Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2012 Vol. 2 (3) July-September, pp.20-28/Dibyendu Talukdar
Research Article

MODULATION OF PLANT GROWTH AND LEAF BIOCHEMICAL PARAMETERS IN GRASS PRA (*LATHYRUS SATIVUS* L) AND FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L) EXPOSED TO NaCl TREATMENTS

***Dibyendu Talukdar**
Plant Cell and Stress Biology Laboratory, Department of Botany, R.P.M. College, University of Calcutta, Uttarpara, Hooghly 712258, West Bengal, India
**Author for Correspondence*

ABSTRACT
Responses of *Lathyrus sativus* L. and *Trigonella foenum-graecum* L., both belonging to the family Leguminosae, were compared on the basis of seed germination, different agronomic traits, and leaf biochemical parameters under three different doses (50, 100 and 150 mM) of NaCl treatments keeping a control (0 mM added NaCl). A pot experiment was conducted in controlled growth environment, and salt treatment was commenced on 12 d old seedlings in each set by watering the plants with equal daily increments of NaCl-supplemented distilled water over three days till 80 d of plant growth. A Petri plate bioassay was conducted separately for germination test. Compared to control, seed germination reduced significantly ($P < 0.05$) in grass pea at 150 mM, while that of fenugreek it occurred at 100 mM. Likewise, growth traits reduced significantly in grass pea at 150 mM and that in fenugreek declined at 100 mM NaCl, but the effect was more severe on fenugreek plants. The reduction in plant height, number of primary branches and delayed onset of flowering in both crops might be attributed to low seed yield plant^{1} (g) under NaCl treatment. Interestingly, seed neurotoxin, β-N-Oxalyl, L α, β-diaminopropionic acid (β-ODAP), increased over control by about 2-fold in grass pea at 100 mM, although it was remained at control level at 150 mM, showing a reverse relationship with elevated salinity. Among the leaf biochemical parameters, Na^{+} increased while K^{+}, total chlorophyll, carotenoids, ascorbate and proline content reduced in both crops. However, comparing control, the decrease became significant at 150 mM for grass pea and at 100 mM for fenugreek. The results revealed that 150 mM NaCl was inhibitory to germination and seedling growth of grass pea genotype, while 100 mM NaCl was found toxic to fenugreek plants, suggesting higher sensitivity of fenugreek seedlings than grass pea to long-term salt treatments.

Key Words: *Ascorbate, Growth, NaCl, Neurotoxin, Lathyrus Sativus L and Trigonnella*

INTRODUCTION
NaCl-induced soil salinity is one of the major abiotic stresses that reduce crop productivity (Boyer 1982). Over 800 million hectares of land throughout the world are salt-affected, and a significant proportion of agricultural land has become saline because of land clearing or irrigation (FAO 2008). In India, nearly one million hectares of land have major problems with salinity, hence lying either unutilized or under-utilized (Hossain *et al.,* 2006). Majority of our present day crops are adversely affected by salinity stress (Munns 2005; Hossain *et al.,* 2006). Salinity induces oxidative stress through the generation of reactive oxygen species within the plant cells (Talukdar 2011b). To mitigate and repair damages triggered by oxidative stress, plants evolved a series of both enzymatic as well as non-enzymatic antioxidant defense mechanism. Ascorbate and carotenoids are two important non-enzymatic defenses against salinity, whereas proline is the most debated osmo-regulatory substances under stress (Anup and Gupta 2003).
Lathyrus sativus L. (grass pea or commonly known as 'khesari') and *Trigonella foenum-graecum* L (fenugreek or commonly as 'methi') are cultivated as annual, winter legumes in wide geographical

regions of India (Pandey *et al.,* 1996; Talukdar and Talukdar 2012). Both the species can be grown easily in marginal environment, and are promising sources of calories, seed proteins, B-vitamins and minerals. In India, grass pea is grown for both food and forage in several states particularly Chhatishgarh, Madhya Pradesh, Eastern UP, Bihar, Maharastra and West Bengal (Pandey *et al.*, 1996). Grown worldwide as a hardy legume crop showing tolerance to different biotic and abiotic stresses (Vaz Patto *et al.,* 2006; Talukdar 2008, 2009a) grass pea has been used as an excellent material in diverse aspects of cytogenetic, mutation genetics and genomic/proteomic research (Talukdar 2008, 2009b, 2010a-d, 2011d, 2012e; Talukdar and Biswas 2007, 2008). In recent years, a robust stock of diploid mutant lines, showing desirable agronomic traits have been characterized in this crop (Talukdar 2009a, b, 2011b, e; Talukdar and Biswas 2005). Significantly, different biochemical mutants exhibiting deficiency in ascorbate, flavonoids, glutathione and over production of glutathione have been isolated and characterized in grass pea very recently (Talukdar 2012a-d). The crop reportedly shows high antioxidant properties (Pastor-Cavada *et al.,* 2009) and activities of different antioxidant enzymes have been elucidated in NaCl-tolerant lines and in lead-induced stress conditions (Brunet *et al.,* 2008; Talukdar 2011b). Fenugreek, on the other hand, is extensively used as spices and in medicine as a carminative, analgesic, anti-inflammatory, anti-diabetic, anti-hypercholesterol, anti-carcinogenic and as an important source of steroidal substance, diosgenin (Amin *et al.*, 2009). Available reports indicate that both grass pea and fenugreek are moderate salt-tolerant (Campbell 1997; Asaadi 2009). Good adaptability to salinity stress was reported in the Mediterranean germplasm (Berger *et al.,* 1999; Patto *et al.,* 2006), while reduction of growth was reported in Iranian germplasms (Mahdavi and Sanavy 2007). Great variations of salinity tolerance were observed in different Indian genotypes of grass pea, and four tolerance levels were subsequently identified (Talukdar 2011f). For fenugreek, the tolerance was reported up to 10 ds m^{-1}salinity level, and a decrease in fresh and dry mass under salinity stress at early growth stage was fond in other cases (Niknam *et al.,* 2006). NaCl causes extensive oxidative damage in different legumes, resulting in significant reduction of different growth parameters, seed nutritional quality and nodulation (Ahmad *et al.,* 2008; Hernandez 2000). Preliminary study also pointed out high sensitivity of the seed neurotoxin, β-ODAP in grass pea under drought stress (Hanbury *et al.,* 2000). Therefore, the possibility of negative impact of salinity on growth and metabolism of these two legumes cannot be ruled out. The main objective of the present work was, therefore, to investigate the modulation of plant growth traits along with leaf biochemical components in grass pea and fenugreek plants in response to different doses (50, 100 and 150 mM) of NaCl.

MATERIALS AND METHODS

Plant Materials

Grass pea var. IC 24 and fenugreek variety 'Azad' were collected from Pulses and Oilseed Research Stations, Berhampore, Murshidabad and a local farm at Chakdaha block of Nadia district, West Bengal, respectively.

Seed Germination and Imposition of NaCl Treatment

Fresh and healthy seeds were surface sterilized by 10% (v/v) sodium hypochlorite for 3 min and allowed to germinate in the dark at 25 °C in two different sets for grass pea and fenugreek with three replications/ treatment. Seeds were considered germinated considering emergence of both plumule and radicle. (Talukdar 2011f). Percentage of germination was calculated, keeping three replicates / treatment for each plant type. Germinated seedlings were immediately transferred to twelve inches earthen pots containing a mixture of soil, vermiculite and farm yard manure (1:1:1). Seedlings were thinned to one per pot (two pots replication-1) and watered evenly for uniform growth until 7 d after first emergence, as reported earlier (Talukdar 2011g). The pots were kept under control condition (temperature day 25 °C, night 20 °C, humidity of 75%, 250 μmol m^{-2} sec^{-1} and a14h photoperiod). Salt treatment was commenced on 12 d old seedlings in each set by watering the plants with equal daily increments of NaCl-supplemented

distilled water over three days till 80 d. This was applied thrice a week for 2-weeks of continuous treatment. Dry weights of the shoots were obtained after drying the samples at 60 °C for 72 h. Different growth traits were recorded during plant growth, and post harvest analysis of seed yield components along with seed neurotoxin was also performed. Seed neurotoxin content was measured by specific colorimetric method (Rao 1978).

Estimation of Leaf Biochemical Parameters

Fully expanded leaves of control and salt-treated plants were analysed for total Na^+ and K^+ contents following the method of Kumar and Sharma (1989). The oven-dried leaf (0.2 g) was ground to fine powder and transferred to a digestion flask (50 ml) containing acid mixture (3 ml) of concentrated H_2SO_4 and $HClO_4$ in the ratio of 9:1 (v/v). The flask was heated gently over a hot plate for 10 to 12 min until the solution became colorless. The cooled digest was then diluted by adding double distilled water and volume was made up as required. The estimation of Na^+ and K^+ contents in acid extracts was carried out using an atomic absorption spectrophotometer (Perkin Elmer, Analyst-300).

Leaf chlorophyll and carotenoid contents were determined by the method of Lichtenthaler (1987). Leaf tissue (50 mg) was homogenized in 10 ml chilled acetone (80%). The homogenate was centrifuged at 4000 g for 12 min. Absorbance of the supernatant was recorded at 663, 647 and 470 nm for chlorophyll a, chlorophyll b and carotenoids, respectively. The contents were expressed as mg chlorophyll or carotenoids g^{-1} FW.

Ascorbic acid content will be estimated following the methods of Mukherjee and Choudhari (1983). Five hundred milligrams of leaf tissue was homogenized in 10 ml of 6% trichloroacetic acid. Four ml of extract was mixed with 2 ml of 2% dinitrophenylhydrazine in acidic medium followed by the addition of 1 drop of 10% thiourea in 70% ethanol. The mixture was boiled for 15 min in water bath and after cooling to room temperature, 5 ml of 80% v/v H_2SO_4 was added in an ice bath (0 °C). The absorbance will be recorded at 530 nm. Concentration of ascorbate will be determined from a standard curve plotted with known concentration of ascorbic acid.

Leaf proline content was estimated according to the method of Bates *et al.*, (1973) from fully expanded leaf samples collected from first formed primary branches.

Statistical Methods

The results are presented as mean ± standard errors (s.e.m) of at least three replicates (n=10). Multiple comparisons among means were performed by ANOVA using SPSS 10 software (SPSS Inc. USA) and separated by DMRT test at significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Effects of Salinity on Seed Germination, Plant Growth and Seed Yield

Seed germination decreased as the doses increased. Germination of grass pea seeds declined below 50% compared to control at 150 mM NaCl, while that of fenugreek occurred at 150 mM (Table 1), indicating different toxicity levels of NaCl on seed germination two crops and higher sensitivity of fenugreek seeds to NaCl treatment. Seed germination of both of these crops are highly sensitive to abiotic stress, as reported earlier in 8 Indian genotypes of grass pea exposed to 50-200.mM NaCl (Talukdar 2011f) and both grass pea and fenugreek genotypes to arsenic treatments (Talukdar 2011c). But the two crops greatly differed in responses, as also confirmed in the present study.

Among the growth parameters, plant height and number of primary branches plant-1 reduced significantly in grass pea at 150 mM and in fenugreek at 100 mM (Table 1). In later case, plant became extremely stunted after 28 d treatment and then died. Reduction in these two prominent vegetative traits along with delayed onset of flowering (20 d in grass pea and 37 d for fenugreek comparing control) might be responsible for significant decrease in pods plant-1, seeds pods-1 and seed yield plant $^{-1}$ (g) in both crops, but the effect was more drastic in fenugreek plants exposed to 100 mM NaCl. Shoot dry weight also decreased markedly, and the reduction was 2-fold for grass pea at 150 mM but about 3.2-fold for

fenugreek at 100 mM NaCl (Table 1). Dry weight of plants has been considered as one of the realistic criteria in determining salt responses in plants (Munns 2005). In grass pea, an increase in height, primary branches and leaf number in association with higher shoot dry weight was reported in NaCl-tolerant mutant lines, while reverse trend was noticed in NaCl-sensitive lines (Talukdar 2011b). Similar observation was reported in *Clitorea ternatea*, a medicinal legume (Talukdar 2011b) and in dwarf mutant population of grass pea (Talukdar *et al.*, 2011). Reduction in plant dry weight under salt stress was also reported in grass pea genotypes grown in Iran (Mahdavi and Sanavy 2007) and in India (Talukdar 2011f) and *Phaseolus* (Bayuelo-Jime´nez *et al.*, 2002).Therefore, high tolerance was found associated with high total plant dry weight, which was increased by positive contribution from other components, least affected by salt induced injury in tolerant lines. In the present case, growth traits and shoot dry weights in both crops were marginally varied at 50 mM, but differential responses of two crops suggested higher tolerance of grass pea to salinity than fenugreek plants under the present experimental condition.

Modulation of Leaf Biochemical Parameters

Compared to control, leaf Na^+ content exhibited a steep hike in grass pea at 150 mM and in fenugreek at 100 mM NaCl. Concomitantly, leaf K^+ content decreased significantly in both crops but in different magnitudes (Table 1). Measurable Na^+ content enhanced by about 1.5-2-fold in grass pea but increased by nearly 3-5-fold in fenugreek plants across the treatment (Table 1). The decrease in K^+ content with concomitant increase in Na^+ level was primarily responsible for declining ratio of K^+/Na^+ in both crops under elevated salt treatment, and the effect was more severe on fenugreek plants than that on grass pea. Higher K^+/Na^+ ratio was attributed to salinity tolerance in grass pea and *Clitorea* mutants (Talukdar 2011b). Excessive accumulation of Na^+ and Cl^- in the leaves has been considered highly harmful for normal metabolism of plants and tolerant genotypes has the capacity of successful salt exclusion (Yeo and Flowers 1986; Munns 2005). The K^+: Na^+ ratio has been used as a discriminating factor between tolerant and sensitive genotypes with greater capacity of former to block or reduce the uptake or exclude the excess amount of Na^+ and associated increase in K^+ content (Munns 2005). In the present material, high accumulation of Na^+ and decrease in K^+ content has led to lowest K^+/Na^+ ratio, rendering both crops highly susceptible to salt treatment. Although decreased, the K^+/Na^+ ratio was close to normal level in grass pea at 100 mM, presumably, providing better protection to plants as evidenced by normal plant growth in this concentration.

Salinity has significant effect on the total chlorophyll, carotenoids and ascorbate content of both crops. All the three traits reduced significantly in grass pea at 150 mM NaCl but in fenugreek plants at 100 mM (Table 1). Similarly, carotenoids, ascorbate and proline content decreased in both crops but the magnitude of reduction was far greater in fenugreek than that in grass pea under 100 mM NaCl. In grass pea, reduction of chlorophyll contents was reported in NaCl-sensitive mutant lines (Talukdar 2011b), which was attributed to the increased activity of chlorophyllase enzyme or due to the disruption of ultrastructure of pigment protein complex by ion toxicity (Saha *et al.*, 2010; Talukdar 2012f).

In plants, carotenoids and ascorbate play a primary role as a non-enzymatic antioxidant defense in combating stress by quenching reactive oxygen species. Reduced levels of these two components in leaves of present NaCl-treated grass pea and fenugreek seedlings indicated their decreased free radical scavenging capacity. A positive correlation between ascorbate contents and anti-oxidant defense enzymes has recently been established in an ascorbate-deficient semi-dwarf mutant line of grass pea (Talukdar 2012b), in *Arabidopsis* (Huang 2005), and also, in other plants (Hernández *et al.*, 2000).

Among the parameters responding to salt treatment, rapid reduction of free proline content is one of the significant events in both crops. Compared with control, endogenous free proline content decreased significantly in grass pea plants at 150 mM NaCl (Table 1), while it was largely unaffected in lower concentrations (Table 1). In fenugreek, free proline started to decrease even from 50 mM NaCl, and the trend became significant in relation to control at 100 mM (Table 1). Proline is an important osmo-regulatory substance, but the exact role of proline in abiotic stress tolerance is being debated (Anoop and

Gupta 2003). Increase in proline content under NaCl stress was reported in *Pisum sativum* (Ahmad *et al.*, 2008) and *Phaseolus aureus* (Misra and Gupta 2005) as a symptom of tolerance. In contrast, proline accumulation was regarded by some workers as a symptom of stress in less-salinity-tolerance species, and its contribution to osmotic adjustment was found negligible as compared with K^+ (Wang *et al.*, 2004). In grass pea, increased proline level might confer protection to mutant lines from salinity-induced growth inhibition, as the marks of tolerance (Talukdar 2011b). In the present study, lower level of proline was, presumably, associated with higher sensitivity to salinity.

Table 1: Seed germination (%), growth traits and leaf biochemical parameters of grass pea (*L. sativus* cv. IC 24) and fenugreek (*T. foenum-graecum* L.) under control (0 mM NaCl added) and three different concentrations (50, 100 and 150 mM) of NaCl. Data are ± s.e.m of three independent experiments (*n*=10). Different lowercase letters after s.e.m indicate significant differences at *P* < 0.05 by DMRT test. DW-dry weight, FW-fresh weight, Nm-could not measure as plants died.

Traits	*L. sativus* L.				*T. foenum-graecum* L.			
	0	50	100	150	0	50	100	150
Seed Germination	99±0.9a	95±1.1a	89±0.8a	44±1.1b	90±0.8a	88±1.1a	45±0.7b	20±0.9c
Plant Height (cm)	51.21±2.6a	52.33±2.2a	49.73±3.1a	27.88±4.0b	35.65±2.8a	35.0±4.1a	11.9±0.8b	3.9±0.1b
Primary Branches Plant^{-1}	16.98±2.6a	17.11±3.3a	15.87±1.6a	7.89±0.9b	12.0±0.8a	11.8±0.8b	3.57±0.7c	1.6±1.2d
Days to Flowering	26.8±6.9c	28.0±7.8c	45.8±10.5a	36.6±8.1b	20.8±5.5b	21.5±6.1b	58.1±11.6c	Nm
Pods Plant^{-1}	88.6±4.9a	80.9±8.7a	77.67±7.7b	59.22±6.0c	13.45±6.9a	13.26±8.5a	8.67 ±7.8b	Nm
Seeds Pod^{-1}	5.5±0.9a	5.3±1.2a	4.9±1.3a	3.1±0.8b	15.80±0.9a	15.65±0.4a	6.77 ±0.5b	Nm
Seed Yield Plant^{-1} (g)	16.7±6.8a	17.23±8.0a	14.8±2.6a	7.67±4.2b	36.80 ±10a	35.90±9.2a	12.70±9.7b	Nm
Seed Protein %	24.87±0.1a	25.11±0.1a	17.65±0.2b	11.89±0.1b	18.70± 0.8a	18.90±0.9a	10.61±1.5b	Nm
Seed ODAP %	0.19±1.5b	0.21±2.3b	0.33±2.2a	0.12±1.0c	Nm	Nm	Nm	Nm
Shoot DW (mg Plant^{-1})	105.8±5.5a	98.8±4.0a	96.9±5.0a	54.4±3.6b	56.8±6.1a	61.7±6.5a	18.2±5.5b	1.6±1.2c
Na^+ Content (mg g^{-1} FW)	1.83±0.9c	1.91±1.2c	2.73±0.8b	3.65±0.7a	1.90±0.8c	2.05±1.2c	5.7±0.8b	9.1±1.5a
K^+ Content (mg g^{-1} FW)	4.15±1.5a	4.01±1.2a	3.95±1.5a	2.76±1.3b	3.87±1.6a	3.71±1.7a	2.03±0.9b	0.98±0.9c
K^+/Na^+	2.22±3.6a	2.10±3.5a	1.45±2.6b	0.79±1.5c	2.06±2.0a	1.82±0.9b	0.38±0.9c	0.11±0.7d
Total Chl (mg g^{-1} FW)	4.88±1.1a	4.91±0.5a	4.80±0.9a	3.11±0.8b	3.59±0.8a	3.61±0.9a	1.89±0.7b	1.17±0.6c
Total Carotenoids (mg g^{-1} FW)	1.75±0.2a	1.80±0.7a	1.68±1.4b	0.95±0.8c	2.12±0.9a	1.98±1.1a	0.67±1.3b	0.38±0.7c
Total Ascorbate (µmol g^{-1} FW)	1.92±3.5a	1.85±4.1a	1.88±3.3a	0.98±4.1b	1.87±1.6a	1.79±2.0a	1.04±1.7b	0.67±0.6c
Proline (µg g^{-1} FW)	3.16±6.1a	3.09±7.5a	3.20±7.0a	2.37±5.5b	1.03±5.6a	1.10±6.0a	0.67±0.5b	0.27±0.8c

Changes in Seed Protein and Neurotoxin Content under NaCl Treatments

Total soluble seed protein content in both crops varied marginally at 50 mM NaCl. However, protein content decreased in both grass pea and fenugreek plants from 100 mM, and this time the effect was more severe on grass pea rather than on fenugreek. Seed neurotoxin, β-ODAP, in grass pea increased by about 1.5-fold at 100 mM, but decreased below control level at 150 mM NaCl (Table 1). Declining/variations in seed protein content suggested negative effect of salinity on nutritional quality of legume edible parts, although information in this regard is extremely limited in other legumes (Niknam *et al.,* 2006).

Efforts are being initiated in the present study to widen the base information regarding effect of environmental stress on stability of seed neurotoxin (β-ODAP) in grass pea genotypes. Since ODAP is an amino acid it may have a role in the stress tolerance that has made the grass pea such a useful species over the long period of its domestication (Lambein *et al.,* 2000).Variations in seed ODAP content were also reported in Australian and Chinese accessions of grass pea under water stress (Cocks *et al.,* 2000; Gengsheng *et al.,* 2001). Remarkably, seed ODAP level in the present grass pea genotype behaved in three different ways upon imposition of salt treatment; it was largely unchanged at 50 mM, increased at 100 mM, and then decreased below control level at 150 mM. As the NaCl-induced growth inhibition in grass pea was observed only at 150 mM, it seems likely that ODAP conferred some degree of protection at 100 mM NaCl, the potential of which decreased with declining level of ODAP accumulation. The result is in agreement with an earlier report on three dwarf mutants of grass pea which differed from each other in seed ODAP level under un-stressed condition but exhibited significant variations in response to salinity towards tolerance (Talukdar 2011a). Furthermore, as ODAP biosynthetic, although not fully known, pathway share cysteine metabolism and cysteine is an important component of glutathione, a thiol-antioxidant compound, it is noteworthy that β-ODAP in grass pea may play significant role in thiol-metabolism for remarkable hardiness of grass pea in diverse climatic conditions (Talukdar 2012b,c).

ACKNOWLEDGEMENTS

Financial assistance in the form of MRP (Grant no-PSW-047/11-12 ERO) from University Grants Commission, New Delhi is gratefully acknowledged.

REFERENCES

Ahmad P, John R, Sarwat M and Umar S (2008). Responses of proline, lipid peroxidation and antioxidative enzymes in two varieties of *Pisum sativum* L. under salt stress. *International Journal of Plant Production* **2** 353-366.

Amin A, Alkaabi A, Al-Falasi S and Daoud SA (2009). Chemopreventive activities of *Trigonella foenum-graecum* (Fenugreek) against breast cancer. http:// www. pubmedcentral. nih. gov/.

Anoop N and Gupta AK (2003). Transgenic indica rice cv IR-50 overexpressing *Vigna aconitifolia* delta(1)-pyrroline-5-carboxylate synthetase cDNA shows tolerance to high salt. *Journal of Plant Biochemistry and Biotechnology* **12** 109–116.

Asaadi AM (2009). Investigation of Salinity Stress on Seed Germination of *Trigonella foenum-graecum*. *Research Journal of Biological Science* **4** 1152-1155.

Bates LS, Waldren RP and Teare ID (1973). Rapid determination of free proline for water stress studies. *Plant and Soil* **39** 205-207.

Bayuelo JJS, Debouck DG and Lynch JP (2002). Salinity tolerance in *Phaseolus* Species during early vegetative growth. *Crop Science* **42** 2184-2192.

Berger JD, Siddique KHM and Loss SP (1999). Cool season grain legumes for Mediterranean environmrnts: The effect of environment on non-protein amino acids in *Vicia* and *Lathyrus* species. *Australian Journal of Agricultural Research* **50** 403-412.

Boyer JS (1982). Plant productivity and environment. *Science* **218** 443–448.

Brunet J, Repellin A, Varrault G, Terryn N and Zuily-Fodil Y (2008). Lead accumulation in the roots of grass pea (*Lathyrus sativus* L.): a novel plant for phytoremediation systems? *Comptes Rendus Biologies* **331**(11) 859-864.

Campbell CG (1997). Grass pea. *Lathyrus sativus* L. Promoting the conservation and use of underutilized and neglected crops. *Institute of Plant Genetics and Crop Plant Research. Gatersleben/ International Plant Genetic Resources Institute (Rome, Italy)* **18**.

Cocks P, Siddique K and Hanbury C (2000). Impact of stress on neurotoxins. In: *Lathyrus: A new Grain Legume*, edited by P. Cocks *et al.,* (RIRDC, Australia) 4-15.

FAO (2008). *Quarterly Bulletin of Statistics*. *FAO*, Rome, Italy.

Gengsheng X, Kairong C, Ji L, Yafu W and Zhixio Li (2001). Water Stress and accumulation of β-N-Oxalyl-l-α,β-diaminopropionic acid in grass pea (*Lathyrus sativus*). *Journal of Agricultural and Food Chemistry* **49** 216–220.

Hanbury CD, White CL, Mullan BP and Siddique KHM (2000). A review of the potential of *Lathyrus sativus* L. and *L. cicera* L. grain for use as animal feed. *Animal Feed Science and Technology* **87** 1-27.

Hernández JA, Jiménez A, Mullineaux P and Sevilla F (2000). Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. *Plant Cell Environment* **23** 853-862.

Hossain Z, Mandal AKA, Datta SK and Biswas A K (2006). Isolation of a NaCl-tolerant mutant of *Chrysanthemum morifolium* by gamma radiation: in vitro mutagenesis and selection by salt stress. *Functional Plant Biology* **33** 91-101.

Huang C, He W, Guo J, Chang Z, Su P and Zhang L (2005). Increased sensitivity to salt stress in an ascorbate-deficient Arabidopsis mutant. *Journal of Experimental Botany* **56**(422) 3041-3049.

Kumar V and Sharma DR (1989). Isolation and characterization of sodium chloride-resistant callus culture of *Vigna radiata* (L.)Wilczek var. *radiata*. *Journal of Experimental Botany* **210** 143-147.

Lichtenthaler HK (1987). Chlorophyll and carotenoids:pigments of photosynthetic biomembranes. *Methods in Enzymology* **148** 350-382.

Mahdavi B and Sanavy S A M M (2007). Germination and seedling growth in grass pea cultivars under salinity conditions. *Pakistan Journal of Biological Sciences* **10** 273-279.

Misra N and Gupta AK (2005). Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. *Plant Science* **169** 331-339.

Mukherjee SP and Choudhary MA (1983). Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiologia Plantarum* **58** 166-170.

Munns R (2005). Genes and salt tolerance: bringing them together. *The New Phytologist* **167** 645-663.

Niknam V, Razavi N, Ebrahimzadeh H and Sharifizadeh B (2006). Effect of NaCl on biomass, protein and proline contents, and antioxidant enzymes in seedlings and calli of two *Trigonella* species. *Biologia Plantarum* **50** 591-596.

Patto M, Skiba B, Pang E, Ochatt S, Lambein F and Rubiales D (2006). *Lathyrus* improvement for resistance against biotic and abiotic stresses: From classical breeding to marker assisted selection. *Euphytica* **147** 133-147.

Pandey RL, Chitale MW, Sharma RN and Rastogi N (1996). Status of *Lathyrus* research in India. In: *Lathyrus Genetic Resources in Asia: Proc. Regional Workshop*, edited by R. K. Arora *et al.,* 27-29 Dec, 1995, (IGAI, Raipur, India) 45-53.

Pastor-Cavada E, Juan R, Pastor JE, Girón-Calle J, Alaiz M and Vioque J (2009). Antioxidant activity in *Lathyrus* species. *Grain Legume* **54** 10-11.

Rao SLN (1978). A sensitive and specific colorimetric method for determination of α, β-diaminopropionic acid and the *Lathyrus sativus* neurotoxin. *Analytical Biochemistry* **86** 386-395.

Saha P, Chatterjee P and Biswas AK (2010). NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mung bean (*Vigna radiata* L. Wilczek). *Indian Journal of Experimental Biology* **48** 593-600.

Talukdar D (2008). Cytogenetic characterization of seven different primary tetrasomics in grass pea (*Lathyrus sativus* L.). *Caryologia* **61**(4) 402-410.

Talukdar D (2009a). Dwarf mutations in grass pea (*Lathyrus sativus* L.): Origin, morphology, inheritance and linkage studies. *Journal of Genetics* **88**(2) 165-175.

Talukdar D (2009b). Recent progress on genetic analysis of novel mutants and aneuploid research in grass pea (*Lathyrus sativus* L). *African Journal of Agricultural Research* **4**(13) 1549-1559.

Talukdar D (2010a). Reciprocal translocations in grass pea (*Lathyrus sativus* L.). Pattern of transmission, detection of multiple interchanges and their independence. *Journal of Heredity* **101**(2) 169-176.

Talukdar D (2010b). Cytogenetic characterization of induced autotetraploids in grass pea (*Lathyrus sativus* L.). *Caryologia* **63**(1) 62-72.

Talukdar D (2010c). Allozyme variations in leaf esterase and root peroxidase isozymes and linkage with dwarfing genes in induced dwarf mutants of grass pea (*Lathyrus sativus* L.). *International Journal of Genetics and Molecular Biology* **2**(6) 112-120.

Talukdar D (2010d). Fluorescent-banded karyotype analysis and identification of chromosomes in three improved Indian varieties of grass pea (*Lathyrus sativus* L.) *Chromosome Science* **13**(1) 3-10.

Talukdar D (2011a). Flower and pod production, abortion, leaf injury, yield and seed neurotoxin levels in stable dwarf mutant lines of grass pea (*Lathyrus sativus* L.) differing in salt stress responses. *International Journal of Current Research* **2**(1) 46-54.

Talukdar D (2011b). Isolation and characterization of NaCl-tolerant mutations in two important legumes, *Clitoria ternatea* L. and *Lathyrus sativus* L.: Induced mutagenesis and selection by salt stress. *Journal of Medicinal Plants Research* **5**(16) 3619-3628.

Talukdar D (2011c). Effect of arsenic-induced toxicity on morphological traits of *Trigonella foenum - graecum* L. and *Lathyrus sativus* L. during germination and early seedling growth. *Current Research Journal of Biological Sciences* **3**(2) 116-123.

Talukdar D (2011d). Cytogenetic analysis of a novel yellow flower mutant carrying a reciprocal translocation in grass pea (*Lathyrus sativus* L.). *Journal of Biological Research-Thessaloniki* **15** 123 – 134.

Talukdar D (2011e). Bold-seeded and seed coat colour mutations in grass pea (*Lathyrus sativus* L.): Origin, morphology, genetic control and linkage analysis. *International Journal of Current Research* **3**(4) 104-112.

Talukdar D (2011f). Morpho-physiological responses of grass pea (*Lathyrus sativus* L.) genotypes to salt stress at germination and seedling stages. *Legume Research* **34**(4) 232-241.

Talukdar D (2011g). The aneuploid switch: Extra-chromosomal effect on antioxidant defense through trisomic shift in *Lathyrus sativus* L**.** *Indian Journal of Fundamental and Applied Life Sciences* **1**(4) 263-273.

Talukdar D (2012a). Flavonoid-deficient mutants in grass pea (*Lathyrus sativus* L.): Genetic control, linkage relationships, and mapping with aconitase and *S* nitrosoglutathione reductase isozyme loci. *The Scientific World Journal*. Article ID 345983, 11.

Talukdar D (2012b). Ascorbate deficient semi-dwarf *asfL1* mutant of *Lathyrus sativus* exhibits alterations in antioxidant defense. *Biologia Plantarum* **56**(4) 675-682.

Talukdar D (2012c). A glutathione-overproducing mutant in grass pea (*LATHYRUS SATIVUS* L.): alterations in glutathione content, modifications in antioxidant defense response to cadmium stress and genetic analysis using primary trisomics. *International Journal of Recent Scientific Research* **3**(4) 234-243.

Talukdar D (2012d). An induced glutathione-deficient mutant in grass pea (*Lathyrus sativus* L.): Modifications in plant morphology, alteration in antioxidant activities and increased sensitivity to cadmium. *Bioremediation, Biodiversity and Bioavailability* **6**(1) 75-86.

Talukdar D (2012e). Meiotic consequences of selfing in grass pea (*Lathyrus sativus* L.) autotetraploids in the advanced generations: Cytogenetics of chromosomal rearrangement and detection of aneuploids. *NUCLEUS* **55**(2) 73-82.

Talukdar D (2012f). Arsenic-induced oxidative stress in the common bean legume, *Phaseolus vulgaris* L. seedlings and its amelioration by exogenous nitric oxide. *Physiology and Molecular Biology of Plants* DOI: 10.1007/s12298-012-0140-8.

Talukdar D and Biswas AK (2005). Induced seed coat colour mutations and their inheritance in grass pea (*Lathyrus sativus* L.). *Indian Journal of Genetics and Plant Breeding* **65**(2) 135-136.

Talukdar D and Biswas AK (2007). Seven different primary trisomics in grass pea (*Lathyrus sativus* L.). I Cytogenetic characterization. *Cytologia* **72**(4) 385-396.

Talukdar D and Biswas AK (2008). Seven different primary trisomics in grass pea (*Lathyrus sativus* L.). II. Pattern of transmission. *Cytologia* **73**(2) 129-136.

Talukdar D and Talukdar T (2012). Floral diversity and its indigenous use in old basin (Khari) of river Atreyee at Balurghat block of Dakshin Dinajpur district, West Bengal. *NeBIO* **3**(2) 26-32.

Wang S, Wan C, Wang Y, Chen H, Zhou Z and Fu H (2004). The characteristics of $Na^{+,}$ K^{+} and free proline distribution in several drought resistant plants of the Alxa desert. *China Journal of Arid Environment* **56** 525-539.

Yeo AR and Flowers TJ (1986). Salinity resistance in rice (Oryza sativa L.) and a pyramiding approach to breeding varieties for saline soils. *Australian Journal of Plant Physiology* **13** 161-173.