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A morphophysiological study in Khamati lahi of rice variety during fluoride stress at seed germination and early seedling stage

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ABSTRACT

The intense behind conducting these studies was to evaluate the toxicological responses of exposing khamti lahi variety of rice to fluoride stress at germination and earlier seedling stage. The experiment was conducting at department of the Botany at Arunachal University of studies Namsai in January 2023.The surface sterilized seed of uniform size were set of germination over the cotton bed in petri plates, treated with the solution of fluoride of various concentration(0,0.5,1mM), prepare from a stock solution NaF(10mM). Results indicated that fluoride could impose significant phyto toxicity during germination and seedling growth during khampti lahi. As compare with the control the germination percentage (55%), germination index (52.2%), seedling vigour index (66.8%), seedling length (33.8%) relative water content (37%) and pigment content was significantly deduced under F stress in the intake 8days old seedling were recorded in khamti lahi variety of rice increasing the dosage of Fluoride.

Keywords: Khamti lahi of rice, Fluoride, germination, seedling growth, pigment

INTRODUCTION

As the primary food crop for 50% of the world's people, rice is farmed on all six continents: Asia, Africa, Australia, Europe, North America, and South America. Though most of the world's rice is grown in Asian countries, India comes in second with 172,580,000 tonnes produced, trailing only China. According to the Food and Agriculture Organization of the United Nations (FAO 2022), Indonesia, Bangladesh, and Vietnam are the top five rice-producing countries in the world. The rice plant belongs to the *Oryza* species of the Poaceae family. Only two of the 24 varieties of the *Oryza* genus are cultivable: *O. sativa* and *O. glaberrima.* There are three further subvarieties of *Oryza sativa: indica, japonica*, and *javanica*. According to Mahajan *et al.* (2017), India produces rice varieties that are a part of the indica subspecies. The main food crop in India is rice, and the production of this crop represents a major part of the nation's economy. It is a vital food for over 65% of India's population. The Ministry of Agriculture and Farmers Welfare of the Government of India broadly categorized India's rice-growing regions into five primary areas during the 2016–17 fiscal year.

Many unusual and ethnic kinds of rice originate in northeastern India. In many places, only specific kind of rice may be found in that location. Due to the high-yielding output, however, the cultivars currently exclusively grow hybrid and common kinds of rice such basmati, black rice, sticky rice, Ranjit, etc. Thailand lahi is a rare species grown in Arunachal Pradesh that is tall, long grained, and sticky in texture. While Khampti lahi is the primary crop in Namsai district, Arunachal Pradesh, Khampti lahi is a tall plant with long, slender grains that are sticky in texture where Arunachal Pradesh is also known as acidic soil (Sharma *et al.,* 2022; Talukdhar and Beka, 2005). Fluoride is a form of fluorine that is an anion. The halogen family of chemicals includes fluoride. According to Greenwood and Earnshaw, (2012), the term "fluoride" can refer to both organic and inorganic fluorine compounds. Fluoride does not occur in nature in its elemental form due to its high reactivity. The oxidative state of the fluoride ion is -1, ranking it as the 13th most prevalent element in the crust of the planet. Numerous environmental, medicinal, and nutritional samples contain it. It is most usually found in the form of chemical compounds like sodium fluoride or hydrogen fluoride in the minerals fluorospar, fluorapatite, topaz, and cryolite (Shashank and Balaaji 2011).

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According to Agalakova and Gusev (2012), fluorides are a prevalent, hazardous, non-biodegradable non-metal pollutant. Fluoride-induced non-metal-induced soil contamination is one of the world's largest problems (Chaudhary and Khan, 2014). Fluoride is necessary for normal plant growth in small doses, but in higher quantities, it can potentially harm both plants and the environment. The manufacturing of aluminum and the mining, processing, and use of phosphate rock as fertilizer for agriculture are the two main human activities that emit fluorides into the environment. Other manufacturing procedures (such as the production of steel, copper, nickel, glass, brick, ceramic, glues, and adhesives) as well as the burning of coal, which contains fluoride impurities, are other sources of fluoride. Once it is in a stable state, fluoride stays in the environment for a reasonable amount of time until it transforms into another chemical or degrades as a result of radiation. The origin and/or absence of clay, the level of organic carbon, the pH, and the soil are the main contributors to fluoride in soils. Additionally, it has been discovered that the fluoride's bioavailability to plants is influenced by the water-soluble fluoride found in saline soils (Al Sabti *et al.,* 2023).

Fluoride generally enters vegetation through two different channels. According to Kamaluddin and Zwiazek, (2003), fluoride initially penetrates the cell walls through stomatal diffusion, permeates them, and then moves to the margins and tips, which are the areas that experience the maximum evaporation. The second channel employs passive diffusion to allow soil and water to reach plant roots. The apoplastic and symplastic xylem channels are subsequently used to transfer fluoride to the shoots in a unidirectional distal migration (Pant *et al.* 2008). Hydrogen fluoride (HF), which diffuses non-ionically, also transports fluoride through cellular membranes. Because the tiny neutral particle of HF enters cell membranes significantly more quickly than a dissociated fluoride ion (F-), there is a more pronounced intracellular intake. Comparing HF to F-, membrane leakage is increased by five to seven orders of magnitude (Pant *et al.* 2008). Fluoride toxicity has an impact on a variety of morphological, physiological, and biochemical processes, including seed germination parameters, growth, development, mineral nutritional status, photosynthesis, respiration, metabolic activity, yield and yield characteristics, and others (Sahariya *et al.* 2021). Researchers have discovered that fluoride-treated plants have significantly lower growth and related parameters than control plants, including seedling germination %, roots and shoot length, plant height, and fresh and dry biomass (Singh *et al.,* 2013). In this article, mainly focus the effect of fluoride in Local Khamti lai rice variety where the Arunachal Pradesh becomes acidic soil.

MATERIAL AND METHODS

The local variety seeds of *Oryza sativa* L., also known as Khamti lai, were collected from Namsai's local farmers. After being surface sterilized in a 1% (w/v) NaOCl solution for 15 minutes, the seeds were repeatedly rinsed with distilled water. After being sterilized and sized uniformly, the seeds were treated with a sodium chloride solution derived from a stock solution (1.0M) and placed on a cotton bed in a petri plate for germination. Four NaF dosages (0, 0.5 and 1.0 mM) were examined using a randomized approach in three replicates. Each glass petri plate held twenty seeds, to which 10.0 ml of a solution (distilled water or sodium fluoride) was added. The following protocols were used to measure the final germination percentage (FGP), germination index (GI), germination energy (GI), and finally the seedling vigour index (SVI) and seedling length: Li. (2008); Abdul-Baki and Anderson (1973); and Moulick *et al.* (2016). A few specific biochemical parameters, like the amount of carotenoid and chlorophyll, were measured using the Arnon (1949) methodology. The software SPSS 21 (Window version) was used to analyze the data using correlation analysis, mean (n=3), and standard error (mean SE) formats.

RESULTS

Seed germination is an important stage in the life cycle of any plant, and it is greatly influenced by a range of environmental conditions (Chowardhara *et al.* 2020).

Effect of Sodium fluoride on germination of khampti lahi

Under the stress condition NaF, the germination% rate was significantly($p<0.001$)

reduced at 1mM (55%) or discriminated as the days increases with compare to control respectively (Table 1). As a result, it also effects on germination index where the concentration increases the germination index also reduced (25 and 55%) at 0.25mM and 1mM as compare with control respectively Table 1. The germination energy also influences by NaF as compared with control where 55% was reduced at 1mM significantly as well as also Relative germination rate also effected as the concentration decreases to 55% and 25% at 0.5mM and 1mM as compare with control (Table 1). The Relative metal injury index was also measure in khampti lahi under the NaF stress condition as a result the relative metal injury index was significantly hampered(*P<0.001*).

Under NaF stress condition, the moisture content as well as Relative water content was significantly drop in seedling plants as compared untreated plants (Table 1) Along with the Seedling vigour index was also significantly was reduced 67% at higher concentration.

Table 1: Consequences of F toxicity on germination, seedling growth, germination energy, relative injury index, germination index and seedling vigor index (intact seedlings) at 8 DAS

NaF Conc. (mM)	FGP	GE	GI	RGR	RSIR	SVI
0	100±0	10±0	12.5±0	1±0	1	1348.77±0.77
50	75± 1.0***	7.5±0.5*	9.37±0.62*	0.75±0.058	0.25±0.10***	832.38±0.38***
100	45± 1.0***	4.5± 0.5**	5.93±0.31**	0.45±0.18**	0.55±0.22**	446.45±0.45***

Effect of NaF on Morphomatric attribute

Under the NaF stress condition, the seedling length was significantly reduced as a concentration of fluoride in khampti lahi variety due to higher dosage of fluoride which inhibit the cell division. The seedling length at high concentration was significantly decreases (34%) khampti lahi seedling plants as compare to untreated plants (Table 2). Similar result also observed in biomass that is Fresh weight (59%) and Dry weight (78%) was significantly reduced as the dosage increases (at 1mM NaF) whereas compare to control plants.

Table 2: Consequences of NaCl toxicity on seedling growth, fresh weight, turgor weight, dry weight and relative water content (intact seedlings) at 7 DAS (days after sowing)

NaF Conc. (mM)	Seedling length (cm)	FW (gm)	DW (gm)	RWC
0	13.66±0.18	0.88±0.01	0.66±0.01	0.145±0.005
50	10.43±0.03*	0.59±0.01***	0.435±0.02**	0.125 ± 0.005^{ns}
100	8.38±0.54**	0.375±0.005***	0.145±0.005***	0.085±0.005*

Effect of NaF on Chlorophyll pigment

Chl *a* (90%), Chl *b* (87%), total chl (73%), Ratio of Chl *a* and chl *b* (1.03) was significantly deduced in khampti lahi under NaF stress condition (Table 3) as compared with control due to the damage pigment structure damage.

Table 3: Consequences of NaCl toxicity on Chlorophyll a, Chlorophyll b, total chlorophyll, Carotenoid and Chlorophyll a and b ratio (intact seedlings) at 7 DAS (days after sowing)

NaF Conc. (mM)	Chl *a* (mg g^{-1} Fw)	Chl *b* (mg g^{-1} Fw)	Total Chl (mg g^{-1} Fw)	Carotenoid (mg g^{-1} Fw)
0	23.15±0.35	17.72±0.47	41.55±0.45	13.69±40
50	5.94±0.06***	10.89±0.30**	16.87±0.12***	3.89±0.11***
100	2.28±0.07***	2.04±0.06***	4.55±0.05***	3.86±0.02***

Membrane Injury Index

Membrane Injury Index meaning leakage of ions from seedling plants. The result showed that the gradually enhanced the concentration of 0.5mM and 1mM as a consequence of control seedling plants.

Table 3: Correlation coefficients among GP%, GI, GE, RGR, RMIR, SVI, SL, FW, DW TW, RWC, Chl a, Chl b, total chl and cartenoids

Parameters	GP%	GI	GE	RGR	RMIR	SVI	SL	FW	DW	TW	RWC	Chl a	Chl b	Total Chl	carotenoids
GP%	1	0.99***	0.99***	0.99***	-0.99***	0.98***	0.94**	0.06*	0.99***	0.502^{ns}	0.99***	0.956*	0.903*	0.962**	0.81*
GI		1	0.967**	0.995***	-0.995***	0.993***	0.961**	0.986***	0.997***	0.567^{ns}	0.946***	0.933***	0.995***	0.978***	0.850*
GE			1	0.987***	-0.985***	0.967**	0.914*	0.925**	0.454^{ns}	0.984***	0.935**	0.871*	0.987***	0.939***	0.785ns
RGR				1	-0.99***	0.990***	0.49**	0.969***	0.521^{ns}	1.00***	0.952**	0.913*	1.0***	0.969***	0.826*
RMI					1	-0.991***	-0.58**	-0.972**	-0.530^{ns}	-0.999***	-0.950**	-0.917***	1.00***	-0.71***	-0.833*s
SVI						1	0.977***	0.991***	0.636^{ns}	0.0991***	0.961***	0.991***	0.991***	0.911*	0.961**
SL							1	0.981***	0.704^{ns}	0.950**	0.865*	0.970***	0.950***	0.983***	0.925**
FW								1	0.276^{ns}	0.733*	0.867*	0.867*	0.733*	0.333^{ns}	0.333^{ns}
DW									1	0.867*	0.867*	0.867*	0.733*	0.333^{ns}	0.333^{ns}
TW										1	0.247^{ns}	0.138^{ns}	0.414^{ns}	0.414^{ns}	0.828*
RWC											1	0.733*	0.733*	0.600^{ns}	0.200ns
Chl a												1	0.943**	0.657^{ns}	0.429^{ns}
Chl b													1	0.943**	0.657
Total Chl														1	0.714ns
Cartonoids															1

**p<0.05, **p<0.01 and ***p<0.001*

Correlation analysis

The result showed analysis among Germination %,Germination Index, Germination Energy, Relative germination rate, Seedling vigour index, Seedling length, fresh weight, dry weight, turgid weight, Relative water content chlorophyll *a, b* total chl, Relative metal injury most of the parameters are positive correlation ship except RMIR with GP, GR, GE, RGR, SVI, SL, FW, DW, TW, RWC, chl *a*, chl *b*, total chl and carotenoid (Table 4) i.e., the increase of the concentration of the NaF also effect on the others parameters also.

DISCUSSION

Phytase, an enzyme, breaks down phytin during germination to give the developing seedling inorganic phosphate. Fluoride suppresses the dephosphorylation of phytin molecules in tissues, which reduces seedling root growth during germination. Fluoride also inhibits the phytase enzyme. For the metabolism of RNA, orthophosphate sources produced from phytin are necessary. Lack of phytin-derived orthophosphates is one of the factors that could limit the growth of seedlings treated with fluoride. Increases in fluoride concentration were found to reduce seed germination in *Triticum aestivum, Zea mays, Helianthus annuus, and Vicia faba* (Shadad *et al.* 1989), as well as *Cicer arietium* (L), (Kumar *et al.* 2017 and Pelc *et al.* 2020), *Solanum lycopersicum* (Ahmad *et al.* 2018), *Cajanus cajan* L. (Yadu *et al.* 2018), cv Anuradha (Datta *et al.* 2012), *Raphanus sativus* L. (Singh *et al.* 2013), Fenugreek (Burgohain and Chowardhara, 2022).According to Shabl *et al.* (2006), increasing the concentration of NaF considerably reduces *Cyamopsis tetragonoloba* seed germination, seedling growth, and biomass when compared to the control. *Oryza sativa* has also been linked to similar discoveries (Gupta *et al.* 2009; Banerjee and Roychoudhury, 2019a; Singh and Roychoudhury, 2020; Banerjee and Roychoudhury, 2021a). One of the most crucial processes in plants is photosynthesis. The photosynthesis is influenced by a variety of circumstances, including heavy metal impact on plants. Different air contaminants may impair a number of metabolic pathways and mechanisms as well as the production of agricultural crops (Kumar *et al.* 2017). Plant response to pollution is influenced by the chemical element's toxicity, the length of exposure, and the species' sensitivity (Oguchi *et al.* 2005). Due to its electromotivity, electronegativity, and significant phytotoxic potential, F stands out among the pollutants. Above all of these factors, it has the capacity to preferentially enter through the stomata (Franzaring *et al.,* 2007). The cells and tissues in the leaves suffered ultrastructural and structural damage as a result of the F buildup. Plant stomatal conductance and gas exchange will be significantly impacted by the damage to cells and tissues. The epidermis and stomata of young coffee (*Coffea arabica*) and orange (*Citrus X sinensis*) plants are harmed when exposed to hydrogen fluoride (HF) in a semi-open mist chamber, according to Mesquita *et al.* (2011). It might lead to a breakdown in the mechanisms that regulate stomatal opening and closing. F decreases the activity of several enzymes in the chloroplasts, including ATP synthase, ribulose bisphosphate carboxylase-oxygenase (RuBisCo), and sucrose synthase. Fluoride greatly increased in concentration in plants impeded photosynthesis as well. F reduces the production of chlorophyll, destroys chloroplasts, and slows down the Hills response to prevent photosynthesis. A decrease in chlorophyll content also has an adverse effect on plant photosynthetic systems. Finally, this led to a decrease in CO_2 generation and assimilation (Domingues *et al.* 2011). The photosynthetic electron transport pathway in plant thylakoid membranes was examined after F exposure. The electron transport rate of PSII is inhibited as F builds up, and then PS-I's electron transport rate rises as a result. This result suggested state changes as a potential mechanism for F toxicity. F treatment at 190 ppm reduces photosynthetic pigments in plants. *Salicornia brachiate* Roxb showed comparable results in Reddy and Kaur (2008) study. Plants grown in F-polluted soil showed decreased photosynthetic capacity, Chl-*a* and Chl-*b* concentrations, total chlorophyll, carotenoids, and leaf area (Kumar and Rao, 2008; Ram *et al.* 2014). According to Gupta *et al.* (2009), F may have hindered chlorophyll production, lowering plant chlorophyll concentration. The amount and activity of the enzyme chlorophyllase, which breaks down chlorophyll, are anticipated to rise when F accumulation (Ram *et al.,* 2014). When plants were grown on soil contaminated with F in the semi-arid region, similar effects were seen (Baunthiyal and Sharma, 2014). Fluoride concentrations above a particular threshold have an adverse effect on photosynthesis, which leads to a decrease in the formation of dry matter (Zouari *et al.,* 2016). SVI reduced in higher concentration than to control by (33% and 66%) in 0.5mM and 1mM as a same have been studied in *Trigonella foenum-graecum* at 7 DAS that it significantly decreased similar to the khampti lahi by (37.6%,60.6% and 80%) of 2,4,6mM concentration of fluoride.

In other parameters stress has also been found as same like in Cadmium and Zinc on Indian mustard (*Brassica juncea*) (Chowardhara *et al.* 2020; Chowardhara *et al.* 2019). Membrane injury index is almost equal in all the concentration which shows that in control injury has been hampered MII has also studied in other stress like in Auminium which shows gradually increased from control to higher concentration (Panda *et al.*2003).

CONCLUSION

The findings suggested that fluoride may cause serious phytotoxicity during khampti lahi seedling development and germination. Under F stress in the input, there was a substantial decrease in the germination percentage (55%), germination index (52.2%), seedling vigour index (67%), seedling length (34%), relative water content (37%), and pigment content as compared to the control. In the Khampti Lahi type of rice, 8-day-old seedlings were seen when the fluoride dosage was increased. Since khampti lahi is a native rice plant that is grown in a specific area of the Namsai district in Arunachal Pradesh, no research has been done on it under abiotic stress conditions up to this point.

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