Chapter 5

Discussion
DISCUSSION

Phosphorus is one of the essential elements of the plant body and is known to play important role in many physiological processes in plants (Marschner, 1995). Of the common crop plants, growth of root crops, development and maturity of cereals and competitive ability of legumes with forage crops are known to be dependent on phosphorus supply to the crops (Black, 1968). However, not only the use of phosphatic fertilizers is costly but also its supply lags behind its demand. Hence, apart from upgrading the per capita productivity of grain legumes, it is also highly desirable to explore the possibilities of achieving economy of phosphatic fertilizers, without sacrificing the yields, through new and innovative techniques including selection of the most suitable source of phosphorus for each agro-climatic zone. In order to reduce the use of chemical fertilizers, biofertilizers could play a crucial role by fixing the atmospheric nitrogen for the crops and/or by increasing the availability of soil nutrients to the crops. Thus, the biofertilizers could help sustain the crop production in the country in a cost-effective manner. In fact, supply of biofertilizers to the crops, particularly those of nitrogen and phosphorus, could play an important role in this regard. In view of generating the agricultural database in this connection, the present experiment was conducted on chickpea with N and P biofertilizers in a soil poor in N as well as P. Different levels of phosphorus were employed in combination with N and P biofertilizers to assess the agricultural performance of the crop in terms of growth parameters, physiological and biochemical parameters, seed yield and yield contributing attributes and quality parameters of the crop (Tables 1-5). The performance of the crop in respect with these parameters is discussed below:

4.1. Growth parameters

Growth of plant organs results from orderly cell division, cell expansion and cell differentiation. These processes, among other factors, depend on proper supply of mineral nutrients and growth substances as well as on the genetic makeup of the plants (Marschner, 1995; Moorby and Besford, 1983). A suitable
combination of these factors brings about healthy growth and development of plants which, in turn, ensures good crop yield and quality. In the present investigation, increasing levels of phosphorus from $P_0$ (phosphorus control) to $P_{60}$ enhanced all the growth attributes progressively (Table 1). However, in most cases, $P_{30}$ and $P_{60}$ gave statistically equal values, with both of the P levels proving significantly superior to $P_0$ level (phosphorus control). In this connection, the present results resemble with those obtained in case of chickpea by Yahiya and Samiullah (1995), Khanda et al. (2001), Bahadur et al. (2002), Pathak et al. (2003a) and Walley et al. (2005). Similar beneficial effect of P application has also been reported in other legumes (Kasheed and Sabale, 2003; Kumar and Singh, 2009).

Similar to the phosphorus levels, the N and P biofertilizer treatments also showed significantly positive effect on plant growth attributes (number of leaves per plant, fresh weight per plant and dry weight per plant). The maximal values of the growth attributes were generally shown by BNF+BPF compared to the biofertilizer control (BF$_0$). The positive effect of the dual inoculation (N+P biofertilizers) and that of N or P biofertilizer applied alone (single inoculation) on growth attributes of chickpea (Gull et al., 2004; Alagawadi et al., 1988) and various other crops (Sharma, 2001; Mayz et al., 2003; Choudhri et al., 2005; Tiwari and Kulmi, 2005; Zaidi and Khan, 2006) confirm the present findings in this regard.

Interaction between inorganic P fertilizer and biofertilizer was significant for most of the growth parameters. In general, $P_{30}$ along with N+P biofertilizers ($P_{30} \times BPF+BNF$) showed the best results. The similar results were obtained for number of leaves per plant. However for fresh and dry weight per plant, the maximum values were attained with $P_{60} \times BPF$, which was statistically at par with $P_{30} \times BPF$ and/or $P_{30} \times BPF+BNF$. In this regard, the present results are in conformity with those published by Saraf et al. (1997) and Dutta and Protit (2009) in case of chickpea.
4.2. Physiological and biochemical parameters

Among the physiological parameters, net photosynthesis and water use efficiency reached the highest extent with P_{30}; while both P_{60} and P_{30}, giving the highest values, were at par in case of internal CO_{2} concentration, stomatal conductance and transpiration rate (Table 2). Thus, P_{30} proved to be the optimum inorganic P level that exhibited the greatest values of all the physiological parameter. These results confirm the findings of other researchers, who observed a positive effect of phosphorus application on some or all of these physiological parameters regarding groundnut (Hossaini and Hamid, 2007), tea (Salehi and Hajiboland, 2008), clusterbean (Burman et al., 2009) and rapeseed. Improved photosynthesis as a result of phosphorus application in the present study might be due to the positive effect of phosphorus on regeneration of ribulose-1,5-bisphosphate (Rao and Terry, 1989; Fredeen et al., 1990), synthesis of ribulose-1,5-bisphosphate carboxylase and adenosine triphosphate (Dietz and Foyer, 1986) and assimilation of carbon dioxide (Longstreth and Nobel, 1980). Moreover, increased rate of photosynthesis as a result of phosphorus application could be due to the prompt and adequate supply of carbon dioxide to the mesophyll cells of the leaves that is evident by the phosphorus-improved internal CO_{2} concentration and stomatal conductance (Table 2). A higher transpiration rate and water use efficiency noted in phosphorus treated plants compared to the control (P_{0}) could also be due to the enhanced stomatal conductance (Johnson et al., 1987; McMurtrie, 1993) that was significantly improved in the phosphorus treated plants in the present study (Table 2). Of the biofertilizer treatments, BNF+BPF proved most beneficial for most of the physiological parameter. Not many references are available on the effect of biofertilizers on photosynthesis and the related parameters. However, enhancement in photosynthetic efficiency of green gram due to N biofertilizer, as noted by Sharma (2001), could be considered in line with the present results in this regard. As for interaction between P levels and biofertilizer treatments, most of the physiological parameters were improved to the greatest extent with P_{30} \times BPF and/or P_{30} \times BNF+BPF that could accordingly be reflected in the improved seed yield and its components (Tables 2 and 4).
Of the biochemical parameters, NR activity, CA activity, N-content, P-content, and leghemoglobin content attained the highest values with P₆₀, while seed protein and carbohydrate contents had maximum values at P₃₀. Significant increase in N and P content in the leaves due to P application in the present study indicates the positive effect of P application on P-content, as expected, but also reveals the beneficial effect of P application on N-content. The latter could be due to overall improvement of plant growth as a result of P application (Table 1). These results are in conformity with those reporting significant increase in N and/or P content or their uptake in the leaves or straw due to P application in chickpea by Pathak et al. (2003b), Yahiya et al. (1995) and Walley et al. (2005). Similar findings have been reported by other researchers in rice-bean (Khanda et al., 2001), soybean (Tiwari et al., 2005) and pigeonpea (Kumar and Kushwaha, 2006). Application of N and P biofertilizers did not influence the P-content in leaves, while the effect of application of N and P biofertilizers and that of their combination was significant on N-content. A positive effect of N biofertilizer on N-content and/or N-uptake has earlier been reported on groundnut (Elsheikh and Mohamedzein, 1998), mungbean (Singh and Tarafdar, 2001) and cowpea (Mayz et al., 2003). A similar positive effect of P biofertilizer on N content, like that found in the present study, has also been found on rice-wheat cropping system (Sharma, 2003), wheat (Diwivedi et al., 2003) and tomato (Choudhry et al., 2005). In the present study, not only the positive effect of combined application of N and P biofertilizers on N-content was observed, but also there was found a positive effect of interaction of phosphorus and N and P biofertilizers both on N- and P-content (Table 3). This indicates the synergistic effect of phosphorus and N and P biofertilizers on N- and P-content that accordingly resulted in the overall improvement in growth and yield attributes of the crop (Tables 1 and 4). In this context, a synergistic effect of combined application of N and P biofertilizers on N and P uptake of chickpea has been reported by Jat and Ahlawat (2006).

There was a significant effect of inorganic P and N and P biofertilizer application on nodule-lehmemoglobin content in the present study (Table 3). As the interaction P₃₀ × BPF resulted in the highest values both for root nodule
leghemoglobin content and leaf P-content, the increase of nodule-leghemoglobin content in the root nodules might be due to the improved availability of phosphorus to the P$_{30}$ × BPF treated plants. Like that in the present study, there was observed a positive effect on nodule leghemoglobin content due to inorganic P application in *Lablab purpureus* by Santhaguru and Hariram (1998). Besides, there was observed beneficial effect of inorganic P and N and P biofertilizers in chickpea by Dutta and Protit, 2009.

The present study also reveals a positive effect of inorganic P fertilizer and that of N and P biofertilizers on nitrate reductase (NR) activity over the respective control (Table 3). The NR activity in plants is influenced by different growth conditions including not only the environmental factors such as light and temperature, but also the application of mineral fertilizers, particularly phosphorus (Oaks, 1985). It is also supported by the significant increase in leaf-N content due to the application of both inorganic P and P biofertilizer in the present research (Table 3). This might have, in turn, increased the capability of the plants for nitrate assimilation as well. The presence of phosphorus in the nutrient solution has earlier been reported to induce higher nitrate assimilation in corn (de Magalhaes et al., 1998) and beans (Gniazdowaska et al., 1999). These results are also in agreement with those of Naeem and Khan (2005) in the case of *Cassia tora*.

There was a significant positive effect of inorganic P fertilizer and that of N and P biofertilizers on carbonic anhydrase (CA) activity in the present investigation (Table 3). The CA activity is known to have its important role in photosynthesis, which is obvious by its presence in all photosynthesizing tissues (Taiz and Zeiger, 2006). It catalyzes the reversible hydration of CO$_2$, thereby increasing its availability for RuBPCO (Badger and Price, 1994; Khan et al., 2004). The enhancement in CA activity due to soil-applied phosphorus or that due to application of N and P biofertilizers could be as a result of adequate availability of N and P at the site of their metabolism, particularly leaves. The higher N and P contents in the treated plants with respect to the control is supported by the positive interaction effect of soil P application and biofertilizers on N and P.
content in leaves (Table 3). A probable cause for the enhancement of CA activity due to application of inorganic P and P biofertilizers might be due to the positive influence of P availability to plants or the de novo synthesis of CA (Okabe et al., 1980).

4.3. Seed yield and yield parameters

As far as the seed yield and yield contributing attributes are concerned, the trend was the same as that of growth parameters. In most of the cases, the seed yield as well as yield parameters increased with the increase in P level (Table 4). In this context, the present results corroborate the findings of Ayub et al. (1998) and Mitra et al. (2006) on green gram, Singh et al. (1997) and Bahadur et al. (2002) on chickpea, Sharma, et al. (2001) on mungbean, Khanda et al. (2001) on rice-bean (Vigna umbellata) cropping system, Kashid and Sabale (2003) in wheat-pigeon cropping system, Kumar and Kushwaha (2006) on pigeon pea, and Yakadri and Murthy (2006) on black gram - foxtail millet cropping system. However, though the highest seed yield was attained exclusively at P60, the greatest values of most of the yield contributing attributes were attained at P30 as well as at P60, both of the P levels being statistically at par. As for the effect of biofertilizer treatments, the highest seed yield was obtained due to BNF+BPF and BPF, while the greatest harvest index was shown by BPF alone. However, in case of most of the other yield components, BNF+BPF resulted in the highest values. In this context, the present results substantiate those findings which confirm the enhancement of seed yield and improvement in the yield components due to application of BNF (El-Ghandour et al., 1996; Singh and Tarafdar, 2001; Mayz et al., 2003; Hernandez and Cuevas., 2003; Naik et al., 2007), BPF (Sharma, 2003; Dwivedi et al., 2003; Chaudhari and Gavhane, 2005) or BNF+BPF (Alagawadi et al., 1988; Shinde and Bangar, 2003; Tiwari and Kulmi, 2005; Gupta, 2004; Zaidi and Khan, 2006; Afzal and Bano, 2008) in case of various crops including chickpea. There was significant effect of the interaction between P levels and biofertilizer treatments with regard to seed yield and all the yield components. Interaction P30 × BNF+BPF gave the highest value for seed yield, stover yield and
crop biomass. It was equally good for 100 seed weight and number of pods per plant. On the other hand, \( P_{30} \times \text{BPF} \) was statistically at par with \( P_{30} \times \text{BNF+BPF} \) for seed yield and stover yield. It was also at par with \( P_{60} \times \text{BNF+BPF} \), the best interaction regarding number of pods per plant and number of seeds per pod. Thus, \( P_{30} \times \text{BPF} \) and \( P_{30} \times \text{BNF+BPF} \) seem to be the paramount combinations of inorganic P level and biofertilizer for seed yield and yield components. These combinations (interactions) are also substantially economic as they resulted in a better seed yield compared to that obtained due to \( P_{60} \) alone, saving 30 kg P ha\(^{-1}\) just by using biofertilizers with a nominal cost. Thus, the present results do not agree with the investigations which claim that \( P_{60} \) and above (80-90 kg P\(_2\)O\(_5\) ha\(^{-1}\)) could be the best P levels for the highest seed yields of chickpea and other legumes (Singh et al., 1997; Ayub et al., 1998; Sharma et al., 2000; Khanda et al., 2001; Bahadur et al., 2002; Kashid and Sabale, 2003; Tiwari and Pal, 2005; Yakadri and Murthy, 2006; Vashist and Yadav, 2009). In conformation with the present results, Ayub et al. (1998) claimed that they observed significant depressions in the seed yield and yield components of mungbean beyond 75 kg P\(_2\)O\(_5\) ha\(^{-1}\). Moreover, Meena et al. (2003) recorded the highest values of pod number per plant, number of seeds per pod, length of pod and test weight as a result of phosphorus dressing at 45 kg P ha\(^{-1}\) that was statistically at par with 60 kg P ha\(^{-1}\). Similarly, Walley et al. (2005) confirmed that phosphorus application at 40 kg P\(_2\)O\(_5\) ha\(^{-1}\) enhanced the vegetative growth of both the chickpea varieties (Desi and Kabuli), but significant enhancement in seed yield was observed only in case of desi variety. Further, Kumar and Kushwaha (2006) emphasized that application of P\(_2\)O\(_5\) beyond 40 kg ha\(^{-1}\) could be deleterious for pigeonpea. In contrast, the present results are in agreement with the findings which confirm that a combination of inorganic P level and P-biofertilizer could be a better fertilizer dressing to achieve maximum seed yield and improved yield components than by applying higher inorganic P levels (with massive cost) alone (Saraf et al., 1997; Jain et al., 1999; Meena et al., 2003; Singh et al., 2005; Afzal and Bano, 2008; Dutta and Protit, 2009).
4.4. Quality parameters

The increase in seed-protein content due to application of inorganic P and that of N and P biofertilizers in the present investigation might presumably be ascribed to increased N content of leaves as a result of significant positive effect of application of inorganic P and N and P biofertilizers (Tables 3 and 5). The enhanced N-content might have increased the amino acid synthesis and, thereby, could have improved the seed-protein content via their translocation to seeds. Further, application of inorganic P and P biofertilizer might have proved effective due to assured P availability and its continuous utilization in the carbon skeleton and amino acid synthesis as well as in the synthesis of energy rich molecules such as ATP. This might have been responsible for the enhanced synthesis of protein during seed development. A significant effect of inorganic P and and/or N and P biofertilizer application on seed-protein content has been reported in case of chickpea (Gupta et al., 1998; Guhey, et al., 2000; Meena et al., 2003), groundnut (Elsheikh and Mohamedzein, 1998), lentil (Akhtar et al., 1987), Cassia tora (Naeem and Khan, 2005), mungbean (Sharma et al., 2000; Singh and Tarafdar, 2001), soybean (Tiwari et al., 2005), green gram (Mitra et al., 2006). Furthermore, in this study, there was observed a beneficial effect of application of inorganic P and N and P biofertilizer treatments on seed carbohydrate content. The untreated plants recorded lowest value of carbohydrate concentration (Table 5). Since the interaction P30 x BPF and P30 x BNF+BPF resulted in the highest values, the increase of carbohydrate content in seed in this study could presumably be due to the improved phosphorus availability to the treated plants in view of the vital role of phosphorus in carbohydrate metabolism (Taiz and Zeiger, 2006).