



SALINITY STRESS MITIGATION IN *B. JUNCEA* BY PUTRESCINE: A PLEIOTROPIC EFFECT

S.N. MISHRA*¹, NITA LAKRA¹, PUSHPA C. TOMAR¹, KANCHAN MAKKAR¹ AND M.V. RAJAM²

¹Department of Biosciences, M.D. University, Rohtak-124 001, Haryana

²Department of Genetics, South Campus, Delhi University, New Delhi

Received on 18 May, 2009

SUMMARY

The salt stress mitigation by putrescine (Put) was evaluated in terms of endogenous titre of polyamines, proline, Na⁺/K⁺ and changing protein profile of leaf and root tissues of the *B. juncea* cv. RH-30 seedlings under salinity (70 – 175 mM NaCl). Put supplementation controlled the decline in endogenous level of Put and spermidine in salt stressed seedlings. The proline accumulation was considerably high in both tissues of the seedlings at 175 mM NaCl with supplementation of Put. The higher Na⁺ content in root than leaf, increased further with seedling age and salinity level was reduced by Put. However, the salinity caused decline in K⁺ content in both tissues did not respond with Put application. Put increased protein contents in stressed seedlings root and leaf tissues. The Put induction of some new proteins of 23, 26, 29 kDa in leaf and 22,23,40,41,80 kDa in root tissues of the 14day seedlings might be involved in stress mitigation. The Put induction of 26 kDa protein in leaf and root tissues of the seedlings exposed to 175 mM NaCl was observed. Hence, the restoration of diminishing endogenous polyamines level, selective decrease in Na⁺ without changing in K⁺ content in leaf as well as in root, increased level of proline and certain specific proteins by Put application might culminated into stress mitigation in the *B. juncea* RH-30, thereby increased seedling biomass. It is suggested that salinity stress mitigation potential of Put might be a pleiotropic effect.

Key words: *B. juncea*, polyamines, potassium, proline, protein, salinity.

INTRODUCTION

In several cases, the damaging effects of some types of stresses could be mitigated by exogenous application of polyamines (PA). The anti stress role of polyamines (Galston and Kaur sawhney 1995, Mishra *et al.* 2003) and putrescine (Mishra and Sharma 1994, Verma and Mishra 2005) in many plants under stress has been suggested. The PA role in salt tolerance is attributed to increase its endogenous titre in some plants (Galston *et al.* 1997, Mansour 2000). Capell *et al.* (1998) suggested improved tolerance due to high level of Put and other PA in rice transgenic with over

expressing ADC gene. In some of the studies, either no effect or little change in endogenous level is also observed. The differential accumulation of PA is also observed, which depend on developmental conditions (Kumar *et al.* 1997) and age of the plant (Johnson 1992, Lauchli and Epstein 1990). However, the tendency of Put accumulation in plant tissues is also found different in different plants under salinity. It is reported that Put either increased (Willadino *et al.* 1996, Lefevre *et al.* 2001) or decreased in many cases (Lin and Kao 1995, Ali 2000) and/or remained unchanged under salinity stress (Benavides *et al.* 1997). Put incremental pattern in *B. campestris* under hyper salinity was observed under short

*Corresponding author, E-mail: snmishramdu@gmail.com

exposure only, while there was little effect under prolonged exposure (Das *et al.* 1995). The differential accumulation of Put within the wheat variety under salt and drought stress (Galiba *et al.* 1993) and in other adverse conditions (Reggiani *et al.* 1994, Hua and Chong 2002) are also reported. In spite of varied presence, Put is considered as a marker for stress tolerance or sensitivity (Bouchereau *et al.* 1999) and designated as modulator of the plant growth under stress (Krishnamurthy 1991, Mishra and Sharma 1994, Singh *et al.* 2002). These varied responses of Put necessitated the need to examine their precise effects in plants in different environmental conditions. In some of the studies, the salinity stress alleviation by Put (Krishnamurthy 1991, Mishra *et al.* 2003) is attributed to increased antioxidative system in whole plants as well as cultured cells (Verma and Mishra 2005, Tang and Newton 2005) and as a nitrogen source for plant growth (Mishra and Sharma 1994, Singh *et al.* 2002).

Put stress mitigation potential, however, is not widely accepted (Walden *et al.* 1997). It might be due to obscure molecular mechanism of PA protective effects and their biological significance for several plants survival and adaptation. Hence, the Put inextricable regulatory link with the growth of plants under stress is to be advanced further in terms of underlying mechanisms of osmotic maintenance by compatible molecules, like endogenous polyamines, proline, Na⁺/K⁺ balance and its molecular interactions by protein profiling up to an extent.

Though, the pressing demand of understanding is due to differential interaction of these molecules also. Like Put controls the K⁺ deficiency in lemna (Tachimoto *et al.* 1992), while inhibits K⁺ level in cultured rice cell (Shih and Kao 1996). Moreover proline, one of the most widely present osmotic solute in plants (Delauney and Verma 1993, Matysik *et al.* 2002) increases under salinity (Soussi *et al.* 1998) and confer stress tolerance through acting as a nitrogen source (Fukutaku and Yamada 1984, Trotel *et al.* 1996). In addition Shen *et al.* (1997) have suggested proline as reactive oxygen scavenger, whereas increased proline is linked with restricted leaf growth as well (Munns 2002). Hence, Put interaction requires to be elucidated, especially when both need glutamate as ultimate source.

Over and above, the stress related proteins are believed to be involved in the proper folded structure of essentially required proteins. For instance, LEA and dehydrins are considered to be acting as substitute for water satisfying hydrogen bonding for polar amino acids at protein surface and thus maintains folded structure (Dure *et al.* 1989, Close 1996), which is implied in desiccation tolerance. It is also suggested that some proteins might be involved in pH regulation, ionic balance and hormone homeostasis (Zhu 2001). The stress specific proteins could be protease inhibitors as well (Lopez *et al.* 1994). In view of above, present study was planned to establish the precise role of Put in salt stress mitigation.

MATERIALS AND METHODS

Seeds of mustard (*Brassica juncea* cv. RH-30) were surface sterilized with bleaching powder (CaOCl₂) for 5 min and sown in Whatman's filter paper lined petri-plates. The seedlings were grown up to 14 day in controlled condition (light 75 Wm², Temp. 25± 2°C, RH 65%) and watered with half strength Hoagland nutrient solution, containing NaCl (0.70, 175 mM) for simulating salinity stress. Put (1mM) was supplemented to germinating seeds under salinity stress from the first day of sowing. The leaf and root tissues were harvested on 7th and 14th day of seedlings for the studies. Growth in terms of biomass was measured by drying the seedlings for 72 h at 68°C and expressed in mg seedling⁻¹.

The endogenous level of polyamines in the leaf and root tissues were determined by the method of Flores and Galston (1982) with modification suggested by Bajaj and Rajam (1995). The proline was estimated following the method of Bates *et al.* (1973). For estimation of Na⁺/K⁺ content, dried leaf and root tissues (0.1g) were digested in acidic conditions and estimation was done by digital flame photometer and expressed as µg g⁻¹ dw using standard for both ions. The total soluble protein in samples were estimated following the method of Lowry *et al.* (1951). The polypeptides profile was examined through SDS-PAGE (12.5%) following Laemmli (1970). Each protein sample was loaded 50µl in the gel. The molecular weight of the polypeptides was determined by using standard protein markers (lysozyme, carbonic

anhydrase, oval albumin, bovine serum albumin and phosphorylase of 14, 20, 43, 67, and 97 kDa respectively from GENEI, India) by plotting R_f value of respective peptide on antilog graph.

The data given are mean value of at least three replicates with \pm SD. The student t-test was applied to find out the significance of the treatments.

RESULTS

Seedling growth: Seedling dry biomass accumulation declined by 30% under salinity. Put application checked the decline and increased the biomass accumulation up to 10% in stressed seedling (Fig. 1).

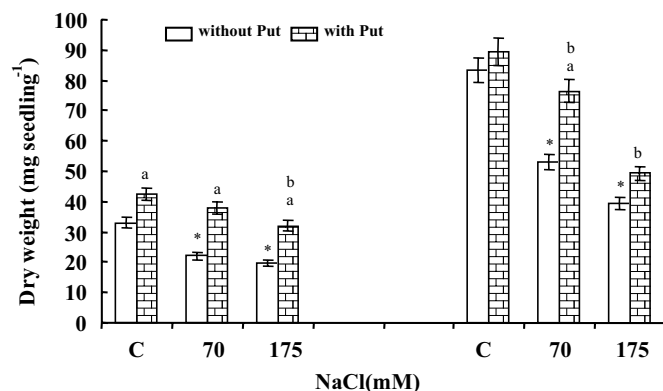


Fig. 1. Accumulation of biomass in 7th and 14th day old seedlings of *Brassica juncea* under salinity as influenced by putrescine. Data are mean value of ($n=3$) \pm SD. The data are significant at $P>0.05$ over control, (*) salinity, (a) Put treatment over salinity, (b) within Put x salinity. No symbol indicates not significant

Polyamines titer in leaf and root tissues: The Put, spermidine (Spd) and spermine (Spm) titer were 455, 498 and 186 mmol g^{-1} fw respectively in the leaf of 7d old control seedling (Fig. 2A), which were considered as basal level of polyamines in tissues and for the comparison of the respective amines in the stressed seedlings. The increase in Put, Spd and Spm level was 34%, 28% and 29% respectively on advancement of the seedlings age (14 d). The salinity (70 to 175 mM NaCl) caused decline in Put and Spd in leaf, except Spm content increased (45-85%) with increasing salinity level.

The Put decline was 64% at 70 mM NaCl and 81% at 175 mM compared with that of control. The exogenous supplementation of Put maintained the PA contents significantly in salt stressed seedlings at both ages, whereas, Spd increased prominently in the tissues with Put application. The PA contents in root tissues were considerably low (Put 73.5, Spd 305, Spm 101 mmol g^{-1} fw) in comparison to leaf tissues (Fig. 2B). The Put and Spd declined in root consistently at both ages of seedlings under salinity. However, Spm in root increased and further elevated under severe saline condition. Put supplementation increased Spd level remarkably in 14-d stressed seedling root. The increase in Spd and Spm content was differential on different salinity level. However, endogenous Put titre in root tissues changed little on supplementation of Put in seedling at 7th day.

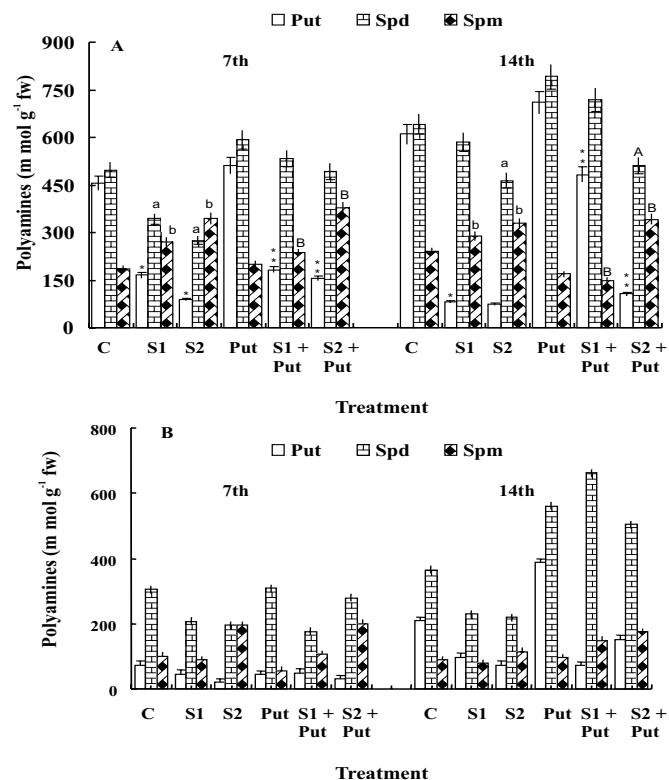


Fig. 2. Contents of endogenous polyamines in (A) leaf and (B) root of seedlings at 7th and 14th day supplied with NaCl and putrescine. C=Control, S1=70mM, S2=175mM NaCl, Put=putrescine, Spd=spermidine, Spm=spermine. Data are mean value of ($n=3$) \pm SD. The significance of treatment at $P<0.05$, between control and salinity (*=Put, a=Spd, b=Spm) and Put treatment under salinity (**=Put, A=Spd, B=Spm). Data without any symbol is not significant.

Proline level in root and leaf tissues: The higher accumulation of proline at 175 mM NaCl was increased further with seedling age (Fig. 3A). The Put application further increased proline level many fold in leaf and root tissues of the seedlings under salinity. The tendency of proline accumulation in root under salinity was same as in leaf, but with lower magnitude at either of the age of the seedling (Fig. 3B).

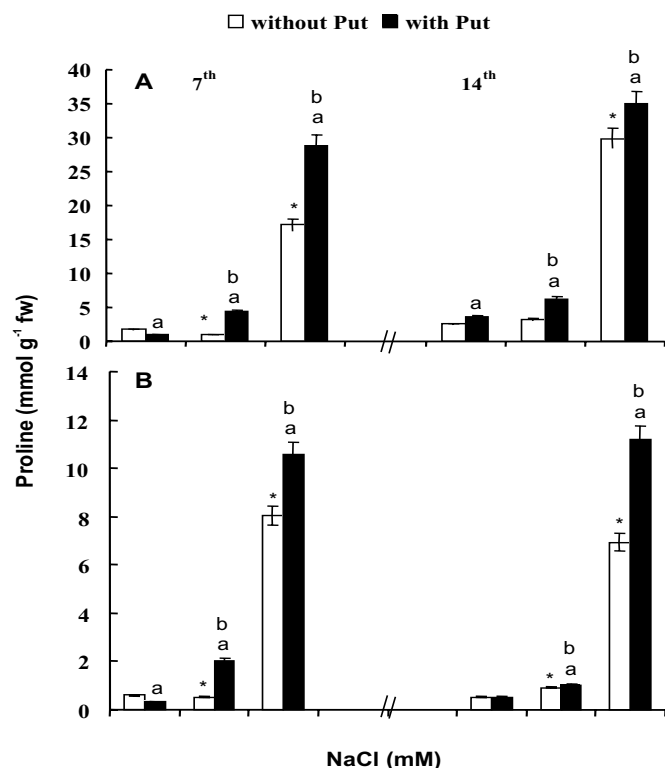


Fig. 3. Proline content in the leaf (A) and root (B) of seedling under salinity at 7th and 14th day, with and without putrescine. Rest legend same as fig. 1.

Na⁺/K⁺ content in the leaf and root tissues: The Na⁺ content increased many fold in the leaf of the seedling at both ages depending on salinity exposure (Fig. 4.1 A, B). The rise in Na⁺ level in leaf was little more on 7th day than on 14th day seedlings under salinity. The increase in Na⁺ content was controlled significantly by Put application. The K⁺ content declined in leaf of the seedlings increasingly with age and under salinity, and remained unaltered by the Put application (Fig 4.1 B). The Na⁺ content accumulation in root tissues was considerably high under salinity and increased with age,

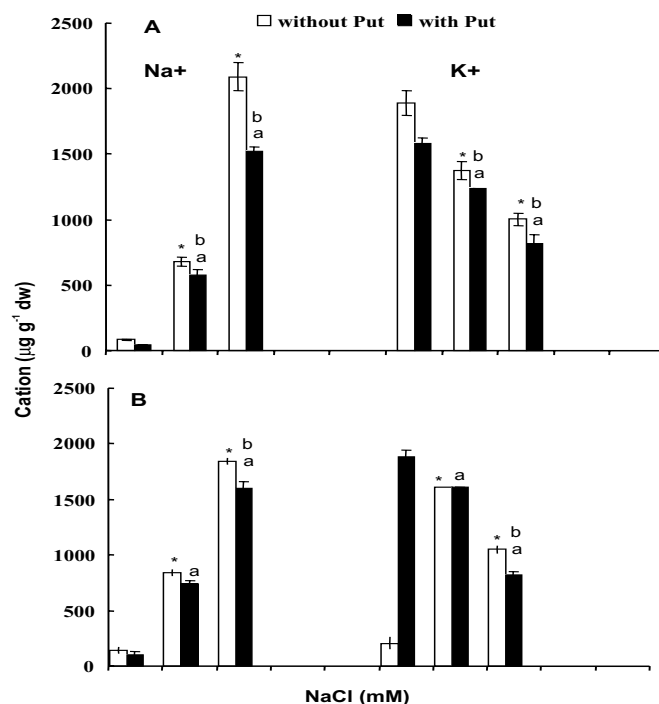


Fig. 4.1. Sodium and potassium content in 7th (A) and 14th (B) day leaves of the seedlings under salinity with and without putrescine. Rest legend same as fig. 1.

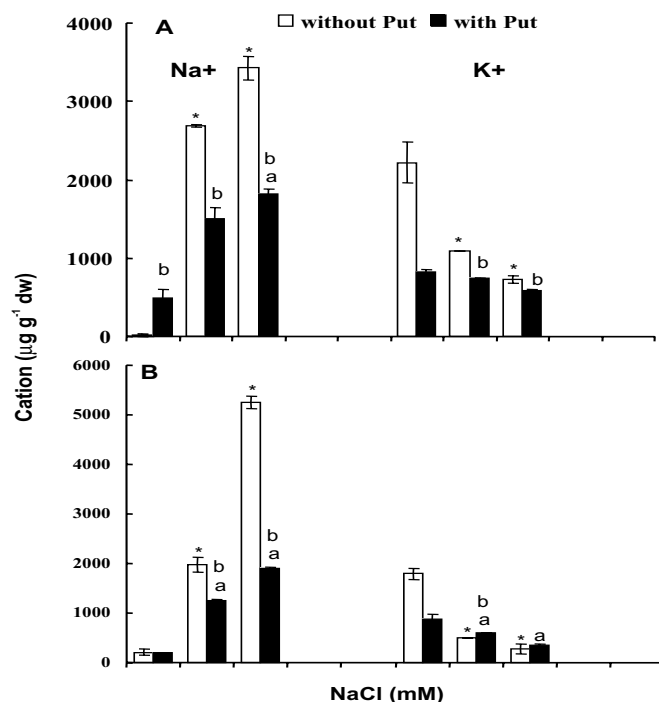


Fig. 4.2. Sodium and potassium content in 7th (A) & 14th (B) day root of the seedlings under salinity with and without putrescine. Rest legend same as fig. 1.

but was controlled by Put (Fig. 4.2A & B).The decline in K⁺ content in root tissue with increasing salinity at both ages of the seedling was observed. K⁺ level did not show any change with Put application.

Protein profile of the seedling: The protein content decreased in the leaf and root tissues of the 7d old seedling at 70 mM NaCl, but slightly increased at 175 mM NaCl (Fig. 5 A, B). Similar protein pattern was also observed in 14th day of the seedling. Put application increased the protein content considerably in leaf and root of stressed seedlings. Put application elevated protein level by 50% in root tissues exposed to high salinity over control. SDS-PAGE examination of protein profile of leaf and root tissues exposed to salinity revealed that the Put application caused either selective accumulation or suppression of few proteins and simultaneously induced some new proteins as well. High salinity (175 mM NaCl) stressed leaf of 7d old seedlings exhibited more accumulation of 37 and 52 kDa peptide over control and 70 mM NaCl stressed leaf (Fig. 6).The

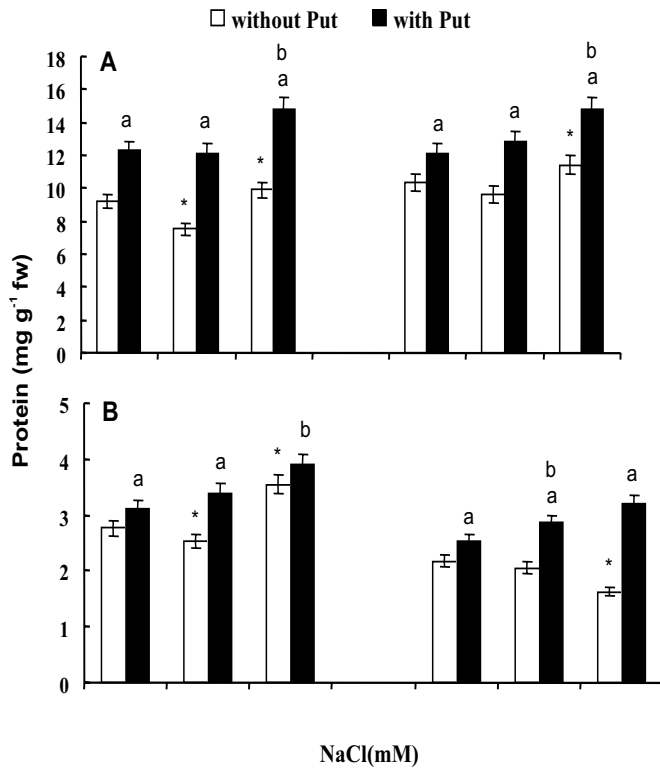


Fig. 5. Protein level in the leaf (A) and root(B) of the seedling under salinity at 7th and 14th day with and without putrescine. Rest legend same as in fig.1.

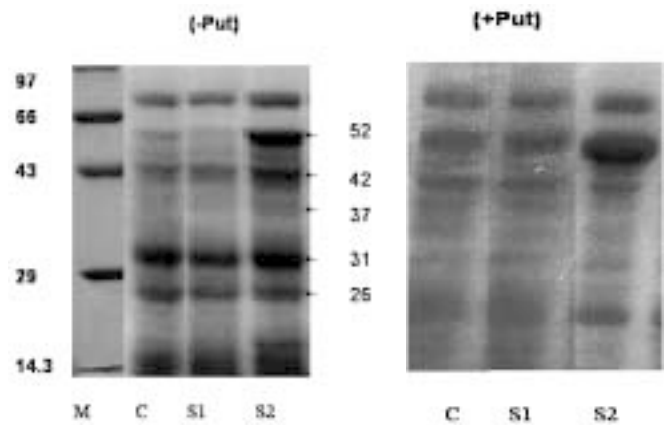


Fig. 6. SDS-PAGE analysis of total soluble protein extracted from leaf tissue of 7 days old seedling of control (C), 70 mM NaCl (S1), 175 mM NaCl (S2), without Putrescine (-Put) and with Putrescine (+Put) treatments. M=Marker

expression of both peptides (37 and 52 kDa) increased with Put also. Put reduced the expression of a 31 kDa peptide transiently in early stage (7th day) of seedling under salinity. The expression of 26 kDa peptide under salinity was also little elevated with Put. At 14th day, the 31 kDa peptide was not observed in control leaf, yet it was accumulated under salt stress and further increased with Put supplementation (Fig. 7). The salinity induced proteins (23 and 29 kDa) in 14-d leaf was expressed prominently in the presence of Put. The 26 kDa protein was also prominent and was more with Put in the 14-d leaf under 175 mM salinity (Fig.7). The root protein profile showed that the expression of 18, 47, 49 and 51

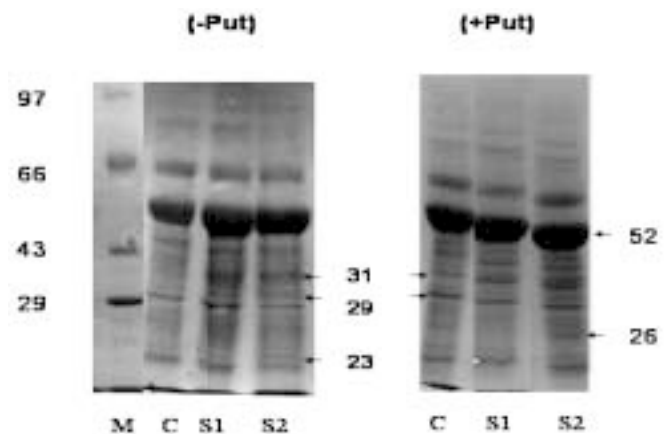


Fig. 7. SDS-PAGE analysis of total soluble protein extracted from leaf tissue of 14 days old seedling. Rest legend same as fig. 6.

kDa peptides in 7th day root were low (Fig.8). The 51 kDa protein disappeared at 70 mM NaCl with respect to that of control. At 175 mM NaCl, the expression of 47 kDa peptide was high compared with 49 kDa peptide (Fig. 8). Put application elevated the expression of 18 and 49 kDa at 175 mM NaCl and reduced the expression of 51 kDa protein. But this was reverted by Put. Put induced some novel proteins of 60, 66 and 70 kDa under

day control root was disappeared under salinity. However, disappearance of 43 kDa peptide in root was marked under both salinity level. The reduction in accumulation of 42 and 49 kDa peptide under salinity was checked by Put (Fig. 9). Further, the expression of 26 kDa peptide in root was like in 7th day leaf, and 29 kDa peptide like in 14th day leaf under high salinity, was found prominent with Put. A high molecular mass (80 kDa) novel peptide was observed with Put application in root under salinity. Salinity lowered accumulation of 22 and 23 kDa peptide was also reverted by Put.

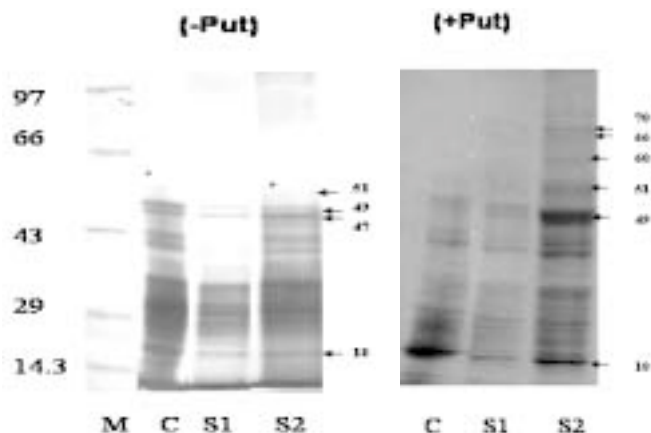


Fig. 8. SDS-PAGE analysis of total soluble protein extracted from root tissue of 7 days old seedling. Rest legend same as fig. 6.

high salinity only. With progression of the seedling age (14 d), root showed accumulation of 42 kDa peptide under 70 mM NaCl stress, while reduced under 175mM NaCl stress (Fig. 9). A 49 kDa peptide observed in 7th

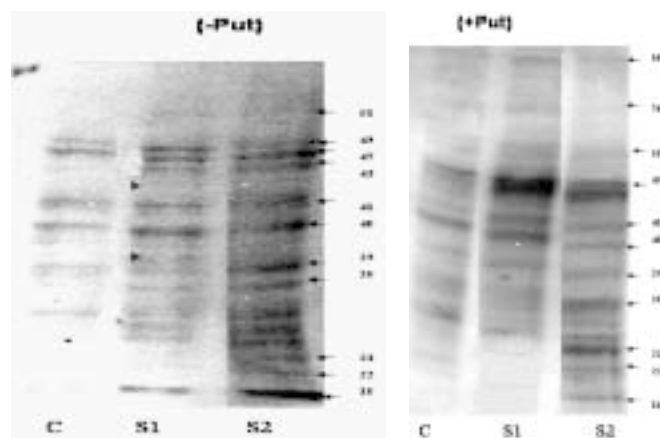


Fig. 9. SDS-PAGE analysis of total soluble protein extracted from root tissue of 14 days old seedling. Rest legend same as fig. 6.

DISCUSSION

The *B. juncea* cv. RH-30 is considered to be relatively salt tolerant based on germinability of seeds and seedling growth under 200 mM NaCl stress condition (Mishra and Sharma 1994). This is substantiated with present study also. The 30% lower biomass accumulation under salinity was reverted significantly by application of Put (Fig.1). The Put potential to neutralize salinity effect has also been reported by others (Kapchina and Foudouli 1991, Singh *et al.* 2002, Mishra *et al.* 2003). It is envisaged that the growth alleviation in stressed plants could be due to check in reduction in endogenous titre of Put under salinity (Fig. 2A & B). The exogenous Put renders osmotic and ionic balance through increased proline and lowering Na⁺ in general and expression of few proteins in specific. The low Put titre under salinity is linked with low growth in rice (Lin and Kao 1995) and in wheat (Reggani *et al.* 1994). The Put decline may lead to imbalance in PA ratio leading to growth retardation (Bajaj and Rajam 1996). The present study showed that PA ratio could be balanced by exogenous Put, (Fig. 2 A,B). Wi and Park (2002) have demonstrated positive correlation between PA contents and stress tolerance, hence, PA level is probably maintained by exogenous application of Put. However, the PA threshold level depends on the stress and developmental condition of specific plant species. The differential accumulation of PA in many other crop plants exposed to salinity has been observed (Das *et al.* 1995, Reggani *et al.* 1994, Hua and Chong 2002). The salinity caused increase in Spm level in Indian mustard corroborates with results observed in rice (Bay *et al.* 1992) and wheat (Galiba *et al.* 1993).

It could be argued that the changed PA ratio in the present study could be due to low titre of endogenous Put, Spd in this cultivar under salinity (Fig. 2A & B). This might be either due to reduced biosynthesis (Aziz *et al.* 1998) or/and increased degradation by diamine and polyamine oxidases (Galston and Kaur-Sawhney 1990). The low level of PA in sensitive cultivars corresponds with the decreased activity of ADC/ ODC and/or increased activity of *diamine oxidase* (Aziz *et al.* 1997, Das *et al.* 1995). The Spm declined in leaf with application of Put (Fig.2A), but was reverse in case of root (Fig.2B). It indicates that the endogenous PA titre might be linked with decrease and/or increase in Put content in tissues, and its incorporation in to PA, may depend on stress sensitivity and physiological status of the plants (EI-Shintinawy 2000). The Put/PA ratios, suggested to be crucial for plant growth (Reggiani *et al.* 1994) may be different in different plants. Chattopadhyay *et al.* (2002) have reported higher spermidine and spermine accumulation in salt tolerant rice plants than sensitive type.

Stress alleviation response of Put is through maintaining ionic and osmotic balance, an essential component of plant ability for salt tolerance (Matysik *et al.* 2002). This could be due to reduced Na⁺ load (Fig. 4.1 & 4.2) and increased proline level in tissues (Fig.3). The trend of Na⁺ increase but K⁺ decrease in leaf and root tissue of stressed seedlings at 7th or 14th day (Fig. 4.1 & 4.2) corroborates with many glycophytes exhibiting salt sensitivity (Zhu 2001). The Put selective effect on reduction of Na⁺ content without changing K⁺ level in leaf and root tissues under salinity further indicates specific regulation of Na⁺ flux, possibly be through modifying fast vacuolar rectifiers on membranes (Chinnusamy *et al.* 2005). This might result in to low cytotoxicity and, not solely, activation of Na⁺/K⁺ ATPase as suggested by Reggiani *et al.* (1992). The least effect of Put on K⁺ level could be explained as high titre of Put (Fig 2 A & B), own bi-cationic status, might be replenishing the K⁺ requirement for various metabolic processes, thereby mitigating the Na⁺/ K⁺ imbalance under salinity. This hypothesis could be in accordance with that of nature of Put replacing K⁺ deficiency (Tachimoto *et al.* 1992).

A consistent elevated level of proline in leaf and root tissues of the plant at both stages (Fig.2 A & B) with Put could further potentiate the stress mitigation. Because proline, apart from being an osmoticum, considered to be acting as nitrogen source for stressed plants (Fukutaku and Yamada 1984), so as the putrescine (Mishra and Sharma 1994). Put also supplemented metabolic process in addition to antioxidant action (Shen *et al.* 2000, Verma & Mishra 2005).

Increase in proline by Put could be by induction of biosynthesis (Aziz *et al.* 1998) and/or decreased degradation under salinity. The correlation between exogenous proline with increased endogenous Put level in leaf of cereals has been reported (Flores and Galston 1984). The experiments here showed that Put caused little change in the proline content in either leaf or root of the control seedlings, while many folds increase in proline under salt stress suggests that Put might be

Table 1. Endogenous PAs and Proline ratio in leaf and root tissues of the seedling under different stress level with and without putrescine supplementation.

Seedling/ age	Treatment (NaCl mM)	Ratio			
		Put/Proline		PAs/Proline	
		Leaf	Root	Leaf	Root
7 th Day	0	257.49	123.5	644.5	805.8
	70	160.1	87.3	754.6	654.0
	175	5.19	2.49	41.3	51.1
	Put	495	28.1	1216	1186.9
	70+Put	41.4	24.4	216	163.5
	175+ Put	5.3	2.92	35.5	48.5
	14 th Day	0	238.2	405	582.8
70		25.4	104.5	299.7	439.7
175		2.51	10.5	29	58.6
Put		191.8	724	451.3	195.2
70+Put		77.2	68.6	216.3	853.4
175+Put		3.06	13.5	27.4	74.26

Data computed from Fig.2 A, B & 3.

influencing proline synthesis only when the biosynthesizing machinery is switched on under stress (Hare *et al.* 1999). This might be due to glutamate availability also, a precursor for both proline and Put at particular physiological stage. The underlying mechanism of cross talk/or link between proline and Put in stress alleviation, apparently could be also visualized with the varying ratio of Put/proline or PAs/proline under different stress level (Table 1). Aziz *et al.* (1998) have suggested that proline accumulation under salinity related with PA metabolism, could be a substrate product relationship. Bouchereau *et al.* (1999) have suggested that, Put catabolism (via DAO) can contribute to proline accumulation under salt stress. It will be interesting to study the relationship between Put catabolism and proline in various plants under stress.

The increased level of protein (Fig. 6) by Put suggests enhanced synthesis of few proteins, evident with high expression (Fig. 6-9). It could be suggested that new proteins (22,23,29,60,66,70 and 80 kDa) induced by Put in salt stressed plant might be involved in stress alleviation through pH regulation, osmo-protection by ionic balance, detoxification of enzymes and hormone homeostasis (Zhu 2001). Further, Put induced 26 kDa peptide in leaf and root tissues of the seedling at both stages under high salinity stress might be acting as a osmotine like in other cases (Hurkman *et al.* 1991). The Put induced other low mass protein 23,29 kDa in leaf and 22,23 kDa in root, could be similar to those in barley roots (Ramgopal 1987, Hurkman *et al.* 1991). However, the 22 kDa protein in mustard root under high salinity, which was further induced by Put could be considered similar to that of leaf tissue of *Raphanus sativus* peptide, suggested to be Kunitz protease inhibitor family protein (Lopez *et al.* 1994). The high expression of 18 kDa peptide in 7th day root and lowered expression in 14th day non-stressed root with Put could be a vegetative storage protein (Corre *et al.* 1996), which was appearing transiently in the mustard (Fig. 7, 8). A similar observation has been noted in *Taraxacum officinale* (Xu *et al.* 2000). The exact nature of Put induced proteins in this plant is to be deduced.

The supplementation of Put, caused differential response on accumulation of PA, proline, Na⁺, K⁺ content

and protein profile of *B.juncea* leaf and root tissues. Exogenous Put relieved the plant from salt stress up to an extent suggests Put pleiotropic effect on plant growth under salinity.

ACKNOWLEDGEMENTS

S.N.M is thankful to U.G.C for funding in the form of project [No.: F 3-114 / 2001 (SRII)].

REFERENCES

- Ali (2000). Role of putrescine in salt tolerance of *Atropa belladonna* plant. *Plant Sci.* **152**: 173-179.
- Aziz, A., Martin-Tanguy, J. and Larher, F. (1997). Plasticity of polyamines metabolism associated with high osmotic stress in rape leaf discs and with ethylene treatment. *Plant Growth Regul.* **21**: 153-163.
- Aziz, A., Martin-Tanguy, J. and Larher, F. (1998). Stress-induced changes in polyamines and tyramine levels can regulate proline accumulation in tomato leaf treated with sodium chloride. *Physiol. Plant.* **104**: 195-202.
- Bajaj, S. and Rajam, M.V. (1996). Polyamine accumulation and near loss of morphogenesis in long term callus cultures of rice. *Plant Physiol.* **112**: 1343-1348.
- Bajaj, S. and Rajam, M.V. (1995). Efficient plant regeneration from long-term callus cultures of rice by spermidine. *Plant Cell Rep.* **14**: 717-720.
- Bates, L.S., Waldern, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil* **39**: 205-207.
- Bay, N.D., Mishra, D.P. and Gupta, R.K. (1992). Mechanism of salt tolerance in rice in relation to sodium, potassium and polyamine content. *Indian J. Agri Biochem.* **5**: 51-55.
- Benavides, M.P., Aizencang, G. and Tomaro, M.L. (1997). Polyamines in *Helianthus annuus* L during germination under salt stress. *J. Plant Growth Regul.* **16**: 205-216.
- Bouchereau, A., Aziz, A., Larher, F. and Martin-Tanguy, J. (1999). Review: Polyamines and environmental changes: recent developments. *Plant Sci.* **140**: 103-125.

SALINITY STRESS MITIGATION BY PUTRESCINE

- Capell, T., Escobar, C., Liu, H., Burtin, D., Lepri, O. and Christou, P. (1998). Over expression of the oat arginin decarboxylase cDNA in transgenic rice (*Oryza sativa* L) effects normal development patterns in *in vitro* and results in putrescine accumulation in transgenic plants. *Theor. Appl. Genet.* **97**: 246-256.
- Chattopadhyay, M.K., Tiwari, B.S., Chattopadhyay, G., Bose, A., Sengupta, D.N. and Ghosh, B. (2002). Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants. *Physiol. Plant.* **116**: 192-199.
- Chinnusamy, V., Jagendorf, A. and Zhu, J.K. (2005). Understanding and improvement salt tolerance in plants. *Crop Sci.* **45**: 437-448.
- Close, T.J. (1996). Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiol. Plant.* **97**: 795-803.
- Corre, N., Bouchart, V., Ourry, A. and Boucaud, J. (1996). Mobilization of nitrogen reserves during regrowth of defoliated *Trifolium repens* L and identification of potential vegetative storage proteins. *J. Exp. Bot.* **47**: 1111-1118.
- Das, S., Bose, A. and Ghosh, B. (1995). Effect of salt stress on polyamine metabolism in *Brassica Campestris*. *Phytochemistry* **39**: 283-285.
- Delauney, A.J. and Verma, D.P.S. (1993). Proline biosynthesis and osmoregulation in plants. *Plant J.* **4**: 215-223.
- Dure, L., Crouch, M., Harada, J., Ho, T.H., Mundy, J., Quatrano, R., Thomas, T. and Sung, S.R. (1989). Common amino acid sequence domains among the LEA proteins of higher plants. *Plant Mol. Biol.* **12**: 475-486.
- EI-Shintinawy, F. (2000). Photosynthesis in two wheat cultivars differing in salt susceptibility. *Photosynthetica* **38**: 615-620.
- Flores, H.E. and Galston, A.W. (1982). Polyamines and plant stress: Activation of putrescine biosynthesis by osmotic shock. *Science* **217**: 1259-1260.
- Flores, H.E. and Galston, A.W. (1984). Osmotic stress-induced polyamines accumulation in cereal leaves. *Plant Physiol.* **75**: 102-113.
- Fukutaku, Y. and Yamada, Y. (1984). Source of nitrogen in water stressed soyabean (*Glycine max*) II fate of ¹⁵N-labelled protein. *Physiol. Plant.* **61**: 622-628.
- Galiba, G., Kocsy, G., Kaur-Sawhney, R., Sutka, J. and Galtson, A.W. (1993). Chromosomal localization of osmotic & salt stress-induced differential alterations in polyamine content in wheat. *Plant Sci.* **92**: 203-211.
- Galston, A.W. and Kaur-Sawhney, R. (1990). Polyamines in plant physiology. *Plant Physiol.* **94**: 406-410.
- Galston, A.W. and Kaur-Sawhney, R. (1995). Polyamines as endogenous growth regulators. In: P.J. Davies (eds.), *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, pp. 158-178. Kluwer Academic Publishers, Dordrecht.
- Galston, A.N., Kaur-Sawhney, R., Altabella, T. and Tiburcio, A.F. (1997). Plants polyamines in reproductive activity & response to abiotic stress. *Bot. Acta.* **110**: 197-207.
- Hare, P.D., Cress, W.A. and Staden van, J. (1999). Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *J. Exp. Bot.* **50**: 413-434.
- Hua, M. and Chong, P.E. (2002). Upregulation of arginine decarboxylase gene expression and accumulation of polyamines in mustard (*Brassica juncea* L) in response to stress. *Physiol. Plant.* **114**: 439-449.
- Hurkman, W.J., Tao, H.P. and Tanaka, C.K. (1991). Germin like polypeptide increase in barley roots during salt stress. *Plant Physiol.* **97**: 366-374.
- Johnson, D.W., Smith, S.E. and Dobrenz, A.K. (1992). Genetic and phenotypic relationships in response to NaCl at different developmental stages in alfalfa. *Theore. Appl. Genet.* **83**: 833-838.
- Kapchina, V. and Foudouli, A. (1991). Effect of growth regulators and polyamines on salinity induced changes of growth and peroxidase activity in *Pisum sativum*. L. *Fiziol. Rast.* **17**: 35-40.
- Krisnamurthy, R. (1991). Amelioration of salt effect and salt tolerance rice (*Oryza sativa* L) by foliar application of putrescine. *Plant Cell Physiol.* **32**: 699-703.

- Kumar, A., Altabella, T., Taylor, N.A. and Tiburcio, A.F. (1997). Recent advances in polyamines. *Trends Plant Sci.* **2**: 124-130.
- Laemmli, U.K. (1970). Cleavage of structural protein during the assembly of head of bacteriophage T4. *Nature* **227**: 680-685.
- Lauchli, A. and Epstein, E. (1990). Plant responses to saline and sodic conditions In: K.K. Tanji (ed.), *Agricultural Salinity Assessment and Management*, pp. 113-137. American Society of Civil Engineering, New York.
- Lefevre, I., Gratia, E. and Lutts, S. (2001). Discrimination between the ionic and osmotic components of salt stress in relation to free polyamine level in rice (*Oryza sativa*). *Plant Sci.* **161**: 943-952.
- Lin, C.C. and Kao, C.H. (1995). Levels of endogenous polyamines and NaCl inhibited growth of rice seedlings. *Plant Growth Regul.* **17**: 15-20.
- Lopez, F., Vansuyt, G., Fourcroy, P. and Delbart, F.C. (1994). Accumulation of a 22-kDa protein and its mRNA in the leaves of *Raphanus sativus* in response to salt stress or water deficit. *Physiol. Plant.* **91**: 605-614.
- Mansour, M.M.F. (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol. Plant.* **43**: 491-500.
- Lowry, J.H., Rosenbrough, N.J., Fair, A.L. and Randall, R.J. (1951). Protein measurement with the foline phenol reagent. *J. Biochem.* **193**: 265-275.
- Matysik, J., Bhalu, B. and Mohnty, P. (2002). Molecular mechanism of quenching of reductive oxygen species by proline under stress in plants. *Curr. Sci.* **82**: 525-532.
- Mishra, S.N., Makkar, K. and Verma, S. (2003). Polyamines in plant growth and development, In: A. Hemantaranjan (eds.), *Advances in Plant Physiology: International Treaties*, pp. 194- 224. Scientific Publisher, India, Jodhpur.
- Mishra, S.N. and Sharma, I. (1994). Putrescine as growth inducers and as a source of nitrogen for mustard seedlings under sodium chloride salinity. *Indian J. Exp. Biol.* **32**: 916-918.
- Munns, R. (2002). Comparative physiology of salt and water stress. *Plant Cell and Environment* **25**: 239-250.
- Ramgopal, S. (1987). Salinity stress induced tissue-specific proteins in barley seedlings. *Plant Physiol.* **84**: 324-331.
- Reggiani, R., Bozo, S. and Bertani, A. (1994). Changes in polyamines metabolism in seedlings of three wheat (*Triticum aestivum* L.) cultivars differing in salt sensitivity. *Plant Sci.* **102**: 21-126.
- Reggiani, R., Zaina, S. and Bertani, A. (1992). Plasmalemma ATPase in rice coleoptiles; stimulation by putrescine and polyamines. *Phytochemistry* **31**: 417-419.
- Shen, B., Jensen, R.G. and Bohnert, H.J. (1997). Mannitol protects against oxidation by hydroxyl radicals. *Plant Physiology* **115**: 527-532.
- Shen, W.Y., Nada, K. and Tachibana, S. (2000). Involvement of polyamines in the chilling tolerance in cucumber cultivars. *Plant Physiol.* **124**: 431-439.
- Shih, C.Y. and Kao, C.H. (1996). Growth inhibition in suspension-cultured rice cells under phosphate deprivation is mediated through putrescine accumulation. *Plant Physiol.* **111**: 721-724.
- Singh, D.B., Verma, S. and Mishra, S.N. (2002). Putrescine effect on nitrate reductase activity, organic nitrogen, protein and growth in heavy metal and salinity stressed mustard seedlings. *Biol. Plant.* **45**: 605-608.
- Soussi, M., Ocana, A. and Lluch, C. (1998). Effect of salt stress on growth, photosynthesis and nitrogen fixation in chickpea (*Cicer arietinum*). *J. Exp. Bot.* **49**: 1329-1337.
- Tachimoto, M., Fukutomi, M., Matsushiro, H., Kobayashi, M. and Takahashi, E. (1992). Role of putrescine in *Lemna* plants under potassium deficiency. *Soil Sci. Plant Nutr.* **38**: 307- 313.
- Tang, W. and Newton, R.J. (2005). Polyamines reduce salt-induced oxidative damage by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation in Virginia pine. *Plant Growth Regul.* **46**: 31-43.
- Trotel, P., Bouchereau, A., Niogret, M.F. and Larher, F. (1996). The fate of osmo-accumulated praline in leaf discs of

SALINITY STRESS MITIGATION BY PUTRESCINE

- rape (*Brassica napus* L) incubated in a medium of low osmolarity. *Plant Sci.* **118**: 31-45.
- Verma, S. and Mishra, S.N. (2005). Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. *J. Plant Physiol.* **162**: 669-677.
- Walden, R., Corderio, A. and Tiburcio, A.F. (1997). Polyamines: small molecules triggering pathways in plant growth & development. *Plant Physiol.* **113**: 709-1013.
- Wi, S.J. and Park, K.Y. (2002). Antisense expression of carnation cDNA encoding ACC synthase or ACC oxidase enhances polyamines content and abiotic stress tolerance in transgenic tobacco plants. *Molecules & Cells* **13**: 209-220.
- Willadino, I., Camara, T., Boget, N., Clarparols, I., Santor, M. and Torne, J.M. (1996). Polyamine, free amino acid variations in NaCl treated embryogenic maize callus for sensitive and resistance cultivars. *J. Plant Physiol.* **149**: 179-185.
- Xu, X.Y., Butler, S.M., Greenwood, J.S. and Bewley, J.D. (2000). Stress mediated effects on the expression of a predominant 18-kDa *Taraxacum officinale* root protein. *Plant Physiol. Biochem.* **38**: 491-497.
- Zhu, J.K. (2001). Plant salt tolerance. *Trends Plant Sci.* **6**: 66-71.