ABSTRACT

Effect of brassinosteroids on the cadmium induced changes in 
Lycopersicon esculentum

SYED AIMAN HASAN

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Six pot experiments were conducted, during 2006-2008 to find out degree of tolerance to cadmium among ten tomato varieties (Lycopersicon esculentum) i.e. K-25, K-21, NTS-9, Kaveri, NBR-Uday, Swarnodya, Sarvodya, NBR-Uttam, Malti and S-22 and to find out one of the most resistant and most sensitive variety. Moreover, the response of tomato varieties towards two brassinosteroid analogues 28-homobrassinolide (HBL) and 24-epibrassinolide (EBL) were also studied. These brassinosteroids analogues were supplemented through two different modes (a) spray to the foliage, (b) percolation through root, to find out suitable mode of application. The silent features in each of the seven experiments are mentioned below.

Experiment 1

The surface sterilized seeds of tomato (Lycopersicon esculentum) varieties K-25, K-21, NTS-9, Kaveri, NBR-Uday, Swarnodya, Sarvodya, NBR-Uttam, Malti, and S-22 were soaked in double distilled water (DDW), 50, 100 or 150 µM CdCl₂ for 0, 4, 8 or 12 hours. The treated seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. Twenty days after sowing (DAS) these treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 30 DAS to assess growth, photosynthetic attributes, SPAD chlorophyll, leaf water potential, activity of nitrate
reductase and carbonic anhydrase, proline content and antioxidant enzymes. All the varieties exhibited significantly different response to the different metal concentration as well as duration of soaking. The variety S-22 could not survive in the presence of any of the concentration of metal. The varieties Sarvodya, NBR-Uttam, and Malti suffered a severe damage. The varieties Kaveri, NBR-Uday and Swarnodya were moderately affected by the cadmium and could not resist the pre-sowing soaking in 150 μM of CdCl₂ for 12 hours. The varieties K-25, K-21 and NTS-9 showed the maximum resistance to cadmium. All the above parameters except antioxidant enzymes and proline content, showed significant decrease in response to the cadmium treatment. However, cadmium treatment resulted in a significant increase in the antioxidant enzymes and proline content, their values increased with the increasing concentration of metal as well as duration of soaking.

**Experiment 2**

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) varieties K-25 (resistant) and Sarvodya (sensitive) were soaked in DDW or 100 μM CdCl₂ for 0 or 8 hours that were selected on the basis of the first experiment. The treated seeds were sown in earthen pots filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedling were subsequently transplanted to the maintained pots. The foliage of fifty nine day old plants was sprayed with DDW/aqueous solution of 10⁻⁸ M of 28-homobrassinolide (HBL) or 24-epibrassinolide (EBL). The samples were collected at 60 and 90 DAS. The parameters studied were the same as in Experiment 1. The rest of the plants were allowed to grow to maturity and were harvested at 180 DAS to study the number of fruits per plants, fruit yield per plant and lycopene, β-carotene and ascorbic acid content in fruit. Both
the varieties showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 60 to 90 DAS. The values for most of the parameters decreased in the plants given pre sowing seed soaking in cadmium, except that of activity of antioxidative enzymes and proline content at the level of leaf and ascorbic acid content of fruit. The followup treatment of either of the brassinosteroid analogues (HBL/EBL) significantly neutralized the ill effect of cadmium in both the varieties but more effectively in K-25 than in Sarvodya. The level of proline and the activity of antioxidative enzymes increased in response to both metal as well as hormone treatment. The variety K-25 showed higher antioxidative enzyme activity and proline content than Sarvodya representing its resistant nature. Out of the two brassinosteroid analogues (HBL/EBL), EBL was more effective than HBL.

**Experiment 3**

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) variety K-25 (resistant) and Sarvodya (sensitive) were soaked in DDW or 100 μM CdCl₂ for 0 or 8 h that were selected on the basis of first experiment. These treated seeds were sown in earthen pots filled with sandy loam soil or farmyard mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedling were percolated with DDW or 10⁻⁸M of HBL or EBL through their roots for 15 min at the time of their transplantation at a rate of 20 cm³ per seedling. These treated seedlings were subsequently transplanted to the maintained pots. The samples were collected at 60, 90 and 180 DAS to asses the parameters studied in experiment 2. The plants obtained from the seeds given pre sowing seed soaking treatment in cadmium possessed significantly reduced growth, net photosynthetic rate, stomatal conductance, water use efficiency, internal CO₂
concentration, transpiration rate, leaf water potential, SPAD chlorophyll value, activity of nitrate reductase and carbonic anhydrase enzyme. The degree of damage caused by metal was more pronounced at the early stage of growth (60 DAS) than at later stage. The dipping of root in hormone effectively neutralized the adverse effect of metal in stressed plants. Variety K-25 was found to be more responsive towards hormone than Sarvodya. The activity of antioxidants and the content of proline were significantly higher in the leaves of stressed plants of both the varieties and the follow up treatment with HBL/EBL increased it further. EBL excelled in its effect over HBL. All the yield attributes i.e. number of fruits per plant, fruit yield per plant and the lycopene and β-carotene content in the fruits exhibited a similar response as that of growth. However, ascorbic acid content of fruit exhibited a trend which was converse to that of all other parameters explained earlier.

**Experiment 4**

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) varieties K-25, K-21, NTS-9, Kaveri, NBR-Uday, Swarnodya, Sarvodya, NBR-Uttam, Malti and S-22 were sown in earthen pots containing 0, 3, 6, 9 or 12 mg CdCl₂ kg⁻¹ soil. These earthen pots were filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedling were subsequently transplanted to the maintained pots. The plant samples were collected at 30 DAS. The parameters studied were the same as in Experiment 1. All the varieties showed significantly different response to different concentrations of metal. The varieties K-25, K-21 and NTS-9 exhibited minimum decrease in response to different Cadmium levels. The highest level 12 mg kg⁻¹ was the most toxic. The varieties K-25, K-21 and NTS-9 show slightly resistance against higher level of metal. The varieties Kaveri,
NBR-Uday and Swarnodya were moderately affected by this level of the metal. However, Sarvodya experienced severe damage at this level. Among others, NBR-Uttam and Malti could not germinate. Moreover, S-22 was most sensitive against metal stress and could not survive in the soil amended with 6 mg kg\(^{-1}\). All the parameters except antioxidant enzymatic activity and proline content in all the varieties showed a linear decrease as the level of the metal in the soil increased (3, 6, 9 or 12 mg kg\(^{-1}\) soil). Among all the varieties, K-25 possessed maximum antioxidative enzyme activity and that of proline content at all the level of metal contamination and Sarvodya possessed minimum values for these parameters representing its sensitive nature towards the metal stress.

**Experiment 5**

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) varieties K-25 (resistant) and Sarvodya (sensitive) were selected on the basis of fourth experiment were sown in earthen pots containing 0, 3, 6, 9 or 12 mg CdCl\(_2\) kg\(^{-1}\) soil. These earthen pots were filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS the treated seedling were subsequently transplanted to the maintained pots. The foliage of fifty nine day old plants was sprayed with DDW/aqueous solution of 10\(^{-8}\)M of 28-homobrassinolide (HBL) and 24-epibrassinolide (EBL). The plant samples were collected at 60, 90 and 180 DAS to study the characteristics studied in experiment 2. All the parameters of both the varieties (K-25 and Sarvodya) increased with the progress of the plant age. The treatment with cadmium generated a decline in the values of most of the parameters in proportion to the concentration of metal except those of proline content and antioxidative enzymatic activity at the level of leaves, and ascorbic acid content in
fruit. Sarvodya experienced comparatively greater damage at all the metal concentration than K-25. However, the treatment with HBL or EBL alone or as a follow up treatment improved these attributes in the plants of both the varieties and also neutralized the damaging effect of the metal, more effectively in K-25 than Sarvodya. The damage caused by the lower two concentration of metal (3 and 6 mg kg$^{-1}$ soil) in most of the parameters was completely neutralized by the brassinosteroids spray in K-25 and partially in Sarvodya. The activity of antioxidants and proline content in both the varieties increased with the increased level of metal in soil and the follow-up treatment with either of the brassinosteroid analogues had an additive effect in their enhancement. Variety K-25 possessed higher values for these attributes in response to all the treatment than Sarvodya. EBL was a better promoter than HBL.

**Experiment 6**

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) varieties K-25 (resistant) and Sarvodya (sensitive) were sown in earthen pots containing 0, 3, 6, 9 or 12 mg kg$^{-1}$ soil. These earthen pots were filled with sandy loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS the treated seedling were percolated with DDW or $10^{-4}$M of HBL or EBL through their roots for 15 min at the time of their transplantation at a rate of 20 cm$^3$ per seedlings. These treated seedlings were subsequently transplanted to the maintained pots. The samples were collected at 60, 90 and 180 DAS to study the characteristics studied in experiment 2. The plants raised in the soil contaminated with metal showed significant reduction in most of the parameter studied except antioxidative enzyme activity and proline content, the decrease was proportionate to the concentration of metal in the soil. The degree of damage caused by the metal was more pronounced at the early stage of
growth than at the later stage. The dipping of roots in either of the brassinosteroid analogues effectively nullified ill effect of the metal more effectively in K-25 than in Sarvodya. Stress generated by the lowest level of metal was completely neutralized by the hormone treatment but partially at highest level. The seedling of the plants grown in metal amended soil showed significant increase in the activity of antioxidant enzymes and proline content at the same rate as the concentration of the metal in the soil increased. However, the application of HBL or EBL caused further increase in its activity, and thus plants received the highest metal level and also received hormone treatment possessed maximum values for these attributes in both the varieties. K-25 possessed greater values than Sarvodya in response to all the treatments. EBL generate better response than HBL.