ABSTRACT

Effect of nitric oxide and brassinosteroids on the salinity induced changes in tomato (Lycopersicon Esculentum)

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Abstract of the thesis, submitted to the Aligarh Muslim University, Aligarh, India, for the degree of Doctor of Philosophy in Botany, 2010.

Eight pot experiments were conducted during 2007-2009 to find out degree of tolerance to salinity in tomato (Lycopersicon esculentum) var. K-21 and to find out the most toxic concentration of salt as seed soaking (50, 100 or 150 mM) or amended in soil (2.9, 5.8 or 8.7 mg kg⁻¹ of soil) of NaCl. Moreover, the response of tomato plant towards two brassinosteroid analogues, 28-homobrassinolide (HBL) and 24-epibrassinolide (EBL), and nitric oxide [source of nitric oxide is sodium nitroprusside (SNP)] were also studied. Brassinosteroid analogues were applied as foliar spray, whereas, nitric oxide through seed soaking. The salient features in each of the eight experiments are mentioned below.

Experiment 1

The surface sterilized seeds of tomato (Lycopersicon esculentum) var. K-21 were soaked in double distilled water (DDW), 50, 100 or 150 mM of NaCl for 8 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 45 and 60 DAS to assess growth, relative water content, photosynthetic attributes, SPAD chlorophyll, activity of nitrate reductase and carbonic anhydrase, proline content and antioxidative enzymes. Plants showed significantly different response to the different salt concentration. 150 mM NaCl concentration was found to be the most toxic. All the
above parameters except antioxidative enzymes and proline content, showed significant decrease in response to sodium chloride treatment. However, NaCl treatment resulted in a significant increase in the antioxidative enzymes and proline content and their values increased with the increasing concentration of salt.

**Experiment 2**

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var K-21 were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg of NaCl/Kg of soil. These earthen pots were filled with study loam soil and farmyard manure mixed in a ratio of 9:1 to create the nursery. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. The tomato plant showed significantly different response to different concentrations of salt. The highest level of salt (8.7 mg Kg⁻¹) was the most toxic. All the parameters except antioxidative enzymes and proline content showed a linear decrease as the level of the salt in the sol increased (2.9, 5.8 or 8.9 mg Kg⁻¹ soil). The highest level of sodium chloride (8.7 mg Kg⁻¹ soil) showed maximum antioxidative enzyme and that of proline content.

**Experiment 3**

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 10⁻⁴, 10⁻⁵ or 10⁻⁶ M sodium nitroprusside (SNP) for 8 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. After 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. Treatment of SNP shows a different response and up to 10⁻⁵M of SNP, most parameters increased. 10⁻⁶M of SNP proved to be inhibitory.
Experiment 4

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW for 8 hours. These 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. After 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The foliage of forty four days old seedlings were sprayed with DDW, $10^{-6}$, $10^{-8}$ or $10^{-10}$ M of 28 homobrassinolide (HBL) or 24-epibrasinolide (EBL). The samples were collected at 45 and 60 DAS. The parameters studied were the same as in experiment 1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. The best response was obtained by $10^{-8}$M of HBL/EBL. Out of the two brassinosteroid analogues (HBL/EBL), EBL was more effective than HBL.

Experiment 5

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 50, 100 or 150 mM NaCl for 8 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. At 20 DAS these treated seedlings were subsequently transplanted to the maintained pots. The foliage of forty four day old plants was sprayed with DDW/aqueous solution of $10^{-8}$ M of HBL or EBL. The samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. Tomato plants showed significantly different response to the treatment. All the parameters studied increased as the growth progressed from 45 to 60 DAS. The follow up treatment of either of the brassinosteroid analogues (HBL/EBL) significantly neutralized the ill effect of salt. The level of proline and the activity of antioxidative enzymes increased in response to salt and hormone treatment. Out of the two brassinosteroid analogues (HBL/EBL), EBL was more effective than HBL.
Experiment 6

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg NaCl 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 forty four 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 45 and 60 DAS to study the parameters same as in experiment 1. All the parameters increased with the progress of the plant age. The foliar spray of HBL or EBL improved the values of all the parameters and neutralized the damaging effect of the salt. EBL was better promoter than HBL.

Experiment 7

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW, 50, 100 or 150 mM NaCl for 8 hours and then transferred to the solution of DDW or 10\(^{-5}\) M of SNP for 8 hours again. The 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 seedlings were subsequently transplanted to the maintained pots. The samples were collected at 45 and 60 DAS. The parameters studied were the same as in Experiment 1. Plants showed significantly different response to the treatment. All the parameters increased as the growth progressed from 45 to 60 DAS. The ill effect generated by the lowest concentration of salt was completely neutralized by SNP.

Experiment 8

The surface sterilized seeds of tomato (*Lycopersicon esculentum*) var. K-21 were soaked in DDW or SNP (10\(^{-7}\)M) for 8 hours. The treated seeds were sown in earthen pots containing 0, 2.9, 5.8 or 8.7 mg
NaCl 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 seedlings were subsequently transplanted to the maintained pots. The plant samples were collected at 45 and 60 DAS to study the characteristics studied in experiment 1. All the parameters increased with the progress of the plant age. The ill effect generated by the lowest concentration of salt was completely neutralized by SNP, whereas medium concentration of salt was partially neutralized.