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ORIGINAL PAPER

Effect of vanadium on germination, growth and activities of amylase and antioxidant enzymes in genotypes of rice

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Abstract
Vanadium causes metabolic interruption with normal growth of plants, and therefore, contamination issues are of major concern. Seed germination is a critical point in seedling establishment and subsequent plant health and vigor. The present study was carried out to assess the physio-biochemical responses: seed germination, seedling growth, activities of antioxidant enzymes and non-enzymes, and amylase contents in rice varieties. The obtained results showed variations in all observed parameters, and the rice varieties respond differently to vanadium treatments. Overall, the protrusion and germination rates of rice seeds were affected and reduced when exposed to vanadium. The fresh biomass and height of shoots and roots were also decreased when seedlings of rice were exposed to vanadium, especially at 30 mg L^{-1}. The results about antioxidant enzymes and non-enzymes indicated that rice varieties respond differently; however, the activities of antioxidant enzymes were increased when treated with vanadium. Furthermore, the contents of antioxidant non-enzymes were also altered in all rice varieties seedlings when exposed to vanadium. The soluble protein contents were also significantly declined in all rice varieties when treated with vanadium. The activities of α, β and total amylase enzymes in rice seeds were also affected when exposed to vanadium; taken as whole, the maximum observed inhibition in amylolytic enzyme activities was observed at 30 mg L^{-1} of vanadium treatment. In summary, rice seedlings were markedly influenced in terms of seed germination, early growth as well as amylase contents when exposed to vanadium. Overall, the toxic effects of vanadium with respect to rice varieties are maximum during seed germination and growth while variations regarding amylase activities were recorded. These results suggested that rice varieties: Chao you 37 and Chao ji 1 hao exhibited maximum losses, while less damage in Tai guo xiang nuo and Qi dao 2000 might be related to strong antioxidant defense system proved them to be tolerant against vanadium.

Keywords Rice · Genotypes · Vanadium · Seed germination · Growth · Antioxidant enzymes · Amylase

Introduction

Globally, the contamination of agricultural land with heavy metals has become a burning issue. Recently, vanadium (V) is widely spreading in soil and biological systems and the transportation of V in different environments increasing continuously due to various anthropogenic activities such as automobiles, combustion of oil, effluents from factories and domestic heating. The chemical and biological weathering processes are also involved in the mobilization of V in soil minerals (Yang et al. 2014). In China, 20 million hectares of agricultural land is polluted with heavy metals and these metals are of major threat to food chain (Cheng 2003). Recent studies indicated that V at higher levels causes toxicity and has been identified as a potential toxic metal and listed in the same class

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as lead, arsenic and mercury (Imtiaz et al. 2015, 2018; Naeem et al. 2007).

The previous studies considered V as a trace element for proper growth and development of plants (Kraepiel et al. 2009) while recent reports challenged its essentiality. Therefore, V yet not listed as an essential element for the plant growth and does not have any evidence that V participates in the cell metabolism. Previously it is reported that higher concentrations of V cause deleterious effects on plants development and metabolism. The plant species showed sharp reduction when exposed to V (Imtiaz et al. 2015). It is also reported that V-contamination poses a serious problem for agriculture production and food chain. The major toxic effects of V on plants are: inhibition of root hairs and growth due to impairment in cell division processes in the root tips, shoots mortality and biomass, and formation of reactive oxygen species (ROS) (Imtiaz et al. 2016, 2018). Generally, plants respond differently when exposed to heavy metal stress in both the value of antioxidants and the activities of antioxidant enzymes. The redistribution of metals within the plants is varietal and metals specific, therefore, the induction of antioxidant metabolism may be critical for metals-tolerance (Sun et al. 2007).

Starch is the major form of carbohydrate that is stored in the grains of cereal crops. Mainly, the hydrolytic enzymes are responsible for the hydrolysis of stored starch through which newly formed sprouting get support (de Andrade and da Silveira 2008). Amylase is the main hydrolyzing enzymes such as α- and β-amylase for the stored starch. Actually, the aleurone layer of seeds contained α-amylase which shares maximally hydrolysis of stored starch into metabolizable sugars through which newly sprouting obtained energy for germination as well as for the growth of shoots and roots (Kaneko et al. 2002). It is reported that metals toxicity causes inhibitor effects on amylase activities during seed germination (Radha and Rajesh 2011). The moderate levels of heavy metals could be important for seed germination as well as enzymes activities while higher levels impair the physiological processes of plants (Bonnet et al. 2000). Currently, there is no study found that describes the effect of vanadium on amylase activity.

Worldwide, rice is the major staple food for people including Chinese. Recently, it is noted that rice production is getting decrease due to various factors, especially heavy metals contamination of agricultural land. Despite that vanadium contamination is getting more attention day by day and poses toxicity for agriculture. However, plant tolerance mechanisms and varietal differences against vanadium are to date not completely understood. Here, in the present study, we have screened 29 varieties of rice for tolerance to the vanadium. Previously, there are no data regarding vanadium and physicochemical studies of rice are available.

Keeping all these issues in mind, the present work was designed to understand the role of vanadium in rice varieties. The main purposes of the present work included: (1) to assess the differential responses of vanadium among various rice varieties, (2) to evaluate the germination ratio and α-amylase activity for rice varieties against vanadium and (3) to find out the level of tolerance among 29 different varieties of rice in varied vanadium concentrations and ultimately to find out the most tolerant and sensitive variety against vanadium. The obtained results are expected to improve our understanding of vanadium responses in rice varieties and will provide future guidance for the better growth of rice in vanadium contaminated soils and/or water.

Materials and methods

Plant materials and growing conditions

The seeds of 29 rice varieties used in the experiment were purchased from Guangxi Guigang and Breeding Professional Co-operatives and Hubei Tianmen Di Long Seed Industry Co. Ltd., the varieties names are given in Table 1.

The experiment was conducted in a controlled room, and the temperature was maintained at 22–25 °C while the relative humidity was 70%. A 14-h photoperiod supported with cool white fluorescent lamps along with an average photon flux density of 820 μmol m^{-2} s^{-1} was also maintained during the experiment period.

Treatments and experimental design

The healthy seeds of rice varieties were surface sterilized with 10% (*v*/*v*) H_2O_2 for 15 min and then rinsed several times with tap water and finally washed three times with deionized water. The overnight soaked seeds were put on the cheese cloth spread on the bottom of plastic boxes.

Part 01—seed germination

A total 30 seeds of each variety for one replicate were spread on the cheese cloths and treated with vanadium (V) solutions with different concentrations: 15 and 30 mg L^{-1} in the form of NH_4VO_3 and a control (distilled water with zero-V) in each variety with three replicates according to complete randomized design (CRD) for the period of 14 days. After every second day, the V-solution was renewed to maintain the V-concentration, and tap water was applied to the respective boxes as per need to supply the moisture for seed germination. The boxes were covered with black polythene for the first four days to provide dark for germination. After the germination, the black covers were removed, and all the boxes were put under the light for the seedling growth.

Table 1 Names of rice varieties used in the experiment

Serial number	Varieties names	Serial number	Varieties names
1	Gao shan 3 hao	16	Song shu dao
2	Y liang you 087	17	Liu sha zhan
3	Chao you 37	18	Shen liang you 5814
4	Chao ji 1 hao	19	Gui hua zhan
5	Chao ji 3 hao	20	Wu bie qing gan dao
6	Tai guo xiang nuo	21	Xiang geng
7	Zhen zhu nuo	22	Tian xiang 208
8	Hai nan wan li xiang	23	Heng sui 9 hao
9	Tian long xiang	24	Chun gui
10	Gao shan 1 hao	25	Shuang you zhan
11	Chao you 2007	26	Mao ya xiang
12	Hei nuo mi	27	Si xiang
13	Shui han liang yong dao	28	Bai xiang 139
14	Qi dao 2000	29	Hua you 665
15	Chao feng han 1 hao		

Observed variables

The protrusion (visible emergence of primary root from seed coat), and germination (primary root size ≥ 2 mm in length) of the rice seeds were observed on daily bases for 7 days thereafter, were observed on 9th, 12th and 14th day. After 14th day, no more protrusion and seed germination were observed for the seed germination experiment. After the 14th day of the experiment, the protrusion percentage (PP) and germination percentage (GP) were calculated according to the method followed by Akinci and Akinci (2010):

$$\text{Protrusion percentage (PP)} = \sum \left(\frac{P}{T}\right) \times 100$$

where P = total number of protruding seeds and T = total number of seeds per petri dish.

$$\text{Germination percentage (GP)} = \sum \left(\frac{G}{T}\right) \times 100$$

where G = total number of germinated seeds and T = total number of seeds per petri dish.

Part 02—rice seedlings

After the recording of seed germination, the germinated seeds with uniform-sized seedlings were transferred into plastic boxes (20 seedlings per box) contained nutrient solution (2.5 L) with 6.5 pH. After 7 days of seedling transplanting, the nutrient solution was renewed along with three vanadium (V) levels: control (zero-V), 15 and 30 mg L^{-1} by using NH_4VO_3. And, the nutrient solution was renewed after every second day and aerated continuously. The seedlings were exposed to nutrient/or vanadium solution for 16 days. Then, the harvested seedlings were washed thoroughly with distilled water and dried with tissue paper. After that, 10 seedlings were selected from each box, and fresh biomass was weighed and recorded using a digital balance (BSA224S-CW, Sartorius), while the length of shoots and roots was recorded with measuring tape. And, remaining harvested seedlings were stored at −80 °C for future analyses.

Analytical methods

Enzyme assays

To determine the antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities, the stored seedlings of rice treated with and without (control) vanadium were used to prepare the enzyme extracts using sodium phosphate buffer (pH 7.8), and the collected supernatant was used for the determination of enzyme assays.

The activity of SOD was measured by using the method described by Beauchamp and Fridovich (1971), the activity of CAT was measured using the method described by Aebi and Bergmeyer (1983), and the activity of POD was determined by using the method described by Putter (1974). The protein concentration was determined using Coomassie Brilliant Blue method as described by Deng et al. (2013).

Non-enzyme assays

To determine the antioxidant non-enzymes: malondialdehyde (MDA), glutathione (GSH) and ascorbic acid (AsA) contents, the stored seedlings of rice treated with and without (control) vanadium were used to prepare the non-enzymes extracts using 5% trichloroacetic acid (TCA), and the collected supernatant was used for the determination of enzyme assays.

Lipid peroxidation was determined by measuring the MDA contents using the method described by Heath and Packer (1968), the contents of GSH were measured using the method described by Sedlak and Lindsay (1968), and the contents of AsA were measured using the method described by Tewari et al. (2006).

Amylase assays

The activities of α- and β-amylase and total amylase were determined using the method by Swain and Dekker (1966). Briefly, the collected homogenate of seeds after grinding with acetate buffer (pH 6.0) was centrifuged, and supernatant was collected into another tube for the determination of total amylase activity. The activity of α-amylase in the supernatant was determined following the procedure for total amylase with the exception of heating step. And, the activity of β-amylase was computed from the difference of total amylase and α-amylase.

The obtained data presented in the figures and tables were subjected to two-factor analysis of variance (ANOVA), and the treatments means comparison and significance ($p \leq 0.05$) level among different treatments were evaluated by Tukey method.

Results and discussion

Results

Seed germination and protrusion

The results regarding percentage of protrusion and germination showed variation when seeds of rice varieties were exposed to different concentrations of vanadium (Table 2). Taken together, both vanadium treatments affected the protrusion and germination percentage of seeds of all rice varieties. As a whole, the maximum reduction in protrusion and germination percentage of seeds was observed when treated with 30 mg L^{-1} of vanadium. Moreover, all the rice varieties showed variations regarding protrusion and germination percentage of seeds when exposed to vanadium treatments. The rice varieties: Tai gua xiang nuo, Qi dao 2000, Hei nuo mi and Gao shan 3 hao showed maximum while Chao ji 3 hao, Chao ji 1 hao, Gui hua zhan, Chao you 37 and Wu bie qing gan dao showed lower protrusion and germination percentage when exposed to vanadium.

Plant height and biomass

The results about the length of shoots and roots are shown (Table S1) which indicated that roots and shoots were decreased with the increasing concentrations of vanadium. The lowest reduction in lengths was recorded in varieties Tai guo xiang nuo and Qi dao 2000 while the maximum reduction was observed in Chao you 37 and Chao ji 1 hao when exposed to different vanadium treatments. However, at higher concentration of vanadium (30 mg L^{-1}) significant decrease in length of shoots and roots was recorded as compared to lower vanadium concentration and control. The varieties: Tai gua xiang nuo and Qi dao 2000 showed resistance against vanadium in which the reduction percentage in shoots length was about 37.20 and 40.5% and in the root was about 76.11 and 81.81% with the application of vanadium 15 mg L^{-1} while at 30 mg L^{-1}, the decreasing percentage in shoots of Tai gua xiang nuo and Qi dao 2000 was about 48.9 and 48.04% and in the shoots was 85.43 and 91.51% as compared to their controls. Similarly, with the increase in the vanadium levels from 15 to 30 mg L^{-1} the reduction percentage of the lengths of the shoots was about 69.66 and 66.33% and in the roots was about 83.61 and 83.5% in the varieties: Chao you 37 and Chao ji 1 hao, respectively, as compared to controls. The same trend of decreasing in fresh weights was recorded in rice varieties: Tai gua xiang nuo and Qi dao 2000, and Chao you 37 and Chao ji 1 hao when treated with vanadium as compared to control (Table 3).

The results about fresh biomass are presented in Table 3, which indicated that vanadium significantly ($p \leq 0.05$) affected the fresh biomass of rice varieties. Generally, the obtained results are same as mentioned above for the varieties; consequently, the maximum fresh weight was attained by the rice varieties: Tai guo xiang nuo, Hei nuo mi, Shui han liang yong dao, Qi dao 2000 and Chao feng han 1 hao when exposed to vanadium in the nutrient medium, while the rice varieties: Chao you 37, Gui hua zhan, Tian xiang 208 and Heng sui 9 hao exhibited the lower fresh biomass when exposed to vanadium. These results about height and fresh biomass are given against 30 mg L^{-1}; however, the results were almost same when exposed to 15 mg L^{-1} of vanadium. Moreover, all the rice varieties showed variations regarding seedlings height and fresh biomass when exposed to vanadium. In addition, both vanadium concentrations gradually stunted the growth (height and fresh biomass) of rice varieties (Table S1 and 3).

Antioxidant enzymes

The obtained findings indicate positive relationship between antioxidant enzyme activities and vanadium concentrations in all rice varieties; however, all the varieties showed variation among each other. The activity of superoxide dismutase (SOD) in all rice varieties was varied when exposed to vanadium as compared to control. The results related to SOD activity of different varieties against vanadium concentrations are illustrated in Fig. 1. Among all the varieties, the activity of SOD enzyme was increased in Tai Guo Xiang nuo and Qi dao 2000 when exposed to different levels of vanadium as

Table 2 Effects of different vanadium concentrations on protrusion and germination percentage of seeds of rice varieties

Rice varieties	Vanadium concentrations (mg L^{-1})					
	0	15	30	0	15	30
	PP (%)			GP (%)		
1	97.3 ± 3a	94.7 ± 3a	94.0 ± 0a	80.7 ± 6a	88.0 ± 4a	92.7 ± 1a
2	97.3 ± 2a	94.7 ± 2a	98.7 ± 3a	88.0 ± 2a	81.3 ± 8a	82.7 ± 6a
3	70.0 ± 2a	65.3 ± 4a	77.3 ± 2a	58.0 ± 12a	52.0 ± 2a	58.0 ± 2a
4	24.0 ± 8ab	50.7 ± 7b	32.7 ± 4a	32.0 ± 9a	32.7 ± 4a	32.0 ± 9a
5	23.3 ± 3a	42.0 ± 9a	38.0 ± 5a	24.7 ± 6a	33.3 ± 8a	32.7 ± 3a
6	92.0 ± 2b	81.3 ± 4a	85.3 ± 6ab	82.0 ± 3b	62.7 ± 10a	63.3 ± 6a
7	84.7 ± 3a	84.7 ± 1a	87.3 ± 1a	79.3 ± 10a	62.7 ± 7a	62.7 ± 10a
8	73.3 ± 10a	73.3 ± 3a	82.0 ± 4a	57.3 ± 16a	62.0 ± 5a	92.7 ± 3b
9	96.7 ± 1b	88.7 ± 2a	96.0 ± 2b	82.0 ± 7b	81.3 ± 4b	66.0 ± 5a
10	98.0 ± 3a	94.7 ± 8a	98.7 ± 2a	78.7 ± 8a	84.0 ± 14a	83.3 ± 5a
11	97.3 ± 1b	99.3 ± 1b	94.0 ± 2a	86.7 ± 10a	91.3 ± 3a	88.0 ± 5a
12	97.3 ± 3a	90.0 ± 5a	98.7 ± 1a	80.0 ± 3ab	89.3 ± 4a	98.0 ± 2b
13	72.0 ± 11a	82.7 ± 3a	73.3 ± 10a	72.7 ± 9a	63.3 ± 2a	91.3 ± 2a
14	94.7 ± 1ab	95.3 ± 2b	92.0 ± 0a	62.0 ± 3a	68.7 ± 4a	72.0 ± 14a
15	83.3 ± 2a	87.3 ± 5a	88.7 ± 1a	64.0 ± 2a	75.3 ± 6a	81.3 ± 4a
16	96.0 ± 2a	88.0 ± 5a	92.0 ± 5a	70.7 ± 6a	74.0 ± 11a	74.0 ± 2a
17	83.3 ± 3a	86.0 ± 1a	94.7 ± 6a	68.7 ± 2a	72.0 ± 11a	86.7 ± 3a
18	94.0 ± 2b	96.7 ± 1b	90.7 ± 1a	78.0 ± 8a	74.7 ± 6a	74.0 ± 8a
19	37.3 ± 9a	61.3 ± 15b	36.0 ± 2a	28.7 ± 6a	26.7 ± 3a	30.0 ± 0a
20	27.3 ± 5a	21.3 ± 1a	26.0 ± 4a	37.3 ± 1b	24.7 ± 1a	28.0 ± 4a
21	98.0 ± 2a	98.0 ± 2a	95.3 ± 1a	92.0 ± 7b	81.3 ± 3ab	73.3 ± 6a
22	99.3 ± 1a	96.0 ± 2a	94.7 ± 4a	68.0 ± 7a	86.7 ± 2a	90.0 ± 4a
23	97.3 ± 1a	96.7 ± 3a	96.7 ± 6a	79.3 ± 4a	84.0 ± 6a	88.7 ± 6a
24	98.7 ± 1a	98.0 ± 0a	98.7 ± 1a	92.7 ± 3b	96.7 ± 3b	85.3 ± 4a
25	97.3 ± 1a	100 ± 0a	98.7 ± 2a	90.7 ± 6ab	94.7 ± 1b	84.0 ± 6a
26	99.3 ± 1a	100 ± 0a	97.3 ± 3a	92.0 ± 3b	82.0 ± 4ab	92.7 ± 3a
27	98.7 ± 1a	99.3 ± 1a	99.3 ± 1a	96.0 ± 2a	92.7 ± 3a	96.0 ± 3a
28	98.0 ± 2a	98.0 ± 0a	98.0 ± 2a	96.7 ± 2a	92.0 ± 3a	88.7 ± 4a
29	90.0 ± 1a	87.3 ± 2a	93.3 ± 2a	66.7 ± 11b	68.0 ± 4ab	61.3 ± 3a

Data means of three replicates and significant ($p \leq 0.05$). Means contained similar letters in row are not significantly ($p \leq 0.05$) different according to Tukey method

PP protrusion percentage, *GP* germination percentage

compared to control, whereas the increase in SOD activity in Chao you 37 and Chao ji 1 hao was lower than Tai guo xiang nuo and Qi dao 2000 but higher than control.

The activity of catalase (CAT) was also significantly ($p \leq 0.05$) altered by the application of vanadium in all varieties than control (Fig. 2). The varieties: Tai gua xiang nuo, Qi dao 2000 and Xiang geng showed increase among all the varieties in the CAT activity while Chao you 37, Chao ji 1 hao and Tian long xiang showed minimum increase among all the varieties when exposed to vanadium treatments. The CAT activity was increased in Tai gua xiang nuo, Qi dao 2000 and Xiang geng by 7.29%, 47.69% and 27.47% with the application of vanadium 15 mg L^{-1} as compared to control, and same increasing trend was observed when treated with vanadium 30 mg L^{-1} than controls. Similarly, the CAT activity of Chao you 37, Chao ji 1 hao and Tian long xiang against vanadium 15 mg L^{-1} was 10.47, 12.63 and 9.67 U mg^{-1} and under vanadium 30 mg L^{-1} was 3.50, 6.04 and 5.98 U mg^{-1} port. Overall with the increase in the vanadium levels the activity of CAT showed variation in all the rice varieties.

The application of vanadium significantly influenced the peroxidase (POD) activity in all rice varieties as compared to control; the POD activity was increased in Tai gua xiang nuo and Qi dao 2000 by 52.52–24.69% and 103.64–30.70% with the application of vanadium at the rate of 15 and 30 mg L^{-1}, respectively (Fig. 3), while there were nonsignificant differences between Chao you 37 and Chao ji 1 hao as compared to control. With the increase in the vanadium levels, the decrease in the POD activity was examined in all the varieties.

Table 3 Fresh biomass of seedlings of rice varieties grown hydroponically under three vanadium concentrations

Rice varieties	Fresh weight (g/10 seedlings)		
	Vanadium concentrations (mg L^{-1})		
	0	15	30
1	1.2467 ± 0.13a	0.9308 ± 0.17a	0.6107 ± 0.05b
2	1.2361 ± 0.22a	0.8424 ± 0.03b	0.5763 ± 0.02b
3	1.7377 ± 0.10a	0.858 ± 0.15a	0.447 ± 0.07b
4	2.1526 ± 0.11a	0.932 ± 0.14b	0.5455 ± 0.04c
5	1.6281 ± 0.27a	0.8558 ± 0.13b	0.558 ± 0.11b
6	1.4866 ± 0.07a	1.0259 ± 0.15a	0.5704 ± 0.02b
7	1.545 ± 0.05a	0.9148 ± 0.02ab	0.6364 ± 0.07b
8	1.7555 ± 0.14a	1.018 ± 0.09b	0.5475 ± 0.03b
9	1.457 ± 0.03a	0.7913 ± 0.10b	0.4847 ± 0.07c
10	1.2382 ± 0.20a	0.9062 ± 0.13a	0.4849 ± 0.08b
11	1.2896 ± 0.23a	0.788 ± 0a	0.4778 ± 0.07b
12	1.2374 ± 0.19a	1.0237 ± 0.05a	0.6264 ± 0.11b
13	1.2901 ± 0.03a	0.8578 ± 0.05b	0.6063 ± 0.01c
14	1.4801 ± 0.25a	0.794 ± 0.12b	0.7933 ± 0.03c
15	1.6141 ± 0.11a	1.1722 ± 0.18b	0.8849 ± 0.04c
16	1.6613 ± 0.13a	0.9312 ± 0.04ab	0.6905 ± 0.04b
17	0.9895 ± 0.18a	0.772 ± 0.02b	0.492 ± 0.07c
18	1.3283 ± 0.24a	0.7007 ± 0.03b	0.5607 ± 0.02c
19	1.3258 ± 0.05a	0.781 ± 0.05b	0.4651 ± 0.01c
20	2.1998 ± 0.05a	1.1946 ± 0.22b	0.7782 ± 0.03c
21	1.664 ± 0.07a	1.0155 ± 0.02b	0.5352 ± 0.10c
22	0.956 ± 0.17a	0.7203 ± 0.04b	0.3917 ± 0.07c
23	1.1286 ± 0.20a	0.7023 ± 0.10a	0.4932 ± 0.06b
24	0.9726 ± 0.15a	0.7274 ± 0.11b	0.4519 ± 0.01c
25	1.1098 ± 0.02a	0.699 ± 0.01b	0.4402 ± 0.01c
26	0.9779 ± 0.16a	0.6471 ± 0.04b	0.2801 ± 0.04b
27	1.2869 ± 0.23a	0.6962 ± 0.09b	0.3884 ± 0.03c
28	1.0988 ± 0.19a	0.6858 ± 0.12b	0.4086 ± 0.02c
29	1.8042 ± 0.16a	0.8902 ± 0.02b	0.5262 ± 0.09c

Data means of ten replicates and significant ($p \leq 0.05$). Means contained similar letters in row are not significantly ($p \leq 0.05$) different according to Tukey method

Antioxidant non-enzymes

The obtained findings indicate positive relationship among antioxidant non-enzymes activities and vanadium concentrations in all rice varieties; however, all the varieties showed variation among each other against vanadium concentrations. The different responses of antioxidant non-enzymes against vanadium among rice varieties possess inherent genetic and biochemical mechanisms to cope up the stressed environment considered a major indicator to demonstrate the potentiality of species. The addition of vanadium significantly ($p \leq 0.05$) enhanced the malondialdehyde (MDA) contents in shoots of rice varieties (Fig. 4). However, the contents of MDA in shoots of rice varieties: Tai guo xiang nuo and Qi dao 2000 were decreased than control, while varieties: Chao you 37 and Chao ji 1 hao exhibited higher MDA contents when exposed to vanadium treatments even higher than control. The MDA contents in Tai guo xiang nuo and Qi dao 2000 were enhanced when exposed to 15 mg L^{-1} while at 30 mg L^{-1} were decreased as compared to control. At the same time, the MDA contents in Chao you 37 and Chao ji 1 hao were increased at both treatments of vanadium as compared to zero-vanadium (control). Overall, all the varieties respond differently to vanadium concentrations.

The results about glutathione reductase (GSH) contents also showed variations among all rice varieties against vanadium treatments (Fig. S1). The GSH contents in shoots of rice varieties: Tai guo xiang nuo and Qi dao 2000 were decreased when exposed to both treatments of vanadium as compared to control. On the other hand, the GSH contents in shoots of rice varieties: Chao you 37 and Chao ji 1 hao were increased in Chao you 37 with addition of 30 mg L^{-1} while in Chao ji 1 hao were increased and then decreased with 15 and 30 mg L^{-1} application of vanadium, respectively. Generally, rice varieties showed variation in GSH contents when treated with vanadium.

The results about ascorbic acid (AsA) contents in all rice varieties exhibited dissimilarity to vanadium concentrations (Fig. S2). The AsA contents in shoots of varieties: Tai guo xiang nuo and Qi dao 2000 were higher at 15 mg L^{-1} while at 30 mg L^{-1} were decreased overall; the AsA contents were increased than control. The same trend of AsA contents was observed in varieties: Chao you 37 and Chao ji 1 hao; however, the AsA contents were higher as compared to Tai guo xiang nuo and Qi dao 2000 varieties when exposed to vanadium treatments.

Soluble protein contents

The results related to the protein contents in different rice varieties under different concentrations of vanadium are illustrated in Fig. S3. Different trends were observed in protein contents in all varieties when exposed to vanadium. The varieties: Tai guo xiang nuo, Qi dao 2000 and Xiang geng were exhibited more protein contents and varieties: Chao you 37, Chao ji 1 hao and Tian long xiang were exhibited less protein contents than others when treated with vanadium. The contents of protein in varieties: Tai guo xiang nuo, Qi dao 2000 and Xiang geng were 1.86, 1.72 and 2.04 g protein L^{-1}, and 1.98, 3.13 and 4.14 g L^{-1} when treated with 15 and 30 mg L^{-1} of vanadium, respectively. Similarly, the contents of protein in varieties: Chao you 37, Chao ji 1 hao and Tian long xiang were 1.85–2.56, 1.86–2.05 and 2.84–3.36 g L^{-1} when treated with 15 and 30 mg L^{-1} treatment, respectively.

The maximum positive correlation coefficients were examined between SOD and POD enzymes, CAT and

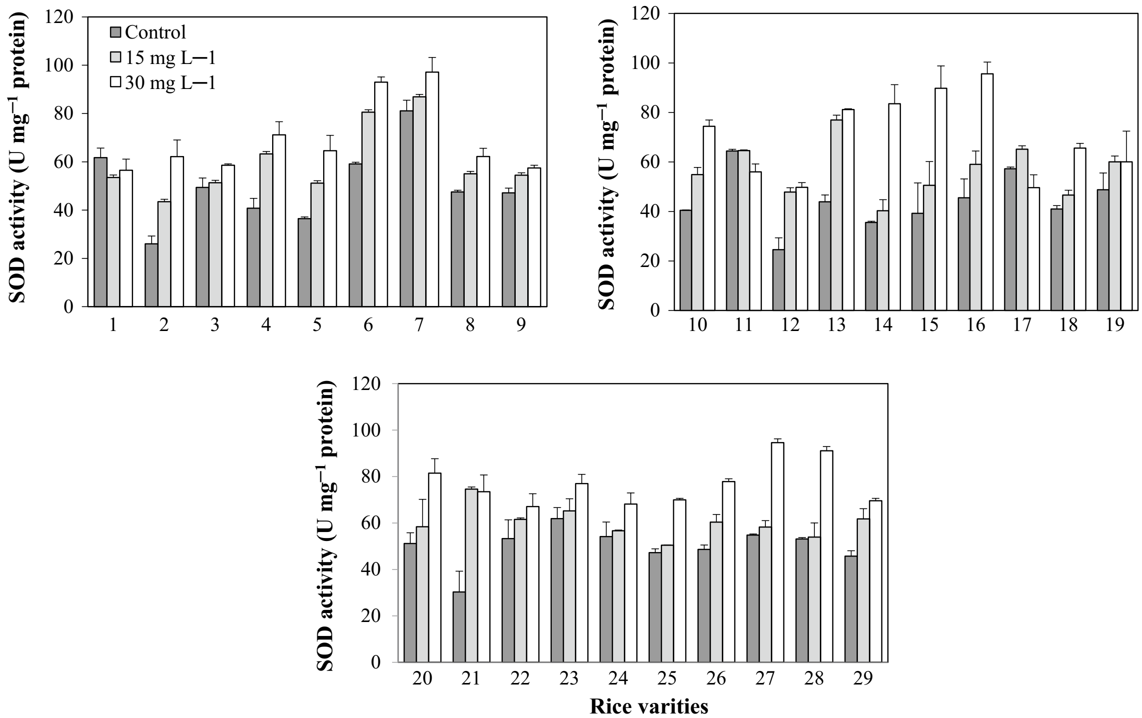

Fig. 1 Effect of vanadium concentrations on superoxide dismutase (SOD) activity in seedlings of rice varieties. Vertical bars represent mean ± SD ($n=3$). ANOVA significant at $p \leq 0.05$

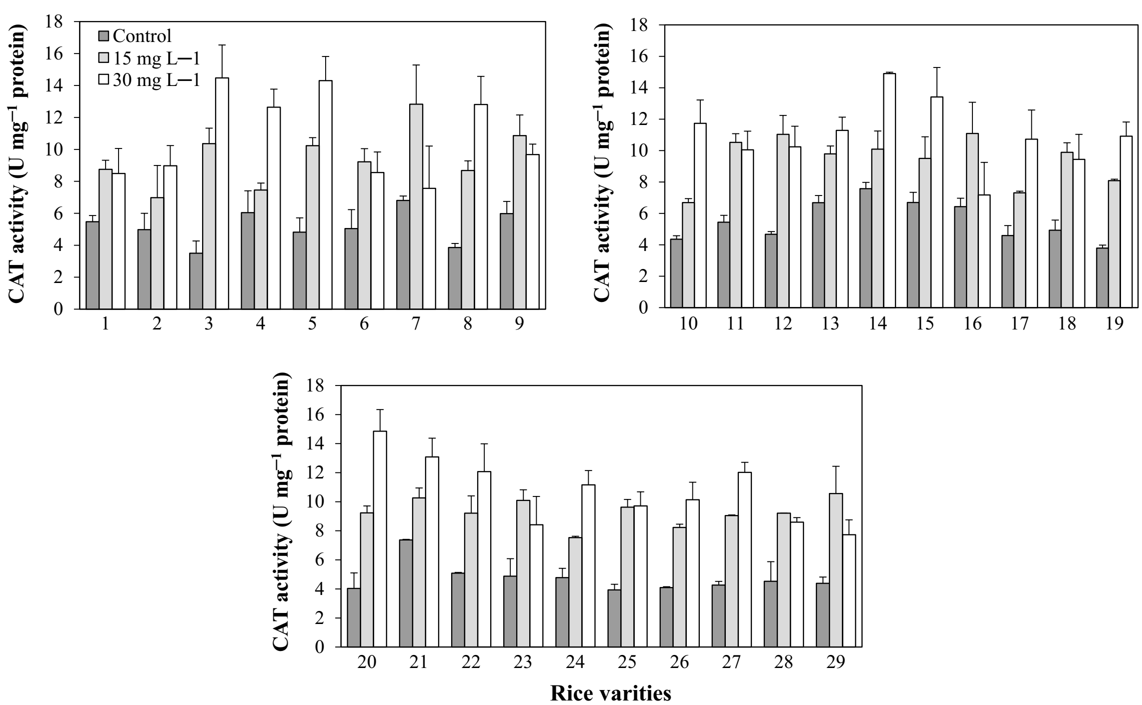

Fig. 2 Effect of vanadium concentrations on catalase (CAT) activity in seedlings of rice varieties. Vertical bars represent mean ± SD ($n=3$). ANOVA significant at $p \leq 0.05$

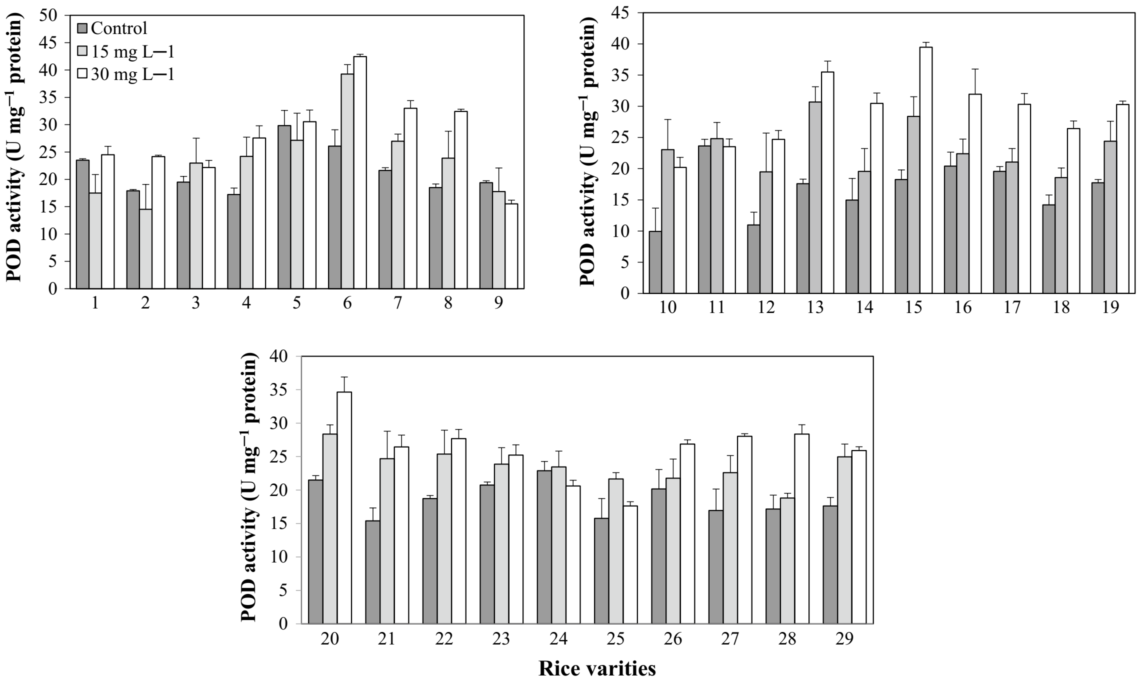

Fig. 3 Effect of vanadium concentrations on peroxidase (POD) activity in seedlings of rice varieties. Vertical bars represent mean ± SD ($n = 3$). ANOVA significant at $p \leq 0.05$

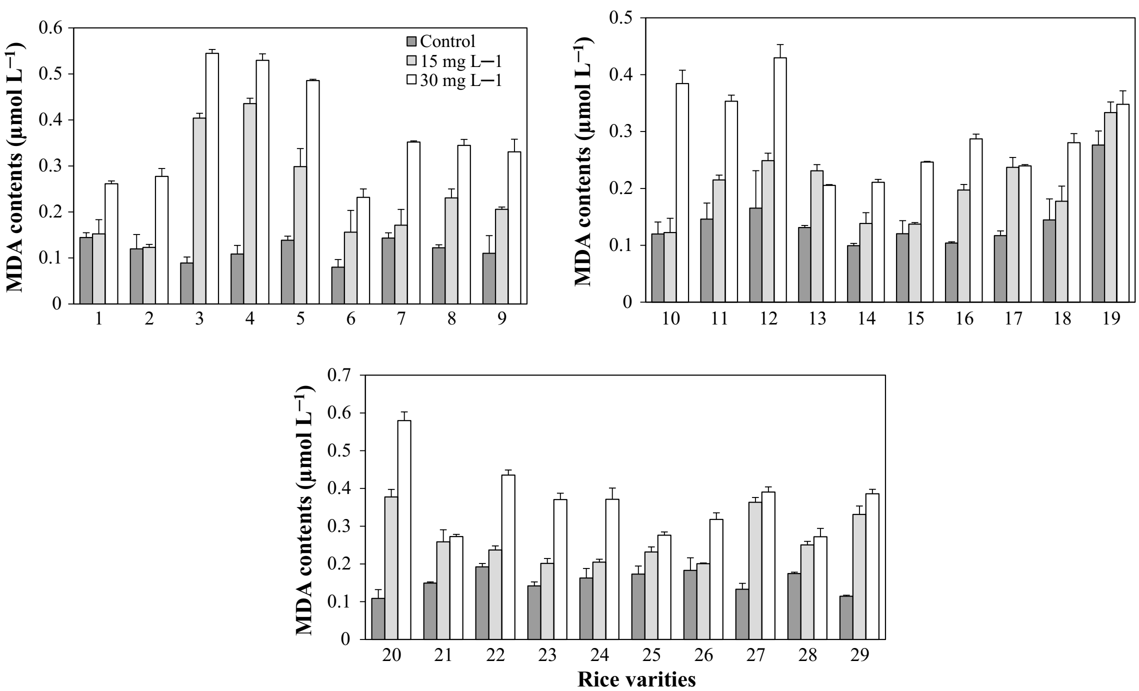

Fig. 4 Effect of vanadium concentrations on malondialdehyde (MDA) contents in seedlings of rice varieties. Vertical bars represent mean ± SD ($n = 3$). ANOVA significant at $p \leq 0.05$

weight, between PP and GP, between SOD and AsA, while the negative correlation was found among protein, CAT and SOD enzymes, among PP, protein and MDA, and other indices (Table S2).

Amylase activity

Figures 5, 6 and 7 show the results about α- and β-amylase, and total amylase activities in germinated seeds of rice

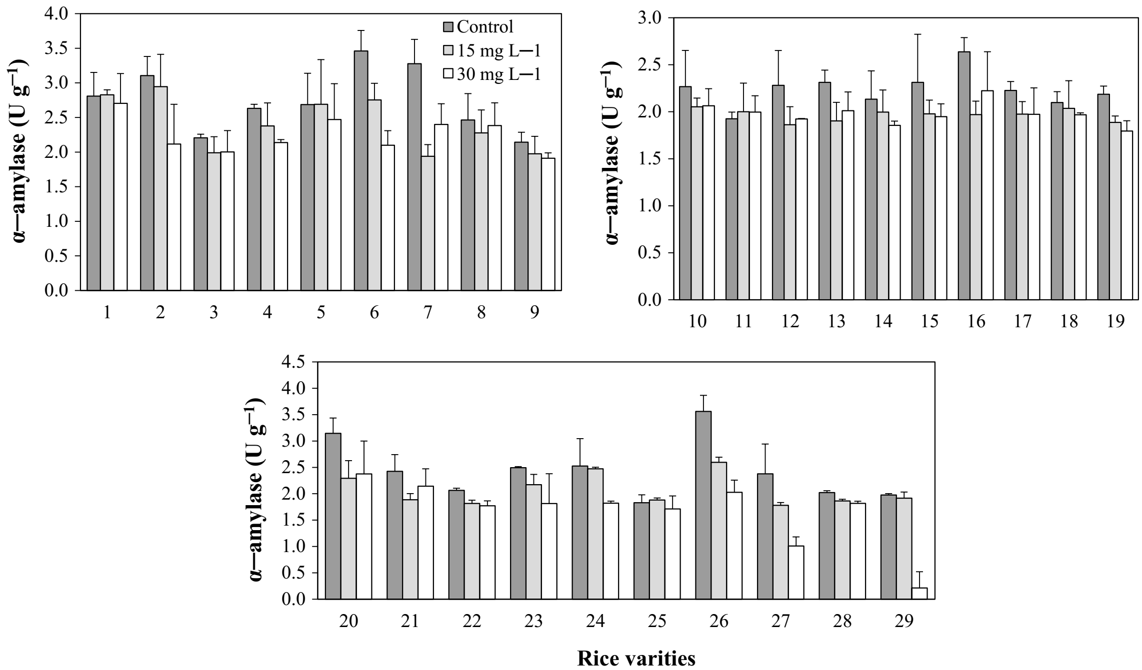

Fig. 5 Effect of vanadium concentrations on α-amylase activity in seedlings of rice varieties. Vertical bars represent mean ± SD ($n=3$). ANOVA significant at $p \leq 0.05$

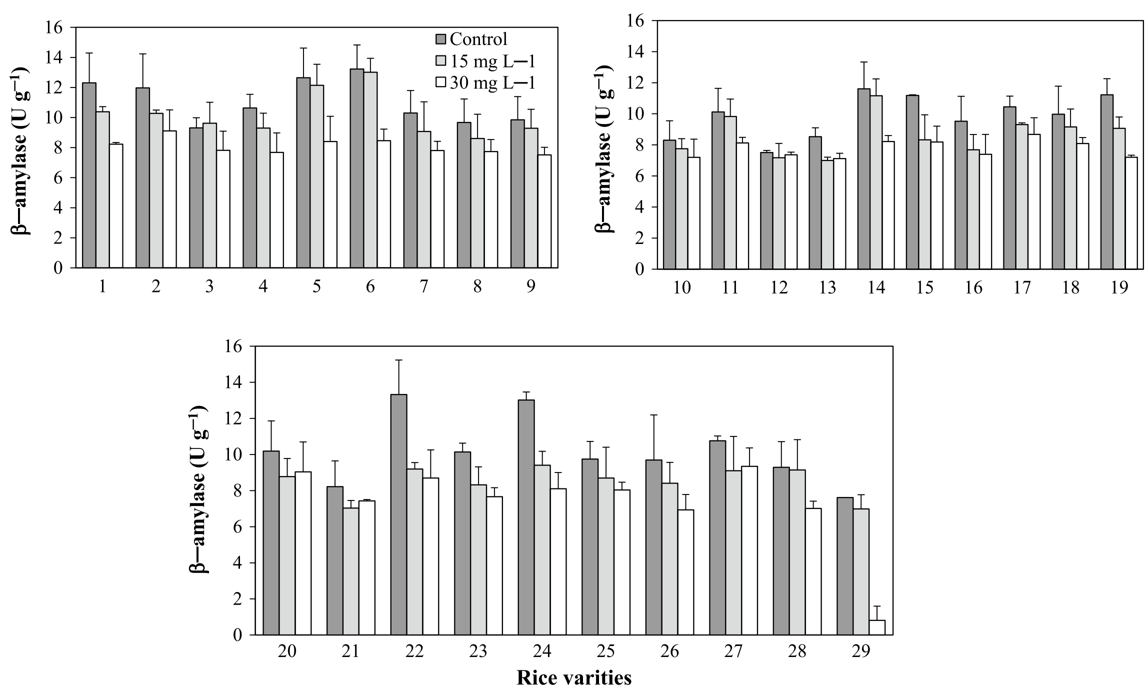

Fig. 6 Effect of vanadium concentrations on β-amylase activity in seedlings of rice varieties. Vertical bars represent mean ± SD ($n=3$). ANOVA significant at $p \leq 0.05$

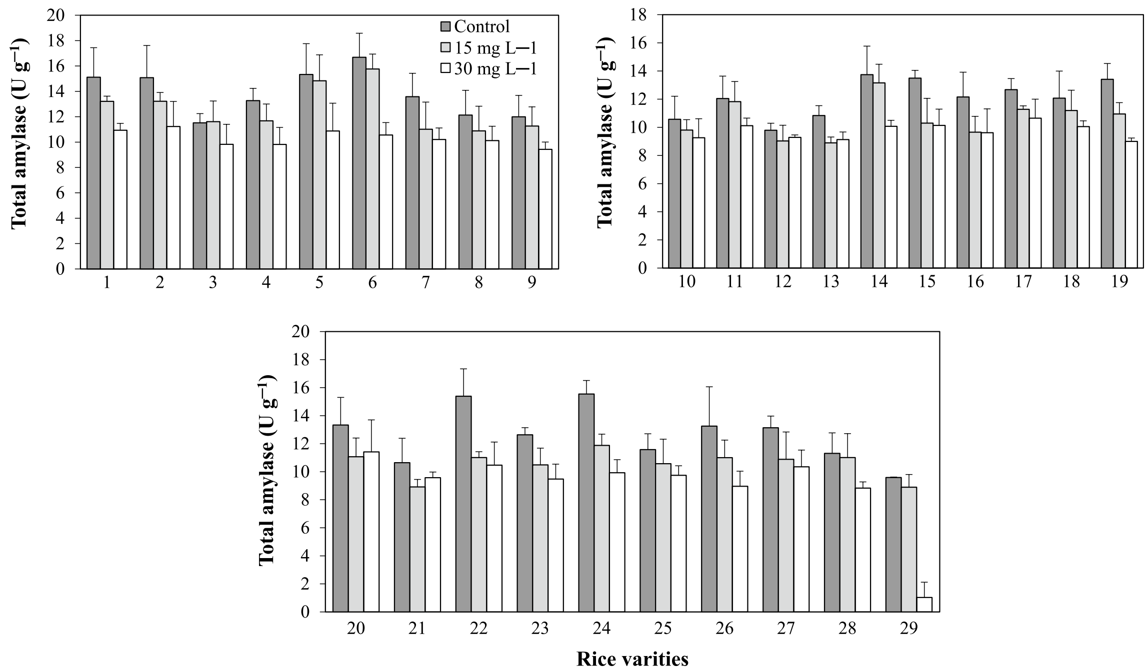

Fig. 7 Effect of vanadium concentrations on total amylase activity in seedlings of rice varieties. Vertical bars represent mean ± SD ($n=3$). ANOVA significant at $p \leq 0.05$

varieties against vanadium treatments. Overall, total amylase activity in seeds of rice varieties showed variation in response to vanadium treatments. The varieties: Tai gua xiang nuo and Chao ji 3 hao showed highest, and Chao you 37 and Chao ji 1 hao showed lowest total amylase activity than remaining varieties. The rice varieties: Tai gua xiang nuo, Chao ji 3 hao and Mao ya xiang showed the maximum, and Chao you 37, Chao ji 1 hao and Si xiang showed the lowest activity of α-amylase when exposed to vanadium. On the other hand, the rice varieties: Tai gua xiang nuo and Chao ji 3 hao and Gui hua zhan showed maximum; Chao you 37, Chao ji 1 hao, Hei nuo mi and Shui han liang yong dao showed lower activity of β-amylase when exposed to vanadium. These results about amylase activities are given against 30 mg L^{-1} of vanadium; however, the results were almost same when exposed to 15 mg L^{-1} of vanadium. Moreover, all the rice varieties showed variations regarding amylase activities when exposed to vanadium in the nutrient medium.

Discussion

Recently, heavy metals contamination has becoming a major environmental concern, and hence, the more agricultural land is being polluted because of this issue. The heavy metals, which mostly share of this pollution, are released from industries such as steel industries, automobiles and chemicals. Vanadium (V) emerging as a major pollutant affects plants, animal and human being. Consequently, it is an urgent need to inspect the paramount processes and mechanisms involved in vanadium toxicity.

The selection of plant species because of inherent ability, and respond differently among the varieties is an approach to cope up the metals toxicity. Moreover, plants have various biochemical mechanisms to alleviate the metals toxicity such as antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) and non-enzymes: malondialdehyde (MDA), glutathione reductase (GSH) and ascorbic acid (AsA) helped to relieve the plants against different metals.

The vanadium concentrations affected the growth parameters all the rice varieties, such as height of plants, germination percentage (GP) and protrusion percentage (PP) are used as indicators against metals stress (Shafiq et al. 2008). Previously it is reported that the presence of metals in nutrient medium affects the cell division in seeds which ultimately affects the protrusion. Shafiq et al. (2008) also reported that metals are involved in disruption of seed coat, and this disruption affects the metabolisms and protrusion of seeds, and furthermore indicated that some seeds showed protrusion while later on, the germination was not appeared/ or completed. In our study, all the rice varieties showed differences in GP when seeds were treated with vanadium concentrations. Some varieties indicated higher PP and GP; however, on later stage, the growth of seedlings and enzymatic activities were reduced such as Gao shan 3 hao, Hai nan wan li xiang, Mao ya xiang and Si xiang. While some varieties exhibited lower PP and GP, however, on later stage, the growth of seedlings and enzymatic activities were increased such as Tai guo xiang nuo, Qi dao 2000, Chao feng han 1 hao which indicated that varieties respond differently to vanadium stress. Shafiq et al. (2008) and de Andrade and da Silveira (2008) reported that the major factors which reduced the PP and GP were depletion of oxygen uptake, disturbance in stored food transportation within seeds and interference in the selection properties of cell membrane because of metals stress; hence, this phenomenon might also be involved in our study because of vanadium stress. The results about PP and GP among rice varieties were not same, and this variation might be due to genetic tolerance and diversity of the varieties. Our results also show an agreement with the findings of Raziuddin et al. (2011) in *brassica,* Aydinalp and Marinova (2009) in *Medicago sativa* for GP when treated with different heavy metals. The obtained results about the reduction in PP are same in line with the results of Seregin and Kozhevnikova (2005) in *Zea mays* and Shafiq et al. (2008) in *Leucaena leucocephala*.

The plant growth parameters such as heights and fresh biomass are considered sensitive to a variety of stresses and also used as markers against metals toxicities. The metal stress caused toxicity in nutrient pool and badly damaged the growth and development including roots and shoots, and biomass yield, and these reductions in growth and development occurred differently in different organs of plants (Liang et al. 2007). In the present study, lengths of roots and shoots, and fresh biomass were noticeably reduced when rice seedlings were exposed to different concentrations of vanadium. Imtiaz et al. (2016) also indicated that growth of chickpea genotypes responds differently when exposed to vanadium, and markedly declined the growth parameters. The growth of lateral roots was badly damaged, and emerging leaves were exhibited chlorotic symptoms when cuphea plants were grown in the presence of vanadium (Hussain et al. 2018). Furthermore, the obtained findings of our experiment indicated that rice growth and vanadium treatment are negatively associated; however, it is also observed that different varieties respond differently to vanadium concentrations. The results of the present study are also same in line with the results which are previously reported by Imtiaz et al. (2015) and Imtiaz et al. (2016) in *Cicer arietinum*.

In response to metals toxicity, reactive oxygen species (ROS) such as H_2O_2, singlet oxygen and hydroxyl radicals are generated inside the plants. On the other hand, plants initiate the antioxidant defense system which consists of enzymes (SOD, CAT and POD) and non-enzymes (MDA, GSH and AsA) to scavenge the toxic effects of metals, and these enzymes and non-enzymes also used as biomarkers against different environmental stresses including metals (Dazy, et al. 2009; Imtiaz et al. 2016). Our results illustrated that the activities of enzymes: SOD, CAT and POD in all the rice varieties were enhanced when exposed to vanadium as compared to control. However, all the rice varieties showed variations regarding the enzyme activities and non-enzymes contents when exposed to vanadium. Some varieties exhibited the enhancement of enzyme activities, while some varieties showed decrease in enzyme activities and non-enzyme contents, but increased than the control when treated with vanadium. Our results are same in line with the findings of Imtiaz et al. (2016) in *Cicer arietinum,* Sun et al. (2007) in *Solanum nigrum,* and Tewari et al. (2006) in *Morus alba*.

The presence of vanadium in nutrient medium markedly affected the amylase activities in seeds of rice varieties. The application of vanadium significantly inhibited the activities of total amylase, α- and β-amylase; in particular, α-amylase might be due to disturbance in the transportation of stored food in seeds during germination and seedling growth (de Andrade and da Silveira 2008). The obtained results indicated that all the rice varieties showed variations regarding amylase activities when exposed to vanadium in the nutrient medium. These results are consistent with the findings of Kaneko et al. (2002) against combined stress of cadmium and mercury. Mikami et al. (1999) also reported that cadmium displaced the calcium which is integral part of enzymes, and hence, α- and β-amylase were inhibited. Resultantly, cadmium disparts the calcium or changing the steric configuration of α- and β-amylase. Moreover, our results show an agreement with the results of Singh et al. (2011) in *Brassica compestris* when exposed to lead and Sfaxi-Bousbih et al. (2010) in *Phaseolus vulgaris* when exposed to cadmium stress.

Conclusion

From the obtained results, it is concluded that addition of vanadium influenced the seed protrusion and germination, growth, activities of antioxidant enzymes and non-enzymes, and activities of α- and β-amylase and total amylase in seeds in all rice varieties. The significant impact of vanadium induced stress was observed by the application of 30 mg L^{-1} at germination and seedling stage. The present results clearly established that antioxidant defense system in all rice varieties underwent changes to cope up the oxidative damage when exposed to vanadium. The main factor in the variation about the results of antioxidant enzymes (SOD, CAT and POD) and non-enzymes (MDA, GSH and AsA) might be due to different threshold limits of tolerance to metals stress. Hence, it can be said that increase or decrease in the activities of enzymes and non-enzymes in rice varieties could be characterized to the tolerance or sensitivity to vanadium stress. The obtained data indicated that Tai guo xiang nuo and Qi dao 2000 varieties showed tolerance, and Chao you 37 and Chao ji 1 hao showed sensitivity against vanadium concentrations than others. However, further study is needed to unveil the relationship between the enzyme activities and vanadium stress.

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Compliance with ethical standards

Conflict of interest None of the authors have any competing of interest. Furthermore, all the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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