Effect of Nitrogen Application on Nodulation in Inoculated Chickpea (Cicer arietinum L.)

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Abstract: A greenhouse experiment was conducted to study the effect of nitrogen application on the growth and nodulation in chickpea (Cicer arietinum L.). Inoculated chickpea variety Punjab-91 was applied with different doses of nitrogen i.e. 0 (control), 50, 100 and 150 kg N/ha in the form of urea. Data regarding the plant height, oven dry root and shoot weights, and number of nodules per plants were recorded every 15 days after the germination (DAG) of seeds up to a period of 60 days. Results showed a marked increase in plant height and oven dry root and shoot weights of chickpea plants due to nitrogen application. Addition of nitrogen at 100 kg/ha caused maximum increase followed by 150 and 50 kg N/ha, respectively. However, this marked increase in plant growth was only evident at the early growth stage i.e. up to 45 DAG, and at the later growth stages, the differences became less evident. A marked decline in nodulation in chickpea was observed at all the levels of nitrogen application.

Key words: Growth, nitrogen application, nodulation, chickpea

Introduction
Pakistani soils are deficient in nitrogen, one of the most abundantly required nutrient elements by plants (Ali et al., 1998). Nitrogen plays an important role in plant metabolism and its deficiency in soil most often results in low crop yields (Buresh et al., 1993; Carangal et al., 1994). Application of chemical fertilizers is the most common practice by which this element is provided in the soil for use by crops. However, the use of nitrogen fertilizers is limited because of their high cost and inadequate credit facilities (Chaudhry et al., 1999).

Biological nitrogen fixation of legumes by forming nodules in association with Rhizobium species is another source of making nitrogen available to plants (Peoples and Creswell, 1992). Estimates show that leguminous plants fix $35 \times 10^6$ metric tons N year$^{-1}$ and the net benefits of legumes are often equivalent to the addition of 60-100 kg N ha$^{-1}$ as fertilizer (Kang et al., 1990; Herridge et al., 1993). Besides this high nitrogen fixing potential of legumes, there is controversy about the nitrogen fertilization of legume crops.

Some scientists believe that before the symbiosis becomes fully functional, there is a need to apply nitrogen fertilizers to legume plants so as to make the nitrogen availability good for the young plants (Vincent, 1982). While the others think that all legumes are well nodulated and the application of nitrogen fertilizers is just the wastage of resources. Chickpea (Cicer arietinum L.) is an important leguminous crop of Pakistan. The present study was therefore conducted to investigate the effect of nitrogen application on nodulation and growth of inoculated chickpea.

Materials and Methods
A greenhouse experiment was conducted at the University of Arid Agriculture, Rawalpindi, Pakistan during the year 1999-2000. The soil was collected at 0-15 cm depth of the field from the research area of the university. The soil was air-dried, ground, passed through a 2 mm sieve and mixed thoroughly. A sub sample of the soil was taken and analyzed for various physico-chemical properties which showed that the soil was loam in texture with EC$_e$, 0.33 dS m$^{-1}$, pH 7.2, organic matter 0.75 %, total nitrogen 1.7 ppm and available phosphorus 7.8 ppm. The standard methods reported by Page et al. (1982) were used for the analyses of the soil.

The experiment was laid out following completely randomized design (CRD) with three replications. Treatments included the application of nitrogen at 0, 50, 100 and 150 kg ha$^{-1}$ in the form of urea. The soil was transferred into polyethylene bags of 19.5 cm in size, at the rate of 2.0 kg per bag. Weighed amounts of urea were applied to soil and mixed thoroughly with hand. Seeds of chickpea variety Punjab-91 were inoculated by compatible strain of Rhizobium before sowing. Six seeds of chickpea were sown in each bag and thinned to 4 seedlings per bag three days after germination. The bags were watered at regular intervals to maintain the soil moisture around field capacity. Tubewell water was used for irrigation purpose throughout the crop growth period.

Data on plant height, number of nodules per plant, fresh and oven-dry shoot weight, and fresh and oven-dry root weight were recorded at 15, 30, 45 and 60 days after the germination (DAG) of seeds. Plant heights were measured just before the removal of plants from the bags at the above mentioned time intervals. Later the plants were taken out completely from the bags along their roots. After washing carefully to remove the adhering soil particles, plant were cut off to separate into roots and shoots and the data on number of nodules and fresh root/shoot weights were recorded. The plant samples were then oven dried at 60°C until a constant weight, and the data on oven dry root and shoot weights were recorded. The statistical analyses of the data regarding analysis of variance (ANOVA) and comparison of means were performed by Statistix 4.1 software.

Results
Effect on plant height: The effect of nitrogen application on the height of chickpea (Cicer arietinum L.) plants is shown in Fig. 1. The application of nitrogen at all the levels caused a significant increase in plant height, compared with the control. The maximum increase was observed in the treatment receiving 100 kg N/ha, followed by 150 and
Effect of nitrogen on nodulation in chickpea

Effect of nitrogen application on the height of chickpea plants

A significant difference in plant height at all the levels of N application was seen during the first 45 days after the germination (DAG). At the later growth stages, only the addition of 100 kg N/ha showed a significant increase, while the other treatments became statistically at par with the control.

Effect on oven dry root and shoot weights: The application of nitrogen showed a significant increase in the oven dry root and shoot weights of chickpea plants at different time intervals up to 45 DAG (Fig. 2, 3). The maximum increase was recorded in the treatment receiving 100 kg N/ha followed by 150 kg N/ha. While the addition of 50 kg N/ha showed only non-significant increase, compared with the control. At the later growth stages, the increase in the oven dry weights of roots and shoots caused by nitrogen application became less evident (non-significant) against the control showing rapid growth of plants in the later treatment.

Effect on nodulation: The data regarding the effect of nitrogen application on nodulation in chickpea plants is presented in Fig. 4. The control treatment where no nitrogen was added showed the maximum number of nodules per plant that were significantly higher than all the nitrogen treatments. The addition of nitrogen caused a
significant decline in number of nodules per plants in chickpea at all the tested levels. The maximum decline was recorded in the treatment receiving 150 kg N/ha, where the nodulation was drastically reduced and the depressing effect of N continued throughout the observed growth period. This was followed by the treatments of 100 kg N/ha and 50 kg N/ha respectively, where the nodulation was also significantly lower than that of control. However, in case of 50 kg N/ha, the nodulation improved at the later growth stages i.e. 60 DAG and became statistically at par with that of the control.

Discussion
Nitrogen plays a very vital role in plant metabolism. It is a constituent of all proteins and nucleic acids and is therefore essential for plant growth. An increase in plant height and oven dry weight of shoots by nitrogen application may be caused by an increase in the leaf size, the size of plant cells, plant protoplasm and the number of secondary branches (Wild, 1988). While, the increase in root weight might be due to high root proliferation, more root hair formation, and more secondary root growth (Anderson and Beltsville, 1983). The present results demonstrated a significant increase in plant growth (plant height and oven dry root and shoot weights) by nitrogen addition to the soil, which is in line with a number of reports published so far (Eardly et al., 1985; Ali and Hussain, 1996; Ali et al., 1998). The effect of nitrogen application on the growth of chickpea plants was significant in early growth period and eventually became less evident at the later growth stages. This explains that chickpea plants in the control did not get enough nitrogen from the soil in the beginning, while the plants in the fertilized soils were getting it, due to which more plant growth was observed in fertilized treatments. However, at the later growth stages, the nodules in the plant roots developed to their maturity and became functional. So enough nitrogen became available for plants in the control, which grew rapidly and the difference in plant growth caused by the addition of nitrogen at the earlier growth period gradually disappeared. Addition of nitrogen to the soil severely depressed the nodulation in chickpea plants. This shows that the presence of adequate nitrogen in the soil decreased the rhizobial activity, which in turn resulted in less rhizobial infection to plant roots and less number of nodules per plant. Similar effect of nitrogen application on nodulation in legume crops has been reported by the other scientists (Eardly et al., 1985; Beck, 1992; Ali et al., 1998). Eardly et al. (1985) concluded that larger or continued application of nitrogen reduced nodulation and the activity of rhizobia. Fairly good agreements have also been found on the effect of nitrogen fertilization on reduced nodulation and high dry matter yields in legumes (Beck, 1992; Ladha et al., 1993).

References