Establishment of sensitive and resistant variety of tomato on the basis of photosynthesis and antioxidative enzymes in the presence of cobalt applied as shotgun approach

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ABSTRACT

Cobalt (Co) affords both beneficial as well as toxic effects to plants. The present study was performed with an aim to find out the varietal differences among five tomato cultivars against the Co induced changes in growth, photosynthesis, nitrate reductase (E.C.1.6.6.1), carbonic anhydrase (E.C.4.2.1.1), antioxidative enzymes i.e. peroxidase (E.C.1.11.1.7), catalase (E.C.1.11.1.6), superoxide dismutase (E.C.1.15.1.1) and that of proline content. Seeds of tomato (varieties, K-25, NTS-9, NBR-Uday, Sarvodya, and Malti) were soaked in 0, 100, 200 or 300 μM CoCl$_2$ for 0, 4, 8, 12 h (shotgun approach) and sampled at 30 days after sowing. All the varieties showed significantly different response to different treatment combinations. Despite substantial varietal difference, increased Co concentration caused concomitant decrease in growth, photosynthesis and the activity of nitrate reductase and carbonic anhydrase. However, the activity of antioxidant enzymes and that of proline content increased with the increased concentration of Co as well as duration of soaking in all the varieties. Out of the varieties, K-25 possessed maximum antioxidative enzyme and proline content that represent its most resistant nature against the toxic effect of Co. The order of susceptibility/sensitivity was K-25 > NTS-9 > NBR-Uday > Sarvodya > Malti.

Key words: antioxidative enzyme, carbonic anhydrase, cobalt, nitrate reductase, photosynthesis, tomato.

INTRODUCTION

Cobalt (Co) is a natural earth element presented in trace amount in soil. Trace elements are necessary for normal metabolic functions in plants, but at higher concentration these metals are toxic and may severely interfere with physiological and biochemical functions (Jayakumar and Vijayarengan, 2006). Similarly, Co also affords both beneficial as well as harmful effects to plants. It has long been applied to plants to raise crops yield (Young, 1979). However, in excess concentration it caused a marked reduction in growth together with chlorosis and necrosis (Vanselow, 1966), inhibits photosynthesis (Van Asshe and Clijsters, 1990), seed germination and seedling growth (Dubey and Dwivedi, 1987). The activities of several enzymes are also disturbed by excessive amount of Co present within the plant (Shalygo et al., 1990) and thus finally reduced the quality of produce (Chatterjee et al., 2006). In general the average level of Co in the soil ranges 30-40 ppm (Kabata-Pendias and Pendias, 1991) and above that it generates toxicity. However, the

concentration of Co in the soil is not the only factors that determine toxicity. Plant species vary in their sensitivity to Co, soil type and soil chemistry greatly influence Co toxicity. One of the most important soil properties is soil acidity and the more acidic the soil, the greater the potential for Co toxicity, at any concentration. Keeping all these points in mind the present piece of work was designed, with an aim to find out the degree of tolerance among different varieties of tomato against differential concentration of Co applied as seed soaking i.e. shotgun approach (Hayat et al., 2010).

MATERIAL AND METHODS

Seeds of tomato (*Lycopersicon esculentum*) cv. K-25, NTS-9, NBR-Uday, Sarvodya, and Malti were purchased from National Seed Corporation Ltd., New Delhi, India. Healthy seeds were surface sterilized with 0.5% (v/v) of sodium hypochlorite solution, followed by repeated washings with double distilled water and were soaked in 0, 100, 200 or 300 μM of Cobalt in the form of cobalt chloride for 0, 4, 8 or 12 h (shotgun approach). These treated seeds were sown in sand, moistened with deionized water in plastic pots of 6 inch diameter. These pots were kept in a plant growth chamber illuminated by incandescent light, with day/night temperature of 25 $\pm$ 2°C on 14/10 h photoperiod. The humidity was maintained at 65 $\pm$ 5%. The plants after their germination in dark for 5 days were supplied with 50 ml of nutrient solution. The composition of nutrient solution was 0.5 μM KNO_3, 0.5 μM Ca $(NO_3)_2$, 0.5 μM $MgSO_4$, 2.5 μM KH_2PO_4, 2 μM NH_4Cl, 100 μM Fe-K-EDTA, 30 μM H_3BO_3, 5 μM $MnSO_4$, 1 μM $CuSO_4$, 1 μM $ZnSO_4$ and 1 μM $(NH_4)_6Mo_7O_{24}$ per liter. The pH of the nutrient solution was adjusted to 5.0 with HCl. The design of the experiment was complete randomized and the position of pots, in the growth chamber was changed daily. The plants were sampled at 30 days after sowing (DAS) to make the various observations. The plants were uprooted and washed under running tap water. The shoot was separated from roots and was weighed for their fresh mass. These were subsequently transferred to an oven run at 80 ^{o}C and left there for 48 hours, and weighed again to obtain their dry mass. Leaf area was determined by gravimetric method where the leaf area of randomly selected leaves from each treatment, was determined by tracing its outline on the graph sheet.

The chlorophyll content in the leaves was measured with the help of a Minolta chlorophyll meter (SPAD-502, Konica Minolta Sensing Inc. Japan). Leaf water potential, was measured in fresh, detached leaves of the sample plants by using PSYPRO, water potential system (WESCOR, Inc. Longman, USA).The rate of photosynthesis and internal CO_2 concentration were measured by using a LI-6400 portable photosynthetic system (LI-COR, Lincoln, NE, USA). The measurements were made in the uppermost fully expanded leaves, between 1100 and 1300 hours.

The activity of NR was determined in fresh leaf samples by the procedure explained by Jaworski (1971). The fresh leaf samples were cut into small pieces and transferred to plastic vials, containing phosphate buffer (pH 7.5) followed by the addition of potassium nitrate and isopropanol solutions. The reaction mixture was incubated at 30 ^{o}C for 2 h followed by addition of sulfanilamide and *N*-1- naphthylethylenediamine dihydrochloride. The absorbance of the colour was read at 540 nm and was compared with that of the calibration curve.

The activity of CA was determined following the procedure described by Dwivedi and Randhawa (1974). The leaf samples were cut into small pieces and suspended in cystein hydrochloride solution. The samples were incubated at 4 ^{o}C for 20 min. The pieces were blotted and transferred to the test tubes, containing phosphate buffer (pH 6.8) followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tube was incubated at 5 ^{o}C for 20 min. The reaction mixture was titrated against 0.01mol/L HCl, after addition of 0.2 mL of methyl red indicator.

For the estimation of antioxidative enzymes, the leaf tissue (0.5 g) was homogenized in 50 mmol/L phosphate buffer (pH 7.0) containing 1% (w/v) soluble polyvinylpyrrolidone. The homogenate was centrifuged at 15000 g for 10 min at 4°C and the supernatant was used as a source of the enzymes catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD). CAT and POX were assayed following the procedure described by Chance and Maehly (1955). CAT was estimated by titrating the reaction mixture, consisting of phosphate buffer (pH 6.8), 0.1 mol/L H_2O_2, enzyme extract and 2 % H_2SO_4, against 0.1 mol/L potassium permanganate

solution. The reaction mixture for peroxidase consisted of pyrogallol, phosphate buffer (pH 6.8), 1% H_2O_2 and enzyme extract. Change in absorbance due to catalytic conversion to pyrogallol to perpurogalline was noted at an interval of 20 s for 2 min at 420 nm on a spectrophotometer. A control set was prepared by using DDW instead of enzyme extract. The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium following the method of Beauchamp and Fridovich (1971). The reaction mixture contained 50 mmol/L phosphate buffer (pH 7.8), 13 mmol/L ethylenediaminetetraacetic acid and 0-50 mL enzyme extract and was placed under a 15 W fluorescent lamp. The reaction was started by switching on the light and was allowed to run for 10 min. The reaction was stopped by switching off the light. Fifty per cent inhibition by light was considered as 1 enzyme unit.

The proline content in fresh leaf and root samples was determined by adopting the method of Bates et al. (1973). Samples were extracted with sulfosalicylic acid and an equal volume of glacial acetic acid and ninhydrine solutions were added. The samples were heated at 100 ºC, to which 5 mL of toluene was added. The absorbance of the toluene layer was read at 528 nm on a spectrophotometer.

Data were statistically analyzed using analysis of variance (Anova) by SPSS (ver. 10; SPSS Inc., Chicago, IL, USA). The least significant difference was calculated for the significant data at $P < 0.05$.

RESULTS

Growth parameters: All the varieties exhibited significantly different response to different concentration of metal as well as duration of soaking. Pre sowing seed soaking treatment caused significant decrease in the fresh and dry mass of shoot and leaf area. The decrease was proportionate to the concentration of the metal (100, 200 or 300 μM) as well as duration of soaking (4, 8 or 12 h). The highest concentration of metal (300 μM) and longest duration of soaking i.e. 12 h caused maximum damage. The variety K-25 was found to be most resistant (Tables 1-3). The order of susceptibility/sensitivity was K-25 > NTS-9 > NBR-Uday > Sarvodya > Malti.

Table 1. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the shoot fresh mass (g) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of Seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		3.10	2.52	2.21	1.89	1.80	2.30	
	0	3.19	2.55	2.24	2.00	1.87	2.37	
	100	2.94	2.32	2.02	1.79	1.50	2.11	V=0.14
4 h	200	2.25	2.11	1.49	1.40	1.31	1.71	C=0.20
	300	2.00	1.70	1.26	1.19	1.09	1.44	VxC=0.39
	Mean	2.59	2.17	1.75	1.59	1.44		
	0	3.22	2.59	2.28	2.06	1.89	2.40	
	100	2.80	2.00	1.86	1.50	1.39	1.91	V=0.25
8 h	200	1.84	1.70	1.39	1.11	1.00	1.40	C=0.10
	300	1.77	1.64	1.23	1.00	0.90	1.30	VxC=0.39
	Mean	2.40	1.98	1.69	1.41	1.29		
	0	3.29	2.58	2.30	2.08	1.70	2.39	
	100	2.46	1.83	1.60	1.30	1.20	1.67	V=0.18
12 h	200	1.66	1.50	1.16	1.00	0.89	1.24	C=0.20
	300	1.40	1.23	1.04	0.80	0.64	1.02	VxC=0.42
	Mean	2.20	1.78	1.52	1.29	1.10		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Table 2. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the shoot dry mass (g) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		0.77	0.63	0.545	0.471	0.44	0.572	
4 h	0	0.78	0.63	0.549	0.473	0.445	0.575	
	100	0.66	0.589	0.46	0.414	0.36	0.497	V=0.048
	200	0.64	0.522	0.42	0.359	0.3	0.448	C=0.045
	300	0.58	0.44	0.36	0.3	0.241	0.384	VxC=0.105
	Mean	0.67	0.545	0.447	0.386	0.336		
8 h	0	0.78	0.63	0.551	0.474	0.446	0.576	
	100	0.64	0.55	0.411	0.36	0.319	0.456	V=0.037
	200	0.58	0.47	0.372	0.302	0.26	0.397	C=0.055
	300	0.54	0.391	0.318	0.243	0.2	0.337	VxC=0.104
	Mean	0.63	0.51	0.413	0.344	0.306		
12 h	0	0.78	0.633	0.584	0.475	0.447	0.577	
	100	0.58	0.49	0.35	0.301	0.25	0.394	V=0.048
	200	0.52	0.444	0.291	0.242	0.19	0.377	C=0.050
	300	0.46	0.384	0.23	0.181	0.18	0.287	VxC=0.110
	Mean	0.59	0.487	0.534	0.299	0.266		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Table 3. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf area (cm^2) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		5.79	5.68	5.4	5.28	4.81	5.39	
4 h	0	5.82	5.71	5.43	5.3	4.85	5.42	
	100	5.64	5.59	5.17	5	4.7	5.22	V=0.17
	200	5	4.8	4.6	3.97	3.4	4.35	C=0.55
	300	4.16	3.8	3.44	2.82	2.36	3.31	VxC=0.81
	Mean	5.15	4.97	4.66	4.27	3.82		
8 h	0	5.84	5.74	5.46	5.32	4.86	5.44	
	100	5.58	5.51	5.1	4.96	4.66	5.16	V=0.14
	200	4.32	4.11	3.92	3.6	3.17	3.82	C=0.20
	300	3.71	3.5	3.3	3	2.56	3.21	VxC=0.14
	Mean	4.86	4.71	4.77	4.22	3.79		
12 h	0	5.87	5.76	5.47	5.34	4.87	5.46	
	100	5.55	5.47	5.06	4.91	4.6	5.11	V=0.14
	200	4.06	3.86	3.61	3.42	2.93	3.97	C=0.30
	300	3.5	3.28	2.97	2.69	2.4	2.96	VxC=0.50
	Mean	4.74	4.59	4.27	4.09	3.7		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Leaf water potential (ψ) and SPAD chlorophyll value: It is evident from table 4 and 5 that the pre sowing seed soaking treatment caused a significant decrease in leaf water potential and SPAD chlorophyll values of the resulting plants. The highest concentration of metal (300 μM) generate maximum inhibition at all the soaking duration (4, 8 or 12 h) irrespective

of varietal difference and decreased the values of ψ by 75.0%, 93.3% and 109.8%; 67.1%, 88.4% and 100%; 50.6%, 85.0% and 105.0%; 64.2%, 96.4% and 111.7%; 64.7%, 98.8% and 114.7% and that of SPAD chlorophyll by 25.3%, 31.3% and 47.1%; 30.1%, 37.6% and 50.4%; 36.8%, 42.9% and 57.9%; 38.8%, 44.8% and 52.5%; 40.1%, 47.4% and 59.8% in K-25, NTS-9, NBR-Uday, Sarvodya and Malti where seeds were pre-treated with 4, 8 or 12 h respectively.

Table 4. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf water potential (Mpa) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		-0.6	-0.69	-0.78	-0.8	-0.82	-0.73	
	0	-0.6	-0.7	-0.81	-0.84	-0.88	-0.76	
	100	-0.7	-0.85	-0.97	-1.08	-1.12	-0.95	V=0.06
4 h	200	-0.9	-1.04	-1.13	-1.21	-1.32	-1.11	C=0.12
	300	-1.1	-1.17	-1.22	-1.38	-1.45	-1.25	VxC=0.20
	Mean	-0.8	-0.94	-1.03	-1.12	-1.19		
	0	-0.6	-0.69	-0.8	-0.84	-0.89	-0.76	
	100	-0.8	-0.91	-1.04	-1.21	-1.33	-1.05	V=0.08
8 h	200	-1	-1.22	-1.23	-1.43	-1.5	-1.25	C=0.18
	300	-1.2	-1.3	-1.48	-1.65	-1.77	-1.47	VxC=0.29
	Mean	-0.9	-1.03	-1.13	-1.28	-1.34		
	0	-0.6	-0.7	-0.79	-0.85	-0.88	-0.76	
	100	-0.8	-0.96	-1.19	-1.4	-1.51	-1.18	V=0.06
12 h	200	-1.1	-1.17	-1.41	-1.63	-1.7	-1.39	C=0.17
	300	-1.3	-1.4	-1.62	-1.8	-1.89	-1.59	VxC=0.26
	Mean	-0.9	-1.05	-1.25	-1.42	-1.49		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Table 5. Effect of3 pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the SPAD chlorophyll in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		42.1	34.2	28.6	26	24.7	31.12	
	0	43.1	35	29	27	25.06	31.83	
	100	40	31.91	26.4	23.6	22	28.78	V=1.50
4 h	200	36.8	27.9	22	20	18.41	25.02	C=3.00
	300	32.2	24.44	18.32	16.52	15	21.29	VxC=5.15
	Mean	38	29.81	23.93	21.78	20.11		
	0	43.7	35.6	29.8	27.2	25.09	32.27	
	100	37.5	30	24.8	22.17	22.11	26.91	V=1.88
8 h	200	35	25	19.97	18.25	16.6	22.96	C=3.19
	300	30	22.2	17	15	13.19	19.47	VxC=5.68
	Mean	36.6	28.2	22.89	20.56	18.74		
	0	44.1	36	30	37.8	25.16	32.61	
	100	32.7	28.4	22.5	20	16.6	23.92	V=1.45
12 h	200	27	23.11	17	15.19	11	18.66	C=3.25
	300	23.3	17.84	12.62	13.18	10.11	15.4	VxC=5.37
	Mean	31.8	26.33	20.53	19.04	15.56		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Net Photosynthetic rate (P_N) and internal CO_2 concentration (Ci): The pre sowing seed soaking in different concentration of Co significantly reduced the P_N and Ci (Tables 6-7) in all the varieties. However, the varieties showed significantly different response to different concentration of metal as well as duration of soaking. Out of the three Co concentrations, the 100 μM was found to be least toxic, irrespective of duration of soaking and did not generate any significant response for Ci in all the varieties. Among the varieties, K-25 showed maximum resistance against all the Co concentrations as well as duration of soaking. The highest concentration of metal 300 μM and longest duration of soaking (12 h) was most detrimental for most of the varieties for P_N and Ci.

Table 6. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the P_N (μMCO$_2$ m^{-2}s^{-1}) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		9.1	7.48	6.81	5.52	4.73	6.72	
	0	9	7.59	6.88	5.57	4.77	6.76	
	100	8.59	7.14	6.12	5	4.12	6.19	V=0.75
4 h	200	8.51	6.42	5.69	4.29	3.6	5.7	C=0.48
	300	7.92	5.96	5.2	3.86	3.1	5.2	VxC=1.48
	Mean	8.5	6.77	5.97	4.68	3.89		
	0	9.04	8.02	6.9	5.6	4.79	6.87	
	100	8	6.66	5.9	4.88	4	5.88	V=0.73
8 h	200	7.5	6.14	5.4	4	3.35	5.27	C=0.58
	300	6.93	5.61	4.85	3.6	2.8	4.75	VxC=1.41
	Mean	7.86	6.6	5.76	4.52	3.73		
	0	9.1	8.06	6.94	5.66	4.82	6.91	
	100	7.6	6.19	5.7	4.17	3.8	5.49	V=0.36
12 h	200	7	5.42	5	3.7	3.1	4.82	C=0.58
	300	6.55	4.94	4.56	3.11	2.49	4.33	VxC=1.04
	Mean	7.56	6.15	5.55	4.16	3.55		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Table 7. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf Ci (ppm) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		310	284	280	273	251	279	
	0	312	286	280	277	254	281	
	100	310	282	280	274	251	279	V=6.00
4 h	200	290	261	256	240	229	255	C=20.00
	300	270	239	222	218	207	231	VxC=30.00
	Mean	295	267	259	252	235		
	0	316	289	281	279	256	284	
	100	313	286	280	277	253	281	V=7.00
8 h	200	280	247	230	221	212	238	C=25.00
	300	242	201	200	208	200	210	VxC=36.00
	Mean	288	255	245	246	230		
	0	318	291	283	280	258	286	
	100	312	289	277	275	251	280	V=6.00
12 h	200	255	224	221	200	189	217	C=21.00
	300	220	201	190	186	180	195	VxC=30.00
	Mean	276	251	242	235	219		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

ESTABLISHMENT OF SENSITIVE AND RESISTANT VARIETY OF TOMATO ON THE BASIS OF PHOTOSYNTHESIS AND
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Nitrate reductase (NR) and carbonic anhydrase (CA) activities: The leaves of the plants raised from the seeds given pre sowing seed soaking treatment in Co possessed significantly lower NR and CA activities (Tables 8-9). Degree of damage caused by the metal was directly proportional to the concentration of metal as well as soaking duration. The lowest duration of soaking i.e. 4h in 100, 200 or 300 μM of Co decreased the activity of NR by 5.6%, 11.9% and 19.4%; 8.4%, 15.2% and 21.6%; 8.6%, 17.9% and 22.5%; 10.4%, 19.2% and 24.0%; 10.5%, 22.7% and 30.4% and that of CA by 6.5%, 17.7% and 22.1%; 7.6%, 20.4% and 34.1%; 17.5%, 26.7% and 38.0%; 14.4%, 18.0% and 40.0%; 19.2%, 40.8% and 46.5% in K-25, NTS-9, NBR-Uday, Sarvodya and Malti respectively over their respective controls. The activity of both enzymes decreased further as the duration of soaking was extended.

Table 8. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf NR activity (n MNO$_2$ g^{-1}h^{-1}F.M.) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		317	291	273	250	242	274	
	0	318	295	279	254	246	278	
	100	300	270	255	230	200	251	V=16.00
4 h	200	280	250	229	205	190	230	C=15.00
	300	256	231	216	193	171	213	VxC=35.80
	Mean	288	261	244	220	201		
	0	320	297	281	256	249	280	
	100	294	255	231	208	190	235	V=15.00
8 h	200	269	234	209	180	160	210	C=11.00
	300	237	229	200	170	149	197	VxC=30.00
	Mean	280	253	230	203	187		
	0	321	299	281	257	250	281	
	100	279	240	228	185	177	221	V=20.00
12 h	200	253	220	195	162	146	195	C=15.00
	300	225	211	180	150	132	179	VxC=40.00
	Mean	269	242	221	187	176		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Table 9. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf CA activity (mol CO$_2$ kg^{-1} (F.M.) s^{-1}) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		3.49	2.86	2.7	2.45	2.2	2.74	
	0	3.52	2.99	2.73	2.5	2.23	2.79	
	100	3.29	2.76	2.25	2.14	1.8	2.44	V=0.11
4 h	200	2.99	2.38	2	2.05	1.32	2.14	C=0.23
	300	2.74	1.97	1.69	1.5	1.19	1.81	VxC=0.38
	Mean	3.13	2.52	2.16	2.04	1.63		
	0	3.56	3.01	2.74	2.53	2.25	2.83	
	100	3.11	2.54	1.97	1.73	1.54	2.17	V=0.17
8 h	200	2.7	2.29	1.74	1.5	1.3	1.9	C=0.25
	300	2.36	1.77	1.5	1.25	1.17	1.6	VxC=0.48
	Mean	2.93	2.4	1.98	1.75	1.56		
	0	3.56	3.02	2.77	2.54	2.26	2.83	
	100	2.92	2.22	1.6	1.4	1.17	1.86	V=0.14
12 h	200	2.5	1.8	1.39	1.15	1.11	1.59	C=0.20
	300	2.12	1.46	1.16	1.09	1.05	1.37	VxC=0.39
	Mean	2.77	2.12	1.73	1.54	1.39		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Antioxidative enzymes and proline content: The data depicted in tables 10-13 clearly indicated that the activity of all the antioxidative enzymes and proline content exhibited a trend which was converse to that of all the other parameter explained earlier. The activity of antioxidative enzymes and proline content increased with the increasing concentration of metal as well as duration of soaking. The leaves of plants that were raised from the seeds received pre sowing seed soaking in 100, 200 or 300 μM of Co for 12 h possessed maximum enzyme activity for all the varieties. However, among variety, K-25 possessed maximum enzymatic activity as well as proline content in response to above said treatment. The values were closely followed by NTS-9 and NBR-Uday.

Table 10. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf CAT activity [μmol H_2O_2 decomposed g^{-1} (F.M.)] in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		417	402	340	393	262	362	
4 h	0	420	407	342	297	269	347	
	100	432	418	343	321	272	357	V=0.90
	200	460	425	370	333	300	377	C=0.65
	300	475	460	388	360	319	400	VxC=1.77
	Mean	446	427	360	327	290		
8 h	0	422	408	345	300	271	349	
	100	457	435	350	331	280	370	V=0.88
	200	481	450	385	340	315	394	C=0.30
	300	493	480	399	375	330	415	VxC=1.98
	Mean	463	443	369	336	299		
12 h	0	425	412	347	302	273	351	
	100	480	456	370	345	291	388	V=0.90
	200	502	487	400	360	328	415	C=0.30
	300	528	512	428	390	345	440	VxC=1.38
	Mean	483	466	386	349	309		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Table 11. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf POX activity (units g^{-1} (F.M.) in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		13.3	12.4	11.7	11.21	10.11	11.74	
4 h	0	13.3	12.45	11.72	11.24	10.1	11.76	
	100	13.8	12.93	12.4	12	10.4	12.31	V=18.23
	200	14.3	13.8	12.63	12.09	10.63	12.69	C=9.10
	300	14.2	14.17	13.12	12.81	11.13	13.17	VxC=30.86
	Mean	14	13.33	12.46	12.03	10.56		
8 h	0	13.4	12.5	11.77	11.29	11.13	11.81	
	100	13.9	13.1	12.38	11.6	10.52	12.3	V=18.00
	200	14.4	13.51	12.85	12.4	10.9	12.81	C=13.80
	300	14.9	14.16	13.4	12.63	11.29	13.28	VxC=36.44
	Mean	14.2	13.31	12.6	11.98	10.71		
12 h	0	13.4	12.51	11.79	11.32	11.16	13.03	
	100	14	13.41	12.66	11.79	10.63	12.49	V=15.39
	200	14.6	13.8	13	12.66	11	13.01	C=22.44
	300	15.1	14.26	13.71	12.74	11.35	13.43	VxC=43.39
	Mean	14.3	13.49	12.79	12.12	11.03		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Table 12. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf SOD activity [units g^{-1} (F.M.)] in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		118	116	111	107	100	110	
4 h	0	125	121	113	110	105	114	
	100	143	127	119	115	109	122	V=4.03
	200	151	139	126	122	113	130	C=5.10
	300	163	147	133	128	119	138	VxC=10.25
	Mean	145	133	122	118	111		
8 h	0	127	122	115	112	104	116	
	100	147	135	125	120	113	128	V=4.60
	200	159	144	133	130	120	137	C=7.05
	300	168	160	154	145	128	151	VxC=12.59
	Mean	150	140	131	126	116		
12 h	0	128	124	116	115	106	117	
	100	164	146	139	128	120	139	V=7.40
	200	173	160	147	135	124	147	C=5.32
	300	179	173	160	140	119	154	VxC=13.80
	Mean	161	150	140	129	117		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

Table 13. Effect of pre sowing seed soaking treatment of cobalt (100, 200 or 300 μM) for 4, 8 or 12 h on the leaf proline content [μmol g^{-1} (F.M.)] in five varieties of tomato (*L. esculentum* Mill.) at 30 days AS.

Duration of seed soaking in Co (μM)	Treatments	Varieties						LSD at 5%
		K-25	NTS-9	NBR-Uday	Sarvodya	Malti	Mean	
Unsoaked control		9.59	7.7	6.32	5.7	4.82	6.82	
4 h	0	9.61	7.77	6.38	5.77	4.86	6.87	
	100	11.5	9.89	7.9	6.1	5.02	8.08	V=0.40
	200	16.1	11.22	8.22	6.59	5.68	9.56	C=0.29
	300	19	14.06	8.93	6.88	5.99	10.97	VxC=0.78
	Mean	14.1	10.73	7.85	6.33	5.38		
8 h	0	9.89	7.9	6.66	5.89	4.99	7.06	
	100	12	10.21	8.11	6.2	5.1	8.32	V=0.60
	200	16.5	11.99	8.8	7	6	10.05	C=0.37
	300	19.9	14.9	10.04	7.15	6.41	11.68	VxC=1.11
	Mean	4.58	11.25	8.4	6.56	5.62		
12 h	0	10	8.1	6.79	5.95	5.08	7.18	
	100	13	10.54	8.46	6.32	5.3	8.72	V=0.65
	200	18.1	13.81	9.17	7.16	6.21	10.89	C=0.34
	300	21.6	17.11	10.3	7.49	6.53	11.21	VxC=1.13
	Mean	15.7	12.39	8.68	6.7	5.78		

AS, after sowing; *V,* varieties; *C,* cobalt concentration.

DISCUSSION

Heavy metals are well known, to generate a large quantity of reactive oxygen species (ROS) in plants that may oxidize protein, lipids and nucleic acid resulting to the abnormalities at the level of the cell (Sanita di Toppi et al., 1999). To maintain metabolic functions under stress conditions, the balance between generation and degeneration of ROS is required; for this purpose plants have well equipped antioxidant system that include antioxidant enzymes (superoxide dismutase, catalase, peroxidase and glutathione reductase) and non-enzymatic low molecular weight antioxidants (glutathione, proline, carotenoids, tocopherols etc.) (Schutzendubell and Polle, 2002). Therefore, an observed increased in the antioxidant enzymes (Tables 10-12) i.e. CAT, POS, SOD and that of proline content (Table 13) in different cultivars of tomato in the present study is a biochemical adaptation of these cultivars to protect themselves against the oxidative stress that burst out in response to Co stress. The literature of the recent years strongly favors this fact further as an increased in the antioxidant system was observed when the plants were exposed to Co (Chatterjee et al., 2006), nickel (Alam et al., 2007), cadmium (Hasan et al., 2008; Hayat et al., 2009), aluminum (Ali et al., 2008) and copper (Fariduddin et al., 2009).

The present investigation reveals that Co stress caused a significant reduction in the NR activity of tomato cultivars. The possible reason that may simplify the cause behind this reduction may be the restriction of NO_2 uptake (Hernandez et al., 1996), the inducer and substrate of NR (Campbell, 1999), as it is a well established fact that Co interfere with the uptake and transport of nutrients (Liu et al., 2000). In addition to this, our results are in consistent with those of Ali et al. (2008) and Hayat et al. (2007), who emphasized that heavy metals sharply decreased NO_3 uptake.

Carbonic anhydrase (CA) is the enzyme that catalyzes interconversion of CO_2 and HCO_3^- and its activity is largely determined by photon flux density, concentration of CO_2, availability of Zn (Tiwari et al., 2005) and the genetic expression (Kim et al., 1994). The stress generated by Co decreased the internal CO_2 concentration (Table 7) and interfere with the availability of Zn (Liu et al., 2000). The cumulative effect of these factors resulted in a decrease in the activity of CA (Table 9).

It is also evident from the present study that SPAD chlorophyll content (Table 5) and the photosynthetic attributes (Tables 6-7) significantly decreased in the tomato cultivars on being fed with Co before sowing. Heavy metals is known to enhance the level of the enzyme chlorophyllase that bring about the degradation of the chlorophyll (Reddy and Vora, 1986) and in particular Co inhibit the activity of the enzymes that involved in the synthesis of chlorophyll synthesis, such as 5-aminolevulinic acid and protoporphyrin (Shalygo et al., 1999). Similar, observations have also been reported earlier in tomato (Gopal et al., 2003) and French beans (Chatterjee et al., 2006) under Co stress. The stress generated by Co also caused a marked reduction in net photosynthetic rate in all the tomato cultivar that may be a direct outcome of reduced stomatal conductance and internal CO_2 concentration (Tables 6-7) in addition to decreased photosynthetic pigment and activity of carbonic anhydrase. The reason behind such belief is the study of Mysliva-Kurdziel et al. (2004) who suggested that heavy metal affect the photosynthetic machinery at multiple levels such as pigment biosynthesis/ degradation, stomatal functioning enzyme inhibition, alteration in membrane structure/ function and photosystem. Decreased in the leaf water potential (Table 14) in the present study in response to Co toxicity may be justify on the ground of physiological drought that was generated by heavy metals (Barcelo and Poschenrieder, 1990).

All these physiological disorders are further expressed in the form of reduced growth (Tables 1-3). The decrease in the growth attributes (fresh and dry mass of shoot and leaf area) is a direct outcome of inhibition of cell division or cell elongation, or a combination of both under Co stress (Jayakumar et al., 2007). Earlier reports also favor this finding that Co toxicity generates marked reduction in growth attributes in radish (Jayakumar et al., 2007).

CONCLUSION

It is concluded from the present study that despite substantial varietal difference among tomato cultivars against Co toxicity, Co induced marked reduction in growth, photosynthesis, and the activity of NR and CA in all the varieties. In addition to this, antioxidative enzymatic activity (POX, CAT, SOD) and proline content emerged out as a good markers for estimating the degree of tolerance among

tomato cultivars, against the toxicity generated by Co in the present study.

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