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RESEARCH ARTICLES

Salt stress in plants and amelioration strategies: alleviation of agriculture and livelihood risks after the Covid-19 pandemic

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Abstract
Agriculture sustains the livelihoods of over 2.5 billion people worldwide. The growing nature of disasters, the systemic nature of risk, a more recent pandemic along with abiotic stress factors are endangering our entire food system. In these stressful environment, it is widely reprimanded that strategies should be encompassed to attain increased crop yield and economic returns which would alleviate food and nutritional scarcity in developing countries. To study the physiological responses to salt stress, *Vigna radiata* seedlings subjected to varying levels of salt stress (0, 25, 50, 100 and 200 mM NaCl) were evaluated by tracking changes in Chl a fluorescence, pigment content, free proline and carotenoids content by HPLC. The ability of plants to adapt to salt stress is related with the plasticity and resilience of photosynthesis. As salt concentration increased, chlorophyll fluorescence indices decreased and a reduction in the PSII linear electron transport rate was observed. Chlorophyll fluorescence parameters can be used for in vitro non-invasive monitoring of plants responses to salt stress. Overall, *Vigna* responded to salt stress by the changes in avoidance mechanism and protective systems. Chl fluorescence indices, enzymatic contents of POD, CAT and free proline were sensitive to salt stress. The study is significant to evaluate the tolerance mechanisms of plants to salt stress and may develop insights for breeding new salt-tolerant varieties.

Keywords Agriculture · *Vigna* · Chl fluorescence · Pigment · Salt

Introduction

COVID-19 pandemic has disrupted agriculture in many ways, already challenged by climate change and abiotic stress factors which were further compounded by lockdown situations. Salinity and drought are major abiotic constraints which need to be combated to ensure global productivity of crops and promote sustainable agriculture (Ma et al. 2020). Metabolic limitations of photosynthesis under (Munns et al. 2006) salt stress results in reduction of photosynthetic enzymes activity, membrane disruption (Meloni et al. 2003), ion toxicity (Ali et al. 2022), osmotic stress (Ali et al. 2019), nutrient deficiencies, physiological and biochemical perturbations and even mortality. Analysis of performance of Chl a fluorescence parameter will compliment growth, water relations and mineral uptake mechanisms in plants subjected to stress (Netondo et al. 2004).

Pigmentation reflects plant photosynthetic light- harvesting capacity (Elfeky et al. 2007) and modification in the composition of leaf pigments could confer salt stress tolerance. A common response to salinity stress is enhancement of sugars and other compatible solutes (Sharma et al. 2019). Proline (amino acid) is a compatible solute which mainly accumulates as an osmostress protectants (Chun et al. 2018). It also protects photosynthetic apparatus, exhibits protein-stabilizing properties (Kavi Kishor et al. 2015), regulate cellular osmotic adjustment and scavenges reactive oxygen species (Ashraf and Foolad 2007) under salt stress.

About 60% of Rajasthan state (34°35′N to 30°10′N latitude and 69°31′E to 76°55′E longitude) falls within of Indian Desert characterized by hot arid conditions (DST 1994). Productivity of legumes are low due to abiotic constraints such as drought and salinity, poor cultural practices

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and unavailability of improved varieties to farmers. *Vigna radiata* (L.) Wilczek, is an nutritious food legume and cash crop cultivated across the globe, native to India, complements cereals as affordable protein pulse crop and is significant for nutritional security and sustainable agriculture (Lambrides and Godwin 2007). Selection of salt tolerant varieties could help in improving productivity and stabilize crop production in stressful environment. The main objective of the study was to understand the response mechanisms of *Vigna* to salt stress which will pave way for production of new salt-tolerant varieties and may also help expansion of agriculture in saline areas. We hypothesize that investigating plant responses like alterations in the morphological and physiological traits under salt stress may lead to deciphering mechanisms to plant tolerance. As a result of intensification of soil salinization, studying effects on the physiological mechanism, evaluation of in-field photosynthetic performance of plants and identifying salt-tolerant varieties could help in combating stress. Thus, the information generated through this research, will be pivotal for agricultural research in arid and semi-arid areas in alike climate and eventually may evolve stratagems for the cultivation of crops in such stress conditions.

Materials and methods

Plant materials and growth conditions

Seeds of *Vigna radiata* 'var. K-851' were procured from Krishi Vighyan Kendra (KVK), Banasthali University, Rajasthan, India and were surface sterilized with 0.1% $HgCl_2$, which were then washed repeatedly with sterile water. Thereafter, seeds were germinated on moistened filter paper, placed in petri dishes in dark at 28˚C, for 3 days and seedlings with well developed roots were transfered to pots which were subjected to salt stress treatment. The experiments were performed in plant growth chamber, Department of Bioscience and Biotechnolgy, Banasthali University, India.

Salt treatment

The petriplate germinated plantlets of *Vigna radiata* were transferred into pots supplemented with Hoagland's nutrient solution (Hoagland and Arnon 1950) and salt treatment was applied to 15 day plants. Analytical grade NaCl were added to pots to provide final concentrations of 0 (control), 25, 50, 100 and 200 mM. Plantlets were grown in plant growth chamber (day/night photoperiod of 14/10 h, day/night temperature 25 ± 2˚C /17 ± 2˚C, relative air humidity 65–70%).

Chl a fluorescence

Chl a fluorescence parameters were measured with a portable Chl fluorescence device (Mini-PAM, Heinz Walz, Effeltrich, Germany) as per the method of Kumari et al. (2005) after 1, 3, 5,7, 9 and 11 days of salt treatment. The light was provided by the internal halogen lamp of Mini-PAM and leaf temperatures were recorded simultaneously with a Ni/NiCr- thermocouple fitted with the Walz leaf clip holder. Leaves were darkened for 30 min and then subjected to actinic light intensity over 4 min in eight steps with increasing levels of light, each 30s apart (Rascher et al. 2000). Light-response curves of $\Delta F/F_m'$ and ETR were obtained were calculated according to Genty et al. (1989).

Proline estimation

The total proline content was measured by acid ninhydrin reaction according to the method of Bates et al. (1973).

Determination of photosynthetic pigments

Photosynthetic pigments (Chl a, Chl b and total Chl) were calculated using Arnon (1949) method.

Determination of antioxidant enzyme activities

Catalase (CAT, EC 1.11.1.6) activity was measured by monitoring the rate of decrease in the absorbance at 240 nm for 2 min (Luck 1970). POD (EC 1.11.1.9) activity was determined by following the decrease in absorbance of guaiacol at 436 nm (Putter 1974).

Estimation of carotenoids (β-carotene and L + Z) via HPLC

Carotenoid concentrations viz. β-carotene, Lutein and Zeaxanthin (L + Z) were determined by HPLC. The method followed was a modified version of Rivas et al. (1989) and Pocock et al. (2004).

Statistical analysis

All data were subjected to analysis of variance (ANOVA), analyzed with SPSS 22.0 statistical software (SPSS Inc., Chicago, IL). The results were presented as mean values of three replicates with lower case letters a ($p \leq 0.001$), b ($p \leq 0.01$) and c ($p \leq 0.05$) which were considered as statistically significant.

Results and discussion

Effect of salt stress on Chl fluorescence

Changes in fluorescence parameters as measured by Mini-PAM for plants under the control and salt treatments are depicted in Table 1. It may be noted that experiments were not continued after 11th day as the leaves wilted due to excessive desiccation imposed by salt stress as corroborated by negligible ETR and yield measurements in the stressed plants.

Chl *a* fluorescence seems to be useful indicator of effects of salt stress on photosynthetic efficiency at high NaCl concentrations (Shin et al. 2020) and can be implemented in elucidation of optimum salt stress ranges for crops grown under controlled environmental conditions. A significant reduction in rate of electron transport (60%) was measured at 200mM concentration in salt stressed plants (Day 3rd) as compared to control. Similarly, at 100 mM a significant reduction (64%) in rate of electron transport was measured with values reaching upto 22.3 ± 2.46 µmol $m^{-2}s^{-1}$ on 5th day of stress. At 50mM NaCl, a significant decline of about 1.88 folds (7th day of stress) and at 25 mM, values reaching upto 20.7 ± 3.20 µmol $m^{-2}s^{-1}$ were observed. At lower NaCl concentrations (25 mM), quantum yield of PSII, $\Delta F/F_m'$ was comparable to control plants. A significant decline of about 65% occurred in $\Delta F/F_m'$ for plants irrigated with 200mM NaCl (3rd day), suggestive for the aggravation of the PSII reaction center at higher stress levels (El-Shintinawy 2000). Physiological responses of plants are increased respiration rate, disrupted mineral supplies, ion toxicity, modifications in growth, diminished photosynthetic rates (Kao et al. 2003), reduced leaf area and decline in Chl fluorescence parameters.

Effect of salt stress on photosynthetic pigments

We measured Chl a, Chl b, total Chl and Chl a:b ratio in experimental leaves and results are depicted in Table 2. Salt stress resulted in significant decline in the total pigment content as compared to that of the unstressed plants. Salt stress at higher concentration (200 mM) caused decline of Chl a by 1.38 folds and Chl b declined by about 22%. Total Chl significantly decreased by 32.32% (100mM conc.). At lower conc. of salt (50mM), a reduction of 17.40% in total Chl was recorded. Total Chl values reached up to 2.55 ± 0.11 mg/g FW (25mM conc.). The Chl a/b ratio increased as salt stress progressed with highest values being observed on last day of stress. Our data exhibited that salt stress caused a significant decline in of Chl a, Chl b and total Chl content in response to salt stress.

Table 1 Cardinal points of the regression lines of saturating photosynthetic photon flux density (PPFDsat) and half saturating PPFDsat, effective quantum yield of PSII at saturating PPFD($\Delta F/F_m'$sat) and at half saturating PPFD (½ $\Delta F/F_m'$) and maximum electron transport rate (ETR_{max}) for var. K-851 of *Vigna radiata* exposed to different conc. of salt for 11 days. The values are expressed as mean ± SD (n=3)

Days	NaCl conc. (mM)	ETR_{max} (µmol $m^{-2}s^{-1}$)	PPFDsat (µmol $m^{-2}s^{-1}$)	½ PPFDsat (µmol $m^{-2}s^{-1}$)	$\Delta F/F_m'$sat	½$\Delta F/F_m'$sat
1	0	63.1±10.98	614 ± 25.51	152 ± 36.09	0.26 ± 0.07	0.48 ± 0.05
	25	64.7 ± 4.65	603 ± 9.17	124 ± 7.02	0.28 ± 0.02	0.58 ± 0.01
	50	63.0 ± 4.47	637 ± 27.06	125 ± 10.60	0.24 ± 0.02	0.57 ± 0.01
	100	47.3 ± 3.06^{bc}	628 ± 28.57	110 ± 19.55^{c}	0.18 ± 0.01^{bc}	0.51 ± 0.09
	200	35.6 ± 2.11^{abc}	631 ± 10.97	94 ± 12.51^{abc}	0.12 ± 0.01^{abc}	0.44 ± 0.09
3	0	60.8 ± 12.90	601 ± 11.93	133 ± 35.09	0.26±0.07	0.52 ± 0.05
	25	65.6 ± 6.07	620 ± 21.00	130 ± 18.15	0.27 ± 0.01	0.56 ± 0.01
	50	55.3 ± 3.40	621 ± 37.51	118 ± 6.43	0.22 ± 0.01	0.53 ± 0.05
	100	40.3 ± 5.57^{abc}	594 ± 27.02	106 ± 24.01	0.16 ± 0.03^{bc}	0.46 ± 0.11
	200	24.5 ± 2.33^{abc}	560 ± 4.51^{c}	90 ± 17.62^{bc}	0.09 ± 0.00^{abc}	0.33 ± 0.10^{abc}
5	0	62.4 ± 10.31	601 ± 11.79	140 ± 27.73	0.27 ± 0.06	0.50 ± 0.06
	25	56.8 ± 5.23	577 ± 12.42	116 ± 6.56	0.26 ± 0.04	0.55 ± 0.02
	50	46.8 ± 6.64^{bc}	575 ±17.67	109 ± 24.56	0.20 ± 0.05^{c}	0.50 ± 0.09
	100	22.3 ± 2.46^{abc}	568 ± 14.84	91 ± 14.84^{bc}	0.09 ± 0.01^{abc}	0.29 ± 0.04^{abc}
7	0	63.9 ± 5.75	623 ± 37.47	123 ± 8.00	0.26 ± 0.04	0.58 ± 0.02
	25	44.0 ± 5.22^{abc}	461 ± 36.72^{abc}	99 ± 4.04	0.24 ± 0.03	0.51 ± 0.07
	50	33.9 ± 4.99^{abc}	435 ± 30.09^{abc}	90 ± 17.58^{c}	0.19 ± 0.05^{c}	0.45 ± 0.09^{c}
9	0	65.6 ± 6.07	620 ± 21.00	130 ± 18.15	0.27 ± 0.01	0.56 ± 0.01
	25	36.2 ± 4.54^{abc}	413 ± 36.29^{abc}	95 ± 13.23^{c}	0.22 ± 0.05	0.47 ± 0.11
11	0	65.5 ± 6.01	601 ± 11.93	137 ± 30.17	0.28 ± 0.04	0.54 ± 0.06
	25	20.7 ± 3.20^{abc}	327 ± 15.59^{abc}	78 ± 5.29^{abc}	0.15 ± 0.02^{abc}	0.31 ± 0.02^{abc}

^{a}significant at $p \leq 0.001$, ^{b}significant at $p \leq 0.01$,^{c}significant at $p \leq 0.05$

Table 2 Pigment content in var. K-851 of *Vigna radiata* exposed to salt stress. The values are expressed as mean ± SD (n=3)

Days	Salt conc. (mM)	Chl a (mg /g FW)	Chl b (mg /g FW)	Total Chl (mg /g FW)	Chl a/b
1	0	3.06 ± 0.04	0.99 ± 0.02	4.05 ± 0.06	3.10 ± 0.03
	25	3.08 ± 0.04	0.99 ± 0.01	4.07 ± 0.05	3.12 ± 0.01
	50	2.59 ± 0.03^{abc}	0.83 ± 0.06^{abc}	3.42 ± 0.09^{abc}	3.13 ± 0.17
	100	2.55 ± 0.12^{abc}	0.81 ± 0.05^{abc}	3.36 ± 0.17^{abc}	3.14 ± 0.08
	200	2.46 ± 0.04^{abc}	0.78 ± 0.03^{abc}	3.24 ± 0.07^{abc}	3.15 ± 0.06
3	0	3.17 ± 0.05	1.02 ± 0.01	4.19 ± 0.04	3.11 ± 0.09
	25	2.84 ± 0.06^{abc}	0.91 ± 0.03^{abc}	3.74 ± 0.08^{abc}	3.13 ± 0.04
	50	2.57 ± 0.02^{abc}	0.82 ± 0.01^{abc}	3.39 ± 0.02^{abc}	3.15 ± 0.06
	100	2.42 ± 0.05^{abc}	0.77 ± 0.05^{abc}	3.19 ± 0.09^{abc}	3.16 ± 0.15
	200	2.29 ± 0.08^{abc}	0.71 ± 0.02^{abc}	3.00 ± 0.10^{abc}	3.24 ± 0.00^{c}
5	0	3.10 ± 0.06	0.99 ± 0.01	4.09 ± 0.06	3.13 ± 0.08
	25	2.75 ± 0.06^{abc}	0.87 ± 0.02^{abc}	3.62 ± 0.08^{abc}	3.14 ± 0.02
	50	2.47 ± 0.05^{abc}	0.78 ± 0.00^{abc}	3.25 ± 0.05^{abc}	3.16 ± 0.07
	100	2.22 ± 0.02^{abc}	0.67 ± 0.01^{abc}	2.89 ± 0.02^{abc}	3.31 ± 0.02^{bc}
7	0	3.04 ± 0.03	0.98 ± 0.02	4.02 ± 0.05	3.11 ± 0.06
	25	2.57 ± 0.01^{abc}	0.81 ± 0.00^{abc}	3.39 ± 0.01^{abc}	3.16 ± 0.01
	50	2.16 ± 0.07^{abc}	0.64 ± 0.02^{abc}	2.80 ± 0.08^{abc}	3.38 ± 0.10^{abc}
9	0	3.03 ± 0.04	0.96 ± 0.04	3.99 ± 0.08	3.16 ± 0.09
	25	2.30 ± 0.06abc	0.72 ± 0.02abc	3.02 ± 0.08abc	3.18 ± 0.03
11	0	2.93 ± 0.07	0.93 ± 0.02	3.86 ± 0.08	3.15 ± 0.02
	25	1.98 ± 0.09abc	0.57 ± 0.03abc	2.55± 0.11abc	3.45 ± 0.03abc

[a]significant at $p \leq 0.001$, [b]significant at $p \leq 0.01$,[c]significant at $p \leq 0.05$

Higher accumulation of Na^+ ions leads to ionic toxicity which in turn induces cell dehydration and membrane dysfunction. Similar results were observed by Benavides et al. (2000) where reduction in the photosynthetic assimilation rate was related to decline of Chl content and 23% decline in Chl content was observed in salt-sensitive potato clones compared to tolerant ones. Salt stress can lead to inhibition of photosynthetic enzymes activity and the disruption of membrane structures (Meloni et al. 2003).

Chl *b* stabilizes light-harvesting antenna proteins and is synthesized from Chl *a* by chlorophyllide a oxygenase (Tanaka and Tanaka 2005). When amount of Chl *b* surpasses the needed amount, excess Chl *b* molecules induce chlorophyllide a oxygenase protein degradation (Yamasato et al. 2005) resulting in suppression of Chl *b* biosynthesis. This is further corroborated by the observation that the first step in the degradation of Chl *b* involves its conversion to Chl *a* (Schumacher et al. 2021).

Effect of salt stresses on carotenoids content

Carotenoids are a type of terpenoids and essential pigments in photosynthesis, synthesized in the plastids of plants' photosynthetic apparatus (Swapnil et al. 2021). β-carotene in photosynthetic tissue has a major protective role by direct quenching of triplet Chl, resulting in photoprotective capacity and mediates a cyclic electron transfer around PSII (Dogra and Kim 2020). Both zeaxanthin and β-carotene (Batra et al. 2014) can act as energy acceptors, quenching excited Chl molecules. The S_1 excited energy state of lutein is believed to be very similar to the Q_y excited state of Chl a and b. β-carotene amounts were reduced, at all concentrations of salt (Table 3). At 100 mM, a decline of

Table 3 Relative concentrations of β-carotene and L+Z obtained through HPLC in fifteen days old plants of *Vigna radiata* exposed to different days of salt stress

Salt conc. (mM)	Days	β-C (µg/g)	L+Z (µg/g)
0	0	63.04	188.22
25	3	62.73	181.12
	5	54.36	172.19
	7	35.93	123.24
	11	27.29	126.97
50	3	50.88	233.57
	5	27.12	146.16
	7	24.22	121.38
100	3	31.54	144.46
	5	26.06	118.44
200	3	26.05	135.89

about 59% was observed. Similar trends were observed for lutein + zeaxanthin contents.

Photoinhibitory effects transpires when the rate of transfer of excitons to the PSII reaction centre surpasses the rate of electron removal from the reaction centre by the primary electron acceptor Qa which leads to accumulation of $P680^+$ which has adequate positive potential to oxidize and destroy Chl 670 of the LHC and β-carotene. This results in significant decline in the photosynthetic capacity. Both Chl a and β-C are bound in the core complex of PSII (Bujaldon et al. 2017), the reaction center which is vulnerable to photoinactivation.

Effect of salt stress on antioxidant enzymes

Salt stress induced ionic toxicity and osmotic stress, triggers accumulation of reactive oxygen species and free radicals inducing oxidative damage (Hasanuzzaman et al. 2020) alter membrane phospholipids and disrupt normal metabolism. Salt stress tolerance mechanisms also depend on the increments of enzymatic and nonenzymatic antioxidant activity. Enzymatic antioxidants which includes catalase (CAT) and peroxidase (POD) constitute the major ROS scavenging systems, quenching oxyintermediates, free radicals and hydrogen peroxide thus, protecting plants from potential cytotoxic effects. The cellular CAT levels in stressed *Vigna radiata* leaves were increased as compared with control plants, with a 2.33, 2.08 and 1.73 folds' increase at 200 mM, 100 mM and 50mM, respectively (Table 4). Similarly, the POD activity enhanced with higher salinity levels (Table 5). The increased levels of CAT and POD measured exhibits that antioxidant mechanisms are presumed to limit cellular damage and to increase the resistance to environmental stresses (Khan et al., 2020). The CAT, as compared to POD, with low affinity towards H_2O_2 but a high processing rate, may act as major enzymatic H_2O_2 scavenger as CAT enzymatic reaction is not saturated even when the cellular H_2O_2 level become several folds higher under salt-stress and its activity is independent of other cellular reductants (Hasanuzzaman et al. 2020). This is indicative of the fact that maintenance of CAT activity could play significant role in imparting stress tolerance in plants. This could be interrelated specifically for C3 plants like rice, where the peroxisomal photorespiratory activity which lead to higher accumulation of H_2O_2 is elevated under salt-stress (Ghannoum 2009). The results of this experiment suggest that antioxidant enzymes activity as POD and CAT could play a pivotal role in salt tolerance mechanisms and could be further exploited in breeding stress tolerant varieties.

Effect of salt stress on free proline content

The increase in proline content was more at higher salt conc. (200 mM and 100 mM). At 100 mM, 11 folds' increase was observed as compared to control (5th day). Similarly, at 50 mM NaCl (day 7), proline content was significantly increased (14.82 ± 0.07 μg g^{-1}FW). The level of proline was increased by 7% as compared to the control (25 mM NaCl). We observed a positive correlation between proline levels and salt stress in *Vigna* (Fig. 1). The increased rate of proline biosynthesis in the chloroplasts contribute to the stabilization of cellular homeostasis by dissipating the excess of reducing potential when electron transport is reduced during stress conditions (Szabados and Savoure 2010).

Conclusion

The differential expression patterns observed in response to salt stress open insights to plant plasticity in response to the multitude of abiotic stressors faced by plants. Generally, the control plants of *Vigna radiata* exhibited higher yield as compared to salt stressed plants. Under stress conditions, decrease in the PSII activity as corroborated by reduction in electron transport which enhances the internal concentration of CO_2 leads to decrease in the carboxylation efficiency. The proline content and enzymatic antioxidants were significantly enhanced in stressed plants over control plants. The amassed knowledge on physiological

Table 4 Changes in catalase activity in *Vigna radiata* exposed to salt stress over a period of 11 days. The values are expressed as mean ± SD (n=3)

Days	Catalase (μ mol H_2O_2 decomposed min^{-1} g^{-1} F.W)				
	0 mM	25 mM	50 mM	100 mM	200 mM
1	4.63 ± 0.05	5.95±1.24	6.13 ± 0.55	7.89 ± 1.60	9.76 ± 1.07
3	5.04 ± 1.16	6.33 ± 1.45	7.95 ± 0.25	9.31 ± 2.60	11.77 ± 1.19
5	5.53 ± 0.03	7.00 ± 1.00	8.80 ± 0.31	11.55 ± 0.55	
7	5.95 ± 0.14	7.36 ± 2.21	10.34 ± 0.68		
9	6.16 ± 0.21	8.29 ± 1.24			
11	6.23 ± 0.14	8.36 ± 2.68			

Table 5 Changes in peroxidase content in in *Vigna radiata* exposed to salt stress over a period of 11 days. The values are expressed as mean ± SD (n=3)

Days	Peroxidase (U mg^{-1} protein)				
	0 mM	25 mM	50 mM	100 mM	200 mM
1	14.21 ± 0.13	15.00 ± 1.01	20.56 ± 0.11	22.54 ± 0.77	23.15 ± 0.27
3	15.03 ± 0.13	20.56 ± 0.19	23.15 ± 0.13	23.61 ± 0.49	29.89 ± 0.23
1	12.75 ± 0.79	14.17 ± 1.20	16.43 ± 0.73	16.19 ± 1.29	18.06 ± 0.36
3	14.54 ± 0.78	17.26 ± 0.76	17.59 ± 1.39	18.15 ± 1.16	26.11 ± 0.74
5	14.76 ± 0.02	19.21 ± 0.55	21.05 ± 1.22	25.6 ± 1.15	
7	15.07 ± 0.66	21.22 ± 0.56	23.03 ± 1.36		
9	15.85 ± 0.45	28.16 ± 1.22			
11	15.95 ± 0.03	29.33 ± 0.67			

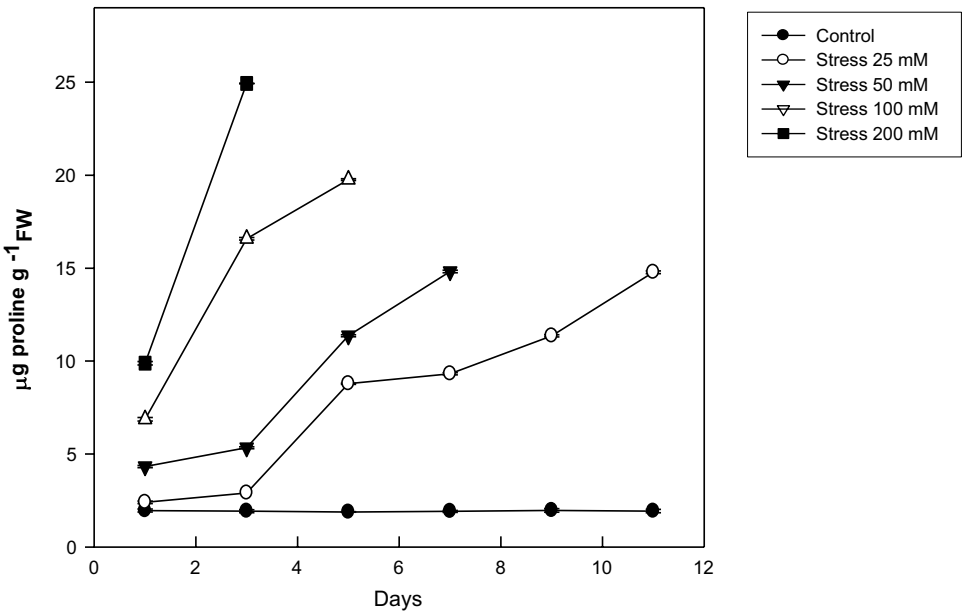

Fig. 1 Changes in proline content in *Vigna radiata* exposed to salt stress over a period of 11 days

and cellular mechanisms underlying plant responses to salt stress, will permit further progress in crop management and breeding.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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