RESEARCH ARTICLE Legume Research- An International Journal, Volume 45 Issue 1: 73-81 (January 2022)

Potential of Seed Halopriming in Mitigating NaCl-induced Adversities on Nitrogen Metabolism in Legume Crops

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ABSTRACT

Background: Salinity is a major threat that impairs legume growth and development worldwide. Therefore, present study was aimed to determine the potential of seed halopriming in relieving NaCl-induced disturbances on nitrogen metabolism of seedlings of six legume crops *viz*., *Lens culinaris*, *Cajanus cajan*, *Cicer arietinum*, *Lathyrus sativus*, *Vigna radiata* and *Vigna mungo* that were detected to have differential sensitivity to NaCl.

Methods: Nonprimed and haloprimed seeds were grown hydroponically under varying NaCl doses for three weeks. Harvested samples were utilised to characterize the toxic effects of NaCl on nitrogen metabolism of nonprimed and haloprimed seedlings.

Result: Nonprimed seedlings exhibited reduced nitrate uptake by virtue of which other assimilatory processes of nitrogen fixation were adversely affected. Haloprimed seedlings experienced lesser toxicity under NaCl stress due to elevated activities of nitrate assimilatory enzymes on account of improved nitrate uptake from solution. Lesser ammonium accumulation and lower glutamate dehydrogenase activity implied lesser cytotoxicity in primed seedlings. Based on the trends obtained from tested parameters, nitrogen metabolism was maximally affected in *Lens* and *Cajanus* followed by *Cicer* and *Lathyrus*. *Vigna radiata* and *Vigna mungo* were least affected and therefore may be suggested for cultivation in saline prone agricultural fields after seed halopriming.

Key words: Halopriming, NaCl stress, Nitrogen metabolism, Legume.

INTRODUCTION

Salinity depresses nutrient uptake causing nutritional deficiency and productivity loss of plants (Ashraf *et al*. 2017). Globally, 45 million hectares of irrigated lands (19.5%) are salt affected (Thu *et al*. 2016) and are unproductive for agriculture. Therefore, to utilize portions of these lands, agriculturists aim at developing salt tolerant crops to grow them in non-arable regions to escalate crop production.

Nitrogen is an integral constituent of proteins, cell construct materials and is crucial for plant growth (Arghavani *et al*. 2017). Nitrogen metabolism is highly affected under salinity and its regulation is crucial for glycophytes to thrive under salinity (Ashraf *et al*. 2018; Teh *et al*. 2016).

Legumes are cheapest source of protein and used in crop rotation to restore soil nitrogen naturally. Being glycophytes, legumes are highly affected under salinity as nitrogen uptake competes with Na^+ and Cl^- entry (Abdelgadir *et al*. 2005).

Present study was aimed primarily to assess the deleterious effects of different NaCl concentrations on the intermediates of nitrogen metabolism in six legume cultivars. Based on tested parameters, partial identification of salt sensitive/tolerant nature of legume cultivars could be deciphered preliminarily. The most tolerant variety could be suggested for cultivation in saline prone agricultural lands. Additionally, all legume cultivars were subjected to halopriming to study whether any improvement occurred in their nitrogen assimilatory ability as compared to those of the nonprimed seedlings under salinity.

MATERIALS AND METHODS

Seeds of *Lens culinaris* Medik. var. Ranjan, *Cajanus cajan* L. var. Rabi, *Vigna mungo* L. var. Sulata, *Cicer arietinum* L. var. Anuradha, *Lathyrus sativus* L. var. Nirmal and *Vigna radiata* L. var. Samrat were collected from Pulse and Oilseed Research Institute, Behrampur, West Bengal. The chosen legume cultivars have not been previously characterized on the basis of physiochemical properties as tolerant/sensitive under salt stress.

Surface sterilized seeds were divided into two fractions. First fractions were lined on glass plates containing blotting papers, inserted into packets containing suitable concentrations of hydroponic solution substituted with 50 mM, 100 mM and 150 mM NaCl (nonprimed sets). Other fractions of seeds were immersed in 50 mM NaCl for 2 hours for halopriming. Thereafter, haloprimed seeds were allowed to germinate in same way as that of nonprimed sets (Biswas *et al*. 2018). Setups were exposed to 16 hours photoperiod, 200 µmol $m^{-2}s^{-1}$ photon irradiance, 27-30°C. After 21 days, samples were harvested to characterize the toxic effects of NaCl on nitrogen metabolism of nonprimed and haloprimed seedlings.

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How to cite this article: Biswas, S., Paul, A. and Biswas, A.K. (2022). Potential of Seed Halopriming in Mitigating NaCl-induced Adversities on Nitrogen Metabolism in Legume Crops. Legume Research. 45(1): 73-81. DOI: 10.18805/LR-4276.

Submitted: 05-11-2019 **Accepted:** 25-02-2020 **Online:** 28-07-2020

Total and soluble nitrogen contents were estimated according to Vogel (1961). Nitrate and nitrite contents were determined according to Zhong *et al*. (2017) and Werber and Mevarech (1978) respectively. Nitrate reductase activity (NR) and nitrite reductase activity (NiR) were done according to Zaghdoud *et al*. (2013) and Ghosh *et al*. (2013) respectively. Dissolved ammonia contents were estimated according to Hoshida *et al*. (2000). Glutamate synthase (GOGAT) activity and Glutamine synthetase (GS) activity was measured according to Chen and Cullimore (1988) and Zhagdoud *et al*. (2013) respectively. Glutamate dehydrogenase (GDH) activity was assayed according to Magalhaes and Huber (1991).

Experiments were performed in triplicates; significant differences among mean values were compared by one-way ANOVA in Sigma Plot 12.0 software. The p-values were considered to be statistically significant at P<0.05.

RESULTS AND DISCUSSION

NaCl exposure decreased total nitrogen contents by 37%, 22%, 20%, 19%, 13% and 4% in root and 14%, 14%, 12%, 8%, 12% and 4% in shoot of *Lens culinaris*, *Cajanus cajan*, *Cicer arietinum*, *Lathyrus sativus*, *Vigna radiata* and *Vigna mungo* respectively. However, in haloprimed root, the decline narrowed down to 12%, 2%, 10%, 7% and 3% in primed root and by 7%, 7%, 5%, 6% and 3% in primed shoot of *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. In haloprimed *Lens*, the said content increased by 1% in root and by 5% in shoot (Table 1). Soluble nitrogen contents decreased by 39%, 22%, 18%, 10%, 5% and 5% in nonprimedroot and by 30%, 18%, 11%, 8%, 4% and 4% in nonprimed shoot of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. Haloprimed seedlings recorded lower reduction in soluble nitrogen contents by 12%, 8%, 2%, 2% and 1% in root of *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. In primed root of *Lens*, the content increased to 6%. In primed shoots, the said inhibition reduced to 3%, 11%, 5%, 5%, 2% and 1% in shoot of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively (Table 2). Nitrate contents decreased by 48%, 43%, 34%,33%, 26% and 26% in nonprimed roots and by 35%, 34%, 30%, 29%, 24% and 25% respectively in nonprimed shoot of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. Depreciation in said contents declined to 30%, 15%, 10%, 11%, 16% and 9% in haloprimed roots and to 14%, 14%, 10%, 10%, 9% and 8% in haloprimed shoots of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively (Table 3). NR activity decreased by 53%, 52%, 31%, 30%, 16% and 3% in root and by 30%, 24%, 25%, 23%, 12% and 2% in shoot of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively under NaCl treatment. Decrement in NR activity was reduced to 22%, 29%, 16%, 15%, 11% and 2% respectively in primed roots of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. In primed shoot of *Lens*, *Cicer*, *Lathyrus* and *Vigna radiata*, the reduction in NR activity narrowed to 6%, 10%, 6% and 7% respectively where as in

Table 1: Effect of NaCl on total nitrogen contents in 21 days old nonprimed and haloprimed legume seedlings.

Treatments	*Lens culinaris* Medik.		*Cajanus cajan* L.		*Cicer arietinum* L.		*Lathyrus sativus* L.		*Vigna radiata* L.		*Vigna mungo* L.	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Nonprimed												
Control	4.69±0.081	5.97±0.010	9.82±0.083	6.96±0.023	6.78± 0.017	7.04±0.091	8.50± 0.017	5.64±0.014	8.04±0.007	7.93±0.008	8.11±0.005	7.55±0.012
50 mM NaCl	3.65±0.012	5.74±0.075	8.78±0.029	6.72±0.015	6.06±0.025*	6.58±0.049	7.82±0.025	5.48±0.050	7.81±0.016	7.30±0.009*	8.03±0.009	7.37±0.002
100 mM NaCl	3.03±0.039	5.19±0.007*	7.62±0.052	5.59±0.006	5.46±0.063*	6.26±0.059*	6.91± 0.063*	5.11±0.003	7.29±0.074*	7.17±0.027*	7.82±0.005*	7.27±0.005
150 mM NaCl	2.17±0.029*	4.52±0.021*	6.51±0.057*	5.56±0.007*	4.81±0.035*	5.73±0.090*	6.02± 0.035*	4.94±0.002	5.75±0.030*	6.67±0.014*	7.47±0.018*	7.08±0.002*
Haloprimed 50 mM NaCl												
Control	5.98±0.512*	6.99±0.084*	9.55±0.137	7.16±0.026*	7.43±0.017*	7.40±0.010*	8.69±0.013*	5.70±0.007	8.37±0.005*	7.97±0.001*	8.13±0.001*	7.57±0.012
50 mM NaCl	4.96±0.045	6.79±0.042*	9.50±0.020	6.68±0.015	7.11±0.025*	6.86±0.031*	8.15±0.035	5.61±0.019	7.93±0.006	7.36±0.016*	8.07±0.002	7.41±0.003
100 mM NaCl	4.50±0.095	6.62±0.066*	8.15±0.019	6.30±0.008	6.19±0.063*	6.28±0.089*	7.25±0.067	5.17±0.017	7.32±0.013	7.09±0.005*	7.85±0.005*	7.31±0.037
150 mM NaCl	3.54±0.065	4.72±0.116*	7.50±0.016*	5.67±0.012	6.00±0.035*	5.79±0.037*	6.45±0.019*	4.95±0.003	6.47±0.002*	7.32±0.004*	7.47±0.009*	7.10±0.003*

Values are mean ± SE with three replicates. * indicates statistically significant at p≤0.05 compared to nonprimed control. Values are expressed as milligram nitrogen per gram dry tissue.

Table 2: Effect of NaCl on soluble nitrogen contents in 21 days old nonprimed and haloprimed legume seedlings.

Treatments	*Lens culinaris* Medik.		*Cajanus cajan* L.		*Cicer arietinum* L.		*Lathyrus sativus* L.		*Vigna radiata* L.		*Vigna mungo* L.	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Nonprimed												
Control	3.83±0.120	5.13±0.011	4.81±0.021	4.51±0.003	6.09±0.015	6.28±0.018	4.54±0.004	4.50±0.004	4.88±0.002	4.72±0.027	4.43±0.003	4.19±0.004
50 mM NaCl	2.81±0.017	4.58±0.044	4.39±0.016	4.18±0.017	5.49±0.014	6.02±0.021*	4.38±0.007	4.21±0.016	4.74±0.016	4.63±0.044	4.31±0.006	4.14±0.007
100 mM NaCl	2.30±0.070	3.33±0.176	3.72±0.008*	3.91±0.023	4.95±0.014	5.82±0.002*	4.29±0.003	4.13±0.012	4.64±0.007	4.53±0.028	4.19±0.033	4.05±0.002*
150 mM NaCl	1.97±0.013	2.90±0.127*	3.13±0.018*	2.98±0.036*	4.48±0.062*	4.84±0.007*	4.09±0.028	4.07±0.013	4.61±0.002	4.46±0.031	4.15±0.028	3.95±0.002*
Haloprimed 50 mM NaCl												
Control	5.10±0.074*	5.91±0.011*	4.84±0.009*	4.65±0.005*	6.34±0.047*	6.48±0.005*	4.61±0.008	4.54±0.004	4.90±0.001*	4.74±0.025	4.48±0.009	4.28±0.001*
50 mM NaCl	4.54±0.026*	5.54±0.012*	4.66±0.008	4.33±0.003	6.01±0.025	6.20±0.002*	4.70±0.013	4.38±0.002	4.88±0.002*	4.72±0.024	4.45±0.002	4.17±0.009
100 mM NaCl	3.87±0.043	5.28±0.238	4.00±0.003	4.21±0.008	5.26±0.033*	6.17±0.026*	4.66±0.008	4.21±0.007	4.80±0.004	4.54±0.023	4.36±0.002	4.08±0.007
150 mM NaCl	2.69±0.018	3.11±0.027*	3.39±0.006*	3.74±0.031	4.92±0.008*	4.94±0.006*	4.50±0.020	4.14±0.016	4.71±0.007	4.47±0.030	4.36±0.008	4.02±0.002*

Values are mean ± SE with three replicates. * indicates statistically significant at p≤0.05 compared to nonprimed control. Values are expressed as milligram nitrogen per gram fresh tissue.

Table 3: Effect of NaCl on nitrate contents in 21 days old nonprimed and haloprimed legume seedlings.

Treatments	*Lens culinaris* Medik.		*Cajanus cajan* L.		*Cicer arietinum* L.		*Lathyrus sativus* L.		*Vigna radiata* L.		*Vigna mungo* L.	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Nonprimed												
Control	473±0.012	417±0.003	458±0.003	515±0.003	417±0.003	346±0.006	486± 0.003	416± 0.004	444±0.005	585±0.003	557±0.003	655±0.001
50 mM NaCl	403±0.005	361±0.005	374±0.003	444±0.005	375±0.003	318±0.005	416±0.005	360±0.016	402±0.005	543±0.003	486±0.005	599±0.003
100 mM NaCl	178±0.003*	276±0.005	290±0.003	346±0.006*	262±0.006	234±0.005*	318±0.001	318±0.012	346±0.003	458±0.006*	444±0.005	500±0.003*
150 mM NaCl	150±0.005*	178±0.006*	122±0.003*	234±0.005*	192±0.001*	178±0.003*	248±0.003*	206±0.013*	234±0.001*	332±0.003*	304±0.003*	374±0.003*
Haloprimed 50 mM NaCl												
Control	473±0.003	445±0.005	515±0.003	599±0.006*	431±0.003	387±0.003	515±0.003*	458±0.004	543±0.003*	655±0.005*	627±0.003*	725±0.003*
50 mM NaCl	417±0.003	375±0.005	444±0.005	515±0.003	388±0.003	361±0.005	500±0.005	402±0.002	458±0.006	585±0.003	571±0.001*	669±0.003
100 mM NaCl	346±0.006	346±0.006	346±0.006	402±0.005	375±0.005	290±0.003	416±0.003	374±0.007	374±0.003	529±0.005	458±0.003	543±0.003
150 mM NaCl	234±0.005	262±0.006	248±0.006	248±0.003*	304±0.003	234±0.005	304±0.003	262±0.016	332±0.010	374±0.003*	374±0.003*	458±0.003*

Values are mean ± SE with three replicates. *indicates statistically significant at p≤0.05 compared to nonprimed control. Nitrate contents are expressed as microgram nitrate per gram fresh tissue.

Cajanus and *Vigna mungo* the activity was promoted by 16% and 1% respectively (Fig 1). Nitrite contents decreased by 55%, 49%, 41%, 40%, 34% and 30% in nonprimed root and by 44%, 47%, 27%, 38%, 31% and 29% in nonprimed shoot respectively of *Lens*, *Cajanus*, *Cicer, Lathyrus*, *Vigna radiata* and *Vigna mungo*. However, reduction in nitrite contents was narrowed to 16%, 16%, 17%, 14% and 11% in haloprimed root of *Lens*, *Cajanus*, *Cicer*, *Lathyrus* and *Vigna mungo* respectively, whereas in *Vigna radiata*, it increased by 5%. In haloprimed shoot, the decrement reduced to 18%, 12%, 8%, 14%, 9% and 3% in *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively (Table 4). NiR activity catalyzes conversion of nitrite into ammonia. The decline in NiR activity was most prominent in *Lens* (37% in root, 24% in shoot) and *Cajanus* (36% in root, 26% in shoot), moderate in *Cicer* (27% in root, 25% in shoot) and *Lathyrus* (28% in root, 22% in shoot) and least in *Vigna radiata* (14% in both root and shoot) and *Vigna mungo* (6% in root, 4% in shoot). Similar effects have been observed in salt stressed *Populus simonii* (Meng *et al*. 2016). In haloprimed seedlings, the inhibition was reduced to 21%, 22%, 19%, 20%, 10% and 3% in roots of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. In primed shoot, the inhibition was reduced to 8%, 16%, 17%, 11% and 2% respectively in *Lens*, *Cajanus*, *Cicer*, *Lathyrus* and *Vigna mungo* respectively whereas in *Vigna radiata*, the activity was promoted by 3% (Fig 2). Dissolved ammonia contents increased in nonprimed root by 81%, 75%, 51%, 46%, 19% and 10% and by 57%, 23%, 46%, 32%, 17% and 9% in nonprimed shoot of *Lens*, *Cajanus*, *Cicer, Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. In haloprimed seedlings, it decreased by 42%, 24%, 16%, 18%, 4% and 3% in root and in shoot the increment narrowed down to 17%, 13%, 16%, 13%, 5% and 3% in *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively (Table 5).

Decline in total nitrogen, soluble nitrogen, nitrate and nitrite contents with reduced activities of NR was recorded in the tested legume cultivars under NaCl stress since, salinity diminished nitrate uptake in tested seedlings. Similar report on reduction of nitrate uptake has been published in salt stressed *Lycopersicon esculentum* (Debouba *et al*. 2006). By virtue of lesser nitrate uptake under NaCl stress, subsequent decline in NR and NiR activities were recorded. This occurred because nitrate content is known to regulate NR and NiR gene expressions (Wang *et al*. 2000). Similar report on decline in NR activity in salt stressed wheat (Ahanger and Agarwal, 2017) evinces that nitrate contents regulate NR activity and determines the flow of nitrate to the active sites of the enzyme. Decreased nitrate uptake in the tested legume cultivars developed an uptake competition between NO_3^- and Cl^- during growth by affecting cellular membranes (Wang *et al*. 2011; Zhang *et al*. 2013). Reduction in nitrate uptake and lower NR activity under salt stress in the tested cultivars decreased corresponding nitrite contents. Amongst the tested cultivars, decrease in the total and soluble nitrogen contents, nitrate and nitrite contents assisted by the NR activity were maximally inhibited in *Lens* and *Cajanus* indicating their salt sensitivity whereas, it was

Table 4: Effect of NaCl on nitrite contents in 21 days old nonprimed and haloprimed legume seedlings.

Treatments	*Lens culinaris* Medik.		*Cajanus cajan* L.		*Cicer arietinum* L.		*Lathyrus sativus* L.		*Vigna radiata* L.		*Vigna mungo* L.	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Nonprimed												
Control	424±0.005	531±0.003	909±0.008	693±0.005	478±0.005	531±0.003	585±0.003	478± 0.005	531±0.003	639±0.010	478±0.003	424±0.005
50 mM NaCl	316±0.003	424±0.006	693±0.008	585±0.012	370±0.006	478±0.003	478±0.006	370±0.003	478±0.003	639±0.005	424±0.003	316±0.003
100 mM NaCl	154±0.005*	316±0.006	531±0.006	424±0.005*	262±0.003	370±0.003	370±0.003*	316±0.001	424±0.003	424±0.006	316±0.003	315±0.003
150 mM NaCl	100±0.003*	154±0.003*	154±0.003*	100±0.003*	208±0.003	316±0.001*	208±0.003*	208±0.003	154±0.003*	262±0.005*	262±0.005*	262±0.003
Haloprimed 50 mMNaCl												
Control	531±0.005*	585± 0.003*	1017±0.005*	748±0.001	585±0.005*	586±0.003*	639±0.005*	531±0.003	639±0.003*	693±0.008	531±0.003	478±0.001
50 mM NaCl	424±0.003	478±0.003	801±0.005	747±0.001	424±0.003	585±0.005*	585±0.005	478±0.003	693±0.005*	639±0.003	478±0.003	424±0.006
100 mM NaCl	262±0.006	424±0.005	747±0.003	479±0.003	316±0.003	478±0.005	531±0.005	370±0.006	585±0.003	531±0.030*	370±0.003	423±0.006
150 mM NaCl	208±0.005*	262±0.003	478±0.006	478±0.003	262±0.003	316±0.005*	262±0.003*	262±0.003	316±0.005*	478±0.003	316±0.005	316±0.003

Values are mean ± SE with three replicates. * indicates statistically significant at p≤0.05 compared to nonprimed control. Nitrite contents are expressed as microgram nitrite per gram fresh tissue.

Table 5: Effect of NaCl on dissolved ammonia contents in 21 days old nonprimed and haloprimed legume seedlings.

Treatments	*Lens culinaris* Medik.		*Cajanus cajan* L.		*Cicer arietinum* L.		*Lathyrus sativus* L.		*Vigna radiata* L.		*Vigna mungo* L.	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Nonprimed												
Control	46±0.003	53±0.003	220±0.015	280±0.015	260±0.001	206±0.001	190±0.011	170±0.001	96±0.001	130±0.005	113±0.006	140±0.008
50 mM NaCl	60±0.003	63±0.003	256±0.008	326±0.017	293±0.001	250±0.001	240±0.011	193±0.003	103±0.001	136±0.005	116±0.005	153±0.001
100 mM NaCl	90±0.008*	80±0.003*	323±0.008*	350±0.010	353±0.002*	290±0.001	276±0.006	223±0.003*	113±0.006	156±0.010	120±0.003	150±0.001
150 mM NaCl	100±0.001*	106±0.005*	576±0.003*	360±0.005	530±0.001*	363±0.004*	320±0.005	256±0.003*	126±0.002	163±0.005*	136±0.003*	156±0.002
Haloprimed 50 mMNaCl												
Control	36±0.005*	36±0.005*	203±0.018*	256±0.006*	236±0.002*	176±0.001*	173±0.008	160±0.005*	83±0.001	116±0.003	106±0.006	133±0.001
50 mM NaCl	56±0.005	56±0.005	240±0.017	310±0.023	253±0.001	156±0.001*	216±0.003	173±0.006	93±0.001	126±0.003	110±0.008	140±0.001
100 mM NaCl	76±0.005	70±0.001	293±0.014	343±0.012	263±0.001	286±0.002	246±0.006	206±0.012	106±0.003	150±0.005	106±0.005	153±0.001
150 mM NaCl	93±0.003*	86±0.006*	356±0.017*	356±0.016	456±0.002*	333±0.002*	263±0.006*	256±0.008*	116±0.002	153±0.003	116±0.006	150±0.002

Values are mean ± SE with three replicates. * indicates statistically significant at $p \le 0.05$ compared to nonprimed control. Values are expressed as micromoles ammonia acid per gram fresh tissue.

moderately affected in *Cicer* and *Lathyrus*. In *Vigna radiata* and *Vigna mungo*, the inhibitory effects were minimal, manifesting their partial salt tolerance. Seed priming helped to overcome such adversities conferring increased nitrate uptake during seedling growth. Present study recorded increased ammonia contents under NaCl stress. Elevated ammonia contents aggravate cytotoxicity, impair osmotic regulation and plant development (Zhang *et al*. 2013). NiR activity in *Lens*, *Cajanus*, *Cicer* and *Lathyrus* was much higher as compared to their respective NR activity whereas, in *Vigna radiata* and *Vigna mungo*, opposite trend was observed, *i.e.* higher NR activity and less of NiR activity. This corresponded to higher accumulation of NH_4^+ in *Lens*, *Cajanus*, *Cicer* and *Lathyrus* indicating more cytotoxic environments. Lower ammonia accumulation due to low NiR activity resulted in lesser cytotoxicity in *Vigna radiata* and *Vigna mungo* indicating that these two cultivars were least salt sensitive.

GOGAT activity decreased by 36%, 32%, 22%, 17%, 12% and 2% in root and by 22%, 20%, 17%, 11%, 5% and 1% in shoot of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. However, in haloprimed roots, the inhibition narrowed down to 34%, 12%, 20%, 9% and 1% in *Lens*, *Cajanus*, *Cicer*, *Vigna radiata* and *Vigna mungo* respectively. In haloprimed *Lathyrus* root, the activity was promoted to 14%. In shoot of primed seedlings, the inhibition narrowed down to 6%, 9%, 16%, 8% and 2% in *Lens*, *Cajanus*, *Cicer*, *Lathyrus* and *Vigna radiata* respectively. In haloprimed shoot of *Vigna mungo*, the activity was promoted to 2% (Fig 3). GS activity declined in root by 42%, 36%, 29%, 27%, 18% and 16% and in shoot by 37%, 26%, 24%, 13%, 8% and 2% respectively in *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. The inhibition in GS activity was decreased to 22%, 18%, 10%, 15%, 12% and 10% in root and by 16%, 14%, 9%, 4%, 1% and 1% in shoot of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* (Fig 4).

Decline in GS and GOGAT activities caused NH_4^+ accumulation under salinity in the tested cultivars. Inhibition was notable in *Lens* and *Cajanus*, followed by *Cicer* and *Lathyrus*. This perhaps generated cytotoxicity because cellular pH was disrupted and photophosphorylation got uncoupled (Ashraf *et al*. 2018). The inhibitory effect of GS and GOGAT was least in *Vigna radiata* and *Vigna mungo*, probably hinting their tolerance towards NaCl. Similar report has been published in arsenic stressed wheat seedlings (Ghosh *et al*. 2013). Seed halopriming helped to alleviate such NaCl induced toxicity, facilitating better nitrate assimilation. Enhanced GDH activity catalyzes conversion of glutamate into ammonia (Skopelitis *et al*. 2006). Salinity provoked significant elevation in GDH activity by 70%, 47%, 35%, 31%, 18% and 4% in nonprimed root and by 58%, 44%, 23%, 28%, 11% and 6% in nonprimed shoot of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. The activity was maximum in *Lens* and *Cajanus*, moderate in *Cicer* and *Lathyrus* and least in *Vigna radiata* and *Vigna mungo*, which could be justified with their respective average percentages of ammonia accumulation

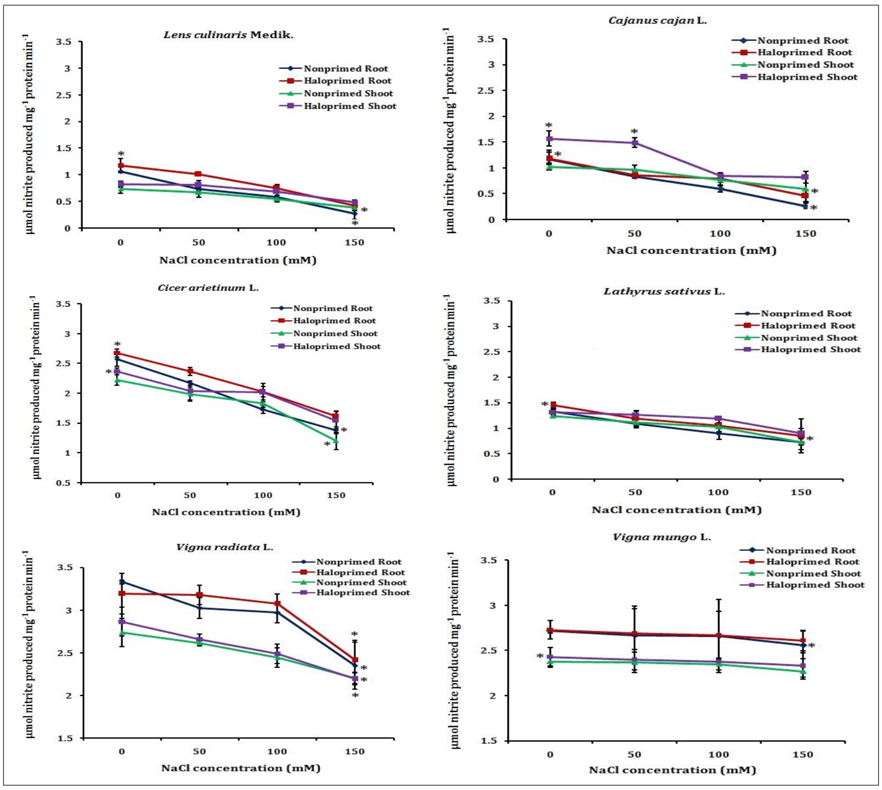

Fig 1: Effect of NaCl on nitrate reductase (NR) activity of 21 days old nonprimed and haloprimed seedlings of legume cultivars. Values are mean ± SE with three replicates. *Indicates statistically significant at p≤0.05 compared to nonprimed control.

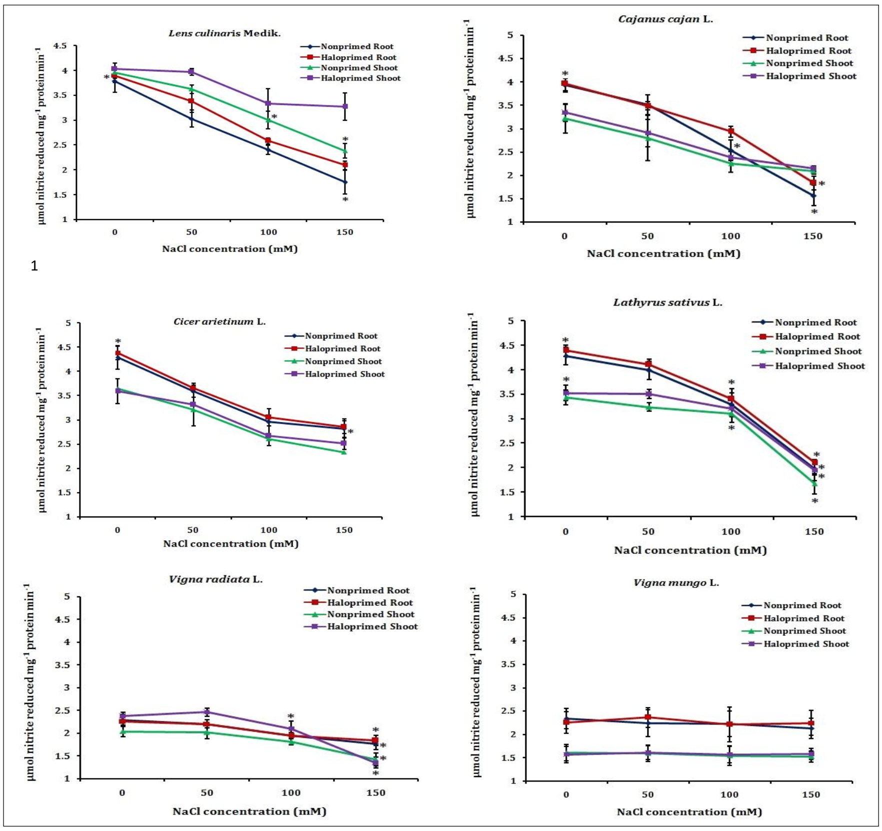

Fig 2: Effect of NaCl on nitrite reductase (NiR) activity of 21 days old nonprimed and haloprimed seedlings of legume cultivars. Values are mean ± SE with three replicates. * Indicates statistically significant at p≤0.05 compared to nonprimed control.

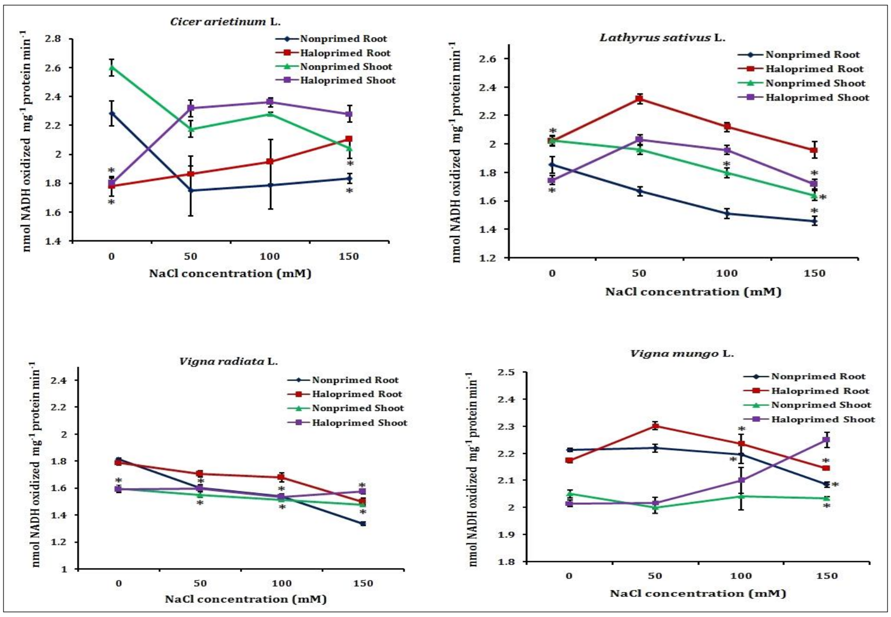

Fig 3: Effect of NaCl on glutamate synthase (GOGAT) activity of 21 days old nonprimed and haloprimed seedlings of legume cultivars. Values are mean ± SE with three replicates. *Indicates statistically significant at p≤0.05 compared to nonprimed control.

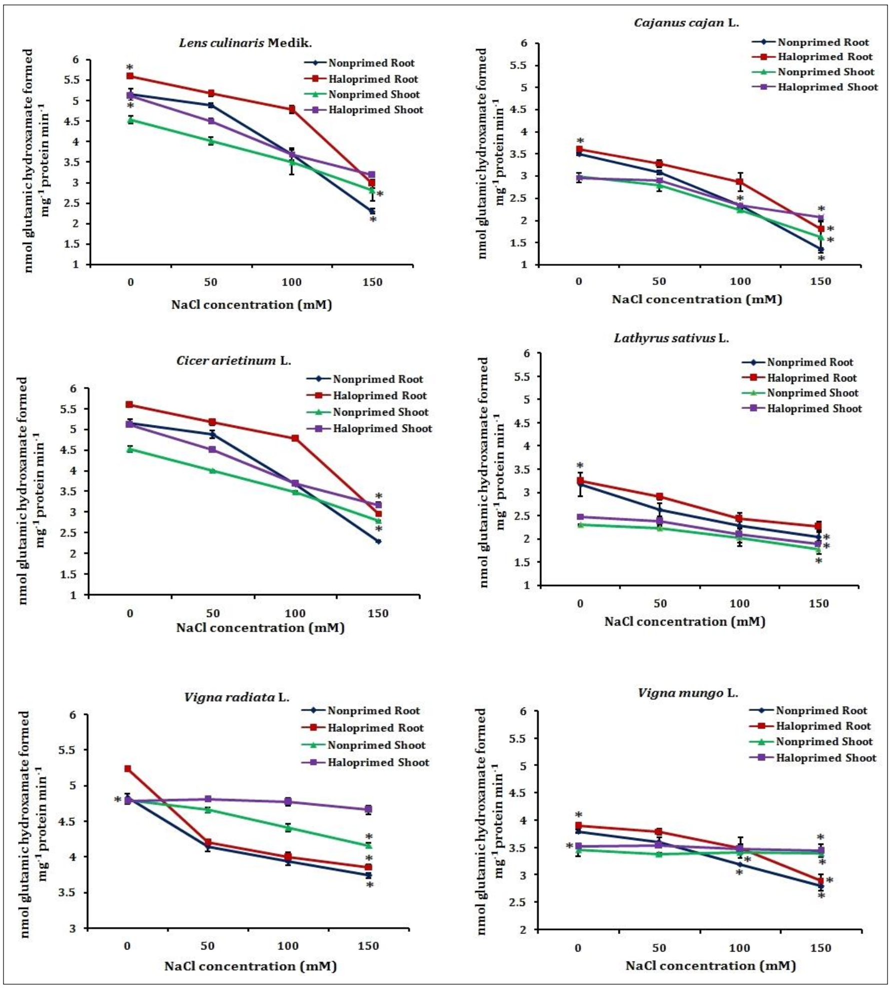

Fig 4: Effect of NaCl on glutamine synthetase (GS) activity of 21 days old nonprimed and haloprimed seedlings of legume cultivars. Values are mean ± SE with three replicates. *Indicates statistically significant at p≤0.05 compared to nonprimed control.

recorded in nonprimed root and shoot. High $NH_4^+$is harmful for cells and needs quick assimilation. Similar changes have been noticed in saline-alkaline stressed tomato (Zhang *et al*. 2013). Seed priming decreased GDH activity to 31%, 18%, 17%, 11%, 12% and 7% in roots and to 23%, 19%, 3%, 10%, 5% and 2% in shoots of *Lens*, *Cajanus*, *Cicer*, *Lathyrus*, *Vigna radiata* and *Vigna mungo* respectively. Present results corroborate with the reduced NH_4^+ accumulation in

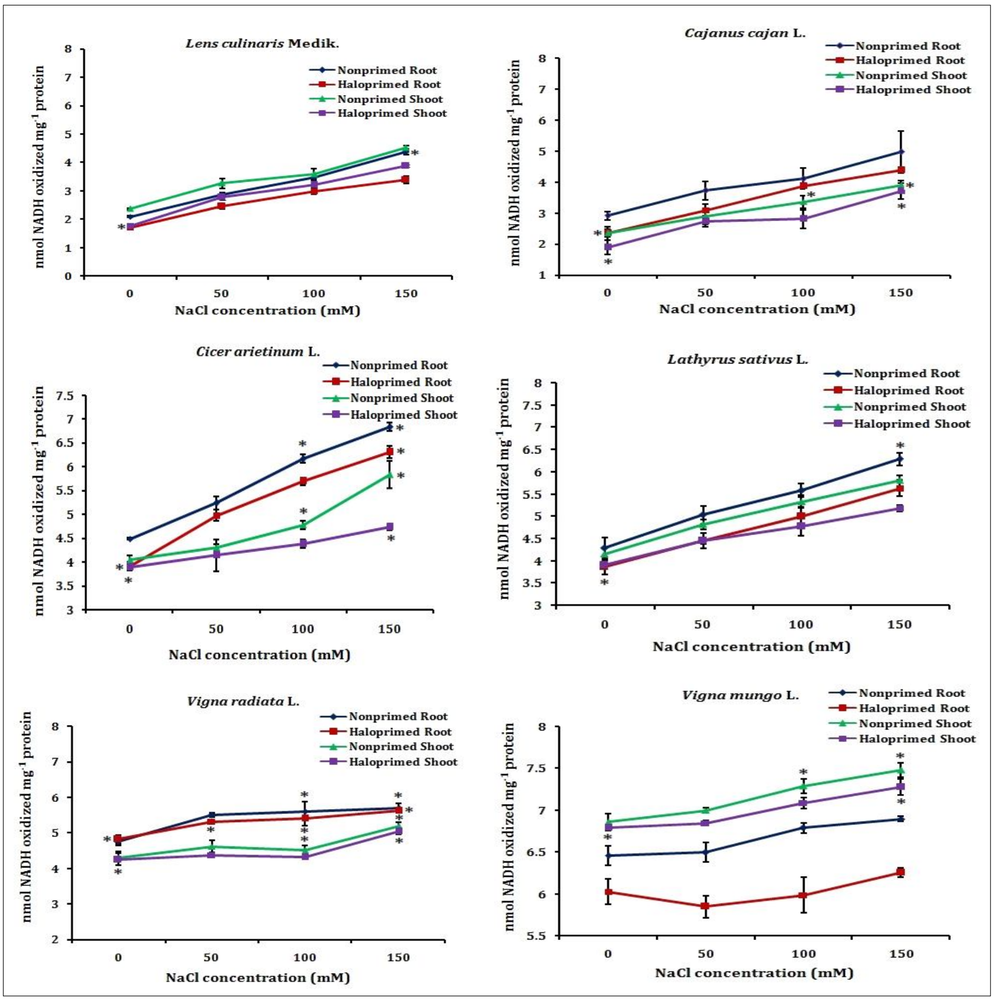

Fig 5: Effect of NaCl on glutamate dehydrogenase (GDH) activity of 21 days old nonprimed and haloprimed seedlings of legume cultivars. Values are mean ± SE with three replicates. *Indicates statistically significant at p≤0.05 compared to nonprimed control.

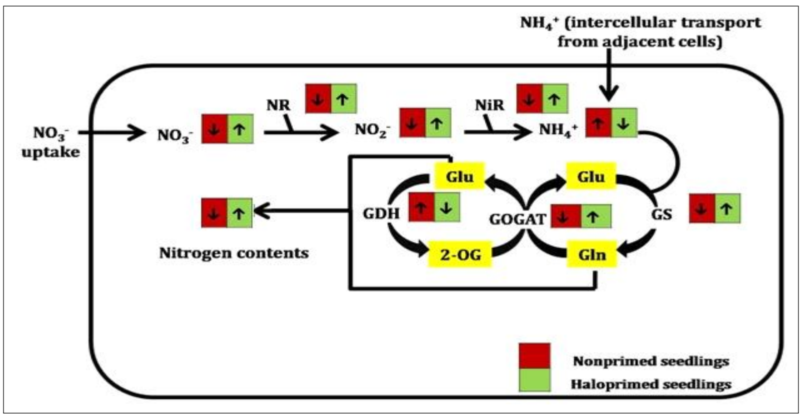

Fig 6: Illustration depicting alterations in intermediates of nitrogen metabolism and its assimilatory enzymes in nonprimed and haloprimed seedlings of legumes cultivars subjected to NaCl stress. ↑ Indicates increase, whilst ↓ indicates decrease in substrate content/enzyme activity.

respective haloprimed seedlings. Similar ameliorative change has been observed in selenate administered arsenic stressed wheat (Ghosh *et al*. 2013) (Fig 5).

Fig 6 schematically illustrates NaCl-induced alterations on nitrogen metabolism in nonprimed seedlings and efficacy of seed halopriming in overcoming such adversities facilitating better nitrate assimilation.

CONCLUSION

The potency of seed halopriming to overcome NaCl-induced disturbances on nitrogen metabolism depended on differential resistance mechanisms of tested cultivars. Pre-germination halopriming probably aroused a 'memory response' in seeds for which better nitrogen assimilation was noted in tested primed seedlings under salinity. The efficacy of halopriming was more effective in salt sensitive cultivars and lesser in partially salt-tolerant cultivars like *Vigna radiata* and *Vigna mungo*. This hinted species-specific efficiency of seed halopriming. Although, the halopriming efficacy was less in partially tolerant cultivars (*Vigna radiata* and *Vigna mungo*) but, it helped them to grow better under NaCl stress, no matter how much less the efficiency was. Based on present results, these two cultivars may thus be haloprimed and suggested for cultivation to increase pulse production in saline prone agricultural fields.

ACKNOWLEDGEMENT

This work was supported by The Department of Science and Technology, Government of West Bengal under sanction no. 1012 (Sanc.) /ST/P/S and T/2G-2/2013.

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