

Role of glutathione reductase and catalase enzyme in antioxidant defense mechanism in controlling fluoride-induced oxidative stress

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ABSTRACT

Fluoride stress is one among the majority of significant abiotic stresses. Fluoride affects plants in negative manner through increasing the level of ROS species and reducing the plant growth. In this studio; *Vigna radiata* L. was exposed to fluoride stress in a half-diluted Hoagland solution. In this study, a concentration-dependent analysis (0, 2.5, 5, 7.5, 10, 12.5, and 15 mM NaF) of antioxidant enzyme activity performed against the most significant inhibition of growth levels and content of malondialdehyde, the reduce in chlorophyll contented be report in seedlings treat by NaF compared to controls. Antioxidant biochemical expression (catalase, glutathione reductase) showed the highest activity at 7.5 mM NaF and showed a significant defense against fluoride stress during the harvest. This study would help to appreciate the responsibility of antioxidants within the survival of plants against fluoride stress.

1. INTRODUCTION

Legumes are the greatest resource of protein and participate in particular essential responsibility in meeting the needs of speedily increasing residents. Mung bean *Vigna radiata* (L.) is an important legume crop in the undersized summer period which is mainly grown up and used for its high protein edibles. Mung beans are alive an extremely affluent basis of simply edible proteins. It contains approximately 24.5% protein, 59.9% carbohydrate, and also 1.2% fat [1]. It has a extremely high-quality capacity in the direction of progress the chemical, physical, and organic properties of the soil; then it is measured a significant element of sustainable crop growing [2]. Mung beans are short-lived and require with a reduction of water than additional summer crop so it be able to be developed inside rainfed area [3].

Legumes belonging to the family of legumes have been used as food used for thousands of time also known as legumes, they are high in protein and easy to digest [4]. Abiotic stress significantly reduces the efficiency of almost all legumes, include mung beans [5]. Although the unfavorable effects induced by stress are variable at different stages of growth, as in mung beans, the negative achieve going on particle give up be additional in the reproductive step than in supplementary stage [6].

A free essential can be alive define like a molecule previous to molecular fragments that contain one or else extra unpaired electrons inside its furthest atomic or else molecular orbital as well as that are able of an autonomous extinction [7]. Reactive oxygen species (ROS) and reactive nitrogen species be free radicals also other non-radical reactive derivatives. The reactivity of the radical be in general stronger that of the non-free radical species, although the radicals be less constant [8]. Free radical be produced since molecules inside the homolytic cleavage of a substance connection and, by redox reaction previously created these greatly immediate radical be able to trigger a chain reaction [9,10]. Oxidative stress can damage all molecular targets. DNA, proteins and lipids, and the primary spot of attack are often not clear because the mechanisms of injury overlap widely the most important cellular goal of oxidative stress. Genetic material be an essential early target of damage [11].

Mung beans are generally considered a salt-sensitive crop [12]. Saline stress causes a substantial reduction in the growth of mung beans. Intended example, salt stress has been establishing to decrease seed germination, fresh with dry biomass, length of shoot and roots, also give here attribute of mung beans [13-15]. This reduction in mung bean growth increases with increasing salt diets [16]. Photosynthetic capability be alive condensed here mung beans in salty regime [17]. The decrease in the rate of photosynthesis is attributed to the decrease of stomatal conductivity and thus to the reserve of CO₂ ease of use for carboxylation [18]. Saline stress affects the accumulation of nutrients in mung beans [19]. More than a few researchers contain report a rapid enhance inside Na⁺ and Cl⁻ levels with a reduce in Ca²⁺ with K⁺ levels in the leaves, stems, and roots of mung beans [20-22]. Antioxidant enzymes activities have been reported it increases in most plants including mung

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beans under the salt stress [23,24]. These better activities of antioxidant enzymes catalase (CAT, superoxide dismutase [SOD], and peroxidase [POD]) in non-enzymatic antioxidants tocopherols, ascorbate also phenolic compound assist out toward defend mung bean plants since the break of ROS induced by salt [25]. As previously reported, compared to most known legumes, mung beans are comparatively extra responsive salty stress. Therefore, it is estimated to their metabolic process will be cruelly affected by means of salty stress. Therefore, the most important object of the present study be just before appraise the salinity-induced inflection in certain key in morphologies.

2. MATERIALS AND METHODS

2.1. Plant Materials, Fluoride (NaF) Treatment, and Experimental Plan

Mung bean (*V. radiate* L.) var. RMG-492 was obtained for experimental purpose from the Rajasthan Agricultural Research Institute (Sri Karan Narendra Agriculture University Jobner), Rajasthan, India, designed for the current study. Seeds were surface cleaned by 0.5% sodium hypochlorite used for 15 min, furthermore, washed in the distilled water. After that, sterilized seed germinates into a Petri plate contain filter paper pre-soaked by the distilled water at 24 h in the dark condition. After 3 days, the appearance of the planula, ten seedlings were transferred to each plastic pot contain half-strength Hoagland nutrient solution with tolerable to produce up and about in a thermostatically proscribed room culture maintained by $28 \pm 2^\circ\text{C}$ and at $500 \mu\text{mol}^{-2} \text{s}^{-1}$ expose to 16 h photo-period. Seedlings of (*V. radiate* L.) 15 day-old acclimated were treated by different concentrations of NaF (0, 2.5, 5, 7.5, 10, 12.5, and 15 mM) for 5 days. The control plant was maintained in a half-strength Hoagland medium without fluoride treatment.

2.2. Physiological Parameters

Physiological parameter was calculated at the same time as root extension rate along with development velocity by measure root length (RL) and fresh weight earlier than also later than conduct with fluoride stress.

Root elongation rate (cm day⁻¹): mean final longest RL–mean initial longest RL/ Δt ($t_2 - t_1$);

(RL: Root length; t_1 : Last day of treatment; t_2 : Initial day of treatment).

$$\text{Growth rate (FW g day}^{-1}\text{)} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

(W1: Fresh weight record initially; W2: Final record fresh weight; T_1 : Period previous to treatment; T_2 : Period after treatment).

2.3. Total Chlorophyll Estimation

The fresh leaves (0.1 g) of *V. radiata* L. in the sample were ground well with 80% chilled acetone in a dark as centrifuged by 10,000 rpm for 10 min at 4°C . The method used by Hiscox and Israelstam [26]. The sample be heated here an oven at 65°C used for 1 h in addition after cool just before room temperature, the absorbance of the extract be recorded at 663 nm, 645 nm, and 480 nm. The chlorophyll content was calculated according to the method given by Arnon [27].

Chl a: $(12.7 \times A_{663} - 2.69 \times A_{545})$.

Chl b: $(22.9 \times A_{645} - 4.68 \times A_{663})$.

Total chlorophyll = $20.2 \times A_{645} + 8.02 A_{663} \times V/W \times 1000$.

2.4. Lipid Peroxidation Estimation

The lipid peroxidation be calculated by the root and leaves in recording malondialdehyde (MDA) level through the method [28]. Tissue from plants extract into 10 ml of 2-thiobarbituric acid at 0.25% (w/v) in 10% trichloroacetic acid. The mix up be incubated at 95°C inside a water bath with stirring use for 30 min by the response was congested up in cool the tube inside an ice-water bath. After that, the sample be centrifuged by 3000 rpm for 10 min along in the absorbance of the supernatant be study on 532 nm and 600 nm (extinction coefficient: $155 \text{ mM}^{-1} \text{ cm}$).

2.5. Proline Estimation

The content of proline was measured of leaves and roots [29]. Plant tissues crushed (0.3 g) in 5 ml of freshly prepared 0.3% sulfosalicylic acid. Then, the sample was centrifuged at 3000 rpm for 20 min. After that, the 1 ml of ninhydrin acid in addition to 1 ml of glacial acetic acid be mixed with the content of proline in the supernatant. The mixture was incubated at 100°C into a water bath with stirring intended for 1 h. The effect was stopped in cooling the tube in a bath of ice and water. The mixture was then added to 2 ml of toluene and then shaken for 1 min. The absorption of the investigated supernatant at a content of 520 nm in proline was expressed in $\mu\text{g/g}$ mass.

2.6. Preparations Estimation Antioxidant Enzyme Assay

2.6.1. Enzyme extract preparation

Fresh leaf material (0.1 g) be homogenized by a mortar and pestle within 3 ml ice-cold conditions 50 mM phosphate buffer pH 6.8 containing 0.05% Triton-x 100, 1 mM EDTA 1 mM ascorbate acid, and also 2% PVP. After homogenate at 14,000 rpm for 20 min with supernatant be used further enzyme assay with stored at -20°C .

2.6.2. (CAT; EC1.11.1.6) activity estimation

The activity of CAT has been determined [30]. Enzyme extract 0.1 ml of in 3 ml of a solution contains 50 mM phosphate buffer (pH 7.0) in addition to 3.125 mm of H_2O_2 in addition to the reduce into absorbance be measured for 5 min at 240 nm.

2.6.3. Glutathione reductase (GR; EC1.8.1.7) activity estimation

The activity of GR has been determined [31]. Enzyme extract 0.1 ml of in 2 ml of a reaction mix contains 0.5 mM EDTA, 0.1 mM phosphate buffer (pH 7.5), 1 mM GSSG, and 0.75mM -5'-dithiobis (acid) 2-nitrobenzoic acid) with 0.1mM NADPH: Absorbance be record for 5 min on 412 nm (extermation coefficient $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$).

2.7. Statistical Analysis

The every part of experiments was performed here in triplicates ($n = 3$). The value in the table, text, and figures signifies mean value \pm standard deviation (SD). The difference between control and treatment was examined statistically in means of the t-test, with the level of significance be $P < 0.05$.

3. RESULTS

3.1. Physiological Parameters of Effect Under the Fluoride Stress

During this study, the rate of root elongation of *V. radiata* (L.) increasing considerably ($P < 0.05$) by decreased fluoride concentration [Table 1]. The growth rate was well significantly reduced ($P < 0.05$) by increased fluoride concentration.

3.2. Chlorophyll Content, Lipid Peroxidation, and Proline Estimation of Effect Under the Fluoride Stress

In the present experiment of chlorophyll estimation, a significant decrease in Chla was recorded significantly ($P < 0.05$) with all treated leaf samples except 5 and 7.5 mM. On the other hand, Chlb also decreased significantly ($P < 0.05$) in all treated leaf samples excluding 5 mM. Total chlorophyll also decreased considerably ($P < 0.05$), except for in a NaF concentration of 5 and 7.5 mM. The highest decrease in Chla and Chlb with total chlorophyll was recorded at 15 mM NaF, approximately 1.8 times compare toward control [Figure 1a].

In fluoride stress, the MDA content was increased considerably ($P < 0.05$) in 5 mM treated root sample. In the case of leaf sample, significant increase was recorded. An exceptionally high level of MDA

be experiential in the leaves, root sample at 15 mM NaF compare to control plants [Figure 1b].

Under fluoride stress, the proline content has increased considerably ($P < 0.05$) inside the leaf and root sample of *V. radiata* (L.). An exceptionally high level of proline be observed inside the root, leaves sample at 15 mM NaF compare to control plants [Figure 1c].

3.3. Response of Usual Antioxidants (CAT and GR) to Fluoride Stress

In leaf and root sample, the CAT activity has increased considerably ($P < 0.05$) toward 7.5 mM NaF, in leaves subsequent to which it increased Figure 2a. In the case of root sample, CAT activity was highest at 7.5 mM.

In the tissues of the leaves, the level of GR significantly ($P < 0.05$) higher than that of the control in the entire concentration Figure 2b. The GR activity was highest with 7.5 mM NaF treated roots and leaves.

Table 1: Change in growth parameters in *Vigna radiata* (L.) at different NaF concentrations

NaF (fluoride) concentration (mM)	Root elongation rate (cm/day)	Plant growth rate (g fresh weight/day)
Control	0.0184±0.0041	0.0176±0.0030
2.5	0.0394±0.0123	0.0194±0.0141*
5	0.0464±0.0094*	0.0531±0.0053*
7.5	0.0709±0.0141*	0.0642±0.0034*
10	0.0526±0.0203*	0.0398±0.0062*
12.5	0.0582±0.0121*	0.0515±0.0009*
15	0.0582±0.0121*	0.05153±0.0030*

Data presented are mean±standard deviation ($n=3$); *significant mean difference from control at $P<0.05$ according to *t*-test

4. DISCUSSION

Increasing concentrations of NaF show phytotoxic effects on the physiology and biochemical parameters of seedling growth. Sodium fluoride can affect some processes in the development of cereals in germination. Weinstein suggested that NaF could inhibit the carbohydrate metabolism of germinating seedlings [32]. Increasing the concentration of sodium fluoride. It shows phytotoxic effects in the parameters that determine the morphology, biochemistry, and phytotoxicity. Fluoride reduces the length of the leaves and root due to the unbalanced absorption of nutrients by the seedlings in the presence of fluoride [33].

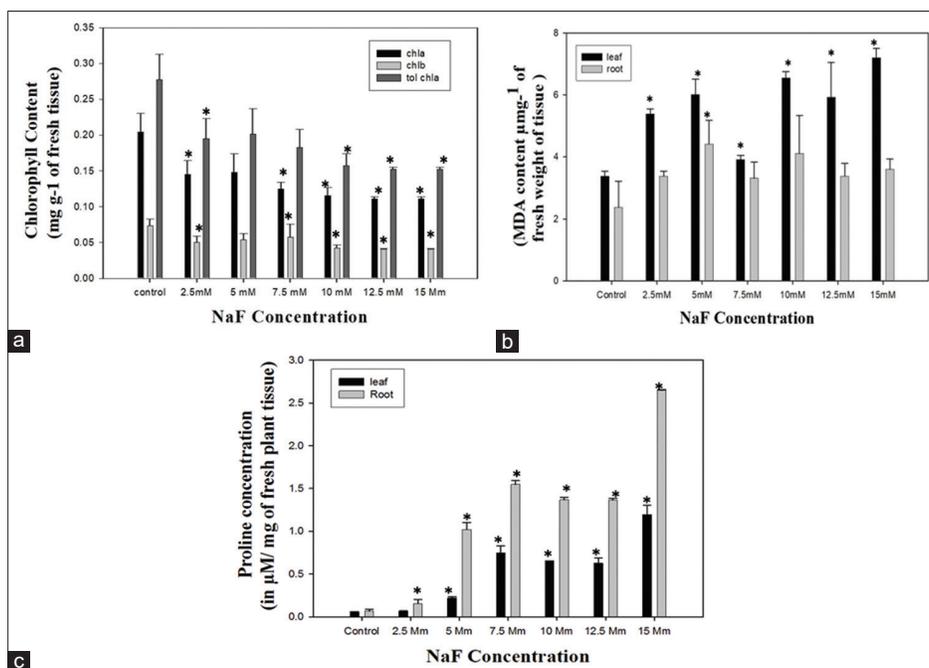


Figure 1: (a) Chlorophyll content of seedling treated by 0–15 mM fluoride (NaF) concentration used for 5 days. Bar shows standard deviation (SD), also statistics point marked by asterisks indicate to mean value be significantly dissimilar among treatment with control ($*P < 0.05$). (b) malondialdehyde (MDA) content of seedling treated by 0–15 mM fluoride (NaF) concentration used for 5 days. Bar shows SD, also statistics point marked by asterisks indicate to mean value be significantly dissimilar among treatment with control ($*P < 0.05$). (c) Proline content of seedling treated by 0–15 mM fluoride (NaF) concentration used for 5 days. Bar shows SD, also statistics point marked by asterisks indicates to mean value be significantly dissimilar among treatment with control ($*P < 0.05$)

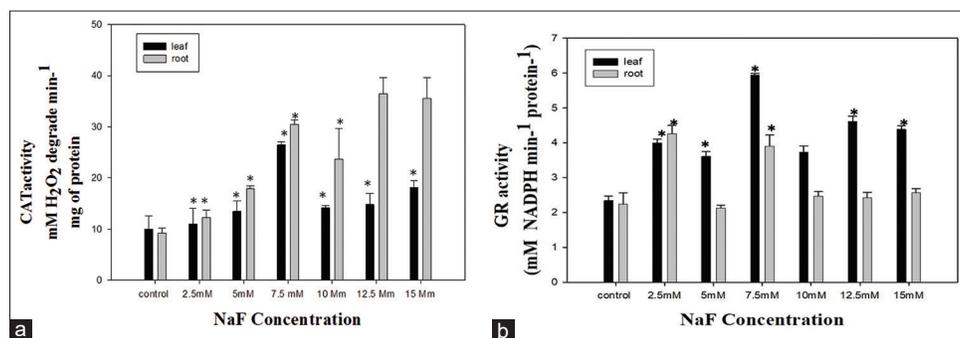


Figure 2: (a) Change inside activity CAT within (*Vigna radiata* (L.) underneath NaF (0–15 mM) concentration value be means of three replicates, in addition to bar explain standard deviation (SD). Statistics point marked by asterisks signify to mean value be significantly dissimilar among treatment with control ($*P < 0.05$). (b) change inside glutathione reductase activity within (*Vigna radiata* (L.) underneath NaF (0–15 mM) concentration value be means of three replicates, in addition to bar explain SD. Statistics point marked by asterisks signify to mean value be significantly dissimilar among treatment with control ($*P < 0.05$)

The current study showed to an enhance in NaF concentration had a negative consequence undergrowth of seedling (*V. radiata* L.). The elongation rates with root development decrease with increasing fluoride concentration. The decrease in the growth rate of the plant is due to poor photosynthesis due to the partial closure of the orifice and the low absorption of water. The hydrolysis of food stocks in storage tissues is limited, interrupting the transport nutrients to the growth alignment [34-36].

The decline content in chlorophyll was observed under fluorinated stress. A higher concentration of NaF has more inhibitory effect than a smaller decrease in pigment content, due to the inhibition of the biosynthesis of chlorophyll during the degradation of chlorophyll [37]. The consequence of fluorine under total chlorophyll with carotenoids of the leaves of three investigational plant life correspondingly. The initial fluoride concentration was higher in the pigments than inside the control, as well as the limit decline inside total chlorophyll in addition to carotenoids was create by 25 mg/l of the preliminary fluoride concentration of 30.15%, 29.44% as well 31.4%, 17% of total chlorophyll with 35.91%. 31.79% with 37.61% of carotenoids be decomposed, correspondingly inside the leaves of *Pistia stratiotes*, *Eichhornia crassipes* with *Spirodela polyrhiza* on a fluoride concentration of 25 mg [38].

This increase in chlorophyll in the presence of F is an exceptional finding and differs from most previous studies in species range 6, including another cultivar (Anuradha) from *Cicer arietinum*. In a study of *Triticum aestivum*, a steady increase in root and shoot length and chlorophyll content was reported in 20 and 40 g/ml NaF. Current observations at *C. arietinum*. Azad corresponds to the results and may be due to a genotypic response to fluoride stress. Unlike barley, in the case of barley, the content of all photosynthetic pigments, i.e., total chlorophyll, chlorophyll a, b, and carotenoids, decreased significantly even at the highest level low concentration of NaF (1.0 mM). This decrease may be due to the inhibition of chlorophyll biosynthesis (because it has been found that a high F reduces the availability of Fe₂₊ + ions necessary for the synthesis of chlorophyll), or the greater degradation of chlorophyll. Chlorophyll during fluorine stress [6]. Protect chlorophyll from oxidative stress. The chlorophyll content of barley has significantly decreased with increasing levels of fluoride. A comparison of the interrelation of Chl with: Chlb/a showed that variations up to 5.0 mM NaF of chlorophyll are mediated by both chlorophyll a and b but at 10.0 mM, mainly by chlorophyll b [39].

The ROS generated in photosynthesis or else sheet respiration tin change the reliability of the membrane, leading to lipid peroxidation [40]. During

this study, the levels of MDA with H₂O₂ increased significantly. The contented of MDA, a produce of lipid peroxidation of the membrane, be regularly used since an indicator of oxidative injury [41]. In this study, the levels of MDA and H₂O₂ increased significantly. The accumulation of H₂O₂ and lipid peroxidation has resulted within a significant reduce inside the stability of the cell membrane. This quantity be calculate since the amount of MDA produced suitable to the peroxidation of polyunsaturated fatty acids into the membrane. Salt stress causes the escape of ions, which causes serious damage to the reliability of the membrane. The increase of result of the lipids peroxidation and H₂O₂ in a significant decrease inside the stability of the cell membrane. The root tissue shows improved retention of ROS along with better avoidance of break to the membrane than to leaf tissue, indicating elevated levels of SOD enzyme here the root tissue. Lipid peroxidation be usually considered an pointer of oxidative stress, in addition, to be quantitatively determined by the content of MDA. Treatment with F causes the formation of superoxide free radicals and ROS in the form of polyunsaturated fatty acid peroxides, which on decomposition form MDA, the most common product of degradation of aldehyde lipids. The root is better protected from oxidative stress than shoots [42].

Excess fluoride is also known for its neurotoxic effects since it increases lipid peroxidation in the brain and reduces the activity of acetylcholine esterase in young people and adults. In addition, it has been reported that increasingly high levels of fluoride reduce the intelligence and memory of children. Chronic exposure to high doses of fluoride causes turbid edema, tubular epithelial degeneration, tissue necrosis, tubular vacuolization, glomerular hypertrophy, and interstitial edema of the kidneys, which leads to nephritis [43].

The higher proline content in an epidemic reflects its best defense mechanism under the root of oxidative accumulation. Proline plays an important role as an osmolyte in activating water uptake to maintain cellular disorder interpretation used for larger membrane integrity here preceding varieties [44].

The content of proline was 27% higher than in controls with a NaF concentration of 1 mM. On the other hand, it was slightly reduced to a higher concentration of seedlings treated with NaF. In *V. radiata*, the accumulation of proline caused by fluorine occurs. This reduces the activity of catabolic enzymes of proline, and the increase in the activity of its biosynthetic enzymes also increases the accumulation of proline under stress conditions [45].

Under fluoride stress surroundings antioxidant enzymatic activity exhibit unlike pattern. The CAT action inside the leaf and root sample

be better than that of the control. Elevated CAT activity is experimental at 7.5 mM NaF with under this concentration the H₂O₂ levels were low. It recovers H₂O₂ separating it directly from water and oxygen. Here be a significant association ($P < 0.05$) connecting the enhance in CAT activity and the concentration of NaF. Similar patterns of increased CAT activity have also been obtained by increasing salinity levels in *T. aestivum* [46]. Elevated CAT the stage a significant role inside the deduction of H₂O₂ formed here peroxisomes by oxidases occupied during β -oxidation of fatty acids, photorespiration in addition to catabolism of purines. It is interesting to note that CAT activity decreases significantly with the use of NaF both in the root and in the induction of a tolerant culture.

Oryza sativa's F-stress also reduced CAT. The data obtained indicate that when the F toxicity of OH[•], bonded to Fe₂ + CAT atoms, is replaced by low molecular weight anions, thereby inhibiting the activity of the enzyme. In contrast, high levels of POD and ascorbate peroxidase (APX) were measured in *C. arietinum* and *O. sativa*. In addition, a dose-dependent decrease in GR was observed in F treated with *Helianthus annuus*. As in the case of candidate enzymes, the AA content initially decreased in response to F, then increased sharply with increasing exposure to F and F concentration in *O. sativa* [47].

The activity of CAT and APX decreased in the shoots of rice seedlings exposed to salinity stress [48].

The reticence of enzymatic behavior on high-stress concentration can be attributed toward changes induce by ROS, such as DNA break transduction changes, protein division along with greater susceptibility toward proteolysis [49]. GR be aggressively involved inside the translation of oxidized glutathione (GSSG) interested in reduced glutathione (GSH) with maintains an elevated proportion of GSH/GSSG. POD be the enzyme that recovers the H₂O₂ produced during the disproportionation of O₂ mediated in SOD. The uppermost activity of these enzymes be record by 7.5 mM NaF into root and leaf sample The increase here POD activity cause in salinity be here glowing recognized [50-52]. As report by Abogadallah[54], POD, CAT, and GR impress a strict control of H₂O₂ concentration. The current study, the entire antioxidant enzymes (CAT and GR) be the lowest on the uppermost concentration of fluoride (15 mM), probably due toward deactivation. Several antioxidant enzymes such since SOD, CAT, APX, and GPX be best study [Figures 3 and 4]. Although in universal each and every one enzyme contain prospective antioxidant resistance activity that helps eliminate ROS inside different conduct reduction of H₂O₂, preservation of the AsA set otherwise adequate levels of GSH with GSSG which in concert preserve the antioxidant sense of balance. During addition, the antioxidant enzymes proceed inside dissimilar subcellular compartment thus contributing just before the detoxification of ROS into organelles such because chloroplasts, mitochondria, peroxisomes, and cytosol. During addition, enzymes that trap ROS in more than a few subcellular compartments may contain a synergistic consequence just before advance stress tolerance here plants [53].

5. CONCLUSION

The previous study has shown that CAT and GR genetic resources play an essential role in the protection of antioxidants against various abiotic stresses. The roots are the first plant in the plant that is resistant to the stress of the average fluorine content or transfer of CAT and GR from the roots and leaves due to the increase in the activity of among the species studied *V. radiata* L. It 15 Mm, CAT and GR decreased compared to 7.5 mM NaF. The 7.5 mm of NaF, very high

activity of certain antioxidant enzymes (CAT, and GR) was observed in different parts of the plant. The results of this study explain that the basic defense response of the plant to stress by fluoride is through the antioxidant enzymes CAT and GR. This study allows us to better understand the complexity of the defensive system include CAT and GR, against NaF stress, which determination of use for future research on the improvement of fluorine-resistant varieties.

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