ABSTRACT

Effect of proline and salicylic acid on the cadmium induced changes in chickpea (Cicer arietinum L.)

QAISER HAYAT

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

Six pot experiments were conducted, during 2007-2009 to elucidate the effect of exogenous proline and/or salicylic acid on the cadmium (Cd) induced changes in chickpea (Cicer arietinum L. cv. Avarodhi). Cadmium was supplemented through soil whereas, proline and salicylic acid was applied as a foliar spray. The salient features of each of the six experiments are mentioned below.

Experiment 1

This experiment was prepared to study the effect of varying doses of cadmium on chickpea. At the start of the experiment, each set of pots was supplemented with different doses (0, 25, 50 or 100 mg per kg of soil) of Cd in the form of CdCl₂. The surface sterilized seeds were inoculated with a uniform layer of Rhizobium ciceri and were sown in these earthen pots (25×25 cm) filled with sandy loam soil mixed with farmyard manure, in a ratio of 6:1. A uniform basal dose of inorganic fertilizers (urea, single superphosphate and muriate of potash) were added at the rate of 40 mg, 138 mg and 26 mg respectively, per kg of the soil to each pot to maintain the fertility of soil. The pots were arranged in a randomized block design technique, in the net house of Department of Botany, Aligarh Muslim University, Aligarh. The plant samples were collected at 60 and 90 days after sowing (DAS) to assess various growth characteristics, nodulation, various enzyme activities viz. nitrogenase, glutamine
synthetase, glutamate synthase, glutamate dehydrogenase, nitrate reductase, carbonic anhydrase, catalase, peroxidase and superoxide dismutase, photosynthetic attributes, contents of proline, carbohydrate and nitrogen and leaf water potential. All the above parameters except antioxidative enzyme activities and proline content, showed a significant decrease in response to the Cd treatment where maximum damage was caused at a Cd concentration of 100 mg per kg of soil. However, Cd treatment resulted in a significant increase in the activity of antioxidative enzymes (catalase, peroxidase and superoxide dismutase) and proline content. The values further increased with the increasing concentration of metal.

Experiment 2

This experiment was prepared with an aim to study the effect of different concentrations (0, 10, 20, 30, 40 or 50 mM) of proline on chickpea. All the agricultural practices including the dosage of inorganic fertilizers were same as in Experiment 1. At the stage of 30 DAS, the resulting plants were sprayed with double distilled water (DDW) serve as control, 10 mM, 20 mM, 30 mM, 40 mM or 50 mM of proline. The plant samples were collected at 60 and 90 DAS to assess various growth characteristics, nodulation, various enzyme activities viz. nitrogenase, glutamine synthetase, glutamate synthase, glutamate dehydrogenase, nitrate reductase, carbonic anhydrase, catalase, peroxidase and superoxide dismutase, photosynthetic attributes, contents of proline, carbohydrate and nitrogen and leaf water potential. Out of the various concentrations of proline tested, lower concentration (20 mM) proved to be most effective. All the above parameters experienced a significant increase under the influence of the exogenous application of 20 mM proline.
Experiment 3

This experiment was prepared with an objective to study the effect of different concentrations ($10^{-4}$, $10^{-5}$ or $10^{-6}$ M) of salicylic acid (SA) on chickpea. All the agricultural practices including the dosage of inorganic fertilizers were same as in Experiment 1. At the stage of 30 DAS, the resulting plants were sprayed with DDW (control), $10^{-4}$ M, $10^{-5}$ M or $10^{-6}$ M of SA. The plant samples were collected at 60 and 90 DAS to assess various growth characteristics, nodulation, various enzyme activities viz. nitrogenase, glutamine synthetase, glutamate synthase, glutamate dehydrogenase, nitrate reductase, carbonic anhydrase, catalase, peroxidase and superoxide dismutase, photosynthetic attributes, contents of proline, carbohydrate and nitrogen and leaf water potential. Out of the various concentrations of SA, lower concentration ($10^{-5}$ M) proved to be the best resulting in a significant increase in the aforesaid parameters.

Experiment 4

This experiment was prepared with an objective to elucidate the effect of exogenous proline on the Cd induced changes in chickpea. At the start of this experiment, five sets of earthen pots (25x 25 cm) were filled with an equal quantity of sandy loam soil mixed with farmyard manure, in a ratio of 6:1. All the agricultural practices including the dosage of inorganic fertilizers were same as in Experiment 1. Out of these five sets of prepared pots, four sets were supplemented with different doses (0, 25, 50 or 100 mg per kg of soil) of Cd, respectively and one set of pots was left untreated serving as control. At the stage of 30 DAS, the foliage of the resulting plants was sprayed with 20 mM proline, except control which received DDW instead of proline. The plant samples were collected at 60 and 90 DAS to assess various growth characteristics, nodulation, various enzyme activities viz. nitrogenase,
glutamine synthetase, glutamate synthase, glutamate dehydrogenase, nitrate reductase, carbonic anhydrase, catalase, peroxidase and superoxide dismutase, photosynthetic attributes, contents of proline, carbohydrate and nitrogen and leaf water potential. The exogenous application of 20 mM proline alleviated the adverse effects generated by Cd (25 or 50 mg per kg of soil) which was expressed in terms of the increase in aforesaid parameters. The mature plants were harvested after 150 DAS to assess the yield characteristics. The exogenous proline treatment proved to be beneficial in improving the yield characteristics of the plants exposed to Cd stress of 25 or 50 mg per kg soil.

**Experiment 5**

This experiment was prepared with an objective to elucidate the effect of exogenous SA on the Cd induced changes in chickpea. At the start of this experiment, five sets of earthen pots (25x 25 cm) were filled with an equal quantity of sandy loam soil mixed with farmyard manure, in a ratio of 6:1. Out of these five sets of pots, four sets were supplemented with different doses (0, 25, 50 or 100 mg per kg of soil) of Cd, respectively while one set was left untreated, serving as control. At the stage of 30 DAS, the foliage of the resulting plants was sprayed with $10^{-5}$ M of SA, except the control set which received DDW instead of SA. The plant samples were collected at 60 and 90 DAS to assess various growth characteristics, nodulation, various enzyme activities *viz.* nitrogenase, glutamine synthetase, glutamate synthase, glutamate dehydrogenase, nitrate reductase, carbonic anhydrase, catalase, peroxidase and superoxide dismutase, photosynthetic attributes, contents of proline, carbohydrate and nitrogen and leaf water potential. The exogenous application of $10^{-5}$ M of SA completely alleviated the adverse effects generated by Cd and resulted in an increase in the above mentioned parameters over their respective controls. The plants were
harvested after 150 DAS to assess the various yield characteristics. The SA treatment completely alleviated the ill effects generated by Cd and resulted in a significant increase in yield characteristics as well.

Experiment 6

This experiment was prepared with an objective to elucidate the interactive effect of the exogenous application of proline (20 mM) and SA (10⁻⁵ M) on the Cd induced changes in chickpea. At the start of this experiment, five sets of earthen pots (25 x 25 cm) were filled with an equal quantity of sandy loam soil mixed with farmyard manure, in a ratio of 6:1. All the agricultural practices including the dosage of inorganic fertilizers were same as in Experiment 1. Out of these five sets of prepared pots, four sets were supplemented with different doses (0, 25, 50 or 100 mg per kg of soil) of Cd, respectively while one set was left untreated, serving as control. At the stage of 29 DAS, the foliage of the resulting plants was sprayed with 20 mM proline, except the control set which received DDW instead of proline and at 30 DAS the plants were sprayed with 10⁻⁵ M of SA, except the control set which received DDW instead of SA. The plant samples were collected at 60 and 90 DAS to assess various growth characteristics, nodulation, various enzyme activities viz. nitrogenase, glutamine synthetase, glutamate synthase, glutamate dehydrogenase, nitrate reductase, carbonic anhydrase, catalase, peroxidase and superoxide dismutase, photosynthetic attributes, contents of proline, carbohydrate and nitrogen and leaf water potential. The exogenous application of 20 mM proline and 10⁻⁵ M of SA exerted an additive effect and completely alleviated the adverse effects generated by Cd resulting in a significant increase in the above mentioned parameters. The increase was also expressed in the yield characteristics of the plants at harvest (150 DAS).