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Research Article

Nitrogen Metabolism in Nitrophilous *Portulaca oleracea* Linn under Water Stress

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Abstract

One month old C_4 succulent *Portulaca oleracea* Linn was water stressed for 4, 8 and 15 days (d). There was significant increase in the activities of enzymes, nitrate and nitrite reductases due to 8 d of water stress as compared to control. Activities of the enzymes glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were also increased in the plants water stressed. Significant accumulation of nitrate and nitrite was observed in the water stressed plants. However, there was no significant change in the proline content of the plants. The amount of total soluble proteins was significantly increased in the root and leaves due to 8 d of water stress. Leaf protein profile (SDS-PAGE) revealed that 15 and 16 kD proteins were exclusive in the leaves of plants water stressed for 4 and 15 d. These alterations in the nitrogen metabolism of this nitrophilous plant for the adaptation to water stress have been discussed.

ABBREVIATIONS

GOT: Glutamic oxaloacetic transaminase; GPT: Glutamic pyruvic transaminase; NR: Nitrate reductase; NiR: Nitrite reductase.

INTRODUCTION

Portulaca oleracea Linn (Common purslane) belonging to family Portulacaceae is an annual herb with succulent stem and leaves. It may grow erect or prostrate. It is 8th common plant on earth [1] that grows in cultivated fields, gardens and waste places. It has long history of its use as human food, animal feed

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and medicinal purpose. Purslane is identified as an excellent source of omega-3 fatty acids, and contains them more than any other leafy vegetable [2].

Among the various environmental factors, water stress (drought) is an important abiotic factor restricting plant growth and development in many regions of the world. During severe drought, the low water availability for transport of various components leads to changes in the concentrations of many metabolites. For example, water-related metabolic processes like photosynthesis are primarily affected by water stress. Water stress leads to reduced chlorophyll content and increased level of

harmful reactive oxygen species. Photosynthetic capacity of plant is closely associated with leaf nitrogen [3]. The two key enzymes involved in assimilation nitrate into organic compounds are nitrate reductase (NR EC 1.6.6.2) and nitrite reductase (NiR EC 1.6.6.4). Nitrate reductase activity is found to be decreased under water stress [4] while in some cases it is increased [5]. Similarly, water stress also showed inhibitory effect on the activity of NiR [6]. In these situations, accumulation of nitrate and nitrite in plant parts was also observed [7]. The enzymes involved in ammonia assimilation, glutamic oxaloacetic transaminase (GOT EC 2.6.1.1.) and glutamic pyruvic transaminase (GPT EC 2.6.1.2.) also show alterations in their activity under stress conditions [8].

Portulaca oleracea, a C₄ plant, shows weak CAM type of photosynthetic metabolism under stress conditions [9,10]. *P. oleracea* can accumulate about 155 ppm nitrate in the cell sap [11]. It is observed that high nitrate content in this plant lowers the oxalic acid concentration [12]. However, there is little information available on the response of purslane to water stress with respect to nitrogen metabolism along with protein profile. As this plant is nitrophilous, it is possible that nitrogen metabolism may play an important role in its water stress tolerance. Hence, in the present investigation, an attempt has been made to study the effect of water stress on some constituents of nitrogen metabolism along with the protein profile of the leaves of *P. oleracea*.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of *P. oleracea* were collected from field and germinated in plastic pots (32 cm diameter × 12 cm height) containing 6 kg acid free silica sand. Plants were grown for 1 month with the supply of ½ strength Hoagland solution (1:1) alternating with water under natural conditions (Light intensity: 30000 lux; Temperature: 30±2°C) in botanical garden of Shivaji University, Kolhapur (MS). After one month of their growth, plants were watered at an interval of 4, 8 and 15 d while, control plants were watered every alternate day. Fresh plants from garden were brought to the laboratory, washed with running water and then with distilled water. Root, stem and leaves were cut into pieces, weighed accurately and immediately used for analysis. All operations were done at room temperature 28±2°C.

Moisture content determination

Moisture content of plant parts was determined by the method described in AOAC [13]. The root, stem and leaves of purslane were collected randomly, washed and blotted dry. 10g of this material was kept in an oven at 70°C for drying till a constant weight was obtained. The loss in weight per 100 g was expressed as moisture content and calculated as follows:

$$\text{moisture content (\%)} = \frac{(\text{fresh weight} - \text{dry weight})}{\text{fresh weight}} \times 100.$$

Nitrate reductase assay

In vivo activity of enzyme NR was determined following the method described by Jaworski [14]. Plant material (fresh root, stem and leaves) was cut into small pieces of about 0.5 cm²

and incubated in 10 mL incubation medium containing 1 mL 1 M KNO₃, 2 mL 5% n-propanol, 5 mL 0.2 M phosphate buffer, pH 7.5 and 2 mL 0.5% Triton-X-100 for 1 h in dark. After incubation, 1 mL of the reaction medium was taken and mixed with 1 mL each of 1% sulfanilamide in 1 M HCl and 0.02% NEEDA [N-1-(naphthyl)-ethylene diamine dihydrochloride]. The absorbance was read at 540 nm on Shimadzu UV-1900 spectrophotometer (Japan) using reagent blank. The standard curve was prepared from 0.03M KNO₂ in the range of concentrations from 0.3 mM to 1.5 mM KNO₂. The soluble proteins in the enzyme extract were determined according to the method by Lowery *et al.* [15]. The activity of enzyme is expressed as μg NO₂⁻ liberated h⁻¹mg⁻¹ protein.

Nitrite Reductase assay

The activity of NiR was determined following the method by Jaworski [14], already described for nitrate reductase except that 1 M KNO₃ was replaced by 0.03 M KNO₂ in the incubation medium and the incubation was done in light. The standard curve of KNO₂ was prepared as described earlier. The activity of enzyme is expressed as μg NO₂⁻ reduced h⁻¹mg⁻¹ protein.

Nitrate (NO₃⁻) content

The method used is based on the reaction between diphenylamine-H₂SO₄ reagent and nitrate [16]. Plant parts were homogenized in chilled 0.2 M phosphate buffer, pH 7.5. The reaction mixture contained 1 mL plant extract, 1 mL distilled water and 1.8 mL diphenylamine-H₂SO₄ reagent (1 g diphenylamine in 100 mL H₂SO₄). After vigorous shaking, the blue colour was allowed to develop for 10 min. The absorbance was recorded at 590 nm on spectrophotometer. Instead of extract, distilled water was used to prepare blank. The standard curve was prepared from 0.1 M KNO₃ (concentrations ranging from 10 mM to 50 mM KNO₃) and from this, NO₃⁻ content was calculated and expressed as mg kg⁻¹ FW.

Nitrite (NO₂⁻) content

NO₂⁻ content was determined following the method by Jaworski [14]. Plant parts were homogenized in chilled 0.2 M phosphate buffer, pH 7.5. The reaction mixture contained 5 mL plant extract to which 1 mL sulfanilamide (1% in 1 M HCl) and 1 mL 0.02% NEEDA were added. After 15 min the absorbance was read at 540 nm. By using different concentrations ranging for 0.3 mM to 1.5 mM KNO₂ prepared from 0.03 M KNO₂, the standard curve for NO₂ was prepared. The blank was prepared with distilled water in place of nitrite. The nitrite content is expressed as mg. kg⁻¹FW.

Transaminases assay

Activities of GOT and GPT were determined according to the method developed by Harper and Paulsen [17]. Five hundred milligrams of plant material was homogenized in chilled medium containing 33 mM Tris-HCl buffer (pH 7.2), 3.3 mM cystein and 0.1 mM sodium salt of EDTA. The resultant suspension was filtered through four layered cheese cloth and centrifuged in refrigerated centrifuge at 10000 rpm for 15 min. Supernatant served as an enzyme source. For GOT, 1 mL of crude enzyme was incubated

with 1 mL reaction mixture containing 0.02 M aspartate and 0.02 M α -ketoglutarate in 0.2 M K-phosphate buffer (pH 7.5). After 0 and 60 min the reaction was stopped with 1 mL of colour reagent (1 mg 2, 4-dinitrophenylhydrazine in 1 mL of 1.35 N HCl). After 30 min, 10 mL of 0.4 N NaOH was added followed by 10 mL distilled water. The absorbance of oxaloacetate phenyl hydrazone formed was read at 504 nm. The enzyme activity is expressed as mM oxaloacetate formed $\text{min}^{-1} \text{mg}^{-1}$ protein.

Activity of enzyme GPT was assayed in similar manner except aspartate was replaced by alanine in reaction mixture and absorbance of pyruvate phenyl hydrazone formed was read at 504 nm. The enzyme activity is expressed as mM pyruvate formed $\text{min}^{-1} \text{mg}^{-1}$ protein.

Free proline content determination

Proline was estimated from the roots, stem and leaves, following the method described by Bates *et al.* [18] and expressed as mg 100 g^{-1} FW.

One dimensional gel electrophoresis for proteins: Leaf protein extraction

Total leaf protein was extracted by the acetone-TCA (trichloroacetic acid) precipitation method [19]. Leaf tissue (0.5 g) was homogenized in a pre-chilled mortar with pestle and extracted in 10% ice-cold TCA and incubated overnight at 4°C. The homogenate was centrifuged at 10,000 rpm for 10 min. The pellet was washed with 100% acetone in order to remove the pigments. The washed pellet was suspended in a known volume of 0.1 N NaOH for estimation of protein following the method by Lowery *et al.* [15]. Proteins in the unknown sample were estimated at 750 nm using de-fatted bovine serum albumin (fraction V) as standard and expressed as mg 100 g^{-1} FW.

Analysis of protein profile of leaf by SDS-PAGE

The pellet obtained from above procedure was dissolved in 1 mL of solubilizing buffer [62.5 mM Tris-HCl, pH 6.8; 20% (w/v) glycerol; 2% (w/v) SDS; 5% (v/v) 2-mercaptoethanol and 0.01% bromophenol blue], heated for 4 min at 95°C and cooled on ice before loading on 12.5% polyacrylamide slab gels. Separating (12.5%) and stacking (4%) gel were prepared according to Laemmli [20]. Electrophoresis was accomplished at 35 mA for 4 h using a Genei Bangalore electrophoresis system. The gels were stained with 0.25% Coomassie Brilliant Blue, R-250 (Sigma). The gels were scanned using a photo scanner and analyzed with GelAnalyzer 2010 software. The low range protein molecular weight marker from Sisco Research Laboratory (SRL), India was used.

Statistical analyses

The experiments were laid out in a completely randomized design (CRD). The analyses were repeated with minimum of three independent biological samples. The data were analyzed by one-way analysis of variance (ANOVA) using the statistical software SPSS 10.0 and the treatment means were compared by using Duncan's multiple range test (DMRT) at $P \leq 0.05$. Data were expressed as mean \pm standard deviation (SD).

RESULTS AND DISCUSSION

Effect of water stress on moisture content of the root, stem and leaves of *P. oleracea*

The effect of water stress on the moisture content of the root, stem and leaves of *P. oleracea* is shown in (Figure 1). From figure, it is clear that *P. oleracea* has maintained the water level normal even under water stressed conditions. The moisture content was increased by 30, 7 and 8% in the root, stem and leaves respectively, in the 8 d water stressed plants over the control. As compared to 8 d of water stress, there was slight decrease in moisture content of root and leaves due to 15 d of water stress.

Moisture content is closely related to water content of plant. Many crop species are distinguished as sensitive and tolerant on the basis of relative water content of the tissue [21]. In the present investigation, it is observed that *P. oleracea* is able to maintain higher level of moisture in all plant parts even under water stress conditions which suggests that *P. oleracea* is a water stress tolerant or drought tolerant species. It is already confirmed that *P. oleracea* has an ability to conserve its water status by shifting from C_4 to CAM type of photosynthesis under stress conditions [10].

Behaviour of NR under water stress

The activity of enzyme NR was increased significantly in the root, stem and leaves (0.065 ± 0.0017 , 0.090 ± 0.0011 and $0.096 \pm 0.0029 \mu\text{g NO}_2^-$ liberated $\text{h}^{-1} \text{mg}^{-1}$ protein, respectively) of *P. oleracea* water stressed for 8 d in comparison to the control (0.059 ± 0.0031 , 0.046 ± 0.0031 and $0.040 \pm 0.0020 \mu\text{g NO}_2^-$ liberated $\text{h}^{-1} \text{mg}^{-1}$ protein respectively in root, stem and leaves) (Figure 2A). However, the activity was lowered in all plant parts after 15 d of water stress. As shown in (Figure 2A), activity of NR was higher in the stem as compared to that in the root and leaves of plants stressed for 15 d. It was observed that stem contains more water (moisture content) when the plants were stressed for longer period (15 d). This is suggestive of some positive role of stem in this succulent plant when subjected to stress.

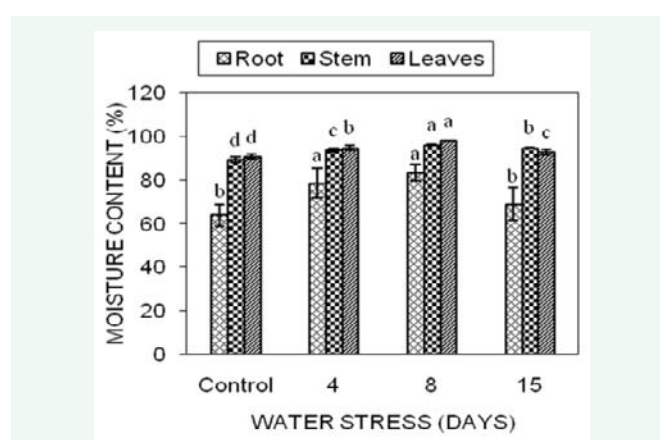


Figure 1 Moisture content of root, stem and leaves of *P. oleracea* Linn. grown under water stress. Different letters indicate significantly different values (DMRT, $p \leq 0.05$; $n = 5$).

A decrease in NR activity due to water stress has been reported in *Leymus chinensis* [22]. NO_3^- concentration in the cell sap is an important factor which induces NR activity. NO_3^- modulates and stabilizes NR transcripts [23]. It is observed that during water stress the decrease in the NR activity was accompanied by a rapid decline in NR transcript levels [24]. In *P. oleracea*, NO_3^- content was increased significantly in the root, stem and leaves which might have induced NR transcripts under mild water stress (4 and 8 d) (Figure 2A). However, during severe drought i.e. 15 d of water stress, there was decrease in NR activity with increased NO_3^- content (Figure 3A). Activity of NR in plants water stressed for 4 and 8 d indicated that *P. oleracea* is able to resist water-deficit upto a certain limit.

Effect of water stress on activity of NiR

The activity of enzyme NiR was increased significantly ($0.010 \pm 0.0001 \mu\text{g NO}_2^-$ reduced $\text{h}^{-1} \text{mg}^{-1}$ protein) in the leaves of *P. oleracea* water stressed for 8 d over that in control leaves ($0.009 \pm 0.0001 \mu\text{g NO}_2^-$ reduced $\text{h}^{-1} \text{mg}^{-1}$ protein) (Figure 2B). But due to 15 d of water stress, its activity in the leaves was decreased ($0.0086 \pm 0.0002 \mu\text{g NO}_2^-$ reduced $\text{h}^{-1} \text{mg}^{-1}$ protein) significantly. There was decrease in the activity of NiR in the root and stem of this plant water stressed. The lowest NiR activity was recorded in the root and stem (0.0029 ± 0.00009 and $0.0043 \pm 0.0002 \mu\text{g NO}_2^-$

reduced $\text{h}^{-1} \text{mg}^{-1}$ protein respectively) of this plant exposed to 15 d of water stress (Fig. 2B).

NiR is second important enzyme involved in nitrate assimilation in plants. Nitrite, the product of enzymatic reduction of nitrate, is further reduced to ammonia by the enzyme NiR. Activity of NiR was decreased in the root nodules of soybean when exposed to water stress due to inadequate supplies of energy and reducing power [25]. It is observed that NiR is more stable than NR under the adverse environmental conditions like drought. This stability of NiR may be associated with its chloroplastic location [26]. Activity of NiR was slightly increased in the leaves of *P. oleracea* while, it was reduced in the root and stem under water stress. Accumulation of NO_2^- in plant tissue leads to reductions of both dry matter per plant, chlorophyll concentrations and the appearance of chlorosis symptoms at the leaf surface of tomato plant [27]. It is possible that to eliminate toxicity of higher NO_2^- concentrations, the activity of NiR was only increased in the leaves. Higher activity of NiR in 8 d water stressed *P. oleracea* leaves is possibly a stress tolerant mechanism to survive under stress conditions.

GOT activity under water stress

Water stress causes an increase in the activity of enzyme GOT in the leaves of *P. oleracea* grown under 8 d water stresses. There

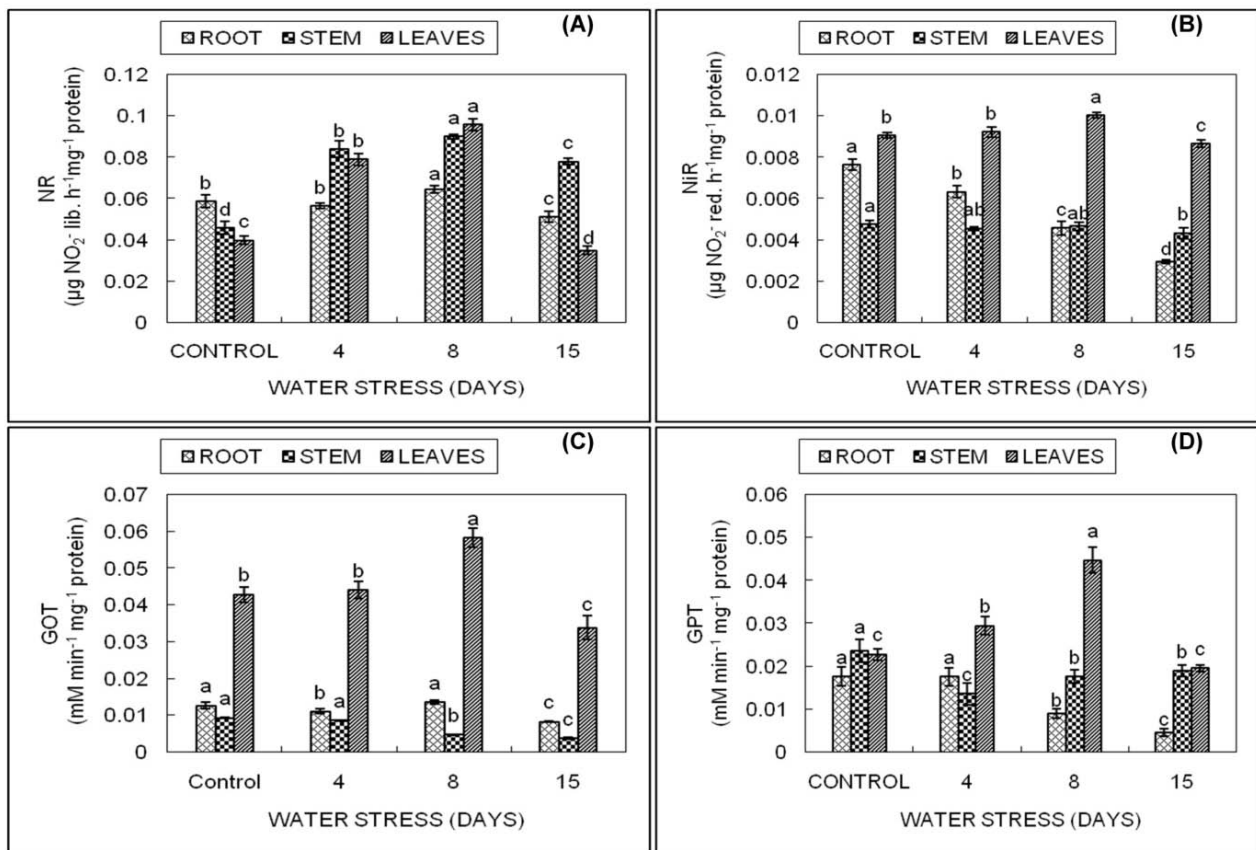


Figure 2 Effect of water stress on some enzymes of nitrogen metabolism in *P. oleracea* L.

A: Nitrate reductase (NR), B: Nitrite reductase (NiR), C: Glutamic pyruvic transaminase (GPT), D: Glutamic oxaloacetic transaminase (GOT). Different letters indicate significantly different values (DMRT, $p \leq 0.05$; $n=3$); Error bars indicate standard deviation (\pm SD).

was an increase in the activity of this enzyme by 2 and 35% in the leaves due to 4 and 8 d of water stress respectively (Figure 2C). After 15 d of water stress, leaves exhibited 21% decrease in the activity as compared to that in control leaves. Activity of GOT was significantly decreased in the stem due to 4, 8 and 15 d of water stress. Significantly low activity i.e. 34 and 59% of control was observed in the root and stem of 15 d water stressed plants.

GOT catalyzes the transamination of oxaloacetate to aspartate, the precursor for asparagine synthesis. Osmotic stress induced by application of polyethylene glycol '6000' and mannitol in rice (*Oryza sativa* L.) resulted in reduced activity of GOT [28]. In the present investigation, activity of this enzyme was increased in the leaves of *P. oleracea* exposed to 4 and 8 d of water stress, which suggested increased transamination of oxaloacetate to aspartate. Aspartate is the metabolic precursor of several amino acids like lysine, threonine, methionine and isoleucine [29]. Therefore, increased activity of GOT in purslane under water stress suggests the induction of synthesis of these amino acids. Positive correlation between availability of nitrogen (NO_3^-) and activity of GOT was noticed in a C_4 plant, *Panicum miliacium* [30]. There was significant accumulation of NO_3^- in the root and shoot of *P. oleracea* under water stress (Figure 3A).

Changes in GPT activity under water stress

The activity of GPT in the leaves of *P. oleracea* was significantly

increased as compared to control by 29 and 96% in the 4 and 8 d water stressed plants respectively (Figure 2D). However, it was significantly declined (14%) in the leaves when plants were water stressed for 15 d. There was significant decrease in the activity of GPT in the root and stem when plants were water stressed. The lowest activity was recorded in the roots ($0.0046 \text{ mM min}^{-1} \text{ mg}^{-1} \text{ protein}$) of 15 d water stressed plants.

GPT also referred to as alanine aminotransferase (AlaAT), is a pyridoxal-5'-phosphate-dependent (PLP) enzyme that catalyzes the reversible transfer of an amino group from alanine to 2-oxoglutarate to form glutamate and pyruvate [31]. Mechanism of GPT is well understood under the abiotic stress like water logging that leads to the condition known as hypoxia [8]. However, there is very little information available on the impact of water-deficit on the activity of GPT. The osmoticum like PEG-6000 and mannitol exerts a negative impact on the activity of GPT [27]. However, in our experiments, there was an elevation in GPT activity in the leaves of *P. oleracea* water stressed for 4 and 8 d. However, after 15 d of water stress, activity of this enzyme was sharply decreased. Higher activity of GPT in the leaves of *P. oleracea* may tend towards accumulation of alanine and 2-oxoglutarate. The increase of alanine in water stressed leaves suggests the predominance of glycolysis and respiration

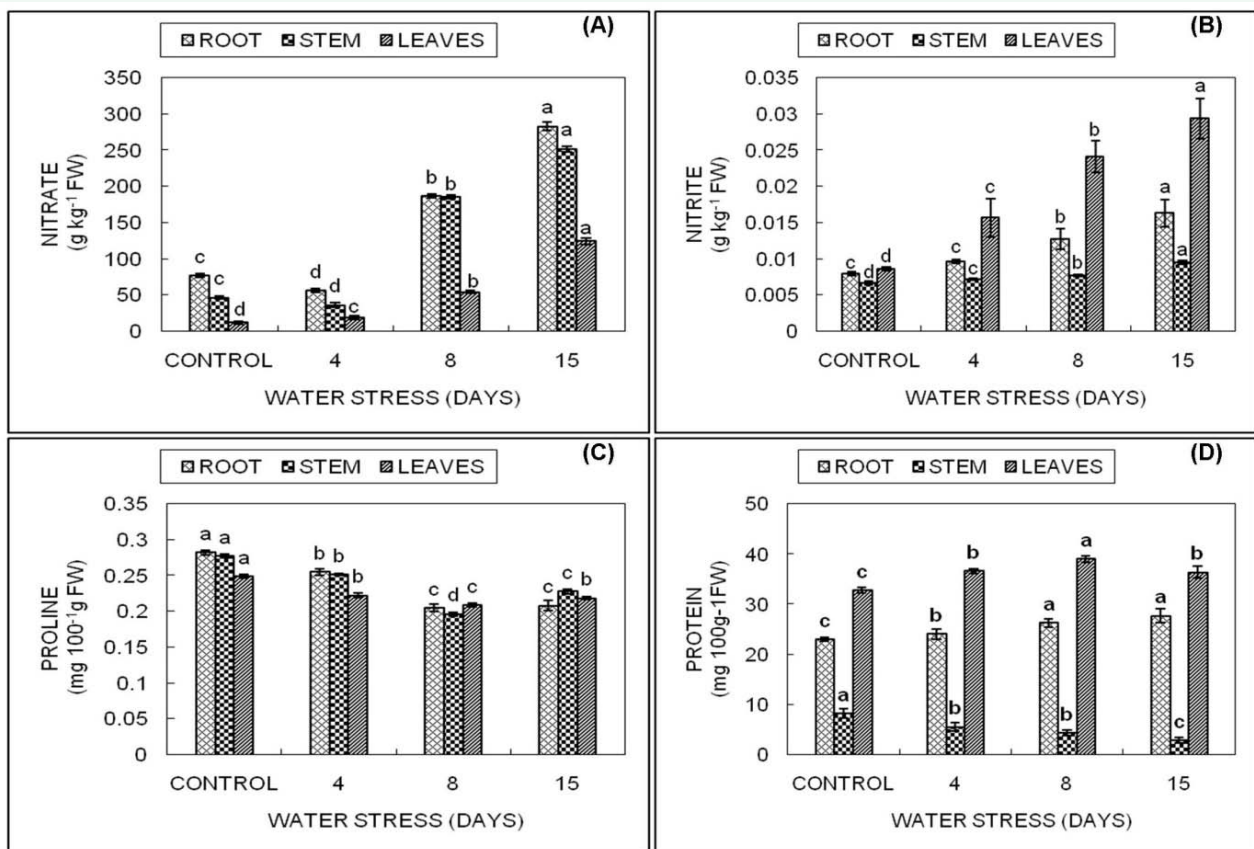


Figure 3 Effect of water stress on some nitrogenous compounds of nitrogen metabolism in *P. oleracea* L.

A: Nitrate, B: Nitrite, C: Proline, D: Protein. Different letters indicate significantly different values (DMRT, $p \leq 0.05$; $n = 3$); Error bars indicate standard deviation ($\pm \text{SD}$).

to sustain the higher energy to furnish carbon skeletons for the photorespiratory cycle [32]. Therefore, increased activity of GPT may be an adaptation of *P. oleracea* to face the water stress conditions.

Accumulation of NO_3^- under water stress

P. oleracea exposed to water stress showed an accumulation of NO_3^- in the root, stem and leaves (Fig. 3A). There was significantly higher accumulation of NO_3^- recorded in the root, stem and leaves (282.70 ± 5.53 , 252.22 ± 4 and 120.11 ± 4.52 mg kg^{-1} FW respectively) of plants stressed for 15 d, (76.63 ± 2.49 , 46.41 ± 1.85 and 12.33 ± 1.86 mg kg^{-1} FW respectively in the root, stem and leaves of well watered plants).

NO_3^- is the major source of inorganic nitrogen for the plants in the cultivated soil. NO_3^- , like other substances is also helpful in osmotic adjustments in the plants under the adverse conditions. It is observed in most of the plants that water stress increases nitrate concentration in plant parts. The drought induced changes in osmotic adjustment in expanded leaves of *Brassica napus* and *B. juncea* were mainly due to the accumulation of nitrate [7]. *P. oleracea* can accumulate high NO_3^- up to 155 ppm [11]. It is possible that under water-deficit conditions purslane accumulates more NO_3^- to adjust osmotic pressure within cell. It is reported that NO_3^- also acts as an inducer of NR in *Spirulina platensis* [33] and NiR in barley seedlings [34]. Hence, accumulation of NO_3^- in *P. oleracea* may be an adaptive measure to combat with water scarcity.

Effect of water stress on NO_2^- content

NO_2^- contents of the root, stem and leaves of *P. oleracea* grown under water stress are exhibited in Figure 3B. It is clear from the results that NO_2^- concentration is increased in the plants grown under water stress conditions. Significant NO_2^- accumulation is recorded in the root, stem and leaves of purslane exposed to 15 d of water stress. Leaves appear to be the major site of NO_2^- accumulation.

Generally, NiR is more active as compared to NR in plant tissues and hence, NO_2^- is never allowed to accumulate in the cells [35]. NO_2^- was reported to acidify the stroma, which may inhibit the enzymatic reactions in the Calvin cycle [36]. It is also observed that it inhibits NR transcripts and its activity in maize [37]. In response to water stress conditions, there was significant NO_2^- accumulation in *P. oleracea*. At the same time, activity of NiR is also found to be increased (Figure 2B). Higher activity of NiR may eliminate the toxic effects of NO_2^- . It is also possible that an increased concentration of NO_2^- in the plant parts due to water stress stimulates NiR activity and detoxification occurs. However, in plants grown under 15 d of water stress there is more accumulation of NO_2^- (Figure 3B) which might have affected the NR and NiR activities (Figure 2A and B).

Changes in proline content

Water stress provoked a gradual and progressive decline in proline content of root, stem and leaves of *P. oleracea* grown under water stress (Figure 3C). As compared to control, 8 d water stressed plants experienced a decrease in proline content by 27, 29 and 16% in the root, stem and leaves respectively. However,

there was significant increase in proline content of the stem and leaves (82.11 ± 0.003 and 87.94 ± 0.002 $\text{mg } 100^{-1}$ g FW respectively) of 15 d in comparison to 8 d water stressed plants. It appears that proline has some role in this plant only under severe drought conditions.

Proline is considered as an osmoregulatory solute in plants subjected to hyperosmotic stress like drought. It is generally accepted that under conditions of water deprivation or extreme salinity, proline accumulation serves as a defense against osmotic challenge by acting as a compatible solute [38]. *P. oleracea* is considered as one of the highly water use efficient plant. From the present investigation it is evident that probably proline has no role to play in this plant, particularly when grown under water stress conditions. Earlier workers have also reported that the role of proline in *P. oleracea* under water stress is not clear [9,39].

Protein content under water stress

From Figure 3D, it is clear that there was an increase in soluble protein contents by 4, 14 and 20% in the roots respectively due to 4, 8 and 15 d of water stress. An increase in the level of protein in the leaves of 4, 8 and 15 d of water stressed *P. oleracea* was by 11, 19 and 11% respectively. However, there was decline in its level in the stem due to water stress respectively by 32, 46 and 64%.

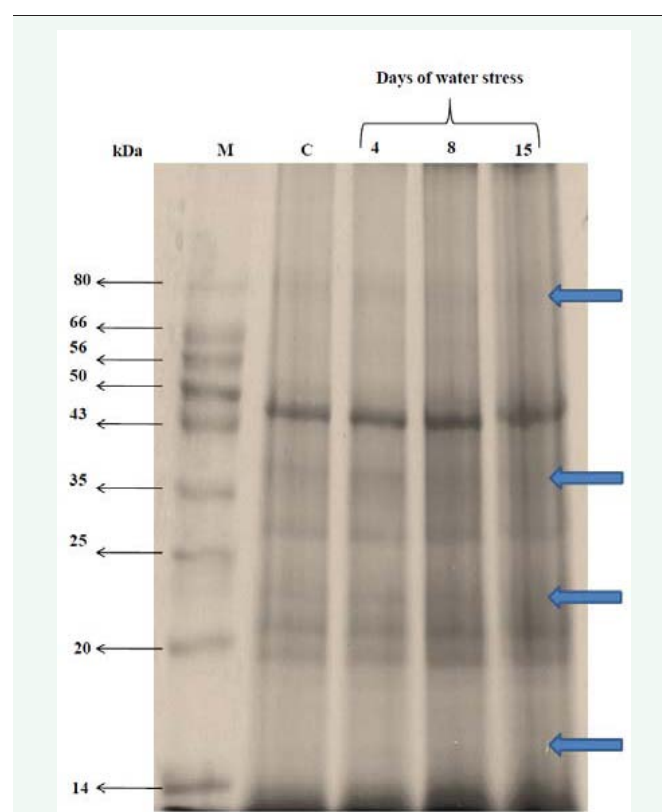


Figure 4 SDS-PAGE of proteins from the leaves of *P. oleracea* Linn. grown under normal and water stressed conditions. M = Molecular weight marker, C = Control (Normal), 4, 8, 15 = Plants water stressed for 4, 8, 15 d respectively. Arrows on the right indicate changes in proteins.

Table 1: Some characteristics of proteins (separated by SDS-PAGE) from the leaves of *P. oleracea* Linn grown under normal and water stressed (4, 8 and 15 days) conditions.

Protein No.	Rm value	Molecular weight kDa	Presence (+) / absence (-) of proteins			
			Days of water stress			
			Control (0)	4	8	15
	0.185	80	+	+	+	-
	0.383	46	+	+	+	+
	0.476	36	+	+	+	-
	0.566	29	+	+	+	+
	0.661	24	+	+	+	-
	0.714	21	+	+	+	+
	0.764	19	+	+	+	+
	0.883	16	-	-	-	+
	0.912	15	-	+	-	-
	0.968	14	+	+	+	+
Total number of protein bands			8	9	8	6

Some workers have reported increased levels of proteins in wheat under drought stress [40]. Protein content of *P. oleracea* was found increased significantly in the root and leaves under water stress. Similar observations are made in drought tolerant *Populus popularis* when exposed to water stress [41]. The increased amount of proteins may include the stress related proteins associated with the water stress tolerance mechanism of this plant.

Protein profile of leaves of *P. oleracea* under water stress

Qualitative analysis of proteins by SDS-PAGE revealed that the intensity of several proteins separated, of the molecular weights, 80, 36 and 24 kDa decreases in the extracts of plants water stressed (Figure 4). The degree of decrease of these proteins seems to be roughly proportional to the intensity of water stress (Table 1). However, proteins of molecular weights 46, 29, 21, 19 and 14 kDa are common in all treatments. However, low molecular weight proteins (15 and 16 kDa) are exclusively observed in the extracts of plants water stressed for 4 and 15 d. The intensity of 46 kDa protein is found to be significantly increased after 8 d of water stress. However, after 15 d of water stress, the visibility of 80, 36 and 24 kDa proteins is not clear (Figure 4), suggesting that these proteins might have disintegrated under the prolonged drought conditions.

It is observed that in plants, there is a specific protein; dehydrins which accumulates in plant tissues under stress conditions [42] but other factors such as salicylic acid decreases them under water stress [43]. The exhaustive work on proteomics analysis of leaves of *Portulaca oleracea* under high temperature and humidity stress revealed that antioxidant defense-related proteins demonstrated marked increase in their activity, which led to lower accumulations of H₂O₂ and O₂⁻ in *P. oleracea* [44]. A change in leaf protein profile of *P. oleracea*, in response to water stress emphasizes role of certain proteins in plant's stress tolerance mechanism. These proteins may be dehydrins or other antioxidant defense related proteins.

CONCLUSION

On the basis of results, it is concluded that the nitrogen metabolism of *P. oleracea* under water stress plays an important role in stress tolerance mechanism. The moisture percentage of the root, stem and leaves of *P. oleracea* under water stress is also significant and suggests higher water use efficiency of this plant. Furthermore, the enhanced activities of NR, NiR, GOT, GPT and accumulation of NO₃⁻ and NO₂⁻ in the root, stem and leaves underlined water stress tolerant nature of this plant. It is noticed that stem of *P. oleracea* also plays an important role in plant metabolism. Analysis of protein profile of leaves revealed some significant changes in individual type of proteins. Further investigations in characterization of these proteins are needed.

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