# Synergy of Photosynthesis and Antioxidant System Potentiate the Growth of Tomato Genotypes under Cadmium Stress

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**Abstract:** Ten varieties of tomato has been screened against four concentration of cadmium (3, 6, 9, 12 mg/kg) amended in soil on the basis of their photosynthetic performance, antioxidant enzyme activities, nitrate reductase and carbonic anhydrase activities to ascertain the resistant and sensitive type. Cadmium inhibited the growth of all the varieties of tomato in concentration dependent manner. The highest concentration (12 mg /kg of soil) was most toxic and at these concentration three varieties namely NBR-Uttam, Malti and S-22 could not germinate. The variety K-25 was proved to be most resistant and grow even at highest concentration and the variety S-22 is the most sensitive one because it could not germinate even at lower concentration.

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## 1. Introduction

Plants reflect a remarkable virtue of expressing toxicological alterations at biochemical and physiological levels in reference to the prevailing environment, therefore, defining environmental health and regarded as biomonitors. The anthropotoxic alterations of environmental fitness not only reflected in these 'easy to grow' phytoindicators but also in chain consumers of different tropic levels (Minissi and Lombi, 1997). However, plants are very sensitive to specific stressors (Wang and Freemark, 1995) such as heavy metal, salinity, high and low temperature and all, including various biotic stresses also. Out of all these abiotic stressors, heavy metal contamination in soil is one of the major threats to biota.

Interestingly, cadmium (Cd) is one of the heavy metal that is not required by the plant but due to its high soluble nature, it is readily absorbed by the plant roots and then transported from root to the other parts (Clemens, 2001). The joint FAO/WHO expert committee on food additives (JASEFA), and the Codex Committee on Food Additives and Contaminants have proposed a limit of 0.1 mg $kg^{-1}$ for Cd in cereals, pulses and legumes due to the risk associated with the long term consumption of Cd contaminated crop (Harris and Taylor, 2001), while the European countries has limit of 0.2 mg $kg^{-1}$ for wheat grain and the maximal tolerable limit of Cd for humans proposed by FAO/WHO is 70 g a day (Ryan, et al., 1982; Wagner, 1993). The phyto-toxicity of the cadmium is well established even though the mechanism involved is still not completely understood. It reduce ATPase activity of plasma lemma fraction (Astolfi, et al., 2004), altered water balance (Hayat, et al., 2010), inhibit activities of several enzymes (Hasan, et al., 2008; Hasan, et al., 2011), slow down the rate of photosynthesis, increase the level of proline and also act on antioxidant enzymes (Hasan, et al., 2008; Hasan, et al., 2011; Shi, et al., 2010). However, most of the information available about cadmium physiology in plants came from the studies with cadmium hyper-accumulator *Thlaspi caerulescens* (J. & C. Presl) (Lombi, et al., 2002) and cadmium tolerant plant such as *Arabidopsis halleri* (L.) (Weber, et al., 2006). Therefore, the present study was planned to assess the effect of soil applied cadmium on the physiology, morphology and biochemical responses in ten different cultivars of tomato to select the resistant and sensitive varieties of Indian origin.

## 2. Material and methods

### 2.1. Plant material and treatment

Seeds of ten varieties of tomato i.e. K-25, K-21, NTS-9, Kaveri, NBR-Uday, Swarnodya, Sarvodya, NBR-Uttam, Malti and S-22 were purchased from National Seed Corporation, Pusa, New Delhi. These were surface sterilized with 5% sodium hypochlorite followed by repeated washings with double distilled water (DDW). These surface sterilized seeds were sown in the earthen pots containing 0, 3, 6, 9, 12 mg $CdCl_2$/kg of soil to create the nursery filled with sandy loam soil and farmyard manure (mixed in the ratio of 6:1).

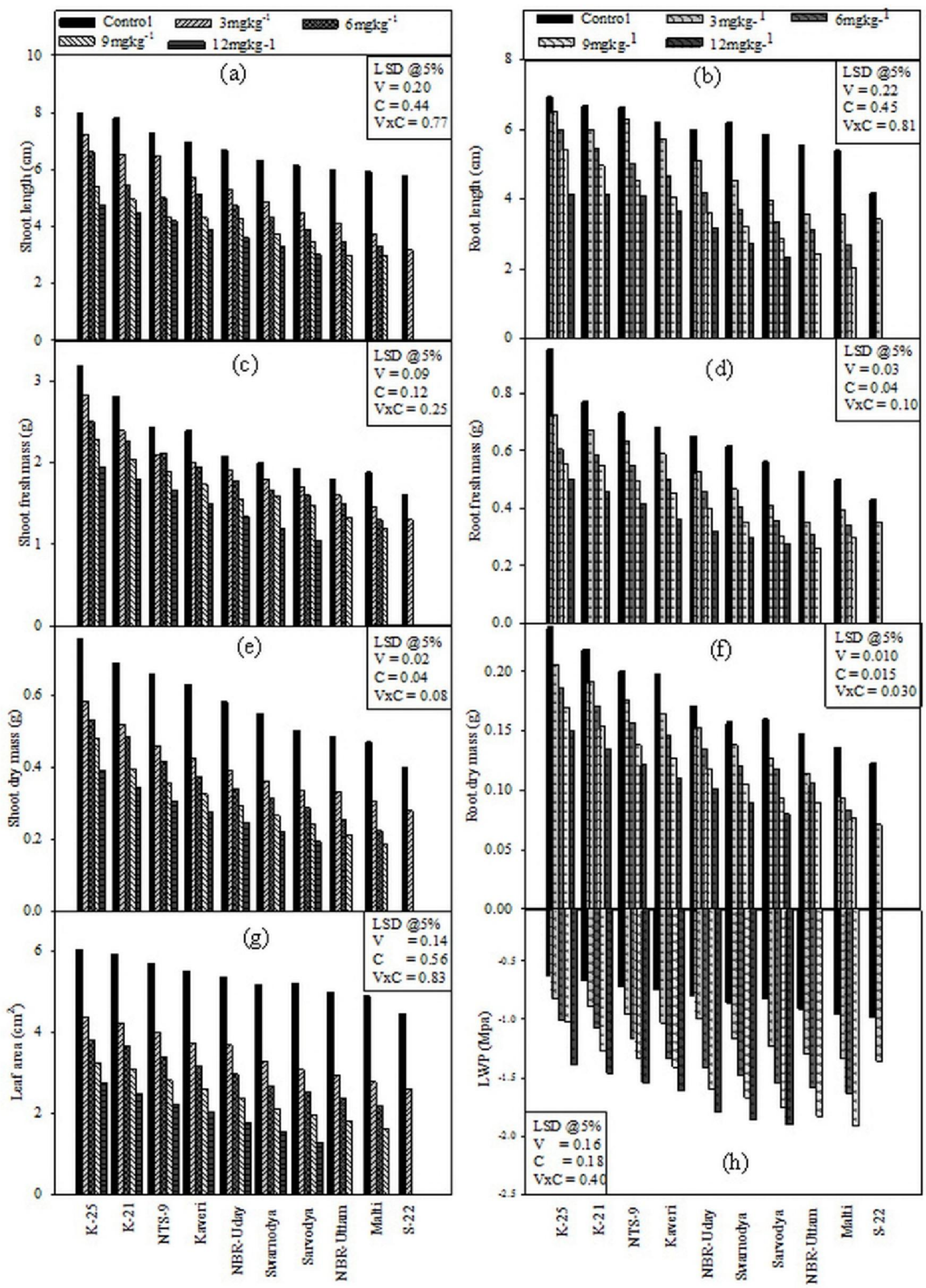


Figure 1. Effect of cadmium (3, 6, 9, 12 mg $kg^{-1}$) induced changes in (a) shoot length, (b) root length, (c) shoot freshmass, (d) root fresh mass, (e) shoot drymass, (F) root dry mass, (g) leaf area, (h) leaf water potential (LWP) in ten different varieties of tomato (*Lycopersicon esculentum* Mill.).

The soil was amended with urea, single superphosphate and muriate of potash at 40, 138 and 26 $mg \cdot kg^{-1}$ of soil, respectively. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots (10 inches in diameter) under similar conditions as in the case of nursery pots. The pots were arranged in a simple randomized design, in a net house under natural environmental conditions during winter season season (October-December) at the Department of Botany of Aligarh Muslim University, Aligarh, India. Each treatment was replicated five times. Irrigation was done with tap water when required. The plants were sampled at 90 days after sowing to assess the various growth and biochemical characteristics. Plants were removed along with the sand and dipped in a bucket, filled with tap water to remove the adhering soil particles while ensuring the integrity of the roots. The plants were blotted on paper and the length of root and shoot was measured, followed by their subsequent weighing to record fresh mass. The roots and shoots were then dried in an oven at 80°C for 72 h and weighed to note their dry mass.

### 2.2. Determination of biochemical characteristics

The chlorophyll content in fresh leaf samples was measured by using a SPAD chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan). Transpiration rate, water use efficiency, internal $CO_2$ concentration, stomatal conductance, and net photosynthetic rate were measured by infra-red gas analyzer (LICOR 6400, Lincoln, NE) between 11:00 and 12:00 hours in full sun. Leaf water potential (LWP) of the fresh leaf sample was measured by PSYPRO, water potential system, WESCOR, Inc, Logan, USA.

The activity of enzymes such as carbonic anhydrase (CA); nitrate reductase (NR); catalase, peroxidase and superoxide dismutase and leaf proline content was analyzed by the method described earlier (Hasan, et al., 2011). The cadmium content in the fruits was analyzed as per the standard procedure using atomic absorption spectrophotometer (Perkin Elmers AAS-300).

### 2.3. Statistical Analysis

Data were subjected to analysis of variance using SPSS (SPSS, Chicago, IL). Least Significant Difference (LSD) was used to separate means.

## 3. Results

### 3.1. Growth attributes

The presence of Cd in the soil caused a significant reduction in all the growth attributes (length, fresh and dry mass of root and shoot and leaf area) and LWP in all the varieties under observation (Fig. 1). The highest (12 mg $kg^{-1}$) level of metal was most toxic and it decreased the dry mass of shoot by 48.1%, 50.3%, 53.6%, 55.9%, 57.2%, 60.1% and 61.5% and that of root by 36.9%, 38.3%, 39.0%, 44.4%, 40.9%, 43.6%, 50.0%, in K-25, K-21, NTS-9, Kaveri, NBR-Uday, Swarnodya and Sarvodya, respectively, over their controls. Moreover, varieties NBR-Uttam, Malti and S-22 could not survive at this level of the metal. The response was closely followed by the next level of the metal (9 mg $kg^{-1}$). Variety NBR-Uttam and Malti experienced maximum damage at this level that was 25.6%, and 36.1% for fresh mass and 55.6% and 60.5% for dry mass of shoot over their control plants.

### 3.2. Photosynthetic attributes

Plants raised in the soil amended with the metal showed significantly lower values for all the photosynthetic attributes over their unstressed control plants (Fig. 2). The degree of damage caused by the metal significantly increased with the level of metal. The variety Malti showed maximum inhibition in $P_N$ which was 27.2%, 35.3% and 43.7% lower in response to 3, 6 and 9 mg $kg^{-1}$ respectively over their controls (Fig. 2b). At 12 mg $kg^{-1}$ this variety could not survive. The variety K-25 showed minimum decrease. The seeds of the varieties NBR-Uttam, Malti and S-22 could not germinate when treated with 12 mg $kg^{-1}$ $CdCl_2$ level. The metal exerted a moderate toxic effect on varieties Kaveri, NBR-Uday, Swarnodya and Sarvodya. The pattern of resistance exhibited by these varieties was Kaveri> NBR-Uday>Swarnodya>Sarvodya.

### 3.3. Leaf nitrate reductase and carbonic anhydrase activity

The activity of NR and CA in all the varieties was severely affected by metal. The lowest level of metal (3 mg $kg^{-1}$) was found to be least toxic for all the varieties. The variety S-22 was highly sensitive and experienced 36.6% and 50.5% decrease in NR and CA respectively over their respective control in response to lowest level (3 mg $kg^{-1}$) of metal, and was unable to survive at higher levels. The two higher levels (6 and 9 mg $kg^{-1}$) generated moderate toxicity in Kaveri, NBR-Uday and Swarnodya. Out of these two, 9 mg $kg^{-1}$ contamination level generated maximum toxicity in NBR-Uttam and Sarvodya. However, K-25, K-21 and NTS-9 showed comparatively better resistance. At highest metal level K-25 was found to be most resistant, among all the varieties (Fig. 3a, b).

### 3.4. Antioxidant system

In all the varieties, the activity of antioxidant enzymes (viz. POX, CAT, and SOD) and proline content showed positive correlation with an increase in the level of metal in the soil. Lowest level of metal showed least activity for all the antioxidative enzymes and that of proline content in all the varieties, the higher two level 9 and 12 showed significantly different response that varies from variety to variety. 9

mg $kg^{-1}$ of the metal increased maximum activity of antioxidative enzymes that is 65.1% and 51.5% POX, 37.1% and 38.0% CAT 28.3% and 26.2% SOD and proline level by 15.3% and 5.6% respectively in NBR-Uttam and Malti over their control. However maximum antioxidative enzymatic activity and proline level in K-25, K-21 and NTS-9, Kaveri, NBR-Uday, Swarnodya and Sarvodya was recorded from the plants raised in the soil amended with 12 mg $kg^{-1}$ of the metal. The trend followed by these varieties was K-25 > K-21 > NTS-9 > Swarnodya > Sarvodya. Among all these varieties Sarvodya was found to be most sensitive and K-25 was found to be the most resistant at all the levels of contamination (Fig. 3c, d, e, f).

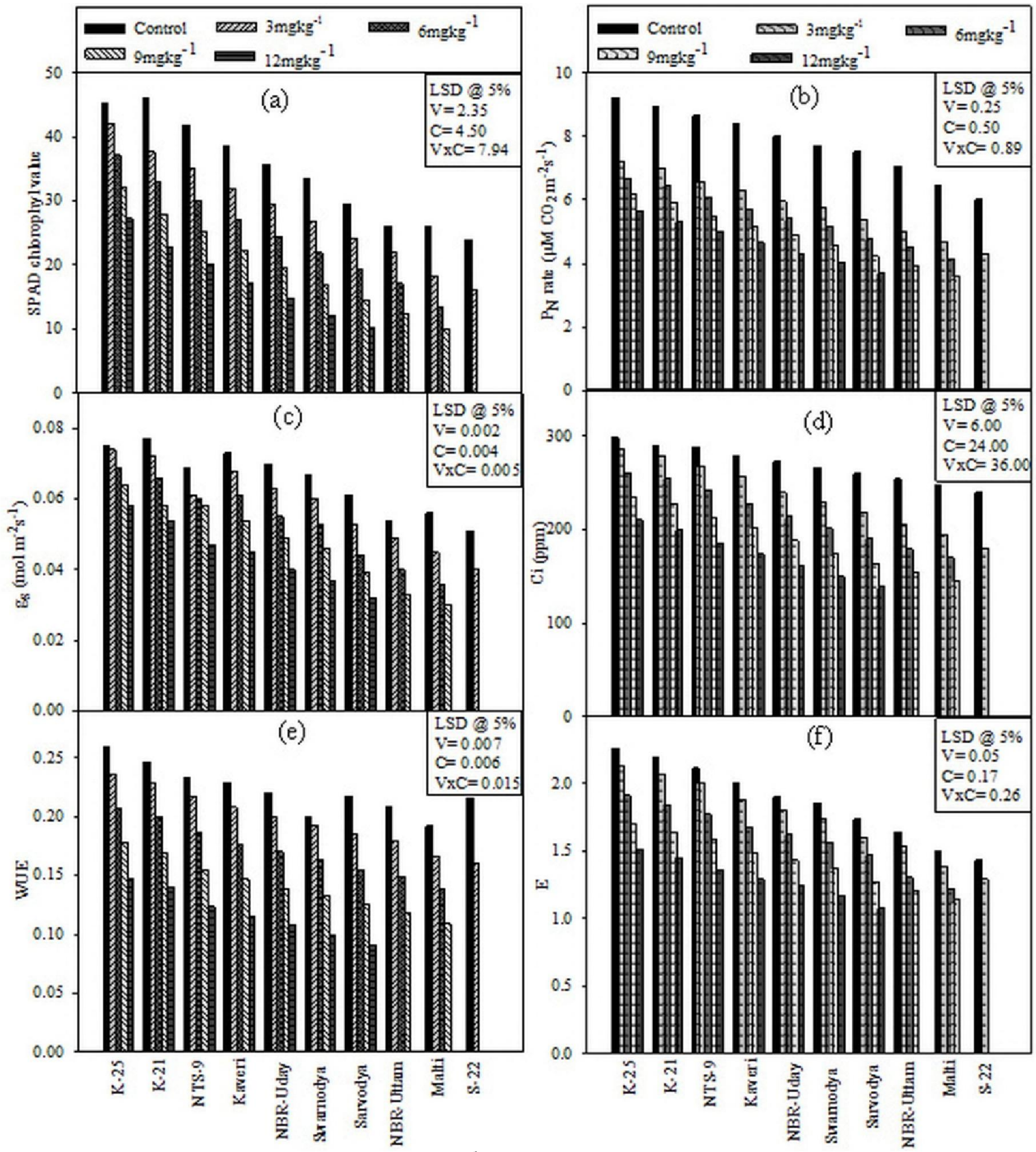


Figure 2. Effect cadmium (3, 6, 9, 12 mg $kg^{-1}$) induced changes in (a) SPAD chlorophyll value, (b) net photosynthetic rate [$P_N$] (c) stomatal conductance [$g_s$], (d) internal $CO_2$ concentration [Ci], (e) water use efficiency [WUE] and (f) transpiration rate [E] in ten different varieties of tomato (*Lycopersicon esculentum* Mill.).

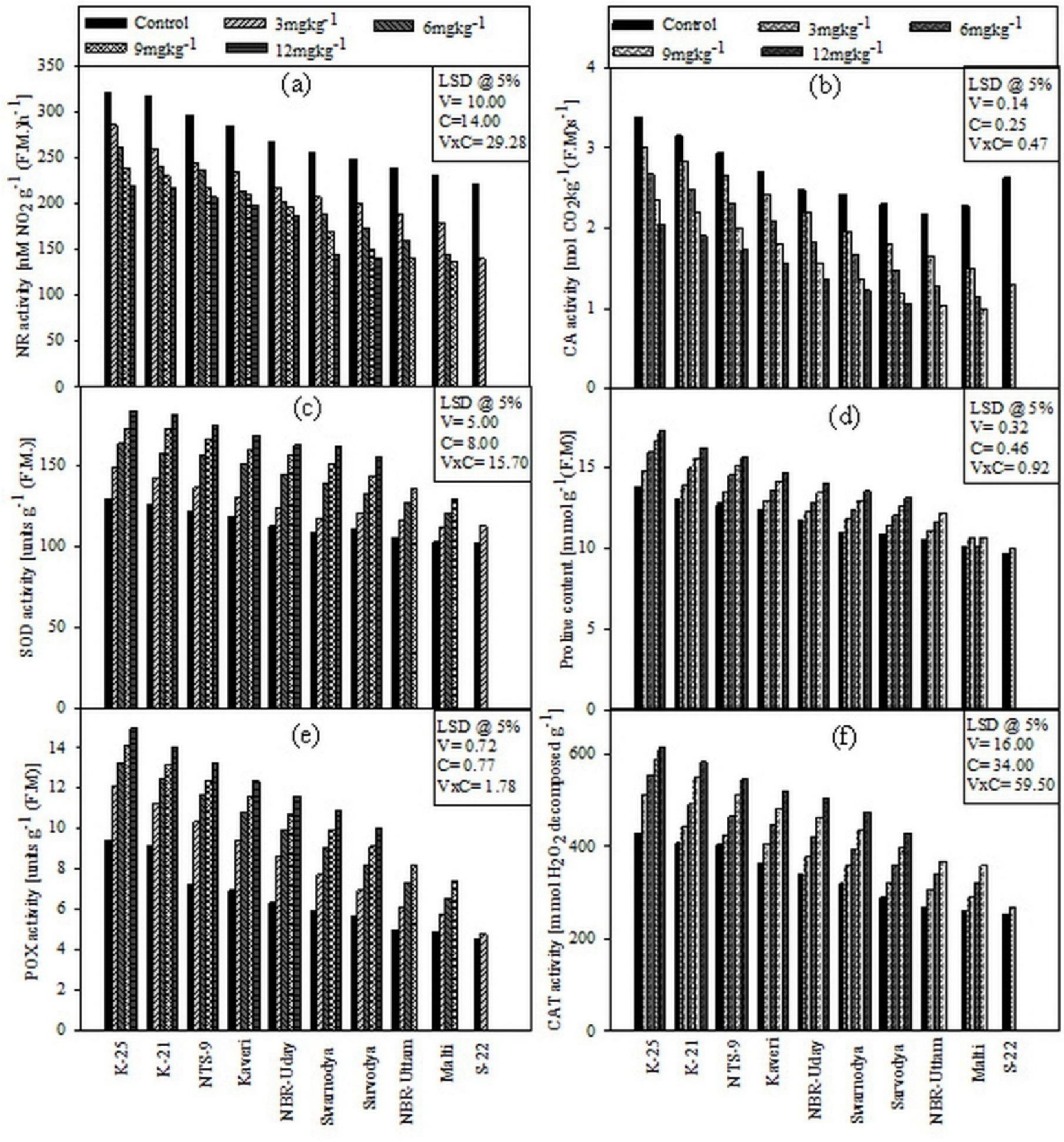


Figure 3. Effect of cadmium (3, 6, 9, 12 mg $kg^{-1}$) induced changes in (a) Nitrate reductase activity [NR], (b) Carbonic anhydrase activity [CA], (c) Superoxide dismutase activity, (d) Proline content, (e) Peroxidase activity, (f) Catalase activity and in ten different varieties of tomato (*Lycopersicon esculentum* Mill.).

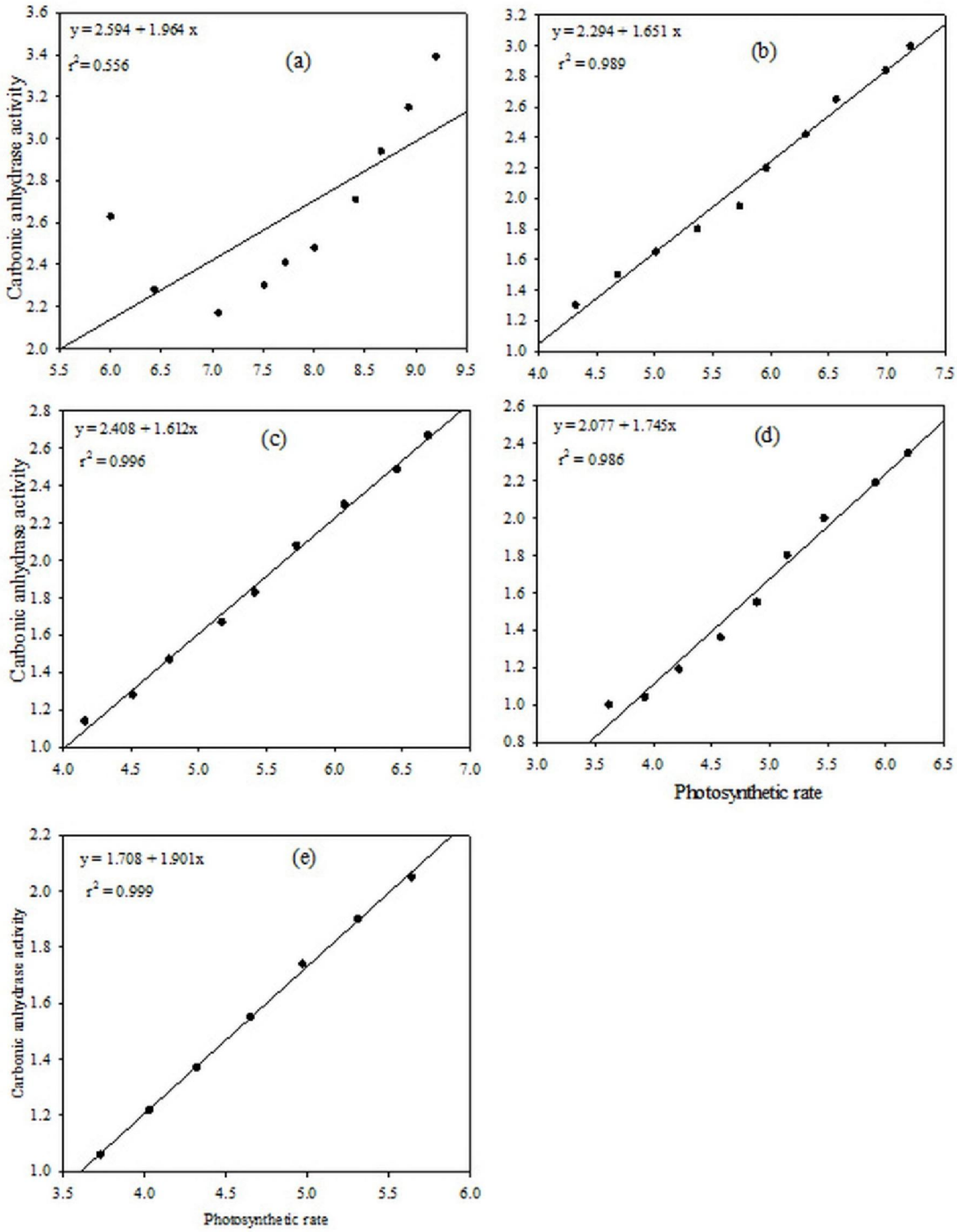


Figure 4. Correlation coefficient values between (a-e) carbonic anhydrase activity and net photosynthetic rate.

Table 1. Effect of different concentrations (3, 6, 9 or 12 mg $Kg^{-1}$ of soil) of cadmium on the accumulation of cadmium (μg $g^{-1}$ DW) in the fruits of ten varieties of tomato (The data are the mean of five replicates ± S.E.)

| Varieties | | | Cadmium concentrations (mg $Kg^{-1}$ of soil) | | |
|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 |
| K-25 | ND | 0.105±0.018 | 0.129±0.012 | 0.141±0.021 | 0.156±0.016 |
| K-21 | ND | 0.135±0.016 | 0.148±0.017 | 0.164±0.018 | 0.179±0.015 |
| NTS-9 | ND | 0.133±0.019 | 0.142±0.009 | 0.160±0.019 | 0.176±0.021 |
| Kaveri | ND | 0.142±0.012 | 0.154±0.013 | 0.168±0.017 | 0.195±0.022 |
| NBR-Uday | ND | 0.147±0.013 | 0.152±0.016 | 0.169±0.012 | 0.192±0.021 |
| Swarnodya | ND | 0.159±0.008 | 0.166±0.018 | 0.182±0.014 | 0.214±0.018 |
| Sarvodya | ND | 0.154±0.013 | 0.163±0.018 | 0.183±0.015 | 0.209±0.019 |
| NBR-Uttam | ND | 0.159±0.015 | 0.168±0.017 | 0.187±0.018 | NF |
| Malti | ND | 0.156±0.016 | 0.165±0.017 | 0.186±0.016 | NF |
| S-22 | ND | 0.163±0.013 | NF | NF | NF |

DW- Dry weight; ND-Non detectable; NF-No fruits formed

### 3.5. Cadmium content in the fruit

The level of the cadmium content in the fruits of all the varieties were increased with the increasing concentration of the heavy metals in the soil (Table 1). In the control sample of all the varieties cadmium content was not detectable. The variety K-25 possess the lowest concentration of cadmium as compare to all the other varieties in all the treatment, whereas, no fruits was formed in NBR-Uttam, Malti and S-22 at the highest concentration.

## 4. Discussion

The growth and survival rate of a plant depends on energy economy, carbon and nitrogen fixation and water relations. Decrease in efficiency of these three leads to the excessive generation of ROS, decreased dry and fresh mass respectively. Cd is known for generating ROS, eliciting water scarcity and decreased biomass production. The present investigation satisfies all these characteristics resulting from Cd toxicity, though responses varies in different varieties depending upon their Cd resistance and increased efficiency. The ten varieties of tomato showed dose (3, 6, 9, 12 mg/kg of soil) dependent variation in growth responses.

Decline in fresh mass (of root and shoot) and length (Fig. 1a, b, c, d) seems due to decreased absorption and assimilation of water which is caused due to Cd toxicity (Barcelo, et al., 1986; Lefèvre, et al., 2010). The equilibrium of water absorption declines against increased electrolyte leakage which renders fall of water potential of absorptive root hair cells. The dry mass of root and shoot (Fig. 1e, d) significantly fall as energy economy for the growth metabolism not only compromised in leaking electrons from respiratory chains, it alternatively generated toxic level of ROS. Heavy metals have strong effect on levels of adenylate nucleotides (Filippis and Pallaghy, 1994). Since ATP and NADPH are the primary requirements for $CO_2$-fixation, it can be inferred that any reduction in ATP synthesis may lead to inhibition of $CO_2$-incorporation (Stratton and Corke, 1979; Wong, et al., 1979). Evidence of increased pyrophosphate contents, decline in ATP pool and availability of NADPH to fix inorganic carbon and nitrogen under stress (Dobrota, 2006) is common consequence. Cd induced decline in cellular adenylate pool appears too laid down the additional cause of compromised carbon and nitrogen fixation and reduced plant growth. Additionally, excess ROS activated defense signaling at the cost of growth and photosynthates assimilated. Our results are in agreement with the observation of Wahid and Ghani (2008) who also find decrease in growth of *Vigna radiata* cultivars on being subjected to differential Cd stresses.

Decreased leaf area (as evident from the Fig. 1g) where on the one hand not only reduced the available surface area of photosynthesis, it additionally limited the availability of minerals viz. Mn (for photolysis of water molecules), Mg (biosynthesis of active chlorophyll molecules) and Zn (activity of carbonic anhydrase). Decreased leaf water potential (Fig. 1h) resulted in lesser availability of water molecules declining water use efficiency (Fig. 2e) for the activity of PSII by declining photolysis of water molecules (Chugh and Sawhney, 1999) and the availability on the active site of key photosynthetic enzymes rendering their activity below optimum viz. rubisco (Chugh and Sawhney, 1999; Siedlecka, et al., 1997), carbonic anhydrase (Hasan, et al., 2008) and enzymes of chlorophyll synthesis. This is further strengthened by the observed positive correlation between CA and photosynthesis (Fig. 4). Cd enhanced the level of the enzyme chlorophyllase that bring about the degradation of chlorophyll (Reddy and Vora, 1986) and also declines the synthesis of α-amino lavulinate and proto-chlorophyllide reductase complex (Stobart, et al., 1985). Another cause of decline in $P_N$ (Fig. 2b) is lowered Ci (Fig. 2d) in the leaf chambers. The signal supposed to travel within leaves under water deficit conditions mediated by Cd toxicity in roots (Hsu and Kao, 2003). The decrease in all the photosynthetic attributes in the present study is in agreement with earlier reports on wheat (Ci, et al., 2010) and tomato (Hayat, et al., 2010; Hasan, et al., 2011).

Although the activity of antioxidant system increased in response to Cd stress (Fig. 3c, d, e, f), the cellular ROS production seemingly generated more oxidative stress, thereby resulted in decreased growth as compared to control plants (Fig. 1) as indicated by the increased accumulation of proline content in the present study (Fig. 3d). It has been hypothesized that the ratio of total antioxidant activity and total cellular reactive radicals ($T_{AA}/T_{CR}$) decreases under heavy metal (Cd) stress. However, this decline in growth parameters was the result of the interaction of Cd and plant genotype. The ratio ($T_{AA}/T_{CR}$) in case of sensitive varieties appears to be lesser as compared to resistant varieties. Similar enhanced antioxidative activity has also been reported earlier in *Vigna radiata* (Ali, et al., 2008), *Cicer arietinum* (Hasan, et al., 2008), wheat (Yusuf, et al., 2011), and tomato (Hasan, et al., 2011) on being exposed to different heavy metal stress.

The majority of the experiment conducted on cadmium stressed plants using seedlings and cell cultures and only in few cases the plants are allowed to grow up to a level of maturity. It has been reported earlier that in tomato cadmium is mostly accumulated in the roots (Delpérée and Lutts, 2008) but can be translocated to the fruits also (Shentu, et al., 2008). In the present observation a low level of cadmium is noted in the fruits sample (Table 1). The level of cadmium is increased with increasing concentration in the soil medium but their level in the different cultivars is different which might be due to the different genetic makeup. The fruits of variety K-25 accumulated less cadmium as compare to other and the level are under permissible limit, therefore is safe for human consumption.

**Conclusion**

It can be concluded from the present investigation that out of the ten tested varieties the variety K-25 was proved to be most resistant and grow even at highest concentration of cadmium (12 mg/kg of soil) and the variety S-22 is the most sensitive one. The variety K-25 also possess lowest amount of cadmium in the fruits, therefore safe for human consumption.

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