Research Article

Effect of phosphorous, rhizobium inoculation and residue types on chickpea productivity

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Abstract

An experiment with the objective to evaluate the effect of phosphorous, rhizobium inoculation and residue types on chickpea productivity was conducted during Rabi 2014-15 at Agronomy Research Farm of The University of Agriculture Peshawar. Randomized complete block design (RCBD) was used for the experiment with four replications. Inoculated and un-inoculated seeds were sown with three phosphorus (P) levels (30, 60, and 90 kg ha⁻¹) and three residue types (cereal, legume and oilseed). A control treatment was also included. Plots supplied with 90 kg P ha⁻¹ induced early flowering and maturity with higher nodules plant⁻¹, plant height, number of pods plant⁻¹ and biological yield which was statistically similar with application of 60 kg P ha⁻¹. Significantly higher number of grains pod⁻¹, thousand grain weight and grain yield was recorded in plots treated with phosphorous at 60 kg ha⁻¹. Residue types showed non-significant effect on all studied parameters. Seeds treated with rhizobium produced maximum nodules plant⁻¹, grains pod⁻¹, pods plant⁻¹, 1000-grains weight, biological yield and grain yield. The interaction between phosphorus and rhizobium inoculation exposed that seeds treated with rhizobium resulted in maximum biological and grain yield when fertilized with 60 kg P ha⁻¹. It can be concluded that chickpea when inoculated with rhizobium and fertilized with 60 kg ha⁻¹ phosphorus resulted in maximum yield and yield attributes of chickpea.

Keywords: Cereal; Grain yield; Legume; Nodules plant⁻¹; Plant height; Pods plant⁻¹

Introduction

Chickpea (Cicer arietinum L.) belongs to family Fabaceae and is grown in many parts of the world with a total production of 9.8 million tons from an area of 11.1 million hectares [1]. Pulses are important crops having high amount of protein in its seed and contribute considerably to dietary protein [2]. Chickpea not only plays an important role in human diet but also improves soil fertility by fixing atmospheric nitrogen [3]. Therefore, pulses have become a key target for
agricultural, environmental and biotechnological research. Among pulses, chickpea is one of the major pulse crop of our country grown on an area under arid and semi-arid conditions. Despite the high yield potential over 4000 kg ha\(^{-1}\) of chickpea [4], the actual yield is still significantly lower in Pakistan. Minimum yield of chickpea is due to combination of biotic and abiotic stresses [5]. In Pakistan during 2014-15 it was cultivated on 0.960 million hectares with total production of 0.484 million tons having an average yield of 504 kg ha\(^{-1}\) [6].

Phosphorus (P) is an essential nutrient of numerous vital plant structural compounds. It acts as a catalyst in several biochemical reactions occurred in plants. It plays an important role in capturing and converting the solar energy into useful plant compounds. These compounds help in the general health and vigor of plants [7]. Phosphorus is connected with some particular plant growth factors that are: root development, vigorous stem, enhanced flower formation and seed production, earlier and more uniform crop maturity, increase nitrogen fixing capacity of legumes, improvement in crop quality and resistance to plant diseases [8, 9]. It is required for higher and sustainable production of grain legumes. Generally, legumes have higher P requisites due to more consumption of energy in the process of symbiotic nitrogen fixation [10]. Low soil P and poor consumption efficiency of P hinders the productivity of most grain legumes [11, 12]. Gupta et al. [13] and Redy et al. [14] reported that P application significantly enhanced the yield in red chickpea. P plays an important role in pod filling and finally improves the grain yield of chickpea.

Soil lacking native rhizobia of chickpea requires artificial seed inoculation for improving root nodulation and yield of the crop [15]. Artificial rhizobium inoculation of chickpea reacts positively when grown in soils that contain its native rhizobia [16]. Application of biofertilizers such as rhizobium can decrease the need for chemical fertilizers and reduce its adverse effects on environment. In the improvement of sustainable agricultural techniques, biofertilizers have a great importance in decreasing environmental pollution and deterioration of nature [17]. Residues are important component for the stability of agricultural ecosystems. Areas where regular mechanical harvesting is practiced, most of the residues are left in the field and can be recycled for nutrients supply. Leaving residues in the field after harvest is one of the way to stabilize soil fertility. As residues are good sources of plant nutrients, both farmers and researchers have shown more curiosity in management of residue types. Usually, it acts as valuable management tool because of high costs of inorganic fertilizers and less yield in monoculture cropping systems [18].

Research studies have revealed that residues enhanced soil fertility, crop productivity, reduced wind and water erosion and prevented nutrients leaching and losses by run-off [19]. Keeping in view the importance of seed inoculation with rhizobium, phosphorus and residues, the present experiment was designed to determine the impact of phosphorous and residues on chickpea inoculated with rhizobium.

**Materials and methods**

To study the impact of rhizobium inoculation with different phosphorus levels and residue types on chickpea productivity, an experiment was conducted at Agronomy Research Farm of The University of Agriculture, Peshawar during Rabi 2014-15. Experiment comprised of three factors i.e. residue types (cereal, legume, oilseed), rhizobium inoculation (inoculated and uninoculated) and phosphorous levels (30, 60 and 90 kg ha\(^{-1}\)). A control treatment was also included for comparison. The experiment was arranged in randomized complete block design having four replications. A sub plot
size of 3 m x 1.5 m was used. Each plot consisted of 5 rows with 30 cm row to row
distance. Nitrogen was applied during sowing at the rate of 25 kg ha\(^{-1}\) as basal dose.
Rhizobium inoculum “BIOZOTE” was used for seed inoculation. Prior to seed sowing, the
chickpea seeds were first sprinkled with sugar syrup and then inoculum was mixed
with seed in shady place. The seed was kept for 20 minutes and then it was sown.
Chickpea variety NIFA-2005 was sown at the rate of 60 kg ha\(^{-1}\) with single row hand drill.
Residue types each at the rate of 5 tons ha\(^{-1}\) were incorporated. Other agronomic
practices were carried out uniformly for all the experimental units throughout the
growing season. Data was recorded for days to first flowering, days to maturity, plant
height, nodules plant\(^{-1}\), number of pods plants\(^{-1}\), numbers of seeds pod\(^{-1}\), 1000-grain
weight, grain yield and biological yield.

**Procedure for recording data**

Number of days to flowering was recorded by counting the total number of days from the
date of sowing to date till appearance of first flower. Days to maturity were recorded as the
total number of days from the date of planting to day when 90% plants in each subplot
turned brown and ready for harvest. Data on plant height (cm) was recorded by measuring
height of ten randomly selected plants in each subplot with the help of meter rod and
averaged. Data on nodules plant\(^{-1}\) were recorded at flowering stage by counting the
number of nodules in five random plants selected in each subplot. For recording nodules plant\(^{-1}\), the spot was irrigated first and when it reached to water (field capacity),
the sampled plants were uprooted with the help of spade and nodules were counted and averaged. Number of pods plant\(^{-1}\) was counted on ten randomly selected plants in
each subplot after harvesting. Ten random pods in each subplot were selected, grains in
these pods were counted and averaged for determining number of grains pod\(^{-1}\). A
random sample of thousand seeds were counted from each treatment and weighed on
sensitive electronic balance to record 1000 grains weight in grams. For biological yield
three central rows in each subplot were harvested, sun dried, weighed and converted
into kg ha\(^{-1}\). Grain yield was recorded by threshing three central rows harvested for
biological yield in each subplot. The threshed seeds were cleaned and weighed to determine
grain yield (kg ha\(^{-1}\)).

**Statistical analysis of the data**

The collected data was analyzed statistically according to analysis of variance procedure
as appropriate for randomized complete block design. Means were compared using
least significant difference (LSD) test at \(P \leq 0.05\) upon significant F-test [20].

**Results and discussion**

**Days to flowering**

Data concerning days to first flowering (Table 1) reflects that phosphorus (P) levels had significantly influenced days to flowering in chickpea. Effect of rhizobium inoculation and residues types with all possible interactions were found non-significant for days to flowering. Mean data indicated that increasing P rate decreased days to flowering with minimum days to flowering was observed in plots treated with higher rate of P (90 kg ha\(^{-1}\)). Control plots showed delayed flowering than rest. In control plots delayed flowering initiation was observed due to prolonged growth period against the treated plots. Our results are supported by the findings of Neenu et al. [21] and Saedullah [22] who reported that flowering was delayed in control plots as compared to phosphorus fertilized plots.

**Days to maturity**

Days to maturity was significantly affected by phosphorous while rhizobium inoculation and residues types had a non-significant effect on days to maturity of chickpea (Table 1). Mean data indicated that increasing P rate decreased days to maturity in chickpea with
less days to maturity recorded for 90 kg P ha\(^{-1}\). An inverse relation was found between P and days to maturity in chickpea. Neenu et al. [21] indicated that phosphorus deficiency delays maturity as compared to phosphorus fertilized plots in chickpea crop.  

**Plant height (cm)**  
Phosphorus had a significant while rhizobium inoculation and residues types had a non-significant effect on plant height of chickpea (Table 1). Control vs rest comparison was found significant for plant height of chickpea while all interactions were found non-significant. Mean values showed that taller plants were recorded for highest level of P (90 kg ha\(^{-1}\)) which was statistically similar with 60 kg ha\(^{-1}\) P while shortest plants were recorded for 30 kg ha\(^{-1}\) P application. Our findings are in accordance with those of Ahmad et al. [23] and Dutta and Bandyopadhyay [24] who reported significant effects of P on plant height of chickpea. Also numerous other studies reported enhanced morphological growth due to direct application of phosphorus [25-27]. Rudresh [28] reported similar results by concluding that plant height was not affected significantly with rhizobium inoculation.  

**Nodules plant\(^{-1}\)**  
Data showing nodules plant\(^{-1}\) are given in (Table 1). Mean data indicated that number of nodules plant\(^{-1}\) were significantly affected by P and rhizobium inoculation while residue types had no significant effect on nodules plant\(^{-1}\). Nodules numbers increased with increasing P rate with more nodules plant\(^{-1}\) counted for highest rate of P i.e. 90 kg ha\(^{-1}\). Seeds inoculated with rhizobium showed higher number of nodules plant\(^{-1}\) than non-inoculated seeds. All possible interactions were found non-significant. Control plots showed less number of nodules plant\(^{-1}\) than rest. The higher number of nodules plant\(^{-1}\) might be associated with enhanced root proliferation due to phosphorus application. More nodules plant\(^{-1}\) with rhizobium inoculation might be due to the reason that inoculation promote rhizobial activities in soil and in turn nodules number. Similar results were reported by [29] who reported that rhizobium inoculation in chickpea increases 27% of nodules plant\(^{-1}\) as compared to control plots. These results are in line with the findings of other scientists [30, 31] who found that number of nodules per plant was the highest in plants inoculated with rhizobium.  

**Number of pods plant\(^{-1}\)**  
Number of pods plant\(^{-1}\) is an important yield determinant in pulse crops. Data concerning number of pods plant\(^{-1}\) is given in Table 1. Data exhibited that number of pods plant\(^{-1}\) significantly increased with P and rhizobium inoculation while residues types and all interactions did not cause any significant variation in number of pods plant\(^{-1}\). Control compared with treated plots was also significant. With increase in P remarkable increase in pods plant\(^{-1}\) was found. Plots treated with 30 kg P ha\(^{-1}\) produced lowest pods plant\(^{-1}\) while highest pods plant\(^{-1}\) was recorded in plots having 90 kg P ha\(^{-1}\). Seeds inoculated with rhizobium produced plants with more pods compared to un-inoculated seeds. Increase in the number of pods plant\(^{-1}\) with the application of P might have resulted from more growth of the plant which in turn had increased number of pods plant\(^{-1}\). These results are supported by the findings of [32] who reported that application of P at higher levels resulted in increased crop growth particularly positive impact was noted on branching, pods, seeds pod\(^{-1}\), seed index and seed yield. The number of pods and seed index was improved under higher P application [33]. Similarly inoculated seeds have more pods plant\(^{-1}\) and these results are supported by the findings of [17] who reported that number of pods plant\(^{-1}\) significantly increased with rhizobium inoculation. These results are also in resemblance with those of [34] who
determined that inoculation positively increased number of pods plant$^{-1}$ in chickpea. The minimum number of pods in the control might have been due to less availability of P and stunted growth.

Table 1. Days to flowering, days to maturity, plant height (cm), nodules plant$^{-1}$ and number of pods plant$^{-1}$ of chickpea as affected by crop residue types, rhizobium inoculation and phosphorous levels.

<table>
<thead>
<tr>
<th>Residue types (RT)</th>
<th>Days to flowering</th>
<th>Days to maturity</th>
<th>Plant height (cm)</th>
<th>Nodules plant$^{-1}$</th>
<th>Number of pods plant$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal</td>
<td>139</td>
<td>189</td>
<td>81.2</td>
<td>71</td>
<td>46</td>
</tr>
<tr>
<td>Legume</td>
<td>139</td>
<td>189</td>
<td>81</td>
<td>72</td>
<td>49</td>
</tr>
<tr>
<td>Oilseed</td>
<td>139</td>
<td>189</td>
<td>81.9</td>
<td>70</td>
<td>49</td>
</tr>
<tr>
<td>LSD$_{(0.05)}$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Rhizobium inoculation (I)**

<table>
<thead>
<tr>
<th>I</th>
<th>Days to flowering</th>
<th>Days to maturity</th>
<th>Plant height (cm)</th>
<th>Nodules plant$^{-1}$</th>
<th>Number of pods plant$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated</td>
<td>139</td>
<td>189</td>
<td>82.2</td>
<td>75 a</td>
<td>49 a</td>
</tr>
<tr>
<td>Un-inoculated</td>
<td>139</td>
<td>190</td>
<td>80.5</td>
<td>67 b</td>
<td>47 b</td>
</tr>
<tr>
<td>LSD$_{(0.05)}$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
</tr>
</tbody>
</table>

**Phosphorous (P) levels (kg ha$^{-1}$)**

<table>
<thead>
<tr>
<th>P levels</th>
<th>Days to flowering</th>
<th>Days to maturity</th>
<th>Plant height (cm)</th>
<th>Nodules plant$^{-1}$</th>
<th>Number of pods plant$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>140 a</td>
<td>190 a</td>
<td>79.6 b</td>
<td>66 b</td>
<td>46 b</td>
</tr>
<tr>
<td>60</td>
<td>139 ab</td>
<td>189 ab</td>
<td>81.9 ab</td>
<td>73 ab</td>
<td>49 ab</td>
</tr>
<tr>
<td>90</td>
<td>138 b</td>
<td>188 b</td>
<td>82.6 a</td>
<td>74 a</td>
<td>50 a</td>
</tr>
<tr>
<td>LSD$_{(0.05)}$</td>
<td>1</td>
<td>1</td>
<td>2.26</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

**Control vs rest**

<table>
<thead>
<tr>
<th>Control vs rest</th>
<th>Days to flowering</th>
<th>Days to maturity</th>
<th>Plant height (cm)</th>
<th>Nodules plant$^{-1}$</th>
<th>Number of pods plant$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>142 a</td>
<td>191 a</td>
<td>76.5 b</td>
<td>59 b</td>
<td>39 b</td>
</tr>
<tr>
<td>Rest</td>
<td>139 b</td>
<td>189 b</td>
<td>81.4 a</td>
<td>71 a</td>
<td>48 a</td>
</tr>
</tbody>
</table>

**Interactions**

<table>
<thead>
<tr>
<th>Interactions</th>
<th>Days to flowering</th>
<th>Days to maturity</th>
<th>Plant height (cm)</th>
<th>Nodules plant$^{-1}$</th>
<th>Number of pods plant$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT x I</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>RT x P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>I x P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>RT x I x P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means of same categories followed by different letters are statistically different at 5% level of probability. NS = Non-significant *= Statistical significance at 5% level of probability ** = Statistical significance at 1% level of probability

**Number of grains pod$^{-1}$**

Data pertaining to number of grains pod$^{-1}$ is presented in Table 2. Analysis of data showed that P and rhizobium inoculation significantly affected grains pod$^{-1}$ while residues types and all possible interactions were found non-significant for numbers of grains pod$^{-1}$. Mean values for P indicated that application of 60 and 90 kg ha$^{-1}$ P produced pods with statistically similar and higher number of grains while less grains were recorded for 30 kg P ha$^{-1}$. Seeds inoculated with rhizobium produced significantly more number of grains pod$^{-1}$ as compared with un-inoculated seeds. The application of phosphorus might have enhanced the photosynthetic activity which resulted in more number of seeds per plant. Similar findings were declared by [35] who recorded more grains pod$^{-1}$ at 60 kg P ha$^{-1}$ as compared with other P levels. Inoculated plots produced more grains pod$^{-1}$ compared to un-inoculated
plots. Increase in the number of grains pod\(^{-1}\) was probably due to availability of more N on account of its uptake by plants due to inoculation. The significant effects of seed inoculation on number of grains pod\(^{-1}\) were also noted by [36]. [29] reported that rhizobium inoculation had a positive effect on growth, yield and yield attributes in chickpea.

**Thousand grains weight (g)**

Analysis of data indicated that thousand grains weight were significantly affected by P and rhizobium inoculation (Table 2). Residue types with all possible interactions did not cause significant variations in thousand grains weight of chickpea. Mean values of the data showed that higher thousand grain weight was recorded in plots that received 60 kg P ha\(^{-1}\) followed by 90 kg P ha\(^{-1}\) with no statistical difference while lower thousand grains weight was recorded in plots treated with 30 kg P ha\(^{-1}\). Heavy grains were recorded in plots where seeds were inoculated with rhizobium than uninoculated seeds. Increasing P levels increased 1000 grains weight by improving photosynthetic activity and better seed formation. Better growth and development of crop plants due to phosphorus supply and nitrogen uptake might have increased the supply of assimilates to seed, which ultimately gained more weight. Our results are supported by the findings of [37] who reported heavier grains due to P application. A positive response of seed weight to inoculation of annual legumes was reported by [17, 27, 38]. Nonetheless, phosphorous either alone or in combination with inoculation, resulted in a significantly maximum weight of thousand seeds of chickpea. These results are in agreement with the findings of some other studies [38, 27]. The reason for maximum 1000 seeds weight recorded for P and inoculation treatment could be due to the enhanced growth and development of plants that resulted from P supply and its positive effect on nitrogen fixation. The resulting nitrogen availability might have promoted the supply of assimilates to seed thereby enabling them to gain more weight. Better growth and development of plants due to rhizobium inoculation might have influenced the nutrient supply to plant growth which resulted in producing more assimilates. These assimilates might have partitioned more efficiently from source to sink and finally added to more seed weight [29]. Different researchers [39, 40] showed that rhizobium inoculation significantly increased 1000 seed weight of chickpea.

**Biological yield (kg ha\(^{-1}\))**

Statistical analysis of the data depicted that biological yield was significantly affected by P and rhizobium inoculation (Table 2). Residue types and all other interactions except P x inoculation was found non-significant. Interaction of P x I revealed that seeds inoculated with rhizobium when fertilized with 60 kg ha\(^{-1}\) P resulted in maximum biological yield while minimum biological yield was recorded in plots where 30 kg ha\(^{-1}\) P was applied to un-inoculated seeds (Figure 1). The positive effect of P on total biomass was in agreement with earlier studies [26, 41, 42] as well as the strongest effect by the combined application of inoculation and P [38]. The substantial increase in biological yield due to P application could be attributed to the increased vegetative growth, possibly a result of its extensive root system under P fertilization. These results are supported by findings of [43] who stated that rhizobium inoculation increases biological yield in chickpea crop. More plant growth, yield and yield components are the possible reasons for higher biological yield in inoculated plots [38]. Un-inoculated treatments and plots with no phosphorous application showed minimum biological yield.

**Grain yield (kg ha\(^{-1}\))**
Significant variations in grain yield was indicated by the individual as well as interactive effect of phosphorous and rhizobium inoculation (Table 2). The residue types and all other interactive effects were found non-significant. Mean data revealed that plots having rhizobium inoculated seeds and fertilized with 60 kg P ha\(^{-1}\) produced maximum grain yield while minimum grain yield was recorded in plots having un-inoculated seeds and treated with lower rate of P i.e. 30 kg ha\(^{-1}\) (Figure 2). Similar effect of P on grain yield of legumes was found by other researchers [24, 41] and attributed to the role of P in the meristematic activity of plant tissues and in the synthesis of other growth components from carbohydrates [44]. The increase in yield due to the more availability of P might have increased the plant growth and better seed development which in turn increased grain yield. These results are in resemblance with [37]. Inoculation of chickpea seed significantly improved its grain yield [45, 46]. The effect was even more pronounced when inoculation and P were applied in combination resulting in a yield advantage over the control. This might have resulted from the positive effects of P on the process of nitrogen fixation where the increased supply of nitrogen through inoculation resulted in enhanced plant growth that eventually leads to higher yield. The increase in yield due to inoculation could be attributed to increase in yield components of the crop in inoculated plots. These findings are identical with those of [29] who reported 22% increase in grain yield by inoculation.

![Interaction of phosphorous and rhizobium inoculation for grain yield (kg ha\(^{-1}\)) of chickpea](image_url)
Table 2. Number of grains pod$^{-1}$, thousand grains weight (g), biological yield (kg ha$^{-1}$) and grain yield (kg ha$^{-1}$) of chickpea as affected by crop residue, rhizobium inoculation and phosphorous levels.

<table>
<thead>
<tr>
<th>Residue types (RT)</th>
<th>Number of grains pod$^{-1}$</th>
<th>Thousand grains weight (g)</th>
<th>Biological yield (kg ha$^{-1}$)</th>
<th>Grain yield (kg ha$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal</td>
<td>1.7</td>
<td>244.2</td>
<td>5093</td>
<td>1374</td>
</tr>
<tr>
<td>Legume</td>
<td>1.7</td>
<td>246.1</td>
<td>5084</td>
<td>1401</td>
</tr>
<tr>
<td>Oilseed</td>
<td>1.6</td>
<td>244.3</td>
<td>5133</td>
<td>1389</td>
</tr>
<tr>
<td>LSD$_{(0.05)}$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Rhizobium inoculation (I)

| Inoculated         | 7 a                         | 247.4 a                     | 5174 a                         | 1439 a                    |
| Un-inoculated      | 1.6 b                       | 242.3 b                     | 5033 b                         | 1337 b                    |
| LSD$_{(0.05)}$      | 3                           | 3.8                         | 114.8                          | 76.7                      |

Phosphorous (P) levels (kg ha$^{-1}$)

| 30                 | 1.6 b                       | 241.6 b                     | 4968 b                         | 1289 b                    |
| 60                 | 1.7 a                       | 248.6 a                     | 5167 ab                        | 1447 a                    |
| 90                 | 1.7 ab                      | 244.5 ab                    | 5175 a                         | 1427 ab                   |
### Conclusion

It can be concluded from the study that yield and yield components of chickpea were found higher at phosphorus application of 60 kg ha\(^{-1}\). Rhizobium inoculated seeds performed well in terms of grain yield than un-inoculated seeds. Therefore, application of phosphorus at the rate of 60 kg ha\(^{-1}\) to seeds inoculated with rhizobium is recommended for higher production of chickpea under the climatic conditions of Peshawar region.

### Authors’ contributions

Conceived and designed the experiments: W Rehan & A Jan, Performed the experiments: W Rehan & A Jan, Analyzed the data: W Rehan, W Liaqat & H Ahmad, Contributed materials/ analysis/ tools: MD Ahmadzai, MF Jan, J Haroon, N Ali & MM Anjum, Wrote the paper: W Liaqat, MF Jan, H Ahmad & W Rehan.

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