Proline Relationship with Protein in Seedlings Under Induced Stress in the Presence of Putrescine

Abstract: Seedlings of Indian mustard (Brassica juncea L.cv. RH-30) grown in controlled condition (irradiance 75 W m^{-2}, RH 60-70% and temp. 25±2°C) for 7d and watered with Hoagland's solution containing different level of NaCl (70, 175 mmol/L NaCl) with or without putrescine (PUT, 1 mmol/L) were examined for PUT amelioration of NaCl induced inhibition in seedling growth. Proline and protein were also examined in leaves as well as in roots to find out their relationship in dark conditions. Salinity caused reduction in seedling growth and biomass accumulation was parallel to increased proline accumulation in leaf and root tissues which were reversed significantly by PUT. This finding suggests that PUT might be activating antioxygenic enzymes and elevating antioxidants there by controlling free radical generation, hence preventing membrane peroxidation and denaturation of bio-molecules resulting into improved seedling growth under salinity.

***Key words:** Indian mustard; Putrescine; Salinity; Proline*

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1. INTRODUCTION

Proline accumulation in plant tissues expose to environmental stresses such as salinity, osmotic, light, temp., mineral deficiency, heavy metal, gaseous pollutants and infection by pathogens [1-3] considered to be one of the plants strategies for stress alleviation demonstrated that salt tolerant cultivars had stronger and faster accumulation of proline than sensitive ones[4]. The proteinogenic amino acid proline function as an osmolite, radical scavenger, electron sink, stabilizer of macromolecules and a cell wall component [5]. Of all amino acids, proline was found to be very high under salinity, which increases with increase in salinity. Plants under saline conditions were able to maintained water potential gradient (osmotic adjustment) by accumulation of inorganic ions and low molecular organic compounds in their tissues [6-7]. Amino acids particularly proline which was found to accumulate in higher quantities under salinity in *S.persica*, thus play an important role in osmotic adjustment and turgor maintenance along with ions like sodium and chloride [8]. Under salinity, proline accumulates to whole tissue concentration upto 1mol 1-1. Increased levels of proline correlate with enhanced salinity tolerance [9]. Proline is an extensively studied molecule in the context of plant responses to abiotic stresses. Many plants accumulate this compatible solute under water deficit [10-12].

Further, it is suggested that the light –induced stimulation of proline is dependent upon photosynthesis, either through the provision of potential energy i.e NADPH or carbohydrate [13], hence chloroplast considered to be site of synthesis as well [14]. The role of proline is attributed in accumulation of either proline reach protein under oxidative stress [5] or acting as an N-storage compound, osmolite and hydrophobic protectant for enzymes and cellular structure [15]. Besides important source of nitrogen in plant metabolism[16], it is also readily available source of energy and reducing power [17]. Proline overproducing transgenic plants exhibit tolerance to high salinity and osmotic stress [18]. Salinity induced free radicals toxicity mitigation by proline is also suggested [19]. The positive effects of proline have been further substantiated by exogenously added proline, which counter acted the salinity effect [19-21]. However, recently it is demonstrated that accumulation of proline in light was higher than in dark in barley leaf [22] as well as in many crops [23-24]. The role of proline in stress mitigation is elusive, however its accumulation in presence of polyamines and relationship between two under stress still remain to be elucidated. The present study is understood the proline accumulation in root and leaf of plant under dark under salinity condition and any co-relation with protein on supplementation of putrescine as this has been implicated in growth and development [25-26].

2 MATERIALS AND METHODS

Proline estimation

Seedlings were raised in petriplates lined with filter paper under control conditions (light 75 wm^2 from

fluroscent tube and bulb of 40 W, Temp.25± 2°) upto 14-days after sowing. Seedlings were kept under different salinity regime (NaCl 70 and 175mM) and maintained in half strength Hogland's solution added with/without putrescine (Put 1mM). The pH 6.4 of the all above solution was kept constant. Proline was estimated in leaf and root tissues of 7th and 14th day old seedlings following methods of Bates *et al.*, [27].The plant material (0.5g) was homogenized in 10ml of 3% sulphosalicylic acid. The homogenate was centrifuge at 5000g for 20 minutes and the supernatant was used for the assay of proline. Reaction mixture contained 2.0 ml aliquot, 2ml glacial acetic acid was added with the 2.0 ml of ninhydrin reagent. This was kept for one hour in boiling water bath. After development of color tubes were placed on ice for termination of reaction, followed by addition of 4.0 ml toluene and intermittent stirring for 20-30 sec. The upper pink layer containing toluene was used for the estimation of proline by reading OD by spectrophotometer at 520nm and expressed as mmol/g/fresh wt. The data is mean value of three replicates with ± SD.

Estimation of total soluble protein

The fresh mass 0.5g of seedling obtained at the specified period contained homogenized in chilled mortar in protein extraction buffer pH 7.0 (Tris- HCl 30mM, DTT 1mM, Ascorbic acid 1mM, PMSF 1mM with PVP 6(mg/ml). The homogenate was centrifuged at 10,000g for 30min in refrigerated centrifuge. Plasto Crafts Super-spin R-V/ F_M. Total soluble protein was estimated by the Lowry method [28].

For the measurement of total protein the reaction mixture was 0.1ml of protein extract, 0.9ml of distilled water and 5ml of alkaline Na_2CO_3 reagent (dissolve 2.0g Na_2CO_3 in 0.1 N NaOH and make up the volume to 100ml with 0.1 N NaOH) and kept for 10-15min followed by adding 0.5ml of Folin's Phenol reagent (half strength). This reaction mixture is allowed to stand for 30 min for the development of color. The bluish green color developed was measured using spectrophotometer (dou-ble beam UV Vis–Cecil at 750nm. The protein content was determined by calculating the standard curve drawn for the pure commercial bovine serum albumin.

3 RESULTS

Proline

Leaf Tissue

Leaf raised in dark for 7-days showed many fold increase in proline at high salinity over control (Fig.1A) and the elevated level of proline remained upto 14- days of growth. Putrescine further increased proline level by 10% over 175mM NaCl at 7th–day while increase was raised 15-24% depending on salinity stress. However, about 20% and 48% decrease in proline content at 7th and 14th day respectively in dark compare with that of light at higher salinity was observed.

Root Tissue

Root proline accumulation was higher than that of leaf tissues (Fig.1B) at 175mM NaCl. Put supplementation further increased the level of proline as compared with light root proline content in dark was lower by 57% and 20% at 7th and 14th day resp. under extreme salinity (175mM NaCl) condition.

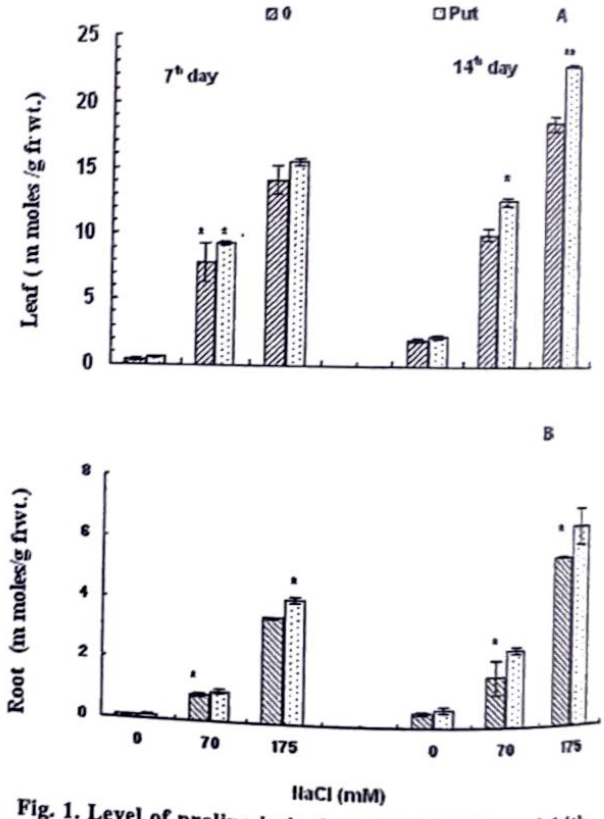

Fig. 1. Level of proline in leaf and root of 7th and 14th day old seedlings.

Protein

Leaf Tissue

Protein level was also determined in leaf and root tissues of seedling raised in dark under salinity allowed to grow for the period of 14th day (Fig. 2A). In RH-30 leaf, protein content was increased to 12.5% at 175mM NaCl over control at 7th day and 28% at 14th day of growth. Put increased protein level up to 30% at 7th day and 20% at 14th day.

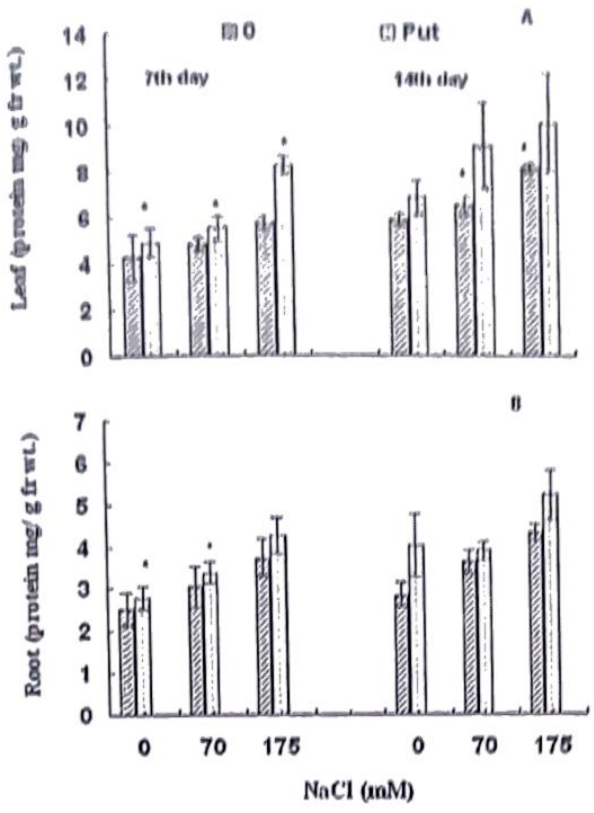

Fig. 2. Level of protein in leaf and root of 7th and 14th day old seedlings.

Root Tissue

Root tissues of seedlings had increased level 26% of protein content at both stages (Fig. 2B). Put induction of the protein level was up to 41% at 7th day and 23% at 14th day over 175mM NaCl.

4. DISCUSSION

Putrescine and proline in stress mitigation

Proline is considered to be of the one major molecule of group of compatible solutes in many plants under stress [29-30], others also involved in normal growth and development). Data showed that proline content increase in salinity (Figs. 1, 2) and the accumulation further elevated with supplementation of Put. Proline accumulation is found increasing rapidly in a variety of crop plants in response to salt stress[31,32,3].This has been implicated in maintenance of osmoticum [33-34] and acting as nitrogen source available for recovery and restoration of growth under stress [35]. Proline accumulation in leaf of barley (*Hordium vulgare* L.cv.alfa) seedlings treated with 70mM NaCl was promoted in light and suppressed in the dark [22]. Moreover, higher level of proline in light than dark is observed in leaf and root tissues suggest that proline biosynthesis under stress might be light mediated. However the dark suppressed of proline synthesis under salinity is suggested in barley[22 24].Though, gradual increase in proline in dark and acceleration under salinity with Put as well indicates towards similar response factor involved in inducing the proline synthesis/or degradation. This might be catalyzed by light. The light promotion of proline accumulation under salinity is observed in higher plants [13, 36-38]. However, free radical generation might be implicated in more proline accumulation depending on stress level in light [39]. Polyamines [40] and proline [41] both are considered to be acting as nitrogenous compounds and our data also indicates a positive correlation of polyamines with proline accumulation under stress conditions. This could be explained as ornithine is the precursor of putrescine at least under stress [42] and ornithine is mainly synthesized from glutamate which is the precursor of proline. This shows that putrescine may lead to proline accumulation via ornithine pathway. Recently, also relationship between free amino acid proline and polyamine content was reported in cereals [43]. Since, the putrescine is increasing proline level, which could be indicator of salt tolerance [44], and inducing protein level thereby increased growth under salinity [40,45]. It could be inferred that its response on stress toxicity mitigation might be due to acceleration of osmotic maintenance and replenishment of nitrogen source for protein synthesis, by increasing proline especially in those of salt associated and simultaneously directly promoting the free radical scavenging machinery as proposed by Verma & Mishra [45] as well. Apparently it seems that proline being amino acid might be incorporated in to protein, their by must be increasing the level of proline is examined. Further, more proline stabilizes the machinery of protein synthesis also [5].

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