



Effect of Vanadium on Growth, Photosynthesis, Reactive Oxygen Species, Antioxidant Enzymes, and Cell Death of Rice

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Abstract

Vanadium (V) as minor concentration is present in various plants and extensively found in soils. The current study was established to assess the response of rice seedlings to different V concentrations and also investigated its toxic effect on growth, photosynthetic assimilation, relative chlorophyll content, SPAD index, ion leakage, enzyme activities, hydrogen peroxide (H₂O₂), and cell death. The rice seeds were sown in Petri dishes for 8 days, and after that, rice seedlings were grown hydroponically in a climate-controlled growth chamber. After 15 days of V-treatment, antioxidant enzyme activities, H₂O₂, protein contents, photosynthetic assimilation, relative chlorophyll content, and cell death were determined by utilizing the Spectrophotometer (*Lambda 25 UV/VIS Spectrophotometer*), and V accumulation (roots and shoots) was determined by GFAAS (GTA 120). The obtained results showed that all V concentrations significantly decreased the biomass (dry and fresh) and root growth as a result of the reduction in total root length, root tips, root fork, root surface area, and root crossing, and V was more accumulated in roots than shoots. Besides this, enzymatic activities were significantly enhanced under V stress. The findings also confirmed that seedling exposed to V stress had lower tolerance indices, photosynthetic activity, and protein contents while the ion leakage was consistently increased by increasing the V concentrations. The viability of plant cells severely damaged in response to high V stress, and H₂O₂ induction might be responsible for cell death. Generally, all V doses had a drastic effect on enzyme activities and caused cell death of rice plants. Moreover, the current study demonstrated that V ≥ 35 mg L⁻¹ caused damaging effects on rice plants.

Keywords Vanadium · Rice · Photosynthesis · Antioxidant · Enzymes · Reactive oxygen species · Cell death

1 Introduction

Globally, vanadium (V) is attracting scientists due to its accumulation in environmental zones and contaminated the agricultural land, eventually injurious to human creatures. Heavy metals contaminated the soil cropping system due to excessive

industrialization and anthropogenic activities (Cui and Du 2011). Vanadium is found an average concentration of 20 to 120 mg kg⁻¹ in soil and categorized 5th most abundant transition metal (Baken et al. 2012). China, Russia, South Africa, and the USA have big deposits of V in the world (Amorim et al. 2007). Among these, China is the largest V producing

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and consuming country worldwide with 57% production of V in the world (GCVIR 2014). Vanadium contaminated 26.49% of soil in the southwest of China as a result of extensive mining and smelting activities (Yang et al. 2017a). The main source of V is parent rocks (Nriagu 1998) such as shale, deposits of phosphate, asphaltic deposits, some uranium ores, and titan ferrous magnetites (Adriano 2001). Besides the natural source, the burning of fossil and numerous industrial processes are also responsible for V addition in the environment (Baken et al. 2012). Vanadium mobilization in soil frequently dissociated from other metals and metalloids and regulated by weathering and redox processes under natural condition (Imtiaz et al. 2015a; Violante et al. 2010).

Many researchers reported that V toxicity, mobility, bioavailability, and bioaccumulation extremely reliant on the V oxidation state (Imtiaz et al. 2015a). Vanadium usually exists in tetravalent (V^{+4}) and pentavalent (V^{+5}) oxidation state in the environmental sample while V^{+5} is generally more mobile and toxic than V^{+4} compound for plants as well as animals (Panichev et al. 2006; Xiao et al. 2015). Vanadate is more toxic among all chemical forms of V; its higher concentration limited the plant growth and development processes by disturbing the physiological and chemical phenomena, alter cellular functions, gene expression (Anke 2004; Imtiaz et al. 2015a; Olness et al. 2005).

Plants easily uptake the V present in the soil but its effect on plants depends upon the concentration. Imtiaz et al. (2015a) reported that a lower concentration of V regulates the plant growth and improves the nitrogen fixation, chlorophyll synthesis, and potassium utilization. As described by Narumol et al. (2011), V at higher concentrations causes stunt growth by decreasing the morphology traits such as the number of leaves, shoot length, and dry weights of leaves, roots, and shoots of tomato, and Chinese green mustard. Similarly, V induces shoot mortality in pickleweed (*Salicornia*) and decreased plant height (Rosso and Stenstrom 2005).

Vanadium adversely affects plant growth and development process by disturbing the enzymatic activity, gene expression, and reactive oxygen species (ROS) production (Reiter et al. 2015). Vanadium behaves as a toxic element when present at high concentration and enhanced the ROS production that caused oxidative stress in plants (Narumol et al. 2011), impaired lipid membranes, and inhibited growth through disrupting the regular metabolic process of the plant (Chary et al. 2008). The plant initiates its ROS detoxifying system which includes antioxidant enzyme such as superoxide dismutase (SOD), catalase (CAT), dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), guaiacol peroxidase (POD), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), and non-enzymatic antioxidants like ascorbate, decreased alkaloids, glutathione, proline, carotenoids, α -tocopherols and phenolic compounds (lignin, flavonoids, and tannins) that perform as scavengers of ROS

(Michalak 2006). Previous researchers like Xiao et al. (2015) just focused on V toxicity on plant physiology through chloroplast injury, root and shoot mortalities, and leaf chlorosis, while the V toxic molecular mechanism is still not studied.

According to Gadjev et al. (2008), for the plants' development, programmed cell death (PCD) is a major biological process, including selective cell death and/or specific mortification of cellular mechanisms, which is being engaged as plant defense plan under heavy metal or abiotic stress (Gadjev et al. 2008). The existence of PCD is relatively the same in animal and plant cells (Havel and Durzan 1996). PCD is directly linked with vacuolization, (deoxyribonucleic acid) DNA fragmentation, and cell shrinkage and chromatin condensation in both plants and animals (Papini et al. 2011; Poór et al. 2013; Wang et al. 2012). Generally, PCD includes, through an apoptotic process, the degradation of DNA fragments from 50 to 200 kb. Previous researchers reported that several enzymatic activities connected with various molecular mechanisms of PCD (Walker and Sikorska 1994) while precise mechanisms still not determined.

Rice is considered a staple food half of the people throughout the world and widely grown worldwide (Reyes and Chin 2009). Rice is a cheap and rich source of protein, fiber which prevents cancer disease in human (Tan and Norhaizan 2017). Vanadium also affects the human health by causing the bodyweight reduction, nose bleeding, weakness, vomiting dehydration, diarrhea (Ghosh et al. 2015). Globally, rice production is 516.3 million tons per annum while China contributed 209 million tons per annum in world rice production (FAO 2019). Phosphorous and potassium fertilizer contain a high concentration of V (Vachirapatama et al. 2002). Excessive uses of phosphate fertilizer lead to increase the V concentration in rice root (Chongkid et al. 2007) while lack of information exists about toxic V concentration in rice plants and at which V concentration rice plants show toxicity resistance.

Vanadium adversely affects plant growth and enters the food chain, causing deleterious effects on human health. There is insufficient information about the physiological and biochemical response of rice against V stress. Consequently, it is necessary to understand the V role in rice plants, especially to find out at which concentration V becomes toxic for rice and causes cell death. In regard of the above-elaborated problems, the objective of the present study was to evaluate the effect of different V concentrations on rice seedlings and at which concentration V starts suppression in the rice growth, and also investigate the effect of V on root morphology, photosynthetic pigments, photosynthetic gas exchange, enzyme activities, protein, electrolyte leakage and cell death.

2 Materials and Method

2.1 Planting Material and Harvest

In this study, rice cultivar “kissan” (*Oryza sativa*) was used to assess the V-toxicity which was purchased from Rice Research Institute (RRI) in Kala Shah Kaku, Sheikhpura, Punjab, Pakistan. The surface of the seeds sterilized prior to sow with 0.1% (v/v) sodium hypochlorite (NaClO) for 15 min, followed by cleaning with running water five times. The seed was sown in dark on filter paper in Petri dishes for 8 days and placed in a climate-controlled growth chamber. After 8 days, uniform rice seedlings were shifted into a 3-L plastic box (eight plants each box) containing Hoagland nutrient solution (Hoagland and Arnon 1950). A day temperature 28 °C, photoperiod 14 h, relative humidity 75%, and photon flux density 820 μmol m⁻² s⁻¹ (cool white fluorescent lamps) while the night temperature 25 °C, photoperiod 10-h relative humidity 75%, and photon flux density 0 μmol m⁻² s⁻¹ were fixed in the growth chamber (RTOP -268B, Bio-Equip) during the whole experiment.

By the addition of the sodium hydroxide (NaOH) and hydrochloric acid (HCl) solution, the nutrient solution pH was kept 6.5. After 6 days of shifting, V was added into Hoagland nutrient solution with different concentrations viz. 0, 15, 25, 35, and 70 mg L⁻¹, using ammonium metavanadate (NH₄VO₃) as defined in Fig. 1. After every 5 days, the nutrient solution was changed along with different V doses. The rice seedlings were treated 15 days with nutrient solution plus V. After that, plants were harvested and separated into roots and shoots after cleaning with deionized water. Harvested shoots were placed at -80 °C after grinding in liquid nitrogen with help of pestle and mortar for further assay. For V determination assay, shoot and roots were dried (at 100 °C) in the oven for 1 h, after that placed at 60 °C till constant weight. The measuring tape was used for measuring the length of shoots. By using an electronic digital balance, fresh biomass for both shoot and root were weighed and noted. Afterward, paper

bags were used for placing roots and shoots, and these bags were put at 105 °C for 15 min in the oven, following at 70 °C for 72 h. Later on, the dry weight (DW) was measured. The experiment design was carried out in a factorial arrangement based on a complete randomized design (CRD) with four replications.

2.2 Ion Leakage and Tolerance Index Determination

From every replication, leaves of four uniform plants were harvested, cleaned with deionized water for the studies of electrolyte leakage (EL). For the estimation of ion leakage, the procedure established by Dionisio-Sese and Tobita (1998) was utilized. By applying the following formula, the EL was calculated:

$$\text{Electrolyte leakage (EL)} = \frac{EC_1}{EC_2} \times 100$$

where

EC₁ = Pre-boiling electric conductivity

EC₂ = Post-boiling electric conductivity

Further, the formula established by Iqbal and Rahmati (1992) was used for calculating the Tolerance index (TI):

Tolerance indices (TI)

$$= \frac{\text{Average root length of vanadium treatment}}{\text{Average root length of control}} \times 100$$

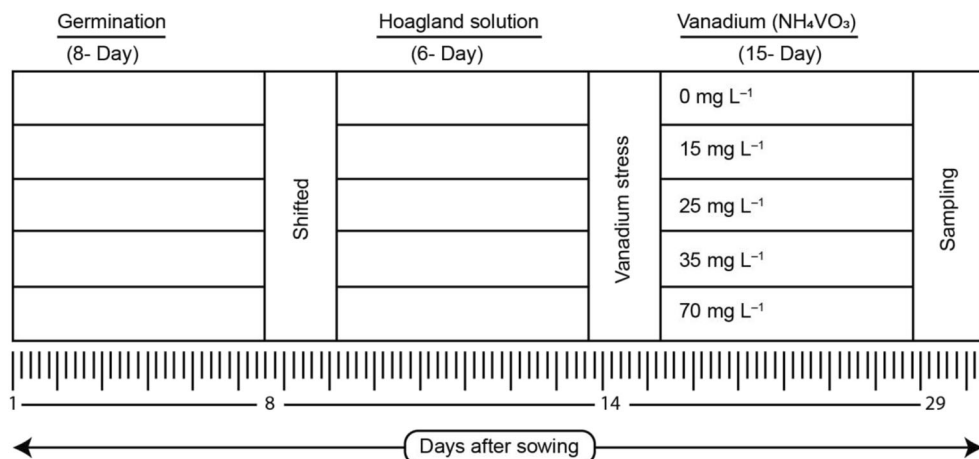
2.3 Photosynthetic Pigments Determination

According to the method established by Lichtenthaler and Wellburn (1983), the photosynthetic pigments, i.e., chlorophyll (a + b) and total carotene (C_{x+c}), were calculated:

Following formula was utilized for estimation of photosynthetic pigment concentration:

$$C_a = 11.75A_{662} - 2.550A_{645}$$

Fig. 1 Experimental setup of the study; vanadium stress treatments were added into Hoagland solution in the form of ammonium metavanadate after 12 days of sowing. The rice seedlings were treated 15 days with different vanadium concentration (0, 15, 25, 35, and 70 mg L⁻¹) under fully environment controlled growth chamber



$$C_b = 18.61A_{645} - 3.960A_{662}$$

$$C_{x+c} = 1000A_{470} - 81.4C_b/227$$

whereas

C_a = chlorophyll a, C_b = chlorophyll b, C_{x+c} = total carotene.

2.4 SPAD Index and Leaf Photosynthesis Parameters

SPAD-502 Chlorophyll Meter manufactured by Minolta Camera Co., Ltd., Japan, was utilized to estimate the relative chlorophyll content of leaves (fully expanded leaves), while portable photosynthesis system (CIRAS-3, Hansatech Co., USA) was used to measure the rice leaves net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration rate (T_r). These parameters were taken upper fully mature leaves with maximum length. At the time of measurements, the growth circumstances of the growth room were controlled as follows: leaf temperature 22 °C, CO_2 concentration 355 $\mu M mol^{-1}$, and photosynthetic photon-flux density 1000 $\mu M m^{-2} s^{-1}$. A total of 4 randomly selected plants, measurements were taken from every treatment (Singh et al. 2007).

2.5 Root Morphology

From every replication, roots of four uniform plants were harvested, cleaned with distilled water for the studies of root characteristics. Imagery Scan Screen (Epson Expression 11000XL, Regent Instruments, Canada) was used for the scanning of root and root measurement was established by WinRHIZO 2003a software (Regent Instruments, Canada).

2.6 SOD, CAT, POD, Malondialdehyde, and Protein Assay

To measure the antioxidant enzyme activities and protein contents, frozen leaf samples (0.5 g) finely ground with help of liquid nitrogen, and homogenized with buffer (4.5 ml), following the description described in the kit. Centrifugation of the homogenate was carried out at 12,000 rpm at a temperature of 4 °C for 15 min, followed by putting the supernatant in another falcon tube for further enzyme analysis.

The SOD and CAT activities were estimated by following the guidelines described in kits (A001-1, A007-1) acquired from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. The SOD and CAT content reading was noted at the wavelength of 550 nm and 405 nm, respectively. POD activities, MDA, and protein contents were measured by exploiting the A084-3-1, A003-3, and A045-2 kits, respectively. The wavelength of POD, MDA activities, and protein contents was recorded at 420 nm, 530, and 595 nm, respectively, by

using the spectrophotometer (*Lambda 25 UV/VIS Spectrophotometer*).

2.7 ROS and Cell Death Detection

The production of H_2O_2 was determined by following the instruction defined in the A064-1-1 kit. H_2O_2 contents were measured at a wavelength of 405 nm (*Lambda 25 UV/VIS Spectrophotometer*). For cell death analysis, V-treated and untreated leaves of rice seedlings were selected and analyzed the cell death by utilizing the Evans blue stain (Baker and Mock 1994).

2.8 Vanadium Determination

Vanadium content accumulated in rice roots and shoots was determined by using the procedure established by Hou et al. (2013). Graphite furnace atomic absorption spectrophotometer (GFAAS-GTA 120) was utilized to determine V-contents.

2.9 Statistical Analysis

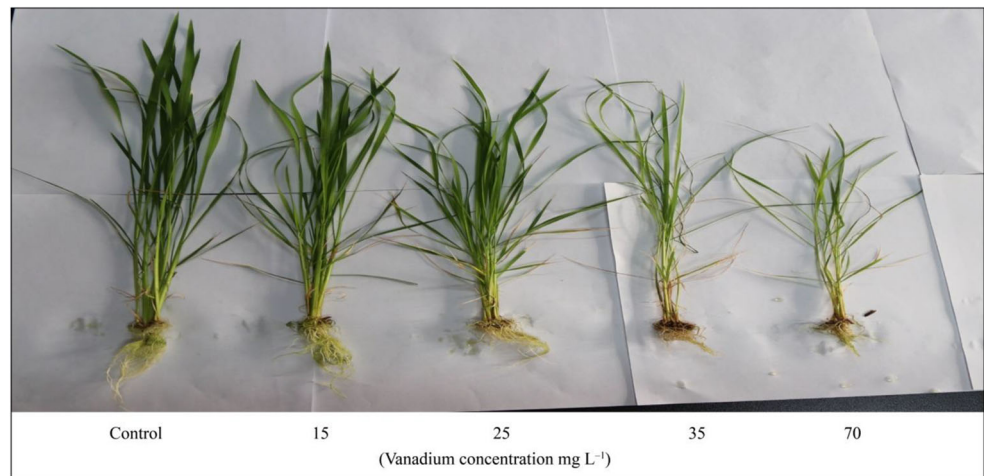
Statistical analysis was accomplished using SPSS software (version 9.1, SPSS Institute Inc. Cary, NC, USA). Different alphabets indicate a significant difference between the V concentrations ($p \leq 0.05$). For four replications of each treatment, the represented data are the means \pm standard errors (SE). Fisher's least significant difference (LSD) test was employed ($p \leq 0.05$), for analyzing the differences among the V concentrations.

3 Results

3.1 Influence of V on Plant Growth

Different V concentrations (15–70 $mg L^{-1}$) significantly ($p \leq 0.05$) caused a decline in the development and growth of roots and shoots of rice seedlings (Fig. 2). Further, we noted that later root development was inhibited by increasing the V concentration. The root and shoot biomass (fresh and dry) of rice seedlings were significantly declined by V concentrations, but the reduction was more noticeable at higher concentrations (35 and 75 $V mg L^{-1}$), compared with those at minor concentrations (15 and 25 $V mg L^{-1}$) (Table 1). The root and shoot biomass (fresh and dry) were declined by 16.55% 27.15%, 58.94, and 73.95% (fresh shoots) and 14.64%, 27.58%, 41.37%, and 63.21% (fresh roots) and 32.07%, 47.16%, 73.58%, and 84.90% (dry shoots) and 17.64%, 29.41%, 52.94%, and 70.58% (dry root) after 15 days upon exposure to 15, 25, 35, and 70 $V mg L^{-1}$, respectively, than untreated. Similarly, all V concentrations significantly decreased the rice shoots and root length (Table 1). Shoot length was decreased

Fig. 2 Rice seedling performance under different vanadium concentrations



by 23.35% to 67.66% while root length was decreased by 7.64% to 32.01% with all V concentration when compared with control. The maximum reduction in shoot and root length was noted by 67.66% and 32.01%, respectively, with 70 V mg L⁻¹ (Table 1).

3.2 Effect of V on TI

The TI was constantly reduced by increasing the V concentrations and indicated the negative correlation among the V concentrations and TI (Table 1). The tolerance index was 82.01%, 70.21%, 50.40%, and 34.89% in rice seedling against 15, 25, 35, and 70 V mg L⁻¹, respectively.

3.3 Influence of V on Root Morphology

In the present study, we found that V concentrations caused a significant decline in root growth of rice seedling, as a result of the decline in total root length, root tips, root fork, root surface area, and root crossing (Fig. 3). Moreover, the root morphology-related

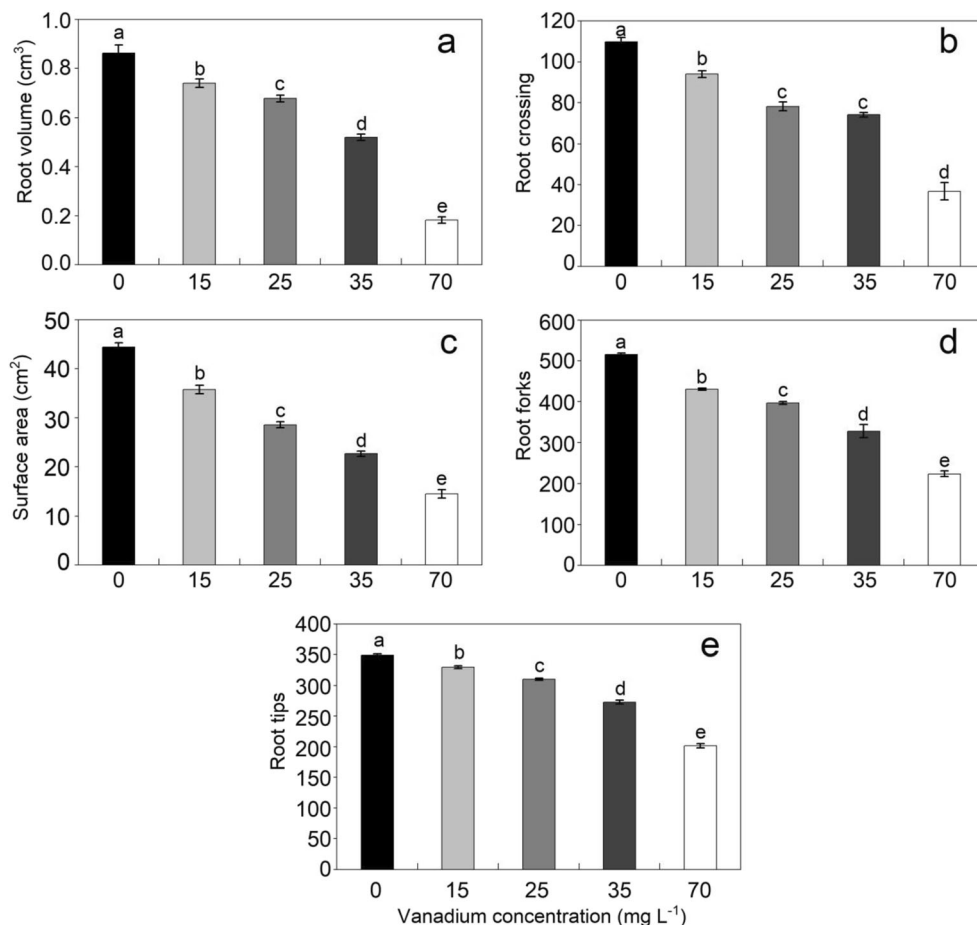
parameters were gradually depressed with increasing the V concentration but the most damaging effect was produced at 35 and 70 V mg L⁻¹ as a comparison with untreated seedlings (Fig. 3).

Root volume in rice seedlings decreased by 14.20% to 78.84% with all V concentrations as compared with control (Fig. 3a). All other V concentrations were comparable with the untreated seedlings (Fig. 3a). The root crossing was reduced 14.35% and 66.51% after the application of 15 and 70 V mg L⁻¹, respectively, as compared with untreated seedlings (Fig. 3b). The rice seedling exposed to 25 and 35 V mg L⁻¹ showed a root crossing statistically similar results (Fig. 3b). All the V concentrations significantly affect the root surface area, root tips, and root forks of rice seedlings (Fig. 3c, d, and e). The root surface area, root forks, and root tips were decreased 19.54%, 35.76%, 48.96%, and 67.27% (Fig. 2c); 16.83%, 22.98%, 36.42%, and 56.59% (Fig. 3d); and 5.15%, 11.03%, 21.84%, and 42.26% (Fig. 3e) after being treated with 15, 25, 35, and 70 V mg L⁻¹, respectively, as compared with untreated seedlings.

Table 1 Effect of vanadium on fresh and dry biomass, and lengths of rice seedlings. Means values ± SE (*n* = 4); different lowercase letters within each column represent statistically significant differences (Fisher’s least significant difference (LSD) test, *p* ≤ 0.05)

Vanadium (mg L ⁻¹)	Biomass yield per plant (g)				Lengths (cm)		Tolerance index (%)
	Fresh		Dry		Shoots	Roots	
	Shoots	Roots	Shoots	Roots			
Control	0.4533 ± 0.0185a	0.0872 ± 0.0007a	0.0532 ± 0.0037a	0.0176 ± 0.0002a	41.75 ± 1.4930a	194.54 ± 3.4920a	100
15	0.3782 ± 0.0062b	0.0747 ± 0.0006b	0.0369 ± 0.0020b	0.0149 ± 0.0004b	32.00 ± 0.9128b	179.66 ± 1.2974b	82.01
25	0.3300 ± 0.0180c	0.0638 ± 0.0019c	0.0282 ± 0.0012c	0.0122 ± 0.0003c	27.25 ± 0.8539c	170.66 ± 2.2173c	70.21
35	0.1867 ± 0.0102d	0.0516 ± 0.0009d	0.0141 ± 0.0016d	0.0086 ± 0.0005d	20.25 ± 0.8539d	152.13 ± 4.1277d	50.4
70	0.1184 ± 0.0070e	0.0323 ± 0.0011e	0.0087 ± 0.0006e	0.0052 ± 0.0003e	13.50 ± 0.9574e	132.26 ± 3.3079e	34.89

Fig. 3 Effect of vanadium levels on **a** root volume, **b** root crossing, **c** root surface area, **d** root forks, and **e** root tips of rice seedlings. Means values \pm SE ($n = 4$); different lower case letters in each subfigure represent statistically significant differences (Fisher's least significant difference (LSD) test, $p \leq 0.05$)

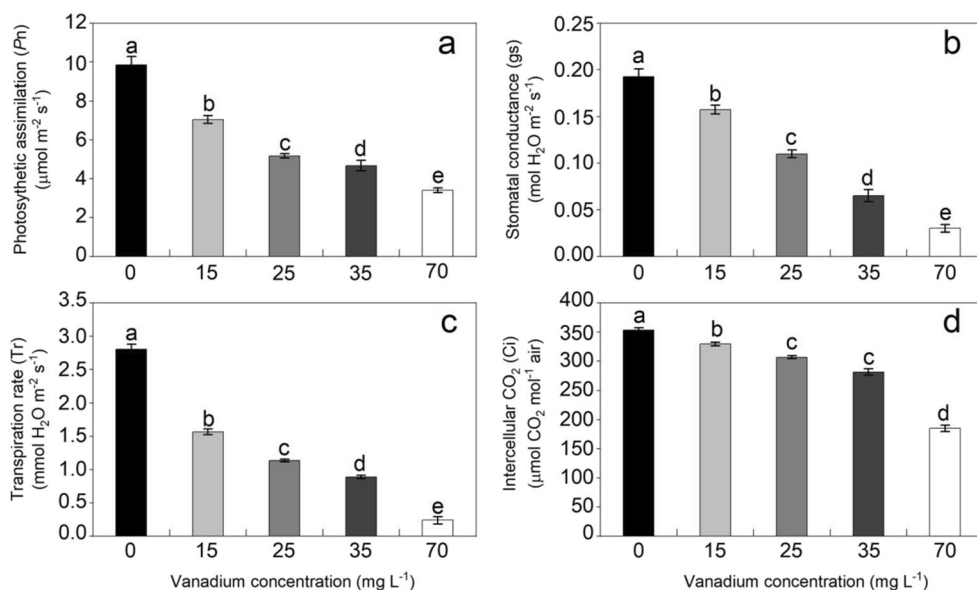


3.4 Change of Photosynthetic Activity in Response to V

Our obtained results clearly showed that all V concentrations produced a drastic effect on photosynthetic

activities of rice seedlings (Figs. 2 and 4). The rate of Pn was lower 52.58 and 65.48% in rice seedlings when exposed to 35 and 70 V mg L⁻¹, respectively as compared with untreated seedlings (Fig. 4a). The gs and Tr were significantly decreased by 84.41% and 91.78%,

Fig. 4 Effect of vanadium levels on **a**) photosynthetic assimilation (Pn), **b** stomatal conductance (gs), **c** transpiration rate (Tr), and **d** intercellular CO₂ (Ci) of rice seedlings. Means values \pm SE ($n = 4$); different lower case letters in each subfigure represent statistically significant differences (Fisher's least significant difference (LSD) test, $p \leq 0.05$)



respectively, following treatment with 70 V mg L⁻¹ (Fig. 4b and c). The seedlings treated with 25 and 35 V mg L⁻¹ showed statistically similar results of an intercellular CO₂ (Fig. 4d).

3.5 Influence of V on Chlorophyll Pigments and SPAD Index

The results demonstrated that photosynthetic pigments and SPAD index significantly reduced by increasing the concentrations of V from 15 to 70 mg L⁻¹ than untreated plants (Fig. 5). The maximum reduction in chlorophyll a and total carotenoids were noted at 70 mg L⁻¹. The addition of 35 and 70 V mg L⁻¹ increased by 51.23% and 57.05% of the chlorophyll a of rice seedling (Fig. 5a). A similar effect was produced with 35 and 70 mg L⁻¹, and an increase of 51.23% and 57.05% was observed for chlorophyll b (Fig. 5b). The carotenoids in the leaves of rice seedlings were decreased up to 19.29%, 40%, 50.87%, and 71.57% after the treatment of 15, 25, 35, 70 mg L⁻¹, respectively, as compared with untreated seedlings (Fig. 5c). The SPAD index was decreased up to 22.32%, 29.16%, 52.84%, and 65.78% when treated with 15, 25, 35, and 70 V mg L⁻¹, respectively, as compared with untreated seedlings (Fig. 5d).

3.6 Response of Antioxidant Enzymes to V

The V concentrations showed significant changes in antioxidant enzyme activities of rice seedlings; the positive relationship was observed between the V addition and

antioxidant enzyme activities when exposed to different V concentrations (Fig. 6). The activities of antioxidant enzymes (SOD, CAT, and POD) exhibited consistent increase with the increase of V concentrations from 15 to 75 mg L⁻¹, while maximum enhancement in antioxidant enzyme activities was pronounced at 35 and 70 V mg L⁻¹. The activities of SOD in rice seedlings were approximately 440.03% and 600.08% higher with the addition of 35 and 70 V mg L⁻¹, respectively, as compared with control (Fig. 6a). Similarly, an increase of 492.37% and 669.88% was observed in CAT activities with 35 and 70 V mg L⁻¹ (Fig. 6b). Besides this, the addition of 15 V mg L⁻¹ produced minimum enhancement 181.87%, while the highest enhancement 748.47% produced with 70 V mg L⁻¹ in POD activities, as compared with control (Fig. 6c).

3.7 Effect of V on H₂O₂ and MDA Contents

The rice seedlings were exposed to different V concentrations that exhibited maximum enhancement in H₂O₂ and MDA contents of rice leaves as compared with untreated plants (Fig. 7). The H₂O₂ production was about 2.26-, 3.33-, 4.89-, and 6.2-folds enhanced when exposed to 15, 25, 35, and 70 V mg L⁻¹, respectively, as compared with control (Fig. 7a). However, H₂O₂ activity was much high at increased concentrations (35 and 75 V mg L⁻¹) than minor concentrations (15 and 25 V mg L⁻¹) in rice seedlings. The obtained results show that MDA contents were enhanced by increasing the V concentrations. The maximum MDA content was noted at 35 and

Fig. 5 Effect of vanadium levels on **a** chlorophyll a, **b** chlorophyll b, **c** carotenoids, and **d** SPAD index (relative chlorophyll content) of rice seedlings. Means values \pm SE ($n = 4$); different lower case letters in each subfigure represent statistically significant differences (Fisher's least significant difference (LSD) test, $p \leq 0.05$)

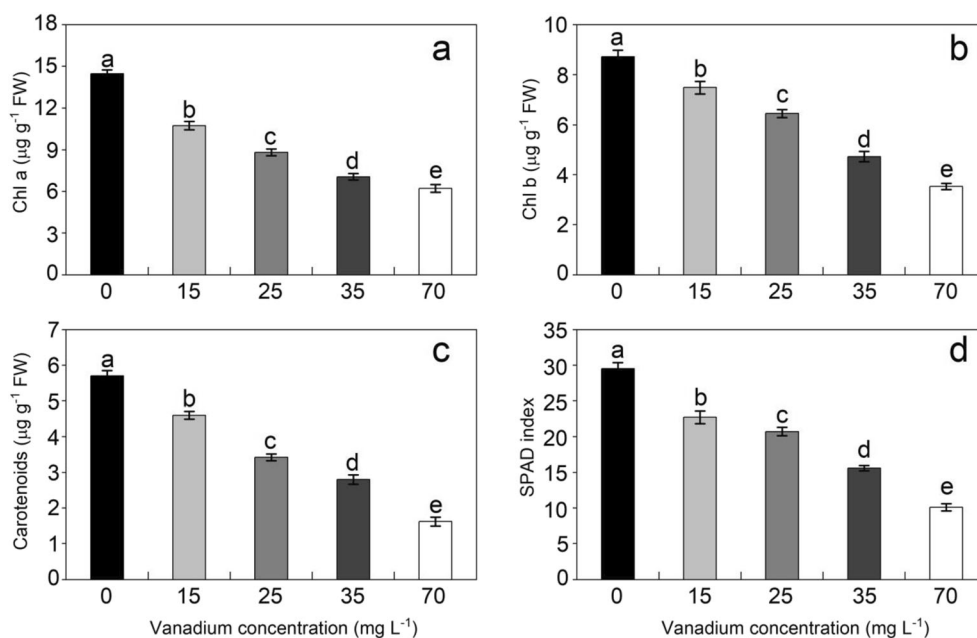
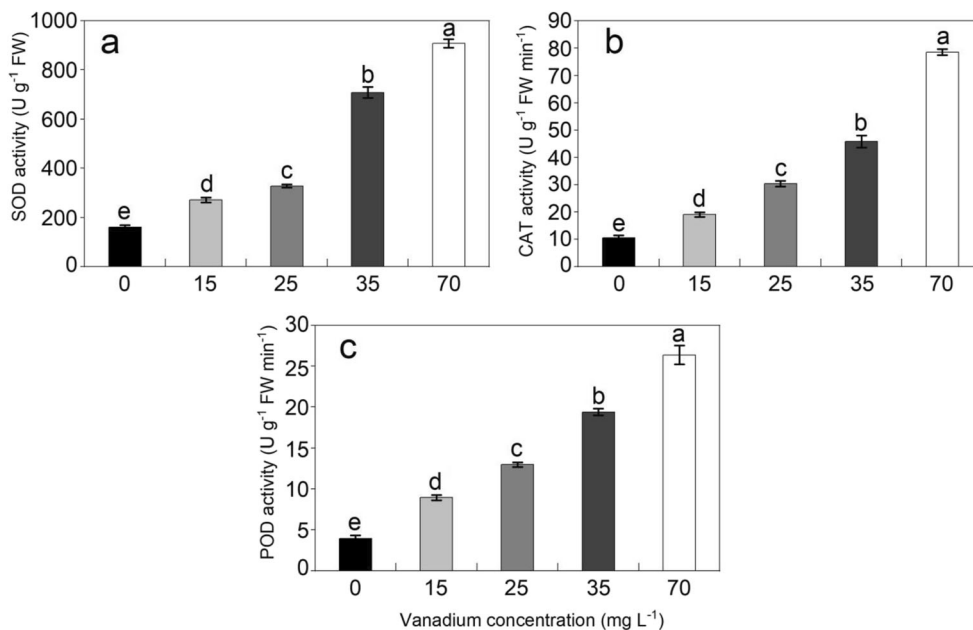


Fig. 6 Response of antioxidant enzymes **a** superoxide dismutase activity (SOD), **b** catalase activity (CAT), and **c** peroxidase (POD) of rice seedlings under different vanadium levels. Means values \pm SE ($n = 4$); different lower case letters in each subfigure represent statistically significant differences (Fisher's least significant difference (LSD) test, $p \leq 0.05$)

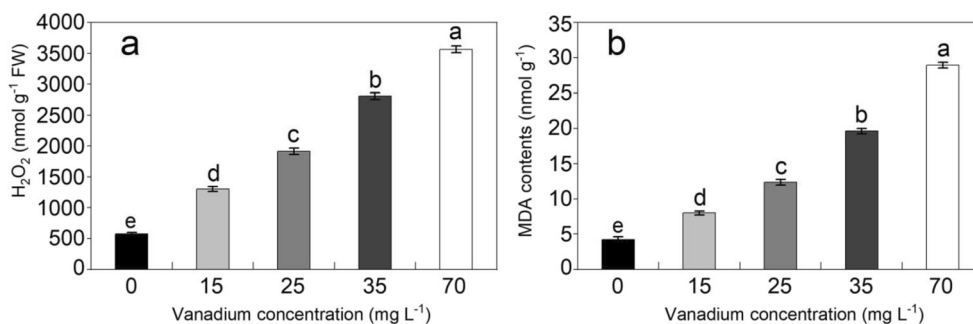


70 V mg L⁻¹, which produced 4.68- and 6.92-folds higher MDA contents than control (Fig. 7b).

3.8 Influence of V on Electrolyte Leakage and Cell Death

The V addition significantly enhanced the values of EL. The values of EL linearly increased by increasing the V concentration; however, the maximum increments 4.18- and 7.81-folds were detected at 35 and 70 mg V L⁻¹, respectively, as a comparison with untreated plants (Fig. 8a). The obtained findings clearly exhibited that V induced the destruction of membrane integrity in rice seedlings. The Evans blue stain was utilized to assess cell death in rice seedling against various V concentrations. The results demonstrated that Evan blue extracted solution absorbance significantly enhanced when rice seedlings interacted with a higher level of V (Fig. 8b). The maximum enhancement (78-folds than untreated plants) was found at 70 V mg L⁻¹ in absorbance of the Evan blue extracted solution in rice seedlings.

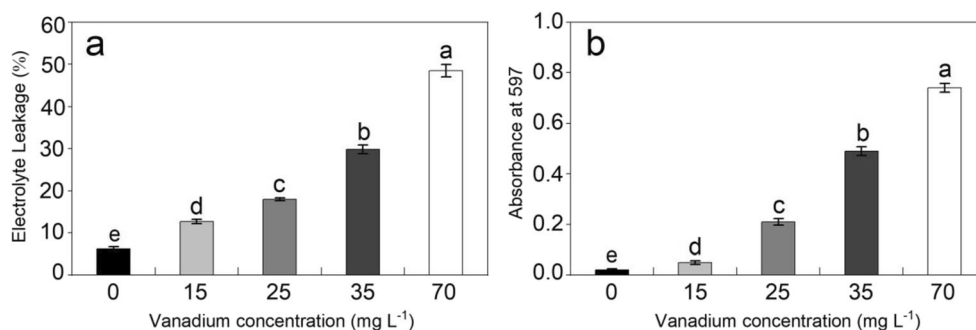
Fig. 7 Effect of vanadium levels on **a** hydrogen peroxide (H₂O₂) and **b** malondialdehyde (MDA) contents in rice seedlings. Means values \pm SE ($n = 4$); different lower case letters in each subfigure represent statistically significant differences (Fisher's least significant difference (LSD) test, $p \leq 0.05$)



3.9 Influence of V on Protein Contents and Its Accumulation in Roots and Leaves

The findings of the present study clearly showed the variation in protein contents of rice seedlings when exposed to different V concentrations (Fig. 9a). The reduction in protein contents consistently increased by increasing the V concentrations from 15 to 70 mg L⁻¹ as a comparison with untreated seedlings, while highest decline 41.48% and 85.18% was noted when rice seedlings were treated with 35 and 70 V mg L⁻¹, respectively, and minimum reduction 22.22% was noted at 15 mg V L⁻¹ than untreated seedlings (Fig. 9a). The V addition resulted in significant accumulation in rice roots and shoots as a comparison with control (Fig. 9b). The obtained findings indicate that V was more accumulated in roots than shoots. The V accumulation in roots and shoots showed a positive correlation with V doses. The highest V 4420.75 μ g g⁻¹ DW was accumulated in root and 2353.75 μ g g⁻¹ DW in shoots as compared with control when treated with 70 V mg L⁻¹ (Fig. 9b).

Fig. 8 Effect of vanadium on electrolyte leakage and cell death in rice seedlings. Means values \pm SE ($n = 4$); different lower case letters in each subfigure represent statistically significant differences (Fisher’s least significant difference (LSD) test, $p \leq 0.05$)



4 Discussion

Recently, agriculture soils are contaminated with different types of soil pollutants from a wide range of sources. Most of the scientists have been reported that large part of the agriculture soil pollutants enters the soil from heavy metals such as V, nickel (Ni), cadmium (Cd), and lead (Pb). Rapid consumption of V in steel industries is the main source in which the V enters the environment and now has become a major issue for the scientists, due to its drastic effects on plant, animal, and human creatures. Therefore, it is necessary to scrutinize the key processes and mechanisms involved in V toxicity.

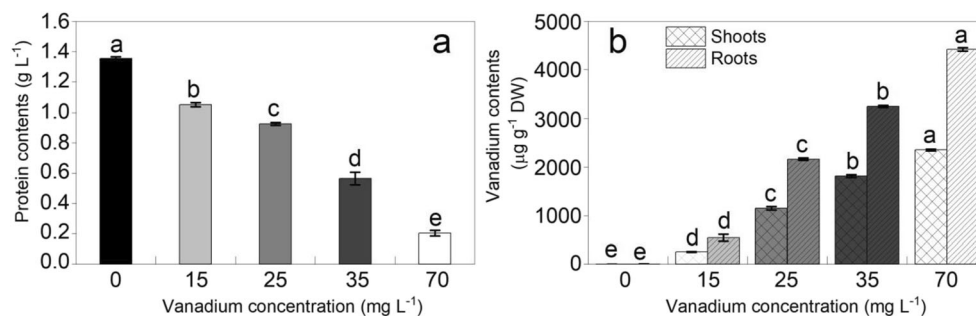
Plant growth traits such as plant heights and fresh biomass are generally considered best indicators to any kind of metal stress and also utilized to estimate the plant tolerance capability against metal stresses. Heavy metals produced toxicity and adversely affected the plant growth trait shoot length, fresh and dry weights (Kaya et al. 2006; Shafeeq-ur-Rahman et al. 2020), and normally such type of decline varied from organ to organ of the plants (Liang et al. 2007) while lower concentrations improve the plant growth (Saldaña-Sánchez et al. 2019). The finding of the current study exhibited a negative correlation between the growth parameters and V concentrations for rice seedling. Similarly, Yuan et al. (2020) reported that the fresh biomass, shoots, and roots length of the rice seedlings were decreased against V stress. Vanadium at higher concentrations markedly decreased the fresh and dry biomass of the roots and leaves (Yang et al. 2017b; Barouchas et al. 2019). Vanadium concentrations produced an imbalance in nutrition

and disturbed their role in anabolic pathways that ceased the normal developmental processes.

The results also indicated that V concentrations limited the root growth, due to the decline of total root length, root surface area, root tips, root forks, root crossings, and root volume. The plants treated with higher V concentration produced shorter, thick, less lateral roots, and coralloid structures (Yang et al. 2017b). The root length and 75% dry weight of *cuphea* were reduced against 7.8 V mg L⁻¹ stress (Olness et al. 2005). The reduction in root length might occur due to mitotic cell division which could restrict root tip development (Meisch et al. 1977). These findings are to be the same as the earlier results described by Imtiaz et al. (2017) in chickpea and Nawaz et al. (2018) in watermelon.

SPAD reflects the leaf relative chlorophyll content (Nawaz et al. 2017). Normally, a high level of chlorophyll represents stronger photosynthetic assimilation and plant growth. Chlorophyll content may represent the plant’s sensitivity against abiotic stress. Generally, the V application reduced the chlorophyll a and b with compared with their respective control. In the current study, the SPAD index, chlorophyll a, and b contents were declined in rice seedlings by increasing the V concentrations. Similarly, Nawaz et al. (2018) demonstrated that relative chlorophyll content dramatically reduced when watermelon seedlings exposed to V stress. Photosynthesis pigments were decreased under V stress, which can attribute to varying the membrane permeability and destruction of the component induced by oxidative stress (Aihemaiti et al. 2019). Chary et al. (2008) demonstrated that initiation of ROS is an important factor that causes a reduction

Fig. 9 Effect of vanadium on a soluble protein contents and b vanadium accumulation in shoots and roots of rice seedling. Means values \pm SE ($n = 4$); different lower case letters in each subfigure represent statistically significant differences (Fisher’s least significant difference (LSD) test, $p \leq 0.05$)



in leaf chlorophyll contents. Similar results were stated in chickpea (Imtiaz et al. 2015b), Soybean (Dos Santos et al. 2020) *Trigonella foenum-graecum* (Choudhary et al. 2012), and *Aeluropus littoralis* (Reijonen et al. 2016) under metal stress.

The Pn reflects plant's capability to harvest CO₂ and involves in several plant metabolic processes. The numerical value represents the plant's capability to produce CO₂ (Yu-chen et al. 2015). Photosynthesis plays a key role in the carbohydrate synthesis process and adversely affected by heavy metal toxicities. The findings of this study indicated that photosynthetic apparatus such as Pn, ci, gs, and Tr rapidly decreased in rice seedling by increasing the V intensity of stress. However, findings of the lateral study by Nawaz et al. (2018) indicated that V at 50 mg L⁻¹ reduced the Pn, ci, gs, and Tr of watermelon seedlings. Vanadium at higher concentration adversely affects the Pn, Tr, and other important physiological processes involved to produce energy, matter, and drive matter translocation, causing in inhibited growth and biomass yield of the plant (Yang and Tang 2015; Abedini and Mohammadian 2018). Vanadium concentrations might be possible to cause damages in chloroplastic and ultra-structure, disturbance in electron transport mechanisms and photosystem I (PSI) electron diversions, and ultimately decreased the photosynthetic activities of plants. The findings of the current study are same with earlier reports that have evaluated the plant growth against various metal stresses, e.g., chickpea under V stress (Imtiaz et al. 2018b), mustard treated against Cd stress (Mobin and Khan 2007), and oilseed rape grown under Pb stress (Tian et al. 2014), turfgrass against mercury (Hg) stress (Yu-chen et al. 2015), wheat under arsenic (As) stress (Maghsoudi et al. 2020), and *Artemisia annua* grown against zinc (Zn) stress (Khudsar et al. 2004).

Plants have developed different biochemical mechanisms to ease oxidative damage caused by heavy metals. Enzymatic antioxidants such as SOD, CAT, POD, and MDA act as a strong antioxidant and also helped to relieve the plants when exposed to V stress. In plants, heavy metal stress stimulates ROS production by causing permanent oxidative damage to several macromolecules (Jain et al. 2010). The plants activate their antioxidant defense machinery to remove oxidative damage caused through mental stress. Antioxidant enzymes comprised SOD, CAT, and POD which are essential components of antioxidant defense machinery and play an important role to reduce and restore the oxidative damage induced by ROS (Jain et al. 2010).

The enzymes are the essential part of antioxidant defense machinery and present in several cellular compartments that stimulate the ROS production in plants. Previously, scientists have conveyed higher anti-oxidant enzyme activity that represents a mechanism to repair oxidative damage in plants (Dazy et al. 2009). The obtained findings of the current study clearly presented a positive correlation among the V and

enzymes. The SOD, CAT, POD, and MDA were significantly higher when treated with higher V concentration. Likewise, SOD, CAT, POD, and MDA activities were increased when exposed to V stress in watermelon (Nawaz et al. 2018) and rice (Yuan et al. 2020). This enhancement in SOD, CAT, POD, and MDA enzymatic activity might be caused as a consequence of the direct effect of heavy metal ions and the indirect effect on free oxide radical production (Zembala et al. 2010). CAT is known as H₂O₂ scavenging enzymes (Wang et al. 2016). The up-regulation of enzyme activities might be ascribed to de novo synthesis of enzyme proteins, although the higher CAT activity allows the plant to remove the H₂O₂ via conversion to oxygen (O₂) and water (H₂O) (Imtiaz et al. 2018b). The enhancement in SOD and CAT decreased the H₂O₂ contents (Nawaz et al. 2018). Vanadium depressed the few antioxidants in corn seedlings which are able to protect the main cellular organs and induced plant tolerance and detoxification under metal stress (Hou et al. 2019). The SOD activity stimulates the superoxide radicals, H₂O, and O₂ yield, consequently holding the superoxide radicals to a stable form. The higher POD activity defends cellular membrane from the oxidative damage induced by metal stress and additionally allows the plants to scavenge H₂O₂ from cells (Miller and Mittler 2008; Monferrán et al. 2009; Verma and Dubey 2003). The results obtained in this study are similar to previous results confirmed by Imtiaz et al. (2018b) in mustard, Imtiaz et al. (2017) in chickpea, Gupta et al. (2009) in Maize, and Yuan et al. (2020) in rice.

Generally, ROS induced in the plant as a result of reaction among fatty acid and heavy metal (Shah et al. 2001). The H₂O₂ is an important component of ROS, and maximum produced when plants interact with heavy metals (Rizwan et al. 2017). Our findings illustrated that H₂O₂ production in rice seedlings was higher in response to increase the V concentration but the highest H₂O₂ production was noted at 70 V mg L⁻¹. The initiation of ROS (H₂O₂) takes place as a result of metabolic processes in plants and primarily damage antioxidant defense system along with metabolic activities and tends to impair the plant cell viability (Dat et al. 2000), ROS are eliminated through antioxidant enzymes such as SOD, CAT, and POD (Alscher et al. 2002). Previously, studies demonstrated that ROS, superoxide anion radical (O₂⁻), and H₂O₂ play important role in plant cell death (Bouchez et al. 2007; Moeder et al. 2002; Overmyer et al. 2003). The ROS, calcium-dependent protein kinase (CDPKs), and phosphatases may participate in the vanadate signaling pathway that caused cell death of rice (Lin et al. 2009). In this study, cell death in rice seedlings under different V concentrations was determined by utilizing the Evans blue stain. The cell death in rice seedling was concentration reliant and enhanced by increasing V concentrations (Fig. 8b). The highest rise in Evan blue extracted solution absorbance was noted at 70 V mg L⁻¹ of rice seedlings. The findings of the current study are similar

with the finding described by Lin et al. (2009) in rice and Imtiaz et al. (2018a) in chickpea.

Besides this, ion leakage results clearly showed a positive relationship with V concentration and confirmed that ion leakage maximally increased when exposed at higher V concentrations. Previously, scientists stated that ROS caused the cell death that can be accessed via absorbance of Evan blue stain and ion leakage (Begum et al. 2011; Kawai-Yamada et al. 2004). Hence, the present findings also confirmed that initiation of ROS due to V could be responsible for cell death in rice seedlings. Similarly, Imtiaz et al. (2018a) demonstrated that V at a higher concentration of 130 mg kg⁻¹ increased the ion leakage and cause cell death of chickpea leaves.

Rice seedlings showed different responses against different V concentrations. A positive relationship was exhibited among the ion leakage and V (Fig. 8a), and the highest electric conductivity was induced in rice seedlings at 35 and 70 mg L⁻¹ as compared with untreated plants. The increment in electrolyte leakage (Fig. 8a) and POD (Fig. 6c) confirmed that V produced toxic effects on rice seedlings. Vanadium induced ion leakage by damaging the cell membrane integrity. The disruption in plant membrane might happen as a result of accumulated V which triggered the ROS yield that might deform the lipid bilayer by cyclic cascade reaction (Fenton reaction), stimulating the membrane disruption against V stress. Besides this, metal stress normally caused the structural interruption of protein via altering the S and N protein groups and change the ionic flow of membrane, which stimulate the electrolyte leakage (Teisseire and Guy 2000). The findings of the current study are consistent with the previous findings reported by Lin et al. (2009) in rice, Tamas et al. (2006) in Chinese cabbage, and Xiong and Wang (2005) in barleys under heavy metals stress.

The production of protein is normally sensitive to metal stress and might be decreased due to metal hindering effects (Hemalatha et al. 1997). In current results, protein content was gradually reduced by increasing the V concentrations (15 to 70 mg L⁻¹); the highest reduction in protein contents was noted at 70 V mg L⁻¹. Generally, the metal stress-induced toxic effects on protein by damaging the functional capability of various enzymes having a functional sulfhydryl group (Tanyolac et al. 2007). The findings of current study accord with the finding of Vassilev and Lidon (2011), Singh et al. (2007), Imtiaz et al. (2018a), and Guo et al. (2004) who confirmed that protein content was decreased in barley, wheat, chickpea, and barley, respectively, when exposed to metal stress.

The TI was consistently reduced in rice seedlings by increasing the V levels in the growth medium and found a negative correlation among the TI and V. The minimum TI in rice seedlings was noted at 70 V mg L⁻¹ and maximum TI at lower concentration 15 V mg L⁻¹. Previously, researchers demonstrated that metal toxicity adversely affects plant growth by

changing the metabolic activities as a result of interaction with enzymes and cause significant variations in plant growth (Gupta et al. 2009). The findings of the current study are similar to the results of Ahmad et al. (2012), Khan et al. (2006), and Imtiaz et al. (2018a).

The uptake and deposition of V in rice roots and shoots were enhanced by increasing the V concentration. Vanadium accumulation in roots was higher than shoots and also showed a positive correlation with V. Vanadium chelation and complexation with a polar compound in the root cell wall, induction of stable compound in root tissues with calcium (Ca), and compartmentalization in vacuoles of the root and stems decreased the free V ion concentration and translocation in the aerial part of plants (Kaplan et al. 1990). This unequal V distribution restricts the V movement to shoots (Imtiaz et al. 2018b) as shown in the current study. Similarly, maximum V accumulation was observed in roots of Chinese green mustard, tomato plants (Narumol et al. 2011), watermelon (Nawaz et al. 2018), mustard (Imtiaz et al. 2018b), and chickpea (Imtiaz et al. 2017) when treated with V.

5 Conclusions

In this study, added vanadium (V) concentrations (0, 15, 25, 35, and 70 mg L⁻¹) reduced the rice seedling growth by altering the morphological, physiological, and biochemical attributes. The results verified that V at 35 and 70 mg L⁻¹ significantly reduced the chlorophyll pigments and photosynthetic gas exchange which ultimately caused a reduction in seedling growth while the less reduction in seedling growth was observed at 15 and 25 V mg L⁻¹. The stunt root growth due to V application was noted and also found that V was more accumulated root than the shoot of rice seedlings. The findings of the present study also indicate the potential involvement of V (35 and 70 mg L⁻¹) in the cell of rice plants. The cell death in rice plants occurred due to oxidative stress produced by V leading to the production of reactive oxygen species (ROS) which augmented the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and *malondialdehyde* (MDA). In the future, further research is needed to explain the mechanism and gene expression involved in cell death caused by V toxicity in rice plants.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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