on relative water content (cm^2) of *Lepidium sativum* L. Data are mean ± SD of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonnferoni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level . Different lower case letters in a table indicate significant difference between control and treatment.

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	26.046±0.301	60.216±0.834	64.636±0.693	76.800±0.545	83.093±0.772
Control Proline (50) µg/l	29.506±0.486a	63.640±0.673a	67.606±0.856a	82.403±0.705a	88.370±0.587a
Proline50 µg/l and -0.01q_wMPa	29.130±0.175	62.900±0.505	66.936±0.652	81.583±0.338	88.036±0.399
Proline50 µg/l and -0.02q_wMPa	28.813±0.277	62.756±0.360	65.231±0.639a	81.070±0.423a	87.400±0.192a
Proline50 µg/l and -0.03q_wMPa	28.676±0.398	62.075±0.398a	64.537±0.640b	80.910±0.325b	87.073±0.405b
Control Proline (100) µg/l	30.173±0.443b	64.076±0.426b	69.023±0.398c	82.946±0.413c	89.043±0.226c
Proline100 µg/l and -0.01q_wMPa	28.770±0.586b	63.623±0.135	68.606±0.809	81.580±0.340c	88.700±0.308
Proline100 µg/l and -0.02q_wMPa	28.676±0.398c	63.523±0.194	67.913±0.663c	81.316±0.066d	88.276±0.156
Proline100 µg/l and -0.03q_wMPa	28.390±0.244d	63.010±0.336b	67.266±0.728d	80.983±0.366e	88.010±0.447c
Control Proline (250) µg/l	31.453±0.425e	64.453±0.295c	70.206±0.043e	83.386±0.250f	90.146±0.013d
Proline250 µg/l and- 0.01q_wMPa	31.086±0.163	64.012±0.475	69.840±0.347	82.936±0.422	89.823±0.336
Proline250 µg/l and -0.02q_wMPa	30.787±0.396	63.782±0.111	69.616±0.328	82.126±0.473f	89.643±0.277
Proline250 µg/l and -0.03q_wMPa	30.493±0.589	63.501±0.195	69.286±0.121	81.813±0.538g	89.266±0.487

(a)

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	26.046±0.301	60.216±0.834	64.636±0.693	76.800±0.545	83.093±0.772
Control Glycine Betaine (50) µg/l	28.773±0.311a	62.743±0.369a	66.273±0.578a	81.880±0.312a	87.366±0.677a
Glycine Betaine 50 µg/l and -0.01q_wMPa	28.440±0.171	62.410±0.111	65.900±0.336	81.256±0.003	86.886±0.319
Glycine Betaine 50 µg/l and -0.02q_wMPa	28.158±0.568	61.869±0.292	65.163±0.038a	80.923±0.331a	86.650±0.400
Glycine Betaine 50 µg/l and -0.03q_wMPa	28.034±0.645	61.537±0.292a	65.013±0.116b	80.403±0.143b	86.096±0.276b
Control Glycine Betaine (100) µg/l	29.328±0.524b	63.153±0.532b	68.646±0.769c	80.270±0.577c	88.440±0.533c
Glycine Betaine 100 µg/l and -0.01q_wMPa	28.935±0.406	62.820±0.543	67.160±0.040c	79.710±0.304	87.890±0.353
Glycine Betaine 100 µg/l and -0.02q_wMPa	28.603±0.357	62.463±0.328	66.650±0.253d	79.473±0.106	87.560±0.110
Glycine Betaine 100 µg/l and -0.03q_wMPa	28.517±0.318	62.313±0.670	66.270±0.613e	79.133±0.301c	87.056±0.398c
Control Glycine Betaine (250) µg/l	30.751±0.348c	63.634±0.808c	69.540±0.646f	80.603±0.328d	89.810±0.330d
Glycine Betaine 250 µg/l and -0.01q_wMPa	29.716±0.763c	63.044±0.511	68.880±0.620	80.036±0.238	89.513±0.324
Glycine Betaine 250 µg/l and -0.02q_wMPa	29.423±0.713d	62.716±0.586	68.263±0.013f	79.663±0.340	89.310±0.170
Glycine Betaine 250 µg/l and -0.03q_wMPa	28.951±0.252e	62.191±0.064c	68.040±0.108g	79.266±0.355d	89.136±0.008

(b)

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	26.046±0.301	60.216±0.834	64.636±0.693	76.800±0.545	83.093±0.772
Control Mannitol (50) µg/l	28.050±0.481a	61.701±0.440a	65.613±0.363a	79.600±0.578a	86.886±0.319a
Mannitol 50 µg/l and -0.01q_wMPa	27.686±0.546	61.376±0.678	65.206±0.043	79.080±0.289	86.543±0.360
Mannitol 50 µg/l and- 0.02q_wMPa	27.135±0.578	60.934±0.352	64.906±0.328	78.833±0.432	86.206±0.033
Mannitol 50 µg/l and -0.03q_wMPa	27.027±0.120a	60.891±0.335	64.303±0.594a	78.313±0.129a	86.170±0.040
Control Mannitol (100) µg/l	28.935±0.406b	62.773±0.561b	68.203±0.612b	79.746±0.426b	88.053±0.330b
Mannitol 100 µg/l and -0.01q_wMPa	28.505±0.370	62.183±0.063	67.826±0.706	78.946±0.413	87.240±0.578
Mannitol 100 µg/l and -0.02q_wMPa	28.143±0.586	61.590±0.360b	67.213±0.561b	78.506±0.308b	86.566±0.441b
Mannitol 100 µg/l and -0.03q_wMPa	27.813±0.326b	61.262±0.082c	67.066±0.564c	78.450±0.190c	86.273±0.095c
Control Mannitol (250) µg/l	30.751±0.348c	63.057±0.916d	69.206±0.578d	80.023±0.251d	89.463±0.328d
Mannitol 250 µg/l and -0.01q_wMPa	30.021±0.473	62.467±0.326	68.840±0.295	79.676±0.301	88.796±0.338
Mannitol 250 µg/l and -0.02q_wMPa	29.495±0.324c	62.377±0.236	68.630±0.410	79.296±0.117	88.156±0.036d
Mannitol 250 µg/l and -0.03q_wMPa	29.150±0.571d	62.140±0.010	68.256±0.580d	78.833±0.352d	87.912±0.227e

(c)

Table 4: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on total chlorophyll content (mg/g) of *Lepidium sativum* L. Data are mean ± SD of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonnferoni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level . Different lower case letters in a table indicate significant difference between control and treatment.

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	36.656±0.463	39.623±0.337	41.613±0.504	42.836±0.211	44.030±0.448
Control Proline (50) µg/l	37.213±0.860	40.596±0.702	41.943±0.847	43.343±0.714	46.090±0.621a
Proline50 µg/l and -0.01q_wMPa	36.880±0.675	39.783±0.215	41.503±0.684	42.643±0.326	45.356±0.148
Proline50 µg/l and -0.02q_wMPa	36.546±0.627	39.613±0.202	40.943±0.513	42.326±0.147	44.720±0.456a
Proline50 µg/l and -0.03q_wMPa	36.213±0.744	39.266±0.481a	40.503±0.248a	42.133±0.323a	44.256±0.632b
Control Proline (100) µg/l	39.226±0.549a	41.266±0.536b	42.046±0.406	44.133±0.491b	46.860±0.621c
Proline100 µg/l and -0.01q_wMPa	38.893±0.527	40.736±0.590	41.036±0.416	43.633±0.498	46.423±0.490
Proline100 µg/l and -0.02q_wMPa	38.560±0.690	39.143±0.570b	40.763±0.339b	43.093±0.472	46.053±0.644
Proline100 µg/l and -0.03q_wMPa	38.226±0.212	39.816±0.754c	40.330±0.655c	42.976±0.434b	45.693±0.344c
Control Proline (250) µg/l	40.786±0.848b	41.633±0.580d	43.140±0.096d	44.566±0.335c	47.523±0.666d
Proline250 µg/l and- 0.01q_wMPa	40.453±0.678	40.863±0.915	43.053±0.079	44.263±0.580	46.386±0.495
Proline250 µg/l and -0.02q_wMPa	40.120±0.650	40.496±0.707	42.770±0.346	44.056±0.385	45.820±0.252d
Proline250 µg/l and -0.03q_wMPa	40.786±0.779	40.033±0.305d	41.898±0.655d	43.033±0.450c	44.946±0.408e

(a)

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	36.656±0.463	39.623±0.337	41.613±0.504	42.836±0.211	44.030±0.448
Control Glycine Betaine (50) µg/l	36.880±0.675	40.173±0.153	41.933±0.722	43.190±0.459	44.716±0.763
Glycine Betaine 50 µg/l and -0.01q_wMPa	36.546±0.627	36.816±0.614a	39.983±0.183a	42.536±0.581	46.050±0.647a
Glycine Betaine 50 µg/l and -0.02q_wMPa	36.213±0.744	36.213±0.746b	39.750±0.187b	42.063±0.433a	44.276±0.612
Glycine Betaine 50 µg/l and -0.03q_wMPa	35.880±0.411	35.483±0.143c	38.696±0.276c	41.240±0.563b	43.643±0.494b
Control Glycine Betaine (100) µg/l	38.893±0.430a	40.443±0.020	42.213±0.546	43.693±0.722	45.823±0.421c
Glycine Betaine 100 µg/l and -0.01q_wMPa	38.560±0.225	39.786±0.638d	39.696±0.538	42.486±0.361c	45.013±0.420
Glycine Betaine 100 µg/l and -0.02q_wMPa	38.226±0.372	39.366±0.655	38.833±0.615	42.130±0.015d	44.680±0.475d
Glycine Betaine 100 µg/l and -0.03q_wMPa	37.893±0.527	38.703±0.944e	37.900±0.345d	41.813±0.343e	45.016±0.421
Control Glycine Betaine (250) µg/l	39.453±0.804b	40.650±0.287	42.226±0.577	44.196±0.572f	46.050±0.647e
Glycine Betaine 250 µg/l and -0.01q_wMPa	38.120±0.525b	39.983±0.630	41.890±0.577	44.120±0.597	45.153±0.289
Glycine Betaine 250 µg/l and -0.02q_wMPa	38.786±0.356	39.350±0.14f	40.976±0.766e	44.001±0.219	44.210±0.611f
Glycine Betaine 250 µg/l and -0.03q_wMPa	38.453±0.447	39.17±0.575g	39.660±0.284f	43.356±0.136f	44.200±0.577g

(b)

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	36.656±0.463	39.623±0.337	41.613±0.504	42.836±0.211	44.030±0.448
Control Mannitol (50) µg/l	36.946±0.627	39.880±0.748	41.830±0.265	43.246±0.557	45.403±0.685a
Mannitol 50 µg/l and -0.01q_wMPa	36.213±0.347	37.783±0.394a	38.883±0.687a	40.633±0.271a	42.596±0.333b
Mannitol 50 µg/l and- 0.02q_wMPa	35.880±0.264	38.346±0.942b	37.446±0.696b	39.700±0.268b	41.170±0.737c
Mannitol 50 µg/l and -0.03q_wMPa	35.546±0.086a	36.026±0.816c	36.580±0.545c	38.950±0.361c	41.453±0.699d
Control Mannitol (100) µg/l	38.560±0.739b	39.930±0.315	41.986±0.761	43.993±0.631	45.746±0.704e
Mannitol 100 µg/l and -0.01q_wMPa	38.226±0.549	38.383±0.600d	39.366±0.581d	41.253±0.479d	43.820±0.195e
Mannitol 100 µg/l and -0.02q_wMPa	37.893±0.527	37.946±0.498e	38.790±0.780e	40.143±0.578e	42.556±0.321f
Mannitol 100 µg/l and -0.03q_wMPa	37.560±0.225	37.170±0.275f	39.020±0.412f	39.816±0.359f	42.343±0.590g
Control Mannitol (250) µg/l	38.820±0.525c	40.976±0.228g	42.346±0.593	44.500±0.365g	45.943±0.143h
Mannitol 250 µg/l and -0.01q_wMPa	37.886±0.356	38.783±0.427g	39.680±0.530g	43.160±0.601g	45.140±0.580h
Mannitol 250 µg/l and -0.02q_wMPa	37.763±0.678	37.323±0.658h	39.253±0.366h	42.176±0.537h	44.816±0.611
Mannitol 250 µg/l and -0.03q_wMPa	37.420±0.650c	37.216±0.571i	38.946±0.890i	41.846±0.319i	44.190±0.871i

(c)

Table 5: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on electrical conductivity (ds-M^{-1}) of *Lepidium sativum* L. Data are mean ± SD of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonnferoni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level . Different lower case letters in a table indicate significant difference between control and treatment.

Treatments	35Days	55Days	75 days	95Days	110Days
Control	1.130±0.030	1.320±0.058	1.376±0.102	1.420±0.085	1.704±0.028
Control Proline (50) µg/l	1.153±0.017	1.486±0.124	1.553±0.093	1.996±0.066a	2.023±0.064a
Proline50 µg/l and -0.01q_wMPa	1.126±0.017	1.390±0.094	1.526±0.054	1.890±0.051	1.926±0.035
Proline50 µg/l and -0.02q_wMPa	1.112±0.006	1.346±0.068	1.416±0.136	1.796±0.054	1.906±0.054
Proline50 µg/l and -0.03q_wMPa	1.106±0.006	1.246±0.027	1.350±0.125	1.633±0.088	1.886±0.047
Control Proline (100) µg/l	1.173±0.006	1.616±0.149	1.716±0.109	2.206±0.052	2.110±0.066
Proline100 µg/l and -0.01q_wMPa	1.160±0.011	1.506±0.148	1.683±0.112a	1.933±0.016b	2.026±0.089
Proline100 µg/l and -0.02q_wMPa	1.140±0.011	1.440±0.183	1.640±0.130	1.830±0.017	1.920±0.030
Proline100 µg/l and -0.03q_wMPa	1.116±0.008	1.360±0.220	1.540±0.202	1.740±0.070	1.870±0.015
Control Proline (250) µg/l	1.220±0.011	1.810±0.049a	1.843±0.056b	2.273±0.115	2.220±0.035b
Proline250 µg/l and- 0.01q_wMPa	1.176±0.012	1.733±0.120	1.776±0.062	2.200±0.173	2.166±0.044
Proline250 µg/l and -0.02q_wMPa	1.153±0.024	1.600±0.115	1.710±0.115	1.833±0.060c	2.136±0.063
Proline250 µg/l and -0.03q_wMPa	1.126±0.037	1.466±0.218	1.610±0.210	1.740±0.075	2.116±0.016

(a)

Treatments	35Days	55Days	75 days	95Days	110Days
Control	1.130±0.030	1.320±0.058	1.376±0.102	1.420±0.085	1.704±0.028
Control Glycine Betaine (50) µg/l	1.148±0.013	1.343±0.033a	1.483±0.036a	1.820±0.075a	1.940±0.023a
Glycine Betaine 50 µg/l and -0.01q_wMPa	1.280±0.211	1.273±0.037	1.410±0.005	1.766±0.033	1.733±0.044
Glycine Betaine 50 µg/l and -0.02q_wMPa	1.123±0.012	1.240±0.070	1.316±0.049	1.666±0.088	1.623±0.043
Glycine Betaine 50 µg/l and -0.03q_wMPa	1.113±0.052	1.200±0.057	1.256±0.132	1.506±0.150	1.990±0.050
Control Glycine Betaine (100) µg/l	1.190±0.050	1.540±0.140	1.593±0.227b	2.006±0.155	1.893±0.052
Glycine Betaine 100 µg/l and -0.01q_wMPa	1.106±0.052	1.440±0.183	1.416±0.242	1.916±0.148	1.700±0.152
Glycine Betaine 100 µg/l and -0.02q_wMPa	1.046±0.037	1.206±0.063	1.396±0.251	1.783±0.056	1.600±0.152
Glycine Betaine 100 µg/l and -0.03q_wMPa	1.033±0.033	1.133±0.033	1.216±0.044	1.663±0.018	1.406±0.058
Control Glycine Betaine (250) µg/l	1.280±0.030a	1.666±0.185b	1.690±0.233c	2.973±0.188b	2.997±0.012b
Glycine Betaine 250 µg/l and -0.01q_wMPa	1.153±0.024	1.433±0.185	1.533±0.202	1.653±0.016	1.820±0.061
Glycine Betaine 250 µg/l and -0.02q_wMPa	1.140±0.030	1.266±0.088	1.433±0.233	1.620±0.085	1.776±0.088
Glycine Betaine 250 µg/l and -0.03q_wMPa	1.133±0.033	1.133±0.033	1.302±0.057	1.560±0.052	1.710±0.155

(b)

Treatments	35Days	55Days	75 days	95Days	110Days
Control	1.130±0.030	1.320±0.058	1.376±0.102	1.420±0.085	1.704±0.028
Control Mannitol (50) µg/l	1.143±0.006	1.373±0.037	1.393±0.078	1.686±0.161a	1.806±0.031a
Mannitol 50 µg/l and -0.01q_wMPa	1.106±0.006	1.233±0.033	1.320±0.047	1.546±0.093	1.646±0.093
Mannitol 50 µg/l and- 0.02q_wMPa	1.066±0.033	1.166±0.088	1.253±0.089	1.456±0.031	1.453±0.033
Mannitol 50 µg/l and -0.03q_wMPa	1.033±0.033	1.100±0.057	1.166±0.044	1.386±0.059	1.373±0.089
Control Mannitol (100) µg/l	1.173±0.006	1.473±0.196a	1.416±0.242	1.883±0.158	1.970±0.100
Mannitol 100 µg/l and -0.01q_wMPa	1.153±0.017	1.340±0.230	1.296±0.122	1.756±0.107	1.403±0.112
Mannitol 100 µg/l and -0.02q_wMPa	1.120±0.020	1.140±0.030	1.153±0.024	1.63±0.0757	1.340±0.077
Mannitol 100 µg/l and -0.03q_wMPa	1.100±0.057	1.230±0.057	1.480±0.057	1.533±0.092	1.600±0.040
Control Mannitol (250) µg/l	1.193±0.037a	1.533±0.145b	1.633±0.233	1.866±0.176b	1.996±0.115b
Mannitol 250 µg/l and -0.01q_wMPa	1.173±0.029	1.204±0.115	1.233±0.033	1.416±0.200	1.429±0.199
Mannitol 250 µg/l and -0.02q_wMPa	1.160±0.030	1.166±0.088	1.236±0.033	1.379±0.080	1.447±0.150
Mannitol 250 µg/l and -0.03q_wMPa	1.130±0.010	1.266±0.066	1.333±0.033	1.476±0.086	1.512±0.025

(c)

Table 6: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on membrane stability index (%) of *Lepidium sativum* L. Data are mean ± SD of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonnferoni multiple comparison post – test P<0.05*, P<0.01**, P<0.001*** significance level . Different lower case letters in a table indicate significant difference between control and treatment.

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	16.466±0.373	18.300±0.378	21.133±0.333	23.333±0.635	24.107±0.305
Control Proline (50) µg/l	16.800±0.152	18.466±0.674	21.566±0.120	23.730±0.333	24.706±0.305
Proline50 µg/l and -0.01q_wMPa	16.226±0.447	18.133±0.470	21.233±0.425	23.333±0.066	24.333±0.088
Proline50 µg/l and -0.02q_wMPa	15.966±0.284	17.903±0.435	20.866±0.480	23.133±0.176	24.066±0.296
Proline50 µg/l and -0.03q_wMPa	15.704±0.351a	17.566±0.317a	20.600±0.305a	22.533±0.466a	23.600±0.450a
Control Proline (100) µg/l	16.987±0.416	18.866±0.378	22.100±0.300b	24.200±0.416b	25.210±0.378b
Proline100 µg/l and -0.01q_wMPa	16.666±0.133	16.966±0.352b	20.833±0.484b	23.766±0.317	24.666±0.328
Proline100 µg/l and -0.02q_wMPa	16.466±0.405	19.566±0.887	20.533±0.338c	23.433±0.202	24.266±0.120c
Proline100 µg/l and -0.03q_wMPa	16.166±0.448b	19.123±0.152	20.333±0.066d	22.666±0.218b	23.992±0.321d
Control Proline (250) µg/l	17.603±0.115c	19.266±0.284c	22.400±0.302e	24.658±0.416c	25.966±0.440e
Proline250 µg/l and- 0.01q_wMPa	16.897±0.152	18.833±0.352	20.833±0.484e	24.200±0.416c	25.466±0.166
Proline250 µg/l and -0.02q_wMPa	16.466±0.284c	18.466±0.120c	20.533±0.338f	23.805±0.513d	24.933±0.317e
Proline250 µg/l and -0.03q_wMPa	16.166±0.491d	17.966±0.233d	20.333±0.066g	23.336±0.057e	24.533±0.384f

(a)

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	16.466±0.373	18.300±0.378	21.133±0.333	23.333±0.635	24.107±0.305
Control Glycine Betaine (50) µg/l	16.600±0.351	18.466±0.448	21.833±0.366	23.520±0.577	24.258±0.404
Glycine Betaine 50 µg/l and -0.01q_wMPa	15.366±0.033	17.733±0.484	20.466±0.366	22.978±0.702	23.633±0.433
Glycine Betaine 50 µg/l and -0.02q_wMPa	15.266±0.088	17.366±0.260	20.200±0.102	22.633±0.284	23.266±0.066
Glycine Betaine 50 µg/l and -0.03q_wMPa	15.033±0.120	17.133±0.120a	20.166±0.066	22.333±0.066a	23.166±0.033
Control Glycine Betaine (100) µg/l	16.720±0.264	18.800±0.378	21.933±0.176	23.805±0.702	24.566±0.176
Glycine Betaine 100 µg/l and -0.01q_wMPa	15.700±0.351	18.366±0.166	20.233±0.088a	23.333±0.581	24.200±0.513
Glycine Betaine 100 µg/l and -0.02q_wMPa	15.500±0.152	18.066±0.375	20.133±0.088b	22.900±0.251b	23.700±0.404a
Glycine Betaine 100 µg/l and -0.03q_wMPa	15.333±0.233	17.900±0.461b	19.900±0.404c	22.666±0.317c	23.333±0.638b
Control Glycine Betaine (250) µg/l	17.102±0.115a	19.266±0.284c	21.529±0.417d	24.266±0.995d	24.687±0.321c
Glycine Betaine 250 µg/l and -0.01q_wMPa	16.995±0.152	18.800±0.378	21.366±0.611	23.933±0.768	24.533±0.202
Glycine Betaine 250 µg/l and -0.02q_wMPa	16.466±0.284a	18.366±0.176c	20.866±0.333d	23.500±0.608	24.200±0.513
Glycine Betaine 250 µg/l and -0.03q_wMPa	16.166±0.491b	17.933±0.366d	20.566±0.328e	23.066±0.352d	23.833±0.545c

(b)

Treatments	**35Days**	**55Days**	**75 days**	**95Days**	**110Days**
Control	16.466±0.373	18.300±0.378	21.133±0.333	23.333±0.635	24.100±0.305
Control Mannitol (50) µg/l	16.600±0.208a	18.630±0.556	22.100±0.450a	23.638±0.284	24.618±0.433
Mannitol 50 µg/l and -0.01q_wMPa	14.533±0.176	17.233±0.033	21.233±0.088a	22.266±0.088	23.200±0.577
Mannitol 50 µg/l and- 0.02q_wMPa	14.333±0.185	16.987±0.435	21.104±0.416b	22.100±0.173	22.866±0.284a
Mannitol 50 µg/l and -0.03q_wMPa	13.904±0.351b	16.733±0.328a	20.333±0.554c	21.133±0.176a	22.144±0.033b
Control Mannitol (100) µg/l	16.666±0.120c	18.723±0.133	22.833±0.284d	23.830±0.375	24.804±0.513
Mannitol 100 µg/l and -0.01q_wMPa	15.300±0.057	17.933±0.266	22.166±0.384	23.366±0.218	23.633±0.433
Mannitol 100 µg/l and -0.02q_wMPa	14.766±0.437c	17.533±0.296b	21.733±0.448d	22.866±0.333b	23.166±0.033c
Mannitol 100 µg/l and -0.03q_wMPa	14.500±0.665d	17.266±0.375c	21.133±0.120e	22.733±0.536c	23.133±0.066d
Control Mannitol (250) µg/l	16.968±0.384	19.266±0.284d	23.400±0.123f	24.376±0.296d	24.931±0.120
Mannitol 250 µg/l and -0.01q_wMPa	14.766±0.775e	18.800±0.378	23.066±0.333	23.933±0.466	24.166±0.491
Mannitol 250 µg/l and -0.02q_wMPa	14.366±0.463f	18.366±0.176d	22.566±0.417f	23.400±0.200d	23.600±0.450e
Mannitol 250 µg/l and -0.03q_wMPa	14.266±0.233g	17.933±0.366e	22.033±0.120g	23.166±0.375e	23.266±0.066f

(c)

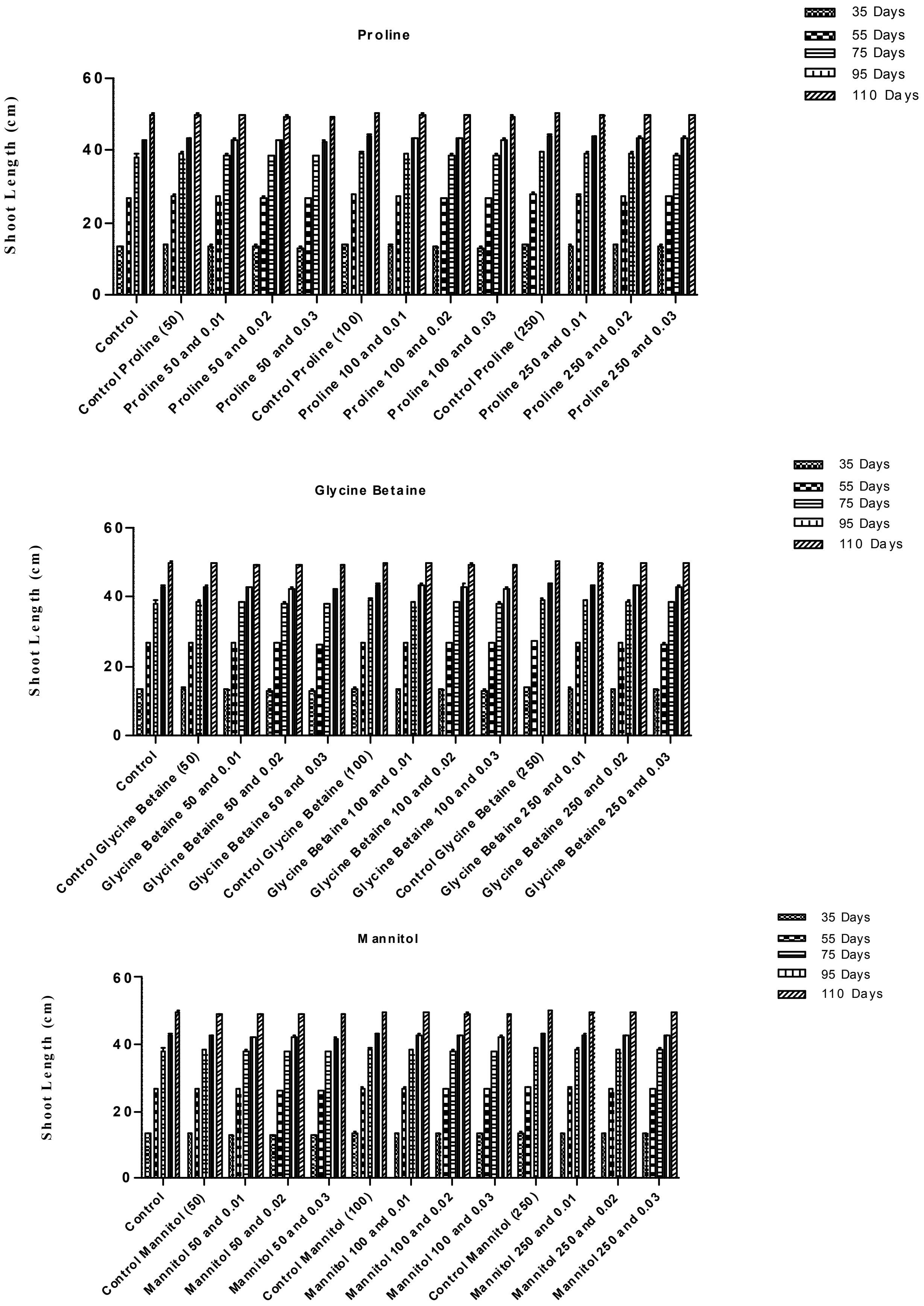

Fig. 1: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on shoot length.

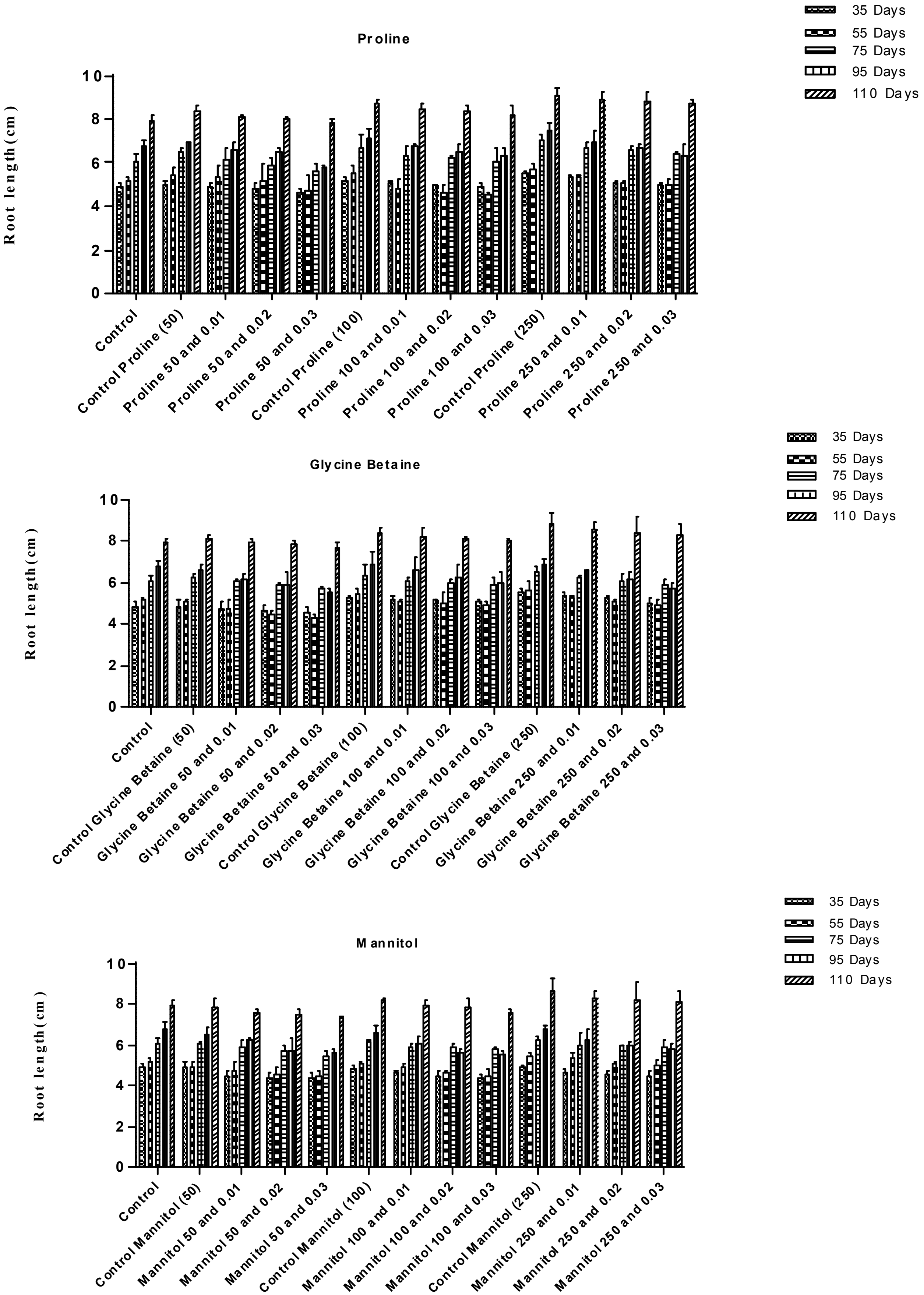

Fig. 2: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on root length.

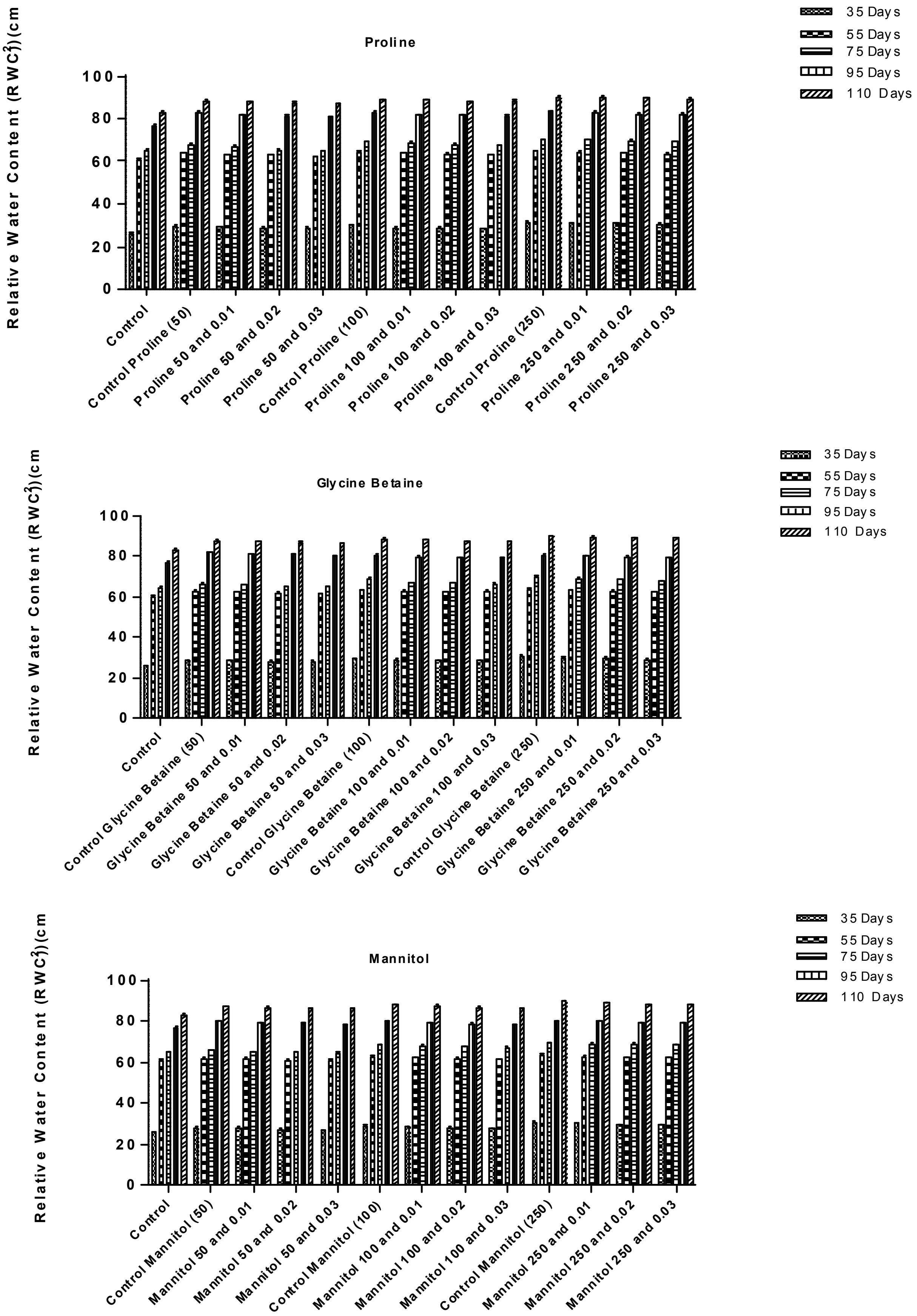

Fig. 3: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on relative water content.

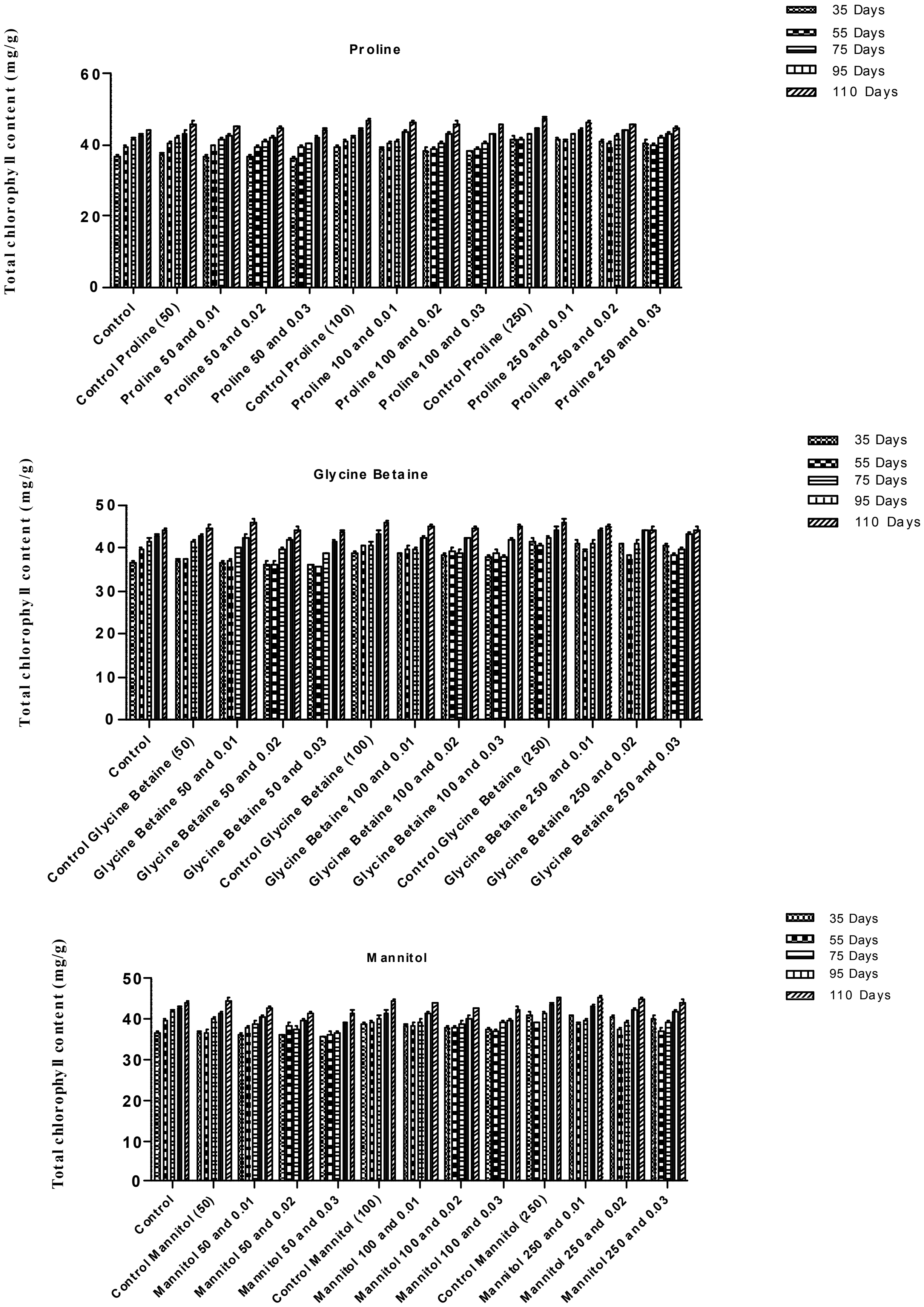

Fig. 4: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on total chlorophyll content.

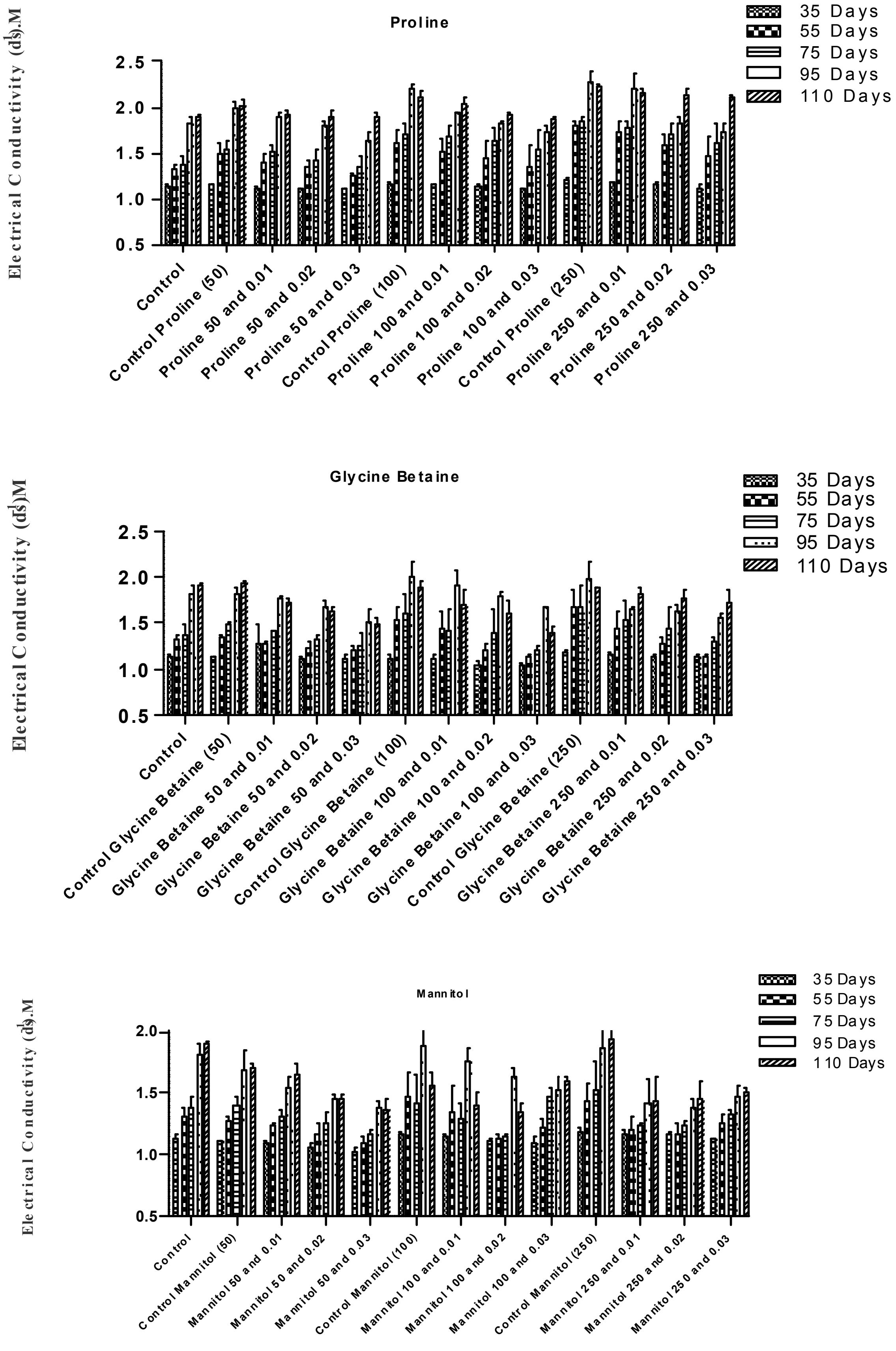

Fig. 5: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on electrical conductivity.

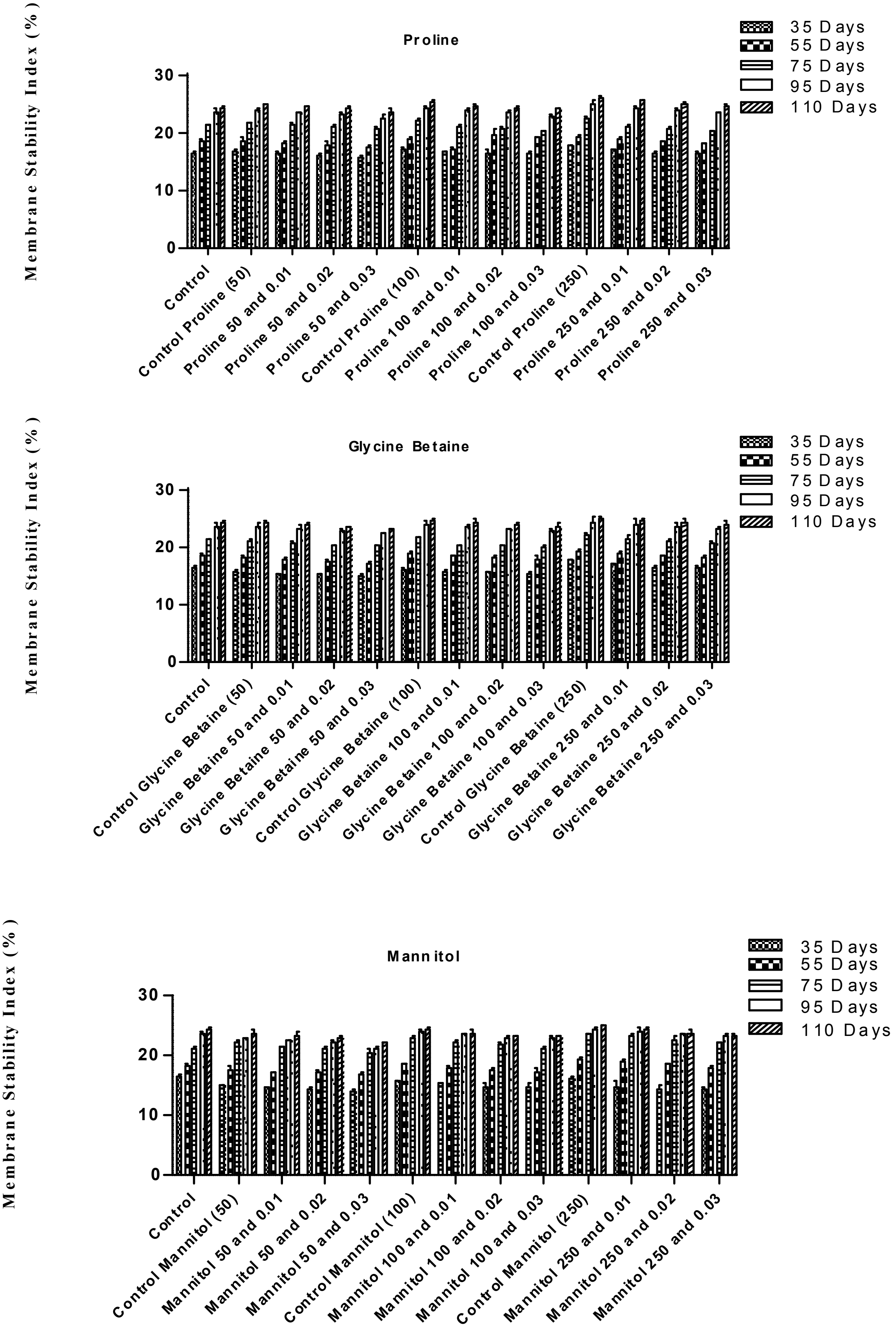

Fig. 6: Effect of proline (a) glycine betaine (b) and mannitol (c) and water stress on membrane stability index.

When exogenous proline, glycine betaine and mannitol was applied the chlorophyll content increased by 2% in 50µg/l, 7% in 100µg/l and 11% in 250µg/l at 35 days. 2% in 50µg/l, 4% in 100µg/l and 5% in 250µg/l at 55 days. By 1% in 50µg/l, 1% in 100µg/l and 4% in 250µg/l at 75 days then by 1% in 50µg/l, 3% in 100µg/l and 4% in 250µg/l at 95 days. By 5% in 50µg/l, 6% in 100µg/l and 8% in proline 250µg/l at 110 days. In case of glycine betaine it is increased by 1% in 50µg/l, 6% in 100µg/l and 8% in 250µg/l at 35 days. 1% in 50µg/l, 2% in 100µg/l and 3% in 250µg/l at 55 days. By 1% in 50µg/l, 1% in 100µg/l and 1% in 250µg/l at 75 days then by 1% in 50µg/l, 2% in 100µg/l and 3% in 250µg/l at 95 days. By 2% in 50µg/l, 4% in 100µg/l and 5% in glycine betaine 250µg/l at 110 days. Then in mannitol it is increased 1% in 50µg/l, 5% in 100µg/l and 6% in 250µg/l at 35 days. 1% in 50µg/l, 1% in 100µg/l and 3% in 250µg/l at 55 days. By 1% in 50µg/l, 1% in 100µg/l and 2% in 250µg/l at 75 days then by 1% in 50µg/l, 3% in 100µg/l and 4% in 250µg/l at 95 days. By 3% in 50µg/l, 4% in 100µg/l and 4% in mannitol 250µg/l at 110 days shown in (Table 4a, b, c & Figs. 4a, b, c respectively). The chlorophyll content was recorded maximum in the case of proline.

Electrical Conductivity

Exogenous proline, glycine betaine and mannitol was applied electrical conductivity enhanced by 2% in 50µg/l, 4% in 100µg/l and 8% in 250µg/l at 35 days. 13% in 50µg/l, 22% in 100µg/l and 37% in 250µg/l at 55 days. By 13% in 50µg/l, 25% in 100µg/l and 34% in 250µg/l at 75 days then by 41% in 50µg/l, 55% in 100µg/l and 60% in 250µg/l at 95 days. By 19% in 50µg/l, 24% in 100µg/l and 30% in proline 250µg/l at 110 days. In case of glycine betaine it is increased by 2% in 50µg/l, 5% in 100µg/l and 13% in 250µg/l at 35 days. 2% in 50µg/l, 17% in 100µg/l and 26% in 250µg/l at 55 days. By 8% in 50µg/l, 16% in 100µg/l and 23% in 250µg/l at 75 days then by 28% in 50µg/l, 41% in 100µg/l and 39% in 250µg/l at 95 days. By 14% in 50µg/l, 17% in 100µg/l and 17% in glycine betaine 250µg/l at 110 days. Then in mannitol it is increased 1% in 50µg/l, 4% in 100µg/l and 6% in 250µg/l at 35 days. 4% in 50µg/l, 12% in 100µg/l and 16% in 250µg/l at 55 days. By 1% in 50µg/l, 3% in 100µg/l and 19% in 250µg/l at 75 days then by 19% in 50µg/l, 33% in 100µg/l and 31% in 250µg/l at 95 days. By 6% in 50µg/l, 16% in 100µg/l and 17% in mannitol 250µg/l at 110 days compared to their respective control shown in (Table 5a, b, c & Figs. 5a, b, c respectively). The electrical conductivity enhancement was observed more in the case of proline.

Membrane Stability Index

Exogenous application of proline, glycine betaine and mannitol was applied then the membrane stability was enhanced by 2% in 50µg/l, 3% in 100µg/l and 7% in 250µg/l at 35 days. 1% in 50µg/l, 3% in 100µg/l and 5% in 250µg/l at 55 days. By 2% in 50µg/l, 5% in 100µg/l and 6% in 250µg/l at 75 days then by 2% in 50µg/l, 4% in 100µg/l and 6% in 250µg/l at 95 days. By 2% in 50µg/l, 5% in 100µg/l and 8% in proline 250µg/l at 110 days. In case of glycine betaine it is increased by 1% in 50µg/l, 2% in 100µg/l and 4% in 250µg/l at 35 days. 1% in 50µg/l, 3% in 100µg/l and 5% in 250µg/l at 55 days. By 3% in 50µg/l, 4% in 100µg/l and 2% in 250µg/l at 75 days then by 1% in 50µg/l, 2% in 100µg/l and 4% in 250µg/l at 95 days. By 1% in 50µg/l, 2% in 100µg/l and 2% in glycine betaine 250µg/l at 110 days. In mannitol it is increased 1% in 50µg/l, 1% in 100µg/l and 3% in 250µg/l at 35 days. 2% in 50µg/l, 2% in 100µg/l and 5% in 250µg/l at 55 days. By 5% in 50µg/l, 8% in 100µg/l and 11% in 250µg/l at 75 days then by 1% in 50µg/l, 2% in 100µg/l and 4% in 250µg/l at 95 days. By 2% in 50µg/l, 3% in 100µg/l and 3% in mannitol 250µg/l at 110 days compared to control shown in (Table 6a, b, c & Figs. 6a, b, c respectively).The membrane stability index was maximum increased in proline as compared to glycine betaine and mannitol was observed.

Discussion

Due to the sessile life cycle, plants have evolved mechanisms to respond and adapt to adverse environmental stresses during their development and growth. Plant growth impaired by severe abiotic stress due to a decrease in stomatal opening, which limits CO2 uptake and hence reduces photosynthetic activity. In order to develop strategies to maintain plant productivity, it is essential to understand the various regulatory mechanisms that control and enhance adaptive responses to stress in different plant species. One of the most common stress response in plants is over production of different types of compatible organic solutes (Serraj and Sinclair, 2002). Compatible solutes are low molecular weight, highly soluble compounds that are usually nontoxic at high cellular concentrations. Generally, they protect plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Yancey *et al.,* 1982; Bohnert and Jensen, 1996). Furthermore, because some of these solutes also protect cellular components from dehydration injury, they commonly referred to as osmoprotectants. These solutes include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alanine betaine, proline betaine, choline O-sulfate, hydroxyl proline betaine, and pipecolate betaine (Rhodes and Hanson, 1993). Glycine betaine is abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (Robinson and Jones, 1986; Genard *et al.,* 1991). GB is known to accumulate in response to stress in many crop plants, including sugar beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and sorghum (*Sorghum bicolor*) (Yang *et al.,* 2003). Exogenous application of GB to low-accumulating or non-accumulating plants may help reduce adverse effects of environmental stresses (Yang and Lu, 2005). For example, foliar application of GB resulted in a significant improvement in salt tolerance of rice plants (Lutts, 2000). In maize (*Zea mays*, L.), exogenously applied GB improved growth, leaf water content, net photosynthesis, and the apparent quantum yield of photosynthesis of the salt-stressed plants (Yang and Lu, 2005). Amino acid proline known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Ozturk and Demir, 2002; Hsu *et al.,* 2003; Kavi Kishore *et al.,* 2005). Proline accumulation also occurs in plants subjected to drought stress. Exogenous application of proline can play an important role in enhancing plant stress tolerance. Proline can also protect cell membranes from salt-induced oxidative stress by enhancing activities of various antioxidants (Yan *et al.,* 2000). For example, growth of tobacco suspension cells under salt stress promoted by exogenous application of 10mM proline, which proposed to be due to proline action as a protectant of enzymes and membranes (Okuma *et al.,* 2000). In soybean cell cultures maintained under salt stress, exogenous application of proline increased activities of superoxide dismutase and peroxidase, which normally contribute to increased salt tolerance (Yan *et al.,* 2000; Hua and Guo, 2002). Mannitol, an important osmolyte, normally synthesized in large amount in many plant species (Mitoi *et al.,* 2009). Although mannitol plays an important role in osmotic adjustment, it acts as an antioxidant to scavenge of hydroxyl radicals (OH-) (Srivastava *et al.,* 2010). Present study focused on the application of different osmolyte involved in the plant responses to water stress and the concomitant growth and physiological adjustment. Understanding these key factors will enable us to improve plant productivity during water stress. Relative water content (RWC) of leaves has reported as direct indicator of plant water contents under water deficit conditions. Water stress lead to reduction of water status during crop growth, soil water potential and plant osmotic potential for water and nutrient uptake, which ultimately reduce leaf turgor pressure which results in upset of plant metabolic activities. Under water stress, condition decrease in water status and osmotic potential in plants is the ultimate outcome of lower relative water content. (Lugojan and Ciulca, 2011). The shoot and root length increased in *Lepidium sativum* L. with the exogenous application of proline, glycine betaine and mannitol. Maximum enhancement were observed in case of proline as compared to glycine betaine and mannitol at their respective concentration as compared to control which is shown in (Table 1a, b, c & Table 2a, b, c) (Figs. 1 a, b, c & Figs. 2 a, b, c). The present study showed enhancement in RWC of *Lepidium sativum* L. maximum observed in case of proline as compared to glycine betaine and mannitol at their respective concentration compared to control which is shown in (Table 3a, b, c) (Figs. 3 a, b, c). Chlorophyll contents is the significantly correlated with photosynthesis and regarded as encouraging selection trait in crop productivity (Teng *et al.,* 2004). Severe water deficit stress restricts the photosynthesis by damaging the chlorophyll components and changing the photosynthetic machinery (Iturbe-Ormaetxe *et al.,* 1998). Decreased photosynthetic amount under water deficit condition is an outcome of Inhibition of RuBisCO (ribulose-1, 5-bisphosphate carboxylase/oxygenase) enzyme activity and development of ATP (Dulai *et al.,* 2006). Higher concentration of chlorophyll is essential for plants because it depicts the low quantity of photo-inhibition of the photosynthetic, which prevents the carbohydrates losses and eventually enhances growth (Farquhar *et al.,* 1989). Similarly, the present study also showed significantly increased in total chlorophyll content more in case of proline as compared to glycine betaine and mannitol which is shown in (Table 4a, b, c) (Figs. 4 a, b, c). Electrical conductivity (EC) showed maximum increased as compared to respective control. The proline enhances the electrical conductivity maximum as compared to glycine betaine and mannitol, which shown in (Table 5a, b, c) (Figs. 5 a, b, c). Membrane stability index (MSI) is of vital important selection criteria of drought tolerant genotypes (Tripathy *et al.,* 2000). High level of accumulation of H_2O_2 under water stress leads to production of hydroxyl radicals, which cause lipid peroxidation and consequently cell membrane rupture (Sairam and Saxena, 2000). The present study also revealed that there is a significant increase in (MSI) in case of proline as compared to glycine betaine and mannitol in *Lepidium sativum* L. which is shown in (Table 6a, b, c) (Figs. 6 a, b, c).

Conclusion

Many plant species naturally accumulate proline, glycine betaine and mannitol as leading organic osmolytes when subjected to different abiotic stresses. These compounds play adaptive roles in intermediating osmotic adjustment and protecting subcellular structures in stressed plants. However, not all plants accumulate these osmolytes in adequate amounts to help in the adverse effects of abiotic stresses. Exogenous application of these osmolytes to plants helpful in growing under stress conditions to enhance their tolerance. Applications of proline, glycine betaine and mannitol to plants during stress proliferation the interior levels of these compounds and generally enhance plant growth and ultimate crop yield under stress conditions. The present study is to determined specific roles of proline, glycine betaine and mannitol in *Lepidium sativum* L. under water stress condition and enhanced the growth and physiological responses.

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