DOI: https://doi.org/10.36094/sc.v86.2020.Alleviation_of_Floride-mediated.Singh.317

Research Communication

Sci. and Cult. 86 (9–10) 320-324 (2020)

Alleviation of Fluoride-Mediated Toxicity via Seed Priming with Calcium Oxide in *Oryza sativa* L. cv. Khitish

Abstract **: The effect of seed priming with calcium oxide (CaO) in the mitigation of fluoride-mediated stress is not yet explored. The present manuscript highlights the amelioration of fluoride toxicity in an indica rice cultivar, viz., Khitish, by initial priming of seeds for 24 h with two different concentrations of CaO (0.3 mM and 0.5 mM). Fluoride toxicity brought about considerable decrease in the seedling emergence, affecting the overall physiology of seedlings. Chlorophyll degeneration, enhanced endogenous malondialdehyde and H_2O_2, and electrolyte leakage due to higher bioaccumulation of fluoride were observed. While the catalase activity was decreased, the other antioxidants like guaiacol peroxidase, proline and total amino acids were elevated. Seed priming with CaO largely improved plant performance under fluoride stress by enhancing germination efficiency, with better physiology of plants, reducing fluoride bioaccumulation and overall oxidative damages. The protective effect of CaO was also evident from restoration of the catalase activity, and lowering the level of guaiacol peroxidase, proline and total amino acids, even under stressed condition. The decrement in the level of carotenoids and total phenolics as a consequence of stress was also overcome via CaO priming. The lower (0.3 mM) concentration appeared to be more potential in stress mitigation. Thus, CaO priming offers a reliable strategy in amelioration of fluoride-mediated injuries in Khitish by inhibiting fluoride uptake and improving the overall plant growth.**

Keywords: **Fluoride stress, calcium oxide, seed priming, antioxidants, osmolytes, rice**

Fluoride contamination is globally recognized as a severe threat to biotic components of the environment, hindering water and mineral transport. In plants, fluoride toxicity affects the overall physiology, causing chlorophyll damage which ultimately leads to chlorosis, leaf margin burn, growth retardation and necrosis of leaf tip. It interferes with the activity of ferric ion-containing enzymes such as catalase, cytochrome oxidase and peroxidase, hinders phosphorylation of proteins and rate of photosynthesis, and generates reactive oxygen species (ROS) which interfere with other metabolic processes[1,2]. Bioaccumulation of fluoride in edible parts can occur through translocation of fluoride ions via root-shoot system, thereby enhancing the endogenous fluoride level[1].

In order to counteract oxidative stress, plants recruit an elaborate defence mechanism involving compatible solutes like proline and amino acids, as well as non-enzymatic and enzymatic antioxidants like phenolics, carotenoids, peroxidase, catalase, etc[3]. In addition to these protective machineries, presence of various divalent elements in the soil like calcium and magnesium has been reported to bind with fluoride ion which results in lower uptake of fluoride from soil. The mitigation of fluoride toxicity by aluminium has been emphasized, but in recent times, several studies have particularly reported the protective role of calcium against fluoride stress. Calcium chelates the fluoride ions forming a CaF_2 complex, ultimately reducing fluoride uptake by plant tissues and reducing the overall damages. 0.6 g L^{-1} of CaO nanoparticles have been used successfully to adsorb 100 mg L^{-1} fluoride ions when present in aqueous solution[4]. The liming of soil with CaO has been shown to decrease the level of fluoride in *Camellia sinensis* L. leaves by 10.5% in comparison with the plants, where Ca was not applied exogenously[5].

Seed priming is an effective and practical technique which accelerates rapid and uniform seedling emergence, high seedling vigor and better yield, and normal metabolic processes under unfavourable environmental conditions. Higher and synchronized germination of primed seeds primarily occurs due to reduction in the lag time of imbibition, build-up of germination-enhancing metabolites, enzyme activation, metabolic repair during imbibition and osmotic adjustment[6]. Although the role of CaO in overcoming fluoride stress has been advocated, there is no available work on the protective action of seed priming

The journal is in the category 'Group A' of UGC-CARE list and falls under the broad category of Multidisciplinary Sciences covering the areas Arts and Humanities, Science and Social Sciences.

with CaO during fluoride toxicity in case of rice plants. Therefore, the present study was aimed to assess the mitigation potentiality of seed priming with CaO, by monitoring germination, seedling biomass, root and shoot length, chlorophyll, H_2O_2 and malondialdehyde (MDA) content, electrolyte leakage, fluoride bioaccumulation as well as the antioxidant action during fluoride-mediated stress in an indica rice cultivar Khitish. To the best of our knowledge, this is the first systematic approach to analyze the mitigation efficiency of CaO in rice plants challenged with fluoride toxicity.

Materials And Methods

Plant material, growth conditions, priming and stress treatment : Seeds of rice variety Khitish were obtained from Chinsurah Rice Research Station, Hooghly, West Bengal. The seeds were washed with 0.1% (w/v) $HgCl_2$ for 10 minutes for surface sterilization. The sterilized seeds were primed with either 0.3 mM CaO or 0.5 mM CaO, and in distilled water for 24 hours at 25°C in dark with constant shaking, keeping the seed weight to solution volume (w/v) in 1:5 ratio as previously standardized[7]. Fresh solution was added after 12 hours. Following priming, the seeds were dried for 24 hours at 25°C to their original moisture content of 8.5 to 9.5% on dry weight basis. The air-dried seeds were placed on filter paper in Petri plates and supplemented with 25 mg L^{-1} NaF for imposing stress, while the seeds treated with distilled water served as control (untreated). The solutions were renewed every two days. The seedlings were grown for 10 days in plant growth chamber as standardized earlier[1]. The 10 day-old seedlings were harvested, dried on blotting paper, and frozen in liquid nitrogen. Each subset was maintained in triplicates.

Estimation of physiological parameters, total chlorophyll, electrolyte leakage, MDA, H_2O_2 and fluoride content : For determination of the percentage of seed germination, 50 seeds were considered from each set of control and treated seeds. Total seedling biomass, shoot length (SL) and primary root length (RL) were measured for randomly selected 50 seedlings from each set. Total chlorophyll was measured following the earlier protocol[3] and was expressed as µg g^{-1} fresh weight (FW). For electrolyte leakage, 0.1 g of fresh tissue was used and the electrolyte leakage was expressed in percentage of total electrolytes leaked from cells[8]. The MDA content of seedlings was measured at 532 nm and total MDA was estimated using the molar extinction coefficient 155 mM^{-1} cm^{-1}. The H_2O_2 content was estimated by recording the absorbance at 395 nm against a standard curve[8]. For fluoride estimation, 0.2 g of seedlings was oven dried, homogenised in 4 mL of ionic strength adjustment buffer (TISAB) pH 5.2. The mixture was boiled in water bath for 15 min and cooled, followed by centrifugation at 10,000 × g for 10 min. Fluoride accumulation in seedlings was measured using a fluoride-sensitive electrode (Cole-Palmer, USA).

Estimation of major osmolytes (proline and total amino acids), non-enzymatic antioxidants (carotenoids and total phenolic content) and activity of enzymatic antioxidants (catalase and guaiacol peroxidase) : The proline content in seedlings was estimated by measuring the absorbance at 520 nm against a standard curve[8]. Total amino acids was estimated using ninhydrin method by measuring the absorbance at 570 against a standard curve[3]. Total carotenoid content was determined[3] using extinction coefficient 139 × 10^3 M^{-1} cm^{-1}. Total phenolic content (TPC) was estimated by measuring the absorbance at 760 nm using Folin–Ciocalteu reagent. TPC of samples was determined using a standard curve[3]. The catalase (CAT, EC 1.11.1.6) activity was determined by monitoring the rate of usage of H_2O_2 at 240 nm[8], using the molar extinction coefficient of 40 mM^{-1} cm^{-1}. The guaiacol peroxidase (GPX, EC 1.11.1.7) activity was estimated by monitoring the change in absorbance at 470 nm for 5 min[3]. The tetraguaiacol formation denoted the activity of GPX which was estimated using the molar extinction coefficient 26.6 mM^{-1} cm^{-1}. Total protein isolation and estimation of protein content was performed as earlier[9].

Statistical analysis : Three replicates (n = 3) for each set were used in a completely randomized design (CRD), and the results were presented as mean ± standard error (SE). All the data obtained were subjected to analysis of variance (ANOVA)[10] by using computer based software XLSTAT v.2017. The means were compared using Tukey's HSD test, where $P \leq 0.05$ was considered as significant.

Results and Discussion

Plants like rice which intensively require water for their growth and development accumulate fluoride in their tissues, leading to fluoride toxicity that causes retardation in plant growth and development and ultimately oxidative stress. Seed priming reprograms the metabolome and generates a 'priming memory' in seeds which facilitates the survival of seedlings under stress condition[6]. There are increasing reports that priming of seeds with Ca provides tolerance by enhancing the internal defence machineries of plants exposed to abiotic stress. The role of Ca ions in causing decrease in fluoride concentration has been reported earlier. Addition of $CaCl_2$ has been shown to be effective in ameliorating fluoride toxicity[11] through the precipitation of CaF_2.

Physiological parameters, total chlorophyll, electrolyte leakage, H_2O_2, MDA and fluoride content : The germination percentage and biomass of seedlings were decreased by 1.3- and 1.4-fold respectively in stressed condition with respect to the control. RL and SL were also decreased by 2.1- and 1.7-fold respectively in fluoride treated seedlings. Priming with 0.3 mM and 0.5 mM CaO increased the germination percentage by 1.4- and 1.2-fold and the seedling biomass was increased by 1.4- and 1.2-fold respectively. Pre-treatment of seeds with 0.3 mM CaO increased the RL and SL by 1.7- and 1.5-fold respectively; whereas 1.6- and 1.3-fold increase in RL and SL was observed in seedlings grown from seeds earlier primed with 0.5 mM CaO.

The chlorophyll content was decreased by 1.4-fold in NaF-treated seedlings along with 2.8-fold higher electrolyte leakage with respect to the unstressed seedlings. Fluoride toxicity enhanced the H_2O_2 content by 2.1-fold in stressed seedlings which led to membrane damage and resulted in 1.7-fold higher MDA level. Seedlings treated with 25 mg L^{-1} NaF for 10 days showed 6.1-fold higher accumulation of fluoride (Table-1). Seeds pre-treated with 0.3 and 0.5 mM of CaO showed 1.4- and 1.2-fold higher chlorophyll level respectively. Stressed seedlings primed with 0.3 mM and 0.5 mM CaO showed 1.7- and 1.2-fold lower electrolyte leakage respectively. Seeds primed with 0.3 mM and 0.5 mM CaO showed 1.6- and 1.4-fold lower MDA level in the stressed seedlings. Priming of seeds with 0.3 mM and 0.5 mM of CaO lowered H_2O_2 content by 1.8- and 1.6-fold respectively with respect to seedlings raised from non-primed seeds. Fluoride bioaccumulation was considerably reduced by 2.0-fold and 1.6-fold when stressed seedlings were raised from 0.3 mM and 0.5 mM CaO-primed seeds respectively (Table-1).

Reduction in germination percentage, seedling biomass, RL and SL, and chlorophyll content during fluoride toxicity was observed, which is similar to the previous studies where fluoride toxicity suppressed the growth of Bengal gram[11] and *Vigna*[12] seedlings. The decrease in chlorophyll content occurs due to breakdown of chlorophyll or reduced synthesis of chlorophyll[1]. Membranes are the major sites of stress injuries, which lead to higher electrolyte leakage due to enhanced membrane permeability. Moreover, lipid peroxidation also exacerbates the oxidative damages through the production of lipid-derived secondary free radicals and generation of a number of cytotoxic by-products namely MDA[1]. The MDA level in our case was significantly increased in rice seedlings exposed to NaF. Increase in MDA level in two varieties of *Triticum aestivum* L. when treated with increasing concentration of NaF has been reported earlier[13]. The increment in H_2O_2 content in fluoride-stressed seedlings is in accordance with earlier studies[1]. The retardation in seedling growth and damages observed in non-primed rice seedlings under fluoride stress is attributed to significant accumulation of fluoride. Earlier observations also showed that exposure of radicles to fluoride caused increased accumulation, thereby causing loss of membrane integrity[1]. Our observation on rice seed priming with CaO showed appreciable promoting effect on fluoride-stressed

Table 1: Percentage of germination, seedling biomass, RL and SL, chlorophyll, electrolyte leakage, MDA, H_2O_2 and fluoride content in rice seedlings. Data are the mean values (n = 3) ± standard error (SE). Data with significant differences obtained against control are labelled with '*' at *$P \leq 0.05$*

Sample	Germination percentage (%)	Seedling biomass (g)	RL (cm)	SL (cm)	Chlorophyll (µg g^{-1} FW)	Electrolyte leakage (%)	MDA (µM g^{-1} FW)	H_2O_2 (µM g^{-1} FW)	Fluoride accumulation (mg kg^{-1} FW)
Control	70.70 ± 0.97	142.50 ± 0.35	1.02 ± 0.54	5.49 ± 0.51	65.58 ± 4.88	14.75 ± 0.59	2.83 ± 0.15	0.16 ± 0.03	32.25 ± 1.12
NaF (25 mg L^{-1})	55.00 ± 0.62*	101.34 ± 0.21*	0.49 ± 0.94*	3.29 ± 0.64*	45.91 ± 5.24*	41.19 ± 0.47*	4.93 ± 0.19*	0.34 ± 0.02*	197.47 ± 1.25*
CaO (0.3 mM)	81.20 ± 0.54*	161.52 ± 0.54*	1.19 ± 0.24*	6.21 ± 0.36*	67.69 ± 4.02	16.61 ± 0.58	2.42 ± 0.24	0.16 ± 0.01	31.71 ± 2.30
CaO (0.3 mM) + NaF (25 mg L^{-1})	75.15 ± 0.31	138.43 ± 0.59	0.82 ± 0.39	5.01 ± 0.87	62.89 ± 4.50	23.65 ± 2.10*	2.99 ± 0.10	0.19 ± 0.04	96.55 ± 3.21*
CaO (0.5 mM)	78.60 ± 0.57	160.34 ± 0.67	0.98 ± 0.54	6.01 ± 0.53	61.54 ± 1.35	19.86 ± 0.98	2.63 ± 0.18	0.16 ± 0.05	29.53 ± 2.34
CaO (0.5 mM) + NaF (25 mg L^{-1})	63.85 ± 0.48*	123.25 ± 0.49*	0.76 ± 0.67	4.19 ± 0.37*	54.05 ± 2.59*	34.63 ± 1.24*	3.41 ± 0.34*	0.20 ± 0.03*	122.12 ± 1.99*

seedlings by improving germination percentage, seedlings biomass, RL and SL, chlorophyll content and decreasing the electrolyte leakage, as well reducing the extent of lipid peroxidation by lowering the MDA and H_2O_2 content, thereby preventing oxidative damages.

Defence machineries of plants: osmolytes (proline and total amino acids), non-enzymatic antioxidants (carotenoids and TPC) and enzymatic antioxidants (CAT and GPX activity) : The defence mechanism against any oxidative stress comprises of triggering the production of compatible solutes and non-enzymatic antioxidants as well as enhancing the activity of antioxidant enzymes. Almost 1.6- and 1.8-fold higher proline and total amino acid level was recorded in stressed seedlings. Fluoride stressed seedlings showed 2.7-fold lower carotenoid content and 1.6-fold lower TPC as compared to that of unstressed seedlings. Treatment with NaF decreased the CAT activity by 1.8 times, while increased the GPX activity by 4.5 times, as compared to that of unstressed seedlings (Table-2). Proline was reduced by 1.3- and 1.2-fold respectively in seedlings exposed to 0.3 mM and 0.5 mM CaO respectively. Pre-treatment of seeds with CaO was effective against damage, bringing down the total amino acid level by 1.5- and 1.3-fold in seedlings in case of 0.3 mM and 0.5 mM primed seeds. The stressed seedlings raised from 0.3 mM and 0.5 mM CaO primed seeds showed respectively 2.4- and 2.3-fold higher carotenoid content, while 1.4- and 1.3-fold higher TPC respectively than that of non-primed stressed seedlings. Stressed seedlings raised from seeds pre-treated with 0.3 mM and 0.5 mM CaO enhanced the CAT activity by 1.4 and 1.3 times respectively, while lowering the GPX activity by 2.0- and 1.5-fold respectively as compared to seedlings generated from non-primed seeds (Table-2).

A number of protective functions such as maintenance of cell turgor and redox equilibrium, stabilization of cellular structures and enzymes, osmoprotection and scavenging of ROS under adverse conditions are attributed to proline[14]. Increase in proline content might be the result of either higher activity of delta-1 pyrroline-5-carboxylate synthetase, a key enzyme involved in proline biosynthesis, or lower activity of catabolic enzymes or breakdown of proline-rich proteins which can be supported by the earlier observations[3]. Likewise, the increase in amino acid content in fluoride-stressed rice is also in accordance with earlier observation which showed increased accumulation of several amino acids in the tolerant cultivar of green gram as compared to the sensitive cultivar under salinization; this can be correlated with salt tolerance ability[15]. The decrease in carotenoid content in our experiment coincides with the earlier report which showed decrease in carotenoid level in Swarno and IR-36 varieties of rice, when grown under increasing concentration of NaF[16]. The reduction of TPC in fluoride-stressed rice is supported by earlier observation in *Cynara scolymus* during high salinity[17]. The TPC of 5- and 7-day old radish sprouts were significantly diminished by moderate salinity as compared with the control[18]. The hydroxyl group bound to the iron moiety present in the active site of CAT is replaced by fluoride ion which results in inhibition of CAT activity[2]. The alteration in the activity of GPX in the present study is in agreement with the earlier findings in As-treated *Glycine max*[19]. Enhanced GPX activity suggests that the enzyme plays a major antioxidative role in the protection of rice plants against fluoride toxicity.

Table 2: Proline, total amino acids, carotenoids, total phenolic content, catalase and guaiacol peroxidase activity in rice seedlings. Data are the mean values (n = 3) ± SE. Data with significant differences obtained against control are labelled with '*' at *P* ≤ *0.05*

Sample	Proline ($\mu g\ g^{-1}$ FW)	Total amino acids ($\mu g\ g^{-1}$ FW)	Carotenoids ($\mu M\ g^{-1}$ FW)	TPC ($mg\ g^{-1}$ FW)	CAT activity ($\mu M\ H_2O_2$ utilized $min^{-1}\ mg^{-1}$ leaf protein	GPX activity (μM tetraguaiacol formed $min^{-1}\ mg^{-1}$ leaf protein)
Control	0.31 ± 0.07	0.32 ± 0.04	29.54 ± 0.99	0.73 ± 0.04	30.24 ± 1.24	6.63 ± 0.58
NaF (25 mg L^{-1})	0.57 ± 0.06*	0.57 ± 0.01*	10.79 ± 0.58*	0.46 ± 0.07*	16.87 ± 1.54*	29.98 ± 1.54*
CaO (0.3 mM)	0.35 ± 0.08	0.28 ± 0.01	29.59 ± 1.05	0.71 ± 0.02	29.87 ± 0.87	7.01 ± 0.98
CaO (0.3 mM) + NaF (25 mg L^{-1})	0.43 ± 0.08*	0.38 ± 0.02	25.89 ± 1.24	0.63 ± 0.04	24.06 ± 0.91	15.19 ± 1.19*
CaO (0.5 mM)	0.39 ± 0.09	0.30 ± 0.07	28.92 ± 0.78	0.69 ± 0.01	28.39 ± 0.84	8.78 ± 0.91
CaO (0.5 mM) + NaF (25 mg L^{-1})	0.49 ± 0.02*	0.44 ± 0.06*	24.60 ± 0.87	0.59 ± 0.02*	22.06 ± 1.43*	19.45 ± 0.87*

Earlier reports have shown that seed priming with $CaCl_2$ mitigated the adverse effects of drought stress in sunflower seedlings by increasing the different growth criteria like SL, leaf area, fresh and dry weight of shoot, increasing the antioxidant enzymes and photosynthetic pigments as well as decreasing the level of H_2O_2, MDA, soluble phenolic and flavonoid contents, as compared to drought-stressed seedlings[20]. Overall, the present study contributes to our mechanistic understanding of Ca priming-mediated attenuation of fluoride accumulation in rice and provides useful information regarding improvement of the adaptability of rice to fluoride stress by seed priming with CaO. The results evidently highlight that CaO priming can be an economic strategy for the farmers engaged in cultivation of rice to maintain usual growth and yield during fluoride stress under field conditions.

Acknowledgments

Financial assistance from Science and Engineering Research Board, Government of India through the grant [EMR/2016/004799] and Department of Higher Education, Science and Technology and Biotechnology, Government of West Bengal, through the grant [264(Sanc.)/ST/P/S&T/1G-80/2017] to Dr. Aryadeep Roychoudhury is gratefully acknowledged. ❐

ANKUR SINGH,
ADITYA BANERJEE,
ARYADEEP ROYCHOUDHURY*

Post Graduate Department of Biotechnology,
St. Xavier's College (Autonomous),
30, Mother Teresa Sarani, Kolkata - 700016,
West Bengal, India

* E-mail: aryadeep.rc@gmail.com

Received : 19 April, 2020

References

1. A. Banerjee, A. Roychoudhury and P Ghosh, *Plant Physiol. Biochem.* **142**, 143-150 (2019).
2. K.A. Kumar, P. Varaprasad and A.V.B Rao, *Res. J. Earth Sci.* **1**, 69-73 (2009).
3. A. Banerjee and A. Roychoudhury, *Plant Cell Rep.* **38**, 1217-1233 (2019).
4. G. Patel, U. Pal and S. Menon, *Sep. Sci. Technol.* **44**, 2806-2826 (2009).
5. J. Ruan , L. Ma , Y. Shi and W. Han, *Ann. Bot.* **93**, 97-105 (2004).
6. A. Banerjee and A. Roychoudhury, In: Advances in seed priming (Rakshit A & Singh HB, Eds) Springer, Singapore, pp 81 (2018).
7. S. Hussain, F. Khan, H.A. Hussain and L. Nie, *Front. Plant Sci.* **7**, 1-14 (2016).
8. A. Roychoudhury, S. Basu and D.N. Sengupta, *Acta Physiol. Plant.* **34**, 835-847 (2011).
9. O. Lowry, N. Rosebrough, A. Farr and R. Randall, *J. Biol. Chem.* **193**, 265-275 (1951).
10. Addinsoft XLSTAT v. 2015.1.01: data analysis and statistics software for Microsoft Excel. Addinsoft, Paris. (2017).
11. U. Dey, N.K. Mondal, K. Das and J.K. Datta, *Fluoride.* **45**, 389-393 (2012).
12. B.R. Gadi, P. Verma and A. Ram A, *J. Chem. Biol. Phys. Sci.* **2**, 1371-1378 (2012).
13. Y. Tak and B. Asthir, *Fluoride,* **50**, 324-333 (2017).
14. A. Roychoudhury, A. Banerjee and V. Lahiri, *Turk. J. Bot.* **39**, 887-910 (2015).
15. N. Misra, A.K. Gupta and U.N. Dwivedi, *J. Plant Sci.* **1**, 56-66 (2006).
16. S. Chakrabarti and P.K. Patra, *Fluoride.* **48**, 56-61 (2015).
17. A. Rezazadeh, A. Ghasemnezhad, M. Barani and T. Telmadarrehei, *Res. J. Med. Plants.* **6**, 245-252 (2012).
18. G. Yuan, X. Wang, R. Guo and Q. Wang, *Food Chem.* **121**, 1014-1019 (2010).
19. V. Chandrakar, A. Dubey and S. Keshavkant, *J. Soil Sci. Plant Nutr.* **16**, 662-676 (2016).
20. M. Madany and R. Khalil, *Egypt. J. Exp. Biol. (Bot.).* **13**, 119-133 (2017).