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REGULAR ARTICLE

Growth, V uptake, and antioxidant enzymes responses of chickpea (*Cicer arietinum* L.) genotypes under vanadium stress

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Abstract
Aims Heavy metals pollution is one of the most challenging problems to the environment and agricultural soils in recent decades. The purpose of the present work was to elucidate the effects of vanadium (V) on growth, V uptake, protein content and enzymes activity to sort out the V-tolerant and the sensitive genotypes of chickpea under hydroponic conditions.
Methods The activities of antioxidant enzymes (SOD, CAT, POD, and GSH, MDA) and protein contents were determined by using UV-1600 Spectrophotometer, and V concentration was determined by using GFAAS (GTA 120).
Results The findings show that V significantly increased the enzymes activities in all chickpea genotypes however, V significantly reduced the protein contents, and more accumulation of V was observed in roots than shoots in all genotypes. The plant biomass and lengths of roots and shoots were also significantly reduced by V. Moreover, NH_4VO_3 caused more toxicity than Na_3VO_4.
Conclusions The previous studies report that higher activities of enzymes increase the tolerance of plants against stress. The obtained data of present study indicated that Noor–2009 and C–44 are tolerant and G–1 and Balkasar are sensitive genotypes of chickpea against V stress.

Keywords Vanadium · Chickpea · Genotypes · Oxidative stress · Antioxidant enzymes

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Introduction

Vanadium (V) is the 5th most abundant element among the transitional metals in the earth crust. Vanadium has been extensively mined in China, South Africa, Russia, and

also in USA (Amorim et al. 2007). Anke (2004) found that soils surrounded by V- enriched mountains contain the amount from 70 to 100 mg/kg. Vanadium is considered one of the most important elements of 21st century due to its very high consumption in industries. Vanadium is extensively dispersed in the environment by different ways like leaching, combustion, use of fertilizers, and waste material from industries, resultantly; V contaminates the soil, water and atmosphere (Kar et al. 2004). The most common form of vanadium is Vanadium pentaoxide (V_2O_5), followed by ammonium metavanadate and sodium orthovanadate. As for as the toxicity of vanadium is concerned, it depends on the nature and oxidation states of compounds, pentavalent is mobile form of vanadium and it is considered to be most toxic (Anke 2004).

Some previous researchers studied the role of vanadium in plants, and considered it as a trace element for proper growth and development of plants (Kraepiel et al. 2009), on the other hand many recent reports challenged the essentiality of V for plants growth. Earlier reports also confirmed that V induces some toxic effects in plants, men and animals (Boulassel et al. 2011) however; the exact role of V in the growth and development of plants is not known (Wang and Liu 1999). Vanadium caused shoots mortality in pickle weed and reduced plant height (Rosso et al. 2005), similarly Olness et al. (2002) observed that V significantly inhibited growth of different plants. The higher concentrations of V inhibit plant growth by significantly reducing shoots length, number of leaves, dry weights of roots, shoots and leaves in Chinese green mustard and tomato plants (Vachirapatama, et al. 2011).

Abiotic stresses like metals toxicity retards growth of plants, animals and indirectly affects human beings. Higher concentrations of heavy metals, both essential (Zn, Fe, Co and Cu) and non-essential (V, Cd, Pb, As and Cr) have a profound impact on plant tissues, and they disturb the metabolic activities of plants cells. The excess of heavy metals is one of the major reason to enhance production of reactive oxygen species (ROS), and higher concentration of ROS causes oxidative damage, destabilizes lipids membranes, and retard plant growth by disturbing normal metabolic activities of cells (Chary et al. 2008). The plants activate its antioxidant defense systems to scavenge ROS which is comprised of enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and low molecular weight gratifiers like glutathione, proline, cysteine, thiols and ascorbic acid (Michalak 2006).

Genotypes differ in response to abiotic stresses, as Wahid and Ghani (2008) reported that uptake of Cd and its internal distribution in roots and shoots induce differences in genotypes and serves as an indicator to study genetic variations for Cd stress tolerance. Guo et al. (2004) observed a great difference in plant species and genotypes for Cu and Cd toxicity. Similarly, Clemens (2006) also reported that different genotypes exhibit variation for the accumulation and distribution of Cd, but no information is available about the differences in the effect of V on uptake and distribution of chickpea genotypes in V toxicity resistance.

Chickpea is one of the leading pulse crops of the world, and ranked 3rd among legumes crops after dry beans and pea, especially in the arid and semiarid regions. More than 50 countries of world grow chickpea, 22 of them cultivate more than 20,000 ha, while 19 grow 10, 000 to 20, 000 ha only (these figures are not so charming). The world production of chickpea exceeds 8.40 million tons per annum. Chickpea is a rich and cheap source of protein supplement and energy in developing countries, and helps to maintain soil fertility by fixing atmospheric nitrogen (Wani et al. 2005).

To the best of our knowledge, no information about the V and physic-biochemical studies of chickpea is available. V and chickpea are closely related to each other because V and chickpea are beneficial for the recovery of diabetes disease. Vanadium compounds are reported to mimic for different metabolic effects of insulin, and might act on insulin target tissues. Vanadium compounds are candidates for oral therapy in diabetes, normalizing plasma glucose homeostasis, but the exact mechanism remains to be determined (Thompson et al. 2009; Cam, et al. 2000). Therefore, this study will provide the basis for understanding toxicity of V in chickpea plants. The main objectives of the present research include:

1) To understand the effect of V on growth, and V uptake of genotypes of chickpea,
2) To illustrate differential response of V stress among various genotypes, and
3) To screen vanadium sensitive and tolerant genotypes of chickpea for further studies.

Materials and methods

Chickpea seeds and growth conditions

The seeds of chickpea cultivars G−1 and Z−1 were purchased from a seed store in Xinjiang, Northwest of China. Chickpea cultivars Pb-2000, C-44, Balkasar and Noor-2009 were collected from Ayyub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The growth room was climate-controlled with a temperature range 22−25 °C and relative humidity 70 %. A 14-h photoperiod with an average photon flux density of 820 mmol m^{-2} s^{-1} was supplied by an assembly of cool-white fluorescent lamps.

Experimental design

A hydroponic experiment with two factors was designed to carry out this study, the first factor was vanadium salts including ammonium metavanadate (NH_4VO_3) and sodium orthovanadate (Na_3VO_4); the'second factor was genotypes of chickpea plants. The experimental design was laid out in factorial form based on complete randomized design (CRD) with 4 replications.

Seedling growth and treatments

Healthy seeds of chickpea were sterilized with 0.1 % sodium hypochlorite solution for 10 min, and washed in running distilled water then sown in garden trays containing sand and irrigated daily with water shower to optimize the water contents. After 12 days, the uniform sized seedlings (4 plants) were transferred into 4 L plastic box containing 20 % Hoagland Nutrient solution (2.5 L). The pH of nutrient solution was adjusted at 6.5 by adding NaOH/HCl solution. The nutrient solution was upgraded to 50 %, and NH_4VO_3 and Na_3VO_4 (25 mg V L^{-1}) were also applied after 5 days of transplanting. In total, the seedlings were remained for 10 days in Hoagland nutrient solution plus vanadium. On 10th days, plants were harvested and washed thoroughly with distilled water then separated into shoots and roots. Harvested shoots were put in the liquid nitrogen and stored at −80 °C for future analysis. Roots and shoots were dried in oven at 100 °C for 1 h and then at 60 °C till constant weight for vanadium determination. Both the fresh and dry weights of the biomass were also weighed and recorded using an electronic digital balance (BSA224S-CW, Sartorius). The measurement of shoot and root lengths were done by using measuring tape.

Analytical methods

To assay the enzymes activities in aerial parts of the treated and control plants, washed fresh above ground part (0.5 g) were ground in liquid N_2 and homogenized in 5 ml of 0.2 mol L^{-1} sodium phosphate buffer (pH 7.8). The homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant was collected in another falcon tube for the determination of enzymes assays.

SOD activity was checked by measuring the ability to reduce the photochemical reduction of nitroblue tetrazolium as illustrated by Beauchamp and Fridovich (1971). CAT activity was evaluated using method demonstrated by Aebi and Bergmeyer (1983). POD activity was assayed using the guaiacol oxidation method by Li (2000). Briefly, fresh leaves (0.5 g) were ground with silica sand in 5 ml sodium phosphate buffer (pH 7.8) on ice. The homogenate was centrifuged at 12,000 rpm at 4 °C for 15 min. The supernatant was used to determine the POD activity. The samples were prepared using 1 ml H_2O_2 (0.2 %), 0.95 ml guaiacol (0.2 %), 1 ml sodium phosphate buffer (pH 7.0) and 0.05 ml enzyme extract. The increase in absorbance was recorded at 470 nm at 30 s intervals up to 3 min with a UV-1600 spectrophotometer. The activity of GSH was determined according to the method explained by Sedlak and Lindsay (1968) and Protein contents were determined by the method of Bradford (1976). The final concentration of soluble protein was calculated by using bovine serum albumin (BSA) standard curve. Lipid peroxidation was estimated by determination of the MDA contents using method described by Heath and Packer (1968). The concentration of MDA was calculated by using the following formula:

$$\mathrm{MDA}\left(\ddot{\mathrm{E}}\mathrm{mol}\Big/\mathrm{l}\right) = 6.45(\mathrm{OD}_{532}-\mathrm{OD}_{600})-0.56\ \mathrm{OD}_{450}$$

The vanadium determination in roots and shoots of treated plants of chickpea was done according to Kashif et al. (2009). The vanadium concentration was determined by using a graphite furnace atomic absorption spectrophotometer (GFAAS-GTA 120).

Statistical analysis

All the collected data were subjected to analyze using SAS software. Firstly, analyzed analysis of variance (ANOVA) to find out the significant ($P \leq 0.05$) vanadium treatment and cultivar effects mean comparison was evaluated by Duncan's Multiple Range Test at 1 %.

Results

Plant biomass

The plant heights were significantly ($P \leq 0.05$) decreased after the addition of vanadium (Table 1). The results showed a reduction of about 58 %, 63 % and 60 %, 64 % in shoots and 61 %, 66 % and 64 %, 66 % in roots lengths of G−1 and Balkasar for NH_4VO_3 and Na_3VO_4 treated as compared to untreated plants, respectively. However, for Noor−2009 and C−44 the reduction was observed about 76 %, 76 % and 75 % and 70 % in shoots and in roots was about 80 %, 81 % and 74 %, 79 % as a result of NH_4VO_3 and Na_3VO_4 application respectively than control. The same reduction trend was examined for percent of V/control for the total plant biomass (fresh) in chickpea cultivars against V stress (Table 2).

Both vanadium salts gradually stunted the growth of plants and roots extensively. Moreover, the plants roots showed significant reduction in the growth of lateral roots production (Fig. 1).

Response of plant antioxidant systems

The obtained results clearly showed positive relationship among the vanadium addition and antioxidant enzymes activities in all cultivars of chickpea but there was significant ($P \leq 0.05$) difference among cultivars in response to V treatment. The activity of SOD in all the cultivars was significantly ($P \leq 0.05$) more in vanadium treated as compared to control (Fig. 2a). The activity of SOD was higher about 5.5 and 4.7 fold for Noor−2009 and 3.6 and 2.9 fold for C−44 against NH_4VO_3 and

Table 1 The lengths of shoots and roots of six genotypes of chickpea seedlings grown hydroponically under the stress of two vanadium salts at the rate of 25 mg L^{-1}. Values are mean±SD (n=4)

Genotypes	Treatments	Shoot length		Root length		Total length	
		cm	Percent (%) of V/control	cm	Percent (%) of V/control	cm	Percent (%) of V/control
G−1	Control	40.56±1.49	100	18.4±0.95	100	58.96±1.90	100
	NH_4VO_3	23.53±2.20	58.00	11.29±1.37	61.34	34.81±1.88	59.04
	Na_3VO_4	25.55±1.71	62.99	12.13±1.13	65.89	37.68±1.81	63.90
Z−1	Control	38.99±1.18	100	18.63±1.07	100	57.61±0.84	100
	NH_4VO_3	24.33±1.83	62.39	11.28±1.44	60.53	35.6±0.80	61.79
	Na_3VO_4	26.55±1	68.10	11.05±1.50	59.32	37.6±1.91	65.26
Pb−2000	Control	37.2±1.14	100	21.33±1.14	100	58.53±1.84	100
	NH_4VO_3	25.33±1.53	68.08	14.64±1.41	68.63	39.96±1.49	68.28
	Na_3VO_4	25.15±1.70	67.61	14.56±1.21	68.28	39.71±1.55	67.85
C−44	Control	37.98±1.55	100	21.64±1.15	100	59.61±2.17	100
	NH_4VO_3	28.31±1.24	74.56	16.03±1.92	74.06	44.34±1.60	74.38
	Na_3VO_4	26.73±1.44	70.38	17.00±0.71	78.57	43.73±0.61	73.35
Balkasar	Control	37.58±1.83	100	20.38±1.60	100	57.95±1.02	100
	NH_4VO_3	22.5±1.86	59.88	13.03±0.70	63.92	35.53±1.59	61.30
	Na_3VO_4	23.89±2.15	63.57	13.38±0.80	65.64	37.26±1.84	64.30
Noor−2009	Control	38.45±1.17	100	20.15±1.42	100	58.6±1.68	100
	NH_4VO_3	29.29±1.72	76.17	16.05±1.55	79.62	45.33±1.22	77.36
	Na_3VO_4	28.29±1.45	76.17	16.29±1.91	80.83	45.58±0.93	77.77

The length represents the mean of 4 replications for each genotype

Table 2 The fresh biomass of shoots and roots of six genotypes of chickpea seedlings grown hydroponically under stress of two vanadium salts at the rate of 25 mg L^{-1}. Values are mean±SD (n=4)

Genotypes	Treatments	Shoot biomass		Root biomass		Total biomass	
		*FW, g/plant	Percent (%) of V/control	FW, g/plant	Percent (%) of V/control	FW, g/plant	Percent (%) of V/control
G−1	Control	0.87±0.017	100	0.72±0.016	100	1.59±0.019	100
	NH_4VO_3	0.42±0.021	47.85	0.23±0.010	31.29	0.64±0.026	40.36
	Na_3VO_4	0.49±0.012	56.16	0.24±0.019	33.65	0.73±0.026	45.98
Z−1	Control	0.83±0.012	100	0.70±0.017	100	1.53±0.068	100
	NH_4VO_3	0.50±0.009	59.55	0.28±0.012	40.02	0.77±0.017	50.65
	Na_3VO_4	0.52±0.018	59.43	0.27±0.014	38.78	0.79±0.020	51.96
Pb−2000	Control	0.81±0.014	100	0.77±0.021	100	1.58±0.018	100
	NH_4VO_3	0.46±0.017	57.21	0.27±0.013	34.91	0.73±0.021	46.33
	Na_3VO_4	0.48±0.029	59.07	0.26±0.012	33.59	0.74±0.022	46.64
C−44	Control	0.91±0.012	100	0.77±0.017	100	1.68±0.031	100
	NH_4VO_3	0.58±0.012	64.01	0.41±0.022	53.20	0.99±0.012	59.08
	Na_3VO_4	0.67±0.012	73.63	0.42±0.013	55.13	1.09±0.052	65.18
Balkasar	Control	0.85±0.026	100	0.82±0.019	100	1.67±0.027	100
	NH_4VO_3	0.38±0.012	44.84	0.22±0.011	26.79	0.60±0.019	35.98
	Na_3VO_4	0.45±0.017	53.25	0.25±0.018	30.93	0.70±0.028	42.29
Noor−2009	Control	0.90±0.015	100	0.80±0.013	100	1.71±0.017	100
	NH_4VO_3	0.58±0.012	64.40	0.43±0.018	53.13	1.01±0.015	59.10
	Na_3VO_4	0.71±0.009	78.53	0.44±0.017	54.88	1.15±0.025	67.40

The weight represents the mean of 4 replications for each genotype

**FW* fresh weight

Na_3VO_4, respectively than control. However, G−1 and Balskasar showed lowest activity which accounted about 2.6 and 2 fold in G−1 and 3.5 and 1.9 fold in Balkasar for the application of NH_4VO_3 and Na_3VO_4, respectively as compared to untreated.

The activity of CAT was significantly ($P \leq 0.05$) increased in C−44 and Noor−2009 than all other cultivars (Fig. 2b). The results of analysis of variance clearly indicated that Noor−2009 and C−44 exhibited about 7.5, 5.5 and 8.4, 6.4 fold higher in treated plants with NH_4VO_3 and Na_3VO_4, respectively than control. But the G−1 and Balkasar resulted 4.3, 2.7 and 4.2, 3.6 fold higher with the application of NH_4VO_3 and Na_3VO_4 than untreated.

The addition of vanadium significantly ($P \leq 0.05$) increased the POD activity as compared to control (Fig. 2c). The obtained data showed that the Noor−2009 about 8.9 and 5.4 fold, and C−44 about 8 and 5.4 fold more POD activity after the addition of

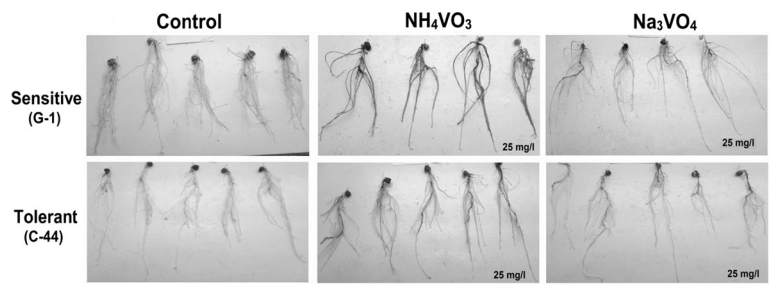

Fig. 1 Effect of vanadium stress on the roots of chickpea genotypes grown hydroponically

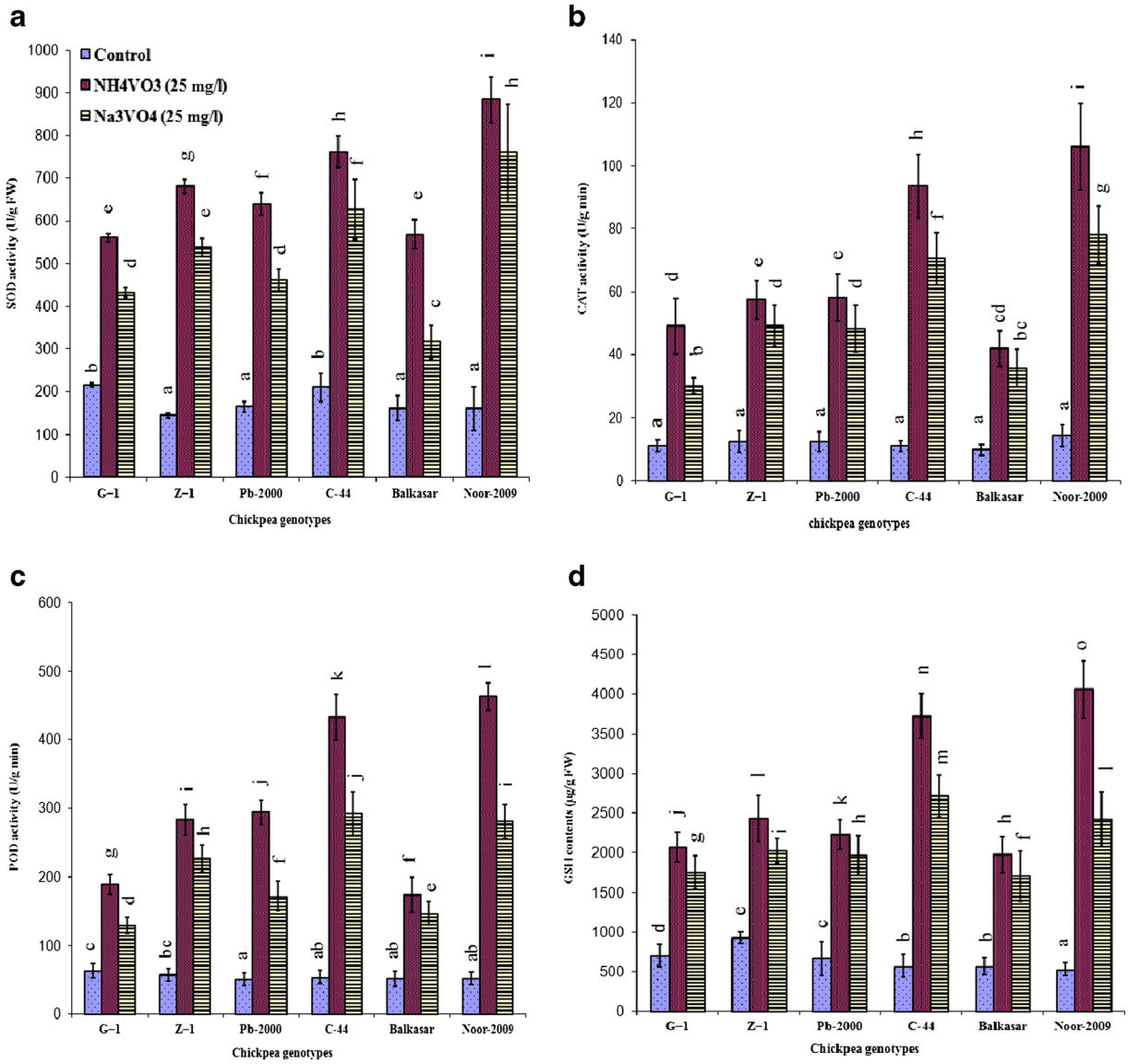

Fig. 2 Effect of vanadium stress on enzymes activities in seedling of chickpea genotypes. (**a**) Superoxide dismutase (*SOD*), (**b**) Catalase (*CAT*), (**c**) Peroxidase (*POD*) and (**d**) Glutathione reductase (*GSH*). Values represent means±S.D. (n=4). Means contained similar letters are not significantly ($P \leq 0.05$) different according to Duncan's multiple range test

NH_4VO_3 and Na_3VO_4 respectively than control. But G−1 exhibited about 3 and 2 fold, and Balkasar accounted about 3.3 and 2.8 fold more POD activity with the NH_4VO_3 and Na_3VO_4 application, respectively than control.

Compared to control, the contents of MDA were found significantly ($P \leq 0.05$) higher with the treatment of V in all cultivars under observation (Fig. 3a). The findings illustrated that G−1 and Balkasar showed about 11.1, 7.3 and 9.7, 7.0 fold increase MDA contents by the application NH_4VO_3 and Na_3VO_4, respectively as compared to control. The MDA contents in Noor−2009 and C−44 accounted about 5.6, 3.6 and 5.2, 3.9 fold more with NH_4VO_3 and Na_3VO_4, respectively than untreated.

The addition of V enhanced the GSH content significantly ($P \leq 0.05$) in Noor−2009 about 7.7, 4.6 fold and in C−44 about 6.5, 4.7 fold, respectively than plants that were not exposed to V treatment, and minimum concentration of GSH, which was recorded about 2.9, 2.5 in

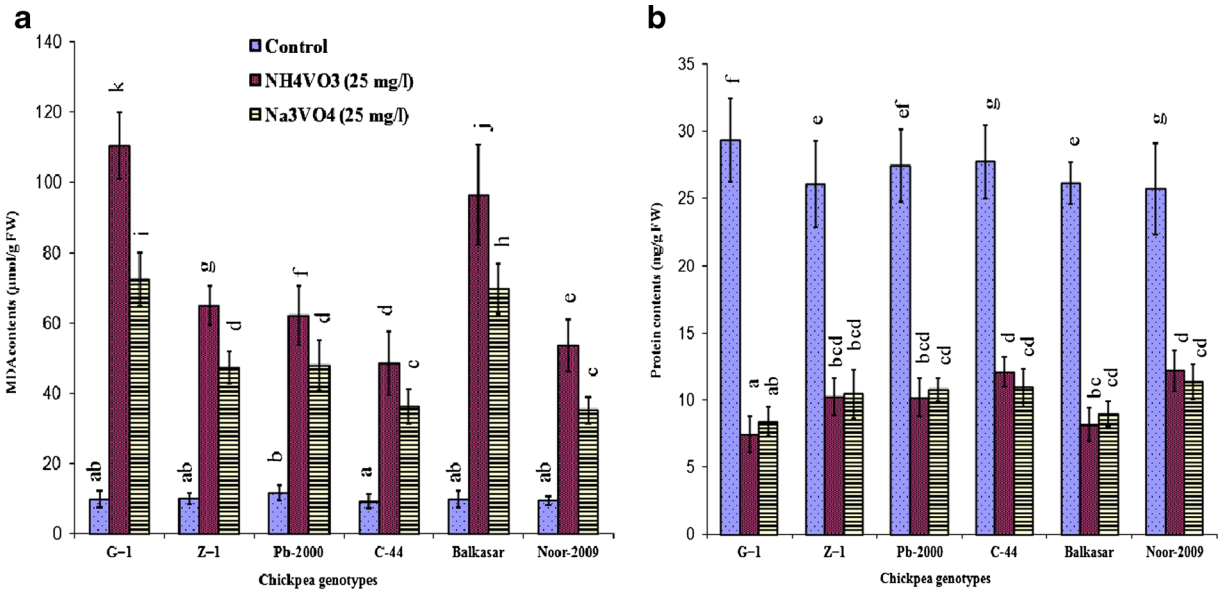

Fig. 3 Effect of vanadium stress on (**a**) Malondialdehyde (*MDA*) and (**b**) Protein Contents in seedling of chickpea genotypes. Values represent means±S.D. (n=4). Means contained similar letters are not significantly ($P \leq 0.05$) different according to Duncan's multiple range test

G−1 and about 3.5, 3 fold higher in Balkasar than control (Fig. 2d).

Protein contents

The obtained results showed that protein contents reduced significantly ($P \leq 0.05$) due to vanadium stress in all chickpea genotypes (Fig. 3b). The maximum reduction was recorded about 75 % and 71 % in G−1 followed by Balkasar about 69 % and 66 % for the application of NH_4VO_3 and Na_3VO_4, respectively. And the lowest reduction was recorded in Noor−2009 about 53 % and 56 % followed by C−44 about 56 % and 61 % with NH_4VO_3 and Na_3VO_4, respectively. The Z−1 and Pb−2000 were also showed about 61 %, 60 % and 61 %, 61 % with NH_4VO_3 and Na_3VO_4, respectively.

Vanadium uptake

The addition of V significantly ($P \leq 0.05$) increased the V concentrations in the tissues of chickpea plants compared to plants having zero V. The concentrations of V were increased in shoots by 1690 %, 1366 % of G−1; 1440 %, 1237 % of Z−1; 1500 %, 1417 % of Pb−2000; 2428 %, 2008 % of C−44; 1920 %, 1535 % of Balkasar and 2224 %, 2078 % of Noor−2009 with the application of NH_4VO_3 and Na_3VO_4 than control (Fig. 4). Similarly, the V concentrations were higher in roots by 518 %, 447 % of G−1, 795 %, 755 % of Z-1, 1494 %, 1392 % of Pb-2000, 1394 %, 1283 % of C−44, 869 %, 794 % of Balkasar and 1355 %, 1231 % of Noor−2009 treated with NH_4VO_3 and Na_3VO_4, respectively than untreated (Fig. 4).

Discussion

Plant biomass, shoots and roots lengths are considered highly sensitive plant response parameters to any stress and used for measuring plant tolerance to metal stress. Application of heavy metals induce toxicity and cause significant reduction in plant growth and development like shoot length, root length, fresh and dry weights of plants (Kaya et al. 2006) and usually such kind of reduction generally occurred differently in different parts of the plants (Liang et al. 2007). For instance, in the present trial, shoots length, roots length, fresh weights and fibrous root system were markedly declined with the addition of NH_4VO_3 and Na_3VO_4. The results of the present experiment also show that NH_4VO_3 caused more toxicity than Na_3VO_4 to chickpea genotypes. Alan et al. (2005) also reported that higher levels of V significantly retarded the production of lateral roots and the younger leaves showed chlorotic

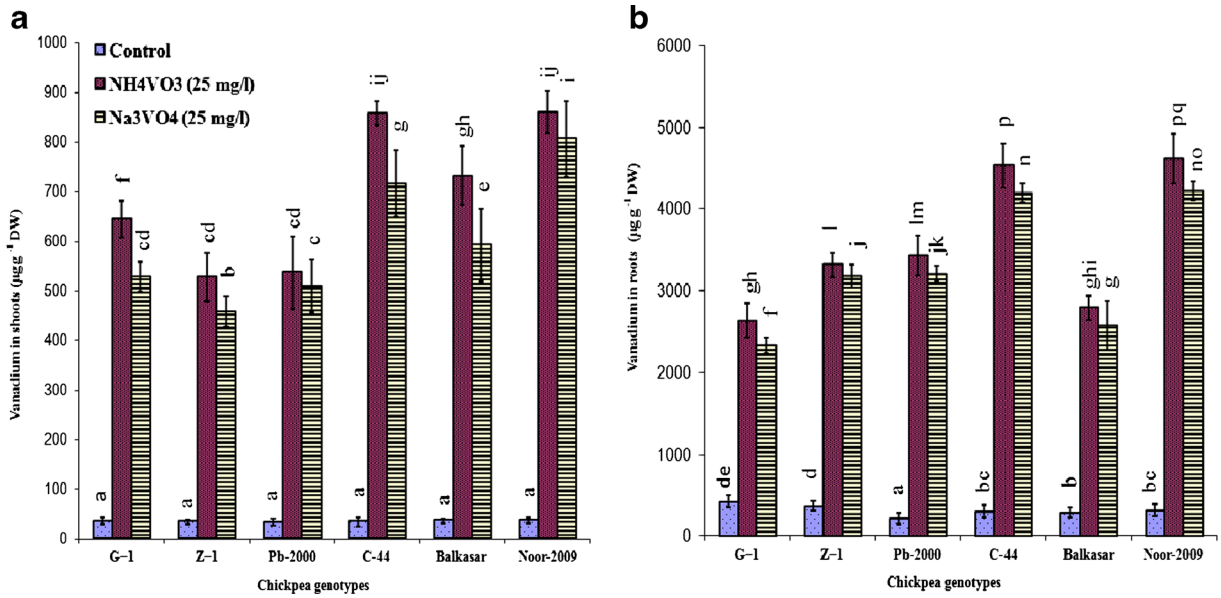

Fig. 4 Vanadium accumulations in different parts of chickpea genotypes. (**a**) Shoots and (**b**) Roots. Values represent means±S.D. (n=4). Means contained similar letters are not significantly ($P \leq 0.05$) different according to Duncan's multiple range test

symptoms in cuphea The outcome of our study strongly revealed a negative association between vegetative growth and V treatment for chickpea genotypes, and these results found to be same with previous studied as Xi-yuan et al. (2012).

In general, Plant protein production is sensitive to heavy metals and reduction in protein production can be due to inhibitory effects of metals (Hemalatha et al. 1997). In the present experiment, the protein for control plants proved a progressive increment whereas, the V stress decreased a noticeable reduction in protein level. Actually, the metals stresses impair the functional ability of a large number of enzymes having functional sulphydryl group, ultimately cause deleterious effects to form protein (Tanyolaç et al. 2007). In the present research, V application also caused reduction in protein contents more in sensitive than tolerant cultivars. The results showed that the addition of NH_4VO_3 induced protein degradation more than Na_3VO_4. Andon and Fernando (2011) reported that Cd toxicity significantly reduced the synthesis of protein in sensitive barley plants. Earlier reports also revealed that Cd stress induced inhibition of protein concentration in plants (Tanyolaç et al. 2007).

Heavy metals toxicity generates superoxide radical, H_2O_2, hydroxyl radicals and singlet oxygen, collectively known as reactive oxygen species (ROS) (Jain et al. 2010) and ROS reduce the work efficiency of antioxidant enzymes. SOD enzyme is regarded as the integral part of the anti-oxidant defense system in plants to regulate the concentration of ROS and present in different cellular compartments and it can be used as biomarker of environmental stress. Therefore, higher activity of SOD in plant tissues reveals its positive role to restore the oxidative damage (Dazy, et al. 2009). Actually the SOD activity in response to toxicity comes out to be due to de-novo synthesis of the enzyme protein (Lozano et al. 1996) and this kind expression maintains the overall defense system of plants under stress environment. Our findings illustrate the enhancement in SOD activity in all plant treated with V than control, moreover, the SOD activity in Noor−2009 and C−44 recorded higher, while G−1 and Balkasar exhibited lower activity. In addition, application of NH_4VO_3 induced more pronounced increase in SOD activity than Na_3VO_4. This is probably due to that the generations of more superoxide free radical resulting more oxidative damage by NH_4VO_3. Higher application of Cu caused increased SOD activity in turfgrass (Ke et al. 2007).

CAT is also one of the main antioxidant enzymes of plant protective system and occurred in peroxisomes and mitochondria, and eliminates the H_2O_2 to water and oxygen (Gupta, et al. 2009). Our findings show higher concentration of CAT activity in Noor−2009 and C−44, whereas, CAT activity was lower in G−1 and Balkasar cultivars. Furthermore, there was

significant difference between NH_4VO_3 and Na_3VO_4 addition for CAT activity. The higher CAT activity was also reported by Hassan and Mansoor (2014) in mung bean plants treated with Pb and Cd. Our results are also similar with the findings of Zhang et al. (2009).

Peroxidase is another vital component of plant antioxidant mechanism and existed in cytoplasm, vacuole, membrane and cell wall of the plants and used as marker for sublethal metal stress (Lagriffoul et al. 1998). In the present trial, the concentration of POD in all genotypes was increased after V application. The maximum activity was recorded in Noor−2009 and C−44 and minimum activity was accounted in G−1 and Balkasar furthermore, NH_4VO_3 caused more POD activity than Na_3VO_4 in all treated cultivars. In different plants species, the induction of POD activity has been observed under heavy metals stress (Tanyolaç et al. 2007), and all reported that POD play central role against a range of stress including metals toxicity in plants.

The MDA is a highly reactive three carbon dialdehyde which is produced as a byproduct of fatty acid peroxidation in the cell membrance due to abiotic stresses in plant cells. MDA is the outcome of the lipid peroxidation, and commonly generated during oxidative stress (Xiao et al. 2012). In the present study, the results indicate that the MDA level was higher in G−1 and Balkasar than remaining four cultivars, while Noor−2009 and C−44 showed lower MDA among all the cultivars. Moreover, NH_4VO_3 induced more MDA contents than Na_3VO_4 in all genotypes. These findings show an agreement with the results reported by Xiao et al. (2012). Similar results were reported by the other researchers in sugarcane (Jain et al. 2010) and *Phaesolus aureus* (Kaur et al. 2012). Our results strongly support with the findings of Hassan and Mansoor (2014) that confirmed that tolerant cultivars had lower capacity of MDA contents than sensitive cultivars.

The GSH is a distinguished and principal component of the antioxidant defense system in plants. And this chemical compound plays important role to control the oxidative stress and mainly against heavy metals toxicity. Actually, GSH production increases by two enzymes termed as γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS), which can be generate by the effect of heavy metals (Mendoza-Cózatl and Moreno-Sánchez 2006). In the present study, the V treatment significantly increased the GSH contents in all examined cultivars than zero V. Our results confirm that Noor−2009 and C−44 attained higher GSH level than G−1, Balkasar, Z−1 and Pb−2000 after V application than control. G−1 and Balkasar exhibited lowest GSH activity after the addition of V among all the cultivars. These findings also confirmed that there was significant difference for GSH contents between NH_4VO_3 and Na_3VO_4 supply in all cultivars. Pereira et al. (2002) reported that the plants showed maximum GSH contents after heavy metals application. Myrene and D'Souza Devaraj (2012) also agreed that leaves of the bean plant showed more GSH contents with Zn pollution.

The obtained data of present study showed that the concentration of V was significantly higher in roots than shoots in all V treated cultivars. The maximum concentration of V was expressed in roots and shoots of Noor−2009 and C−44, but the roots attained higher and much greater than the above ground part of the V treated plants in all genotypes. The values achieved in Z−1 and Pb−2000 showed lowest V concentration in shoots as well as in roots. The pattern of V accumulation was roots>shoots in all chickpea genotypes. However, the maximum accumulation of V in shoots and roots was acquired by the addition of NH_4VO_3 than Na_3VO_4. Narumol et al. (2011) also confirmed that the roots uptake higher amount of V than leaves, shoots and fruits in Chinese green mustard and also in tomato plant treated with NH_4VO_3. The observed values from this study were in agreement with the results reported by Rascio et al. (2008).

Conclusion

In conclusion, V markedly influenced the growth, protein contents and activities of antioxidant enzymes among all the genotypes of chickpea plants. The greatest impact of V induced toxicity was recorded by the application of NH_4VO_3. The present research established that under V stress, antioxidant defense mechanism in genotypes of chickpea underwent changes in chemical processes to remediate the oxidative damage. The main reason in the variation of activities of antioxidant enzymes (SOD, CAT, POD, MDA and GSH) may be due to different threshold levels of tolerance to heavy metals (Hou et al. 2007). Therefore, it can be said that increase and decrease in enzymatic activities could be characteristic to the increased tolerance and sensitivity, respectively to V. The data which is generated in the present study indicated that Noor−2009 and C−44 genotypes

are more tolerant and G−1 and Balkasar are more sensitive than others. However, additional study is required to disclose the relationship between V stress and the response of antioxidant enzymes.

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Conflict of Interest None of the authors have any conflict of interest.

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