



Salinity Induced Abiotic Stress Enhances Nutritional Value of *Vigna radiata* Sprouts

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ABSTRACT

Background: Incorporating edible sprouts into the diet offers a wealth of nutritional benefits for both humans and animals. They are rich in proteins, vitamins, minerals and enzymes, making them a valuable addition to diets. Exogenous pre-harvest treatment can modify the biological activity and metabolite synthesis of sprouts, enhancing their nutritional and functional properties.

Methods: We aimed to determine the effect of different concentrations of NaCl (50 and 200 mM) on *Vigna radiata* sprout growth and health-promoting properties. 24, 48 and 72 h germinated sprouts were treated with NaCl (50 and 200 mM) for 1h incubated till 96h and were harvested for nutritional analysis.

Result: Both sodium chloride treatments to 48 h germinated seeds showed significant increase in protein, sulphur, phenolic and flavonoid content in vitamin C compared to control. While both sodium chloride treatments to 72 h germinated seeds showed significant increase in sulphur, free radical scavenging activity, phenolic and flavonoid content and vitamin C compared to control. The results revealed that NaCl treatment resulted in sprouts with improved nutritional value without disturbing their yield.

Key words: Abiotic stress, Nutrition, Sodium chloride, Sprouts, *Vigna radiata*.

INTRODUCTION

Sprouts are a nutrient-dense food source that can significantly enhance human and animal diets. They provide a rich array of vitamins, minerals, antioxidants and fiber, supporting various aspects of health, including digestion, heart health and weight management. In animal nutrition, sprouted grains improve digestibility, growth performance and disease resistance, while also promoting sustainable feeding practices (Galieni *et al.*, 2020). One of the most consumed vegetables in various Asian countries, mung bean (*Vigna radiata*) sprouts are a good source of minerals and phytochemicals such as vitamins, phytosterols, GABA and flavonoids that support its biological activities (Nair *et al.*, 2013). *Vigna radiata* sprouts are superior than non-sprouted mung beans in terms of protein and antioxidant compounds (Van Hung *et al.*, 2020). During sprouting, legumes create and accumulate bioactive molecules that protect against environmental stress. These compounds are known to have antioxidant, antidiabetic, anti-inflammatory and anti-carcinogenic activities (Šibul *et al.*, 2016). Seed germination increases phenolic content and antioxidant compounds in mung bean, fava, wheat, fenugreek, mustard, alfalfa, sunflower, soybean, broccoli, lentil, radish and kale (Cevallos-Casals and Cisneros-Zevallos, 2010) which showed positive outcome in hen (Mattioli *et al.*, 2016) and rabbit (Dal Bosco *et al.*, 2015).

Exogenous pre-harvest treatment can modify the biological activity and metabolite synthesis of leguminous plants (Liu *et al.*, 2019). Plant growth is highly affected by salinity or abiotic stress. Various findings revealed the effect of salinity stress upon quality and food value of maize (Hichem and Mounir, 2009) and

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wheat sprouts (Cuong *et al.*, 2020). Koodkaew (2019), reported that 100 mM sodium chloride (NaCl) treatment enhances nutritional value of mung bean sprouts with reduction in fresh weight. Salinity stress in *Salvia hispanica* increased vitamin C, phenolic and flavonoid compound with reduction in germination rate (Younis *et al.*, 2021). Salinity stimulate generation of ROS causing oxidative stress resulting increased phenolic compounds and antioxidant activity of plants (Türkan and Demiral, 2009).

An appropriate treatment time can help to achieve nutrient rich sprouts with high phenolic compounds, flavonoids, free radical scavenging ability, mineral nutrients, protein content, sulphur content and vitamin C. However, we aimed to study the effect of NaCl treatment with slight modification in improving the nutritional value of *Vigna radiata* sprouts leaving the fresh weight undisturbed.

MATERIALS AND METHODS

Collection of seeds

The *Vigna radiata* seeds (IPM-2-14) were collected from Indian Institute of Pulse Research Kanpur, India.

The entire experiment was conducted in 2022-2024 at the Applied Biology Division, CSIR- Indian Institute of Chemical Technology, Hyderabad, Telangana.

Seed germination assay

Healthy seeds of *Vigna radiata* IPM-2-14 were cleaned using autoclaved distilled water (ADW), surface sterilized for two minutes with 70% ethyl alcohol and rinsed thrice with ADW to get rid of the alcohol. These seeds were kept in ADW for 14 h at 30°C to initiate germination and moved to sterile petri plate covered with moist tissue paper. Different NaCl solutions (50,100, 200,300,400 and 500 mM) were sprayed over seeds up to day 4 and were observed for germination.

Germination treatment

Data collected from seed germination assay NaCl at 50 mM and 200 mM was selected for further treatment. 48 and 72 h germinated seeds were treated with 50 and 200 mM NaCl and ADW as a control for 1 h in a beaker. After NaCl treatment seeds were washed 5 times with ADW to remove salts from sprouts and kept at 30°C in incubator till harvest (96 h germination), moistening them with ADW daily.

Fresh weight

The weight of sprouts was recorded before and after the treatment for 1 h at 48, 72 h of germination with 50 and 200 mM NaCl.

CHNS analysis

Seeds treated with 50 and 200 mM NaCl for 1h and control, were oven dried at 70°C and crushed using mortar and pestle. This powdered sample was used to perform CHNS analysis.

Free radical scavenging assay, total phenolic content (TPC) and total flavonoid content (TFC)

Extract was prepared according to method of Wei *et al.* (2019), to estimate free radical scavenging activity, TPC and TFC. 1 g Treated seeds were homogenized with 5 ml of 80% ethyl alcohol, centrifuged at 12000g for 15 min at 4°C. Supernatant was collected and used as extract. To estimate free radical scavenging activity 0.5 ml of extract was mixed with 2 ml of 0.2 mM DPPH in ethyl alcohol placed in dark at room temperature for 30 min. Using spectrophotometer at 570 nm absorbance was measured. The above extract was used to estimate TPC in treated and control seeds (Xu and Chang, 2007). Through the calibration curve TPC was expressed as gallic acid equivalents (mg of GAE/ml of sample). TFC was measured as per Shi *et al.* (2017) 0.5 ml extract, 0.1 ml aluminium chloride and 0.1 ml potassium acetate were added and incubated at room temperature for 30 min and absorbance was recorded at 415 nm. Calibration

curve of rutin was used to estimate TFC as Rutin equivalents-RE (µg of RE/ml of sample).

Vitamin C estimation

2.6 g of treated control sprouts were crushed in 5 ml milli Q water and centrifuged for 10 min. at 10000 rpm. at 4°C. Supernatant was used to estimate Vit. C /ml of extract according to Soliman (1979).

Mineral analysis

Seeds of *Vigna radiata* were treated as mentioned above with 50 and 200 mM NaCl. 0.2g powdered samples were incubated (Alkaltham *et al.*, 2020) in 70% nitric acid overnight followed by filtration to analyze various minerals such as potassium (K), sodium (Na) using flame photometry and iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) using ICP-MS (CSIR-IICT, Hyderabad, India).

Statistical analysis

For data analysis One-way ANOVA was used followed by Tukey test using instat 3 software. All experiments were replicated three times and results are expressed as means $\pm$ standard deviation (SD). *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$) indicates significant difference compared to control for same time period. Different letters denote statistically significant difference within the group.

RESULTS AND DISCUSSION

Salinity is a major abiotic stress to a plant. During salt stress, all the major processes such as cell expansion and division, protein synthesis, lipid and energy metabolism are affected causing retarded plant growth, development and seed germination (Carillo *et al.*, 2011). Salinity stress reduced seed germination in rice due to depletion in energy source by reducing activity of gibberellic acid and α -amylase (Liu *et al.*, 2018). Our study showed that NaCl treatment up to 100 mM did not affect seed germination in *Vigna radiata* significantly, but 200 mM NaCl treatment decreased seed germination significantly. At 300 mM and above NaCl concentrations 0% seed germination was observed (Fig 1). Previous study revealed a decrease in germination rate and seedling growth of mung bean (Salahuddin *et al.*, 2017), isabgol (Verma and Solanki, 2022) and soybean (Çirka *et al.*, 2021). On a similar fashion reduced seed germination ratio with increase in NaCl concentration was observed in our study. Salinity stress reduced seed germination, radical and shoot length and fresh weight in *Vigna radiata* (Promila and Kumar, 2000). NaCl treatment reduced fresh weight of sprouts in buckwheat (Lim *et al.*, 2012) and mung bean (Koodkaew, 2019). Outcome of our study did not reveal any negative effect upon salinity stress on weight of NaCl treated sprouts compared to control. No statistically significant change in % weight gain was noted with 50 and 200 mM treated seeds (Table 1).

% Protein and % Sulphur content

Increased protein content in the sprouts was observed at 50 mM and 200 mM of NaCl treatments. Compared to control 48 h germinated seeds after 50 mM NaCl treatment show 20% while 200 mM treatment show 16% increase (Table 1). Our results are in concurrence with the findings of Yan *et al.* (2017) and (Koodkaew, 2019) which showed significant effect on increase in protein content in mung bean sprouts treated with NaCl, CaCl_2 , NaCl- CaCl_2 and NaCl-Glucose. Compared to control 72 and 90 h germinated seeds after 50 and 200 mM NaCl treatment showed statistically insignificant change in % protein content. Proteins from foods like legumes, nuts, dairy and fish offer bioactive peptides that help prevent and manage chronic diseases like diabetes, inflammation and heart conditions (Shahnaz *et al.*, 2024). Research indicates that plant-based protein sources are essential for human health

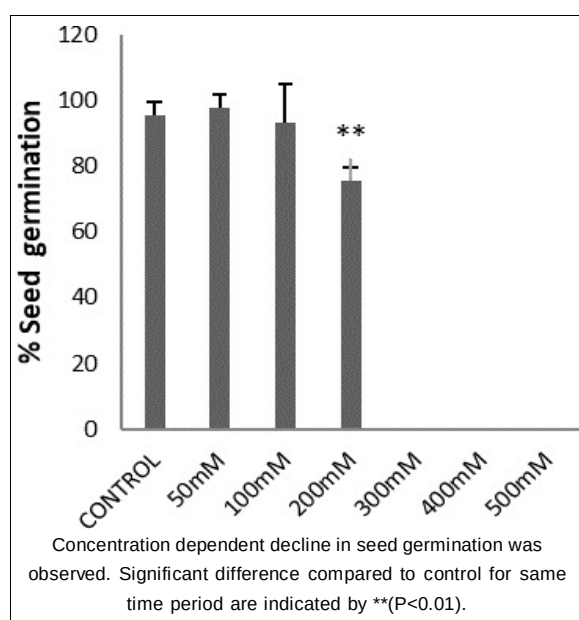


Fig 1: Effect of NaCl treatment on seed germination in *Vigna radiata*.

and environmental sustainability because they promote muscle protein synthesis and offer advantages for the heart, metabolism and digestive tract (Sharma *et al.*, 2025). % nitrogen was converted to % protein by using formula ($\% \text{N} \times 6.25$). A 31%, 11% and 8 and 14% increase % sulphur content due to 50 mM and 200 mM NaCl treatment to 48 and 72 h germinated seeds was noted compared to control (Table 1). Increased protein and sulphur could be due to NaCl stress. There is a positive correlation between sulphur content and net dietary protein value (Miller and Naismith, 1958). Diet with sulphur rich antioxidants helps to maintain health and slow aging process in chronologically and biologically aged subjects (De la Fuente, 2010). Our result shows that 50 and 200 mM NaCl treatment had enhanced sulphur content of sprouts significantly.

DPPH free radical scavenging assay and total phenolic and flavonoid content

DPPH free radical scavenging activity due to 50 mM and 200 mM NaCl treatment to 90 and 72 h germinated seeds was significantly higher than control seeds (Table 2). Similar treatment to 48 h germinated seeds did not show significant difference. Several organic acids, phenolic compounds and flavonoids contribute to the antioxidant activity of plant. Increased antioxidant activity in various NaCl treated sprouts was reported by (Koodkaew, 2019; Lim *et al.*, 2012). TPC of 50 mM NaCl treatment to 48, 72 and 90 h germinated seeds was increased by 40, 57 and 41% compared to control seeds respectively. Similar treatment at 200 mM NaCl showed 36, 28 and 38% higher TPC than control seeds (Table 2). NaCl treatment up to 100 mM enhanced phenolic content while 200 mM NaCl treatment reduced phenolic content in safflower sprouts (Kim *et al.*, 2021). TFC of 50 mM NaCl treatment to 48, 72 and 90 h germinated seeds was increased by 48, 150 and 59% compared to control seeds respectively. Similar treatment at 200 mM NaCl showed 30, 33 and 60% higher TFC than control seeds (Table 2). The maintenance of human health depends on the anti-inflammatory, antioxidant, antiviral, antibacterial and cardio protective properties of phenolic acids and flavonoids (Oluwole *et al.*, 2022). Rich in phenolic compounds and antioxidants, sprouts have several health

Table 1: Effect of NaCl treatment on change in fresh weight, % protein content and % sulphur content in *Vigna radiata* sprouts.

Germination time at treatment	NaCl treatment	% weight gain	% Protein content	% Sulphur content
48 h	Control	24.11±6.04a	30.31±2.28a	0.317±0.007a
	50 mM	35.59±10 a	36.56±0.40b	0.415±0.017b
	200 mM	37.71±10a	35.30±0.87b	0.353±0.003c
72 h	Control	17.09±6.41a	30.31±2.28a	0.317±0.007a
	50 mM	20.64±1.96a	32.58±2.99a	0.342±0.008b
	200 mM	11.99±3.5a	34.23±2.20a	0.361±0.007b
90 h	Control	3.99±1.88a	30.31±2.28a	0.317±0.007a
	50 mM	6.46±3.45a	33.43±1.84a	0.329±0.008a
	200 mM	4.34±3.34a	32.88±1.37a	0.343±0.004b

Significant difference compared to control for same time period are indicated by different letters.

benefits, especially when it comes to controlling diabetes, inflammation, hyperlipidemia, cancer and neurodegeneration (Günel-Köroğlu *et al.*, 2025). Dietary antioxidant supplementation increased the quality of milk production in farm animals (Ponnampalam *et al.*, 2022). Chambial *et al.* (2013) Observed that Raw sprouts are superior in terms of antioxidant activity compared to their processed form of all the treatments, applying 50 mM NaCl to seeds that have germinated for 72 h increases their antioxidant content, which increases the sprouts' ability to fight oxidative stress and has therapeutic potential for the treatment of chronic illnesses.

Estimation of vitamin C

Vitamin C is an antioxidant which guards the body from free radicals and is crucial for bone formation, wound healing and preservation of healthy gums (Chambial *et al.*, 2013). Seed germination enhances vitamin C content in sprouts compared to raw seeds (Fordham *et al.*, 1975). But

some abiotic stresses like sucrose treatment can enhance vitamin C content of sprouts compared to normally germinated sprouts (Wei *et al.*, 2019). Plants show increase in ascorbic acid content in order to scavenge free radicals produced due to salinity stress (García Caparrós *et al.*, 2019). Increased salt stress resistance and reduced lipid peroxidation was noted upon exogenous application of vitamin C (Shalata and Neumann, 2001). In our study 50 mM and 200 mM NaCl treatments to 48 and 72 h germinated seeds enhanced vitamin C content by 2, 2.66 and 3.1 and 3 folds compared to control sprouts (Fig 2). There is a considerable amount of data showing that maintaining adequate vitamin C levels helps protect against age-related cognitive decline and Alzheimer's disease; however, consuming vitamin C appropriately can reduce supplement usage (Harrison, 2012). Vitamin C boosts immune function by enhancing white blood cell activity, improving the skin barrier and aiding in the body's defense against infections, inflammation and even cancer (Alberts *et al.*, 2025). Supplemental vitamin C at 200 mg/kg diet significantly increased eggshell weight, eggshell thickness, shell breaking strength, egg production and improved immunity in White Leghorn (Panda *et al.*, 2008). In animal husbandry, the supplementation of mineral and vitamin C promotes enhanced growth, improves overall health by reducing issues like diarrhea and contributes to stronger, more resilient calves, ultimately leading to increased productivity and better long-term development in dairy farming (Ozkaya and Neja, 2017).

Mineral analysis

Minerals are essential for the body's functions, aiding in processes like enzyme activation, bone health and nerve function. Deficiencies can lead to conditions like diabetes, heart disease, or cancer. A balanced diet ensures adequate intake to prevent these health issues (Gharibzahedi and Jafari, 2017). Flame photometric analysis showed that 50 and 200 mM NaCl treatment to 48 and 72 h germinated seeds increased sodium significantly in all treated groups compared to control but potassium level remained unchanged (Table 3). Data obtained from ICP-MS analysis

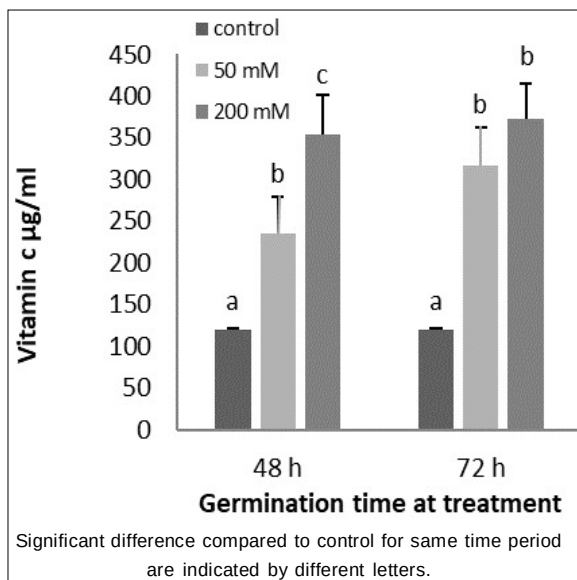


Fig 2: Effect of NaCl treatment on vitamin c content in *Vigna radiata*.

Table 2: Effect of NaCl treatment on % DPPH scavenging activity, total phenolic and flavonoid content in *Vigna radiata* sprouts.

Germination time at treatment	NaCl treatment	% DPPH scavenging activity	TPC mg of GAE/ml	TFC µg of RE/ml
48 h	Control	32.97±1.2a	2.55±0.15a	22.09±0.614a
	50 mM	35.42±2.26a	3.59±0.06 b	32.76±0.77b
	200 mM	37.6±1.37a	3.53±0.2b	28.77±1.34b
72 h	Control	32.97±1.2a	2.55±0.15a	22.09±0.614a
	50 mM	58.96±1.45 b	4.02±0.14b	55.04±2.63b
	200 mM	47.8±2.27 c	3.26±0.09c	29.36±2.71c
90 h	Control	32.97±1.2a	2.55±0.15a	22.09±0.614a
	50 mM	50.6±0.13b	3.57±0.09b	35.12±0.22b
	200 mM	43.92±5.37b	3.47±0.13b	35.37±0.78b

Significant difference compared to control for same time period are indicated by different letters. GAE- Gallic acid equivalent; RE- Rutin equivalent.

Table 3: Effect of NaCl treatment on mineral content in *Vigna radiata* sprouts.

Germination time at treatment	NaCl treatment	Na	K	Mn	Zn	Cu
		(mg/l)				
48 h	Control	584±154a	8900±1519a	14.13±0.39a	39.5±3.5a	12.5±0.5a
	50 mM	1102±30b	8934±1872a	13.07±0.55a	40±10a	14±10a
	200 mM	1296±179b	7157±922a	12.24±0.9a	40.5±2.5a	11.85±0.85a
72 h	Control	584±154a	8900±1519a	14.13±0.39a	39.5±3.5a	12.5±0.5a
	50 mM	1376±99b	8060±100a	12.9±0.3a	40.5±2.5a	13.45±0.45a
	200 mM	2170±369c	7884±231a	12.78±0.16a	44±10a	13.38±0.42a

Significant difference compared to control for same time period are indicated by different letters.

showed insignificant change in copper, zinc and manganese content in all treated groups compared to control group (Table 3). Germination of sorghum grains increase bioavailability of iron, zinc and calcium (Tizazu *et al.*, 2011). Sucrose induced abiotic stress reduces calcium, iron and zinc content in green gram sprouts (Wei *et al.*, 2019). During seed germination, salinity stress can cause physiological and biochemical alterations. The nutritional value of sprouts has changed as a result of these modifications. Such enhanced-nutritional sprouts can be used to meet the dietary needs of livestock, such as poultry and cattle.

CONCLUSION

The search for novel approaches to enhance advantageous dietary attributes has been spurred by the need of nutrient-dense foods with enhanced beneficial value of *Vigna radiata* sprouts without affecting their fresh weight. Edible sprouts have gained popularity for both human and animal consumption due to their high nutritional value. Mung bean sprouts can have their nutritional value increased without compromising upon yield by using an appropriate pre-harvest NaCl treatment. In our study 50 and 200 mM NaCl treatment for 1 h to 48 and 72 h germinated seeds potentially increased health-promoting factors such as protein, sulphur, free radical scavenging activity, vitamin C, phenolic and flavonoid content of the sprouts. From these findings we conclude that employing external salinity stress application technique can prove to be a cheap and effective way to enhance nutritional value of *Vigna radiata* sprouts without effecting their fresh weight.

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Disclaimers

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responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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