



Effect of *Mesorhizobium*, plant growth promoting rhizobacteria and phosphorus on plant biometery and growth indices of desi chickpea (*Cicer arietinum* L.)

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Abstract: The field experiment was conducted during 2015-16 to study the effect of biofetilizer inoculation [control, *Mesorhizobium only, Mesorhizobium* + RB-1 (*Pseudomonas argentinensis*) and *Mesorhizobium* + RB-2 (*Bacillus aryabhattai*)] and four levels of phosphorus (0, 15, 20 and 25 kg P_2O_5 ha⁻¹) on chickpea growth. RB-1 and RB-2 were the plant growth promoting rhizobacteria (PGPR). Biofertilizers could play a crucial role in reducing the dependence on chemical fertilizers by fixing the atmospheric nitrogen for crop and/or by increasing the availability of phosphorus and phytohormones to the crop. The 16 treatment combinations were laid out in Factorial Randomized Complete Block Design and replicated three times. In biofertilizer treatments, *Mesorhizobium* + RB-1 proved superior over control and sole inoculation of *Mesorhizobium* and at par with *Mesorhizobium* + RB-2 with respect to plant height (cm), number of branches (plant⁻¹), shoot and root dry matter (kg ha⁻¹) which were recorded at 30, 60 90, 120 days after sowing (DAS) and at harvest. Application of 25 kg P_2O_5 ha⁻¹ gave the highest values of all the growth attributes *viz.* plant height (60 cm), number of primary (5.3) and secondary (27.2) branches per plant, shoot dry matter (4000 kg ha⁻¹) and root dry matter (354 kg ha⁻¹) which were significantly higher than that of 0 and 15 kg P_2O_5 ha⁻¹ and at par with 20 kg P_2O_5 ha⁻¹. Similar results were observed in case of crop growth rate (CGR) whereas relative growth rate (RGR) was not influenced significantly by various biofertilizer and phosphorus treatments. The dual inoculation with PGPR strains along with phosphorus application have a supplementary effect on the growth of chickpea.

Keywords: Chickpea, growth indices, Mesorhizobium, phosphorus, shoot dry matter

INTRODUCTION

Chickpea (Cicer arietinum L.), also called Bengal Gram or garbanzo bean, is the third most widely grown cool season pulse crop in the world after common beans (Phaseolus vulgaris L.) and fieldpea (Pisum sativum L.) (Dotaniya et al., 2014). In India, chickpea is a premier pulse crop grown on area of 10.2 million ha during 2013-14, contributing 9.5 million tonnes to the national pulse basket with productivity of 967 kg ha⁻¹ (Anonymous, 2016). It holds an important role in the vegetarian diets due to its high protein content (16-20%), carbohydrates (51%), fat (25.8%), total dietary fibre, vitamins and minerals (Hirdyani, 2014). Chickpea is a winter season crop primarily grown in low rainfall areas. Diversified domestic, industrial and other uses of chickpea and its ability to grow better with low inputs under abrasive edaphic factors and arid environments make it an important component of the cropping system of subsistence farmers in the Indian subcontinent.

Biofertilizers are preparations containing living cells or latent cells of efficient strains of microorganisms, which when applied through seed or soil treatment, promote plant growth by increasing the nutrient acqui-

sition to the host plant. These help in enhancing biological nitrogen fixation through promotion of nodule formation, phosphorus solubilisation, production of phytohormones like cytokinins, gibberellins and indole acetic acid (IAA) (Verma et al., 2013) and also show antagonism against phytopathogens by production of siderophores, celluloses and antibiotics (Kaur and Sharma, 2013, Kandoliya and Vakharia, 2013) in chickpea. Seed inoculation with Rhizobium increases the nodulation and its dry weight which is beneficial in improving the growth attributes viz. plant height, root length and root dry weight and hence improves nutrient availability (Das et al., 2013 and Shahzad et al., 2014). The Rhizobium inoculation enhances the biological nitrogen fixation of the plant which not only improves the nitrogen nutrition of inoculated chickpea but also reduces the dependence on inorganic nitrogen fertilizers (Namvar et al., 2011).

Phosphorus (P) is recognized as the second most important element in plant nutrition after nitrogen. It is a key nutrient element required for high and sustained productivity of grain legumes. P is involved in several plant functions including energy transfer reactions, photosynthesis, transformation of sugars and starches and nutrient translocation within the plant (Dotaniya *et*

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al., 2014). It stimulates root development and enhances the development of reproductive parts (Gulpadiya and Chhonkar, 2014). Increasing P levels upto 40 kg P_2O_5 ha⁻¹ resulted in significant improvement in growth, yield attributes and grain yield as compared to lower doses of phosphorus (Pingoliya *et al.*, 2014).

The response to soluble phosphorus fertilizers is very low due to rapid P fixation (Sarawgi et al., 2012). Moreover, phosphatic fertilizers are not only costly but also their supply is lower than their demand. Hence, it is highly desirable to explore the possibilities of saving phosphatic fertilizers without sacrificing economic yields. In these conditions, biofertilizers could play a crucial role by increasing the availability of phosphorus and other nutrients to the crops. The plant growth promoting rhizobacteria (PGPR) inoculants hold great prospects for sustaining the crop production along with balanced P fertilizer application as of PGPR with Psolubilizing ability plays an important role in improving the soil P availability to plants by lowering soil pH and microbial production of organic acids as well as mineralization of fixed phosphorus (Singh and Singh, 2014). Therefore, the experiment was conducted with the aim of improving the performance of chickpea in terms of growth attributes of chickpea in a cost effective manner, employing graded levels of inorganic P fertilizer along with biofertilizers.

MATERIALS AND METHODS

Experimental site: A field experiment was conducted at research farm of Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during the *rabi* 2015-16. The soil of the experimental site was loamy sand in texture with pH of 7.5, low organic carbon (0.35%) and available N (123.8 kg ha⁻¹) and medium available P (15.4 kg ha⁻¹) and available K (177.5 kg ha⁻¹). The meteorological data during the crop period indicated that the total amount of rainfall received during crop season was 74.0 mm. Mean maximum and minimum air temperature was recorded to be 24.9°C and 11.5°C respectively.

Experimental design: The experiment comprised of four biofertilizer treatments [control, *Mesorhizobium* only, *Mesorhizobium* + RB-1 (*Pseudomonas argentinensis*) and *Mesorhizobium* + RB-2 (*Bacillus aryabhattai*)] and four levels of phosphorus (0, 15, 20 and 25 kg P_2O_5 ha⁻¹). The 16 treatment combinations were laid out in randomized complete block design and replicated three times in 5.5 m × 3 m plots. The chickpea cultivar 'PBG 7' was sown on 8 November 2015 at 30 cm row to row spacing. The seeds were inoculated with *Mesorhizobium* sp. *ciceri* (LGR-33), RB-1 (*Pseudomonas argentinensis*) and RB-2 (*Bacillus aryabhattai*), as per the treatments which were applied to the seed before sowing. RB-1 and RB-2 were plant

growth promoting rhizobacteria (PGPR). The microbial cultures were procured from the Microbiology laboratory of Pulses Section, Department of Plant Breeding and Genetics, PAU, Ludhiana. After the procurement of cultures, the sub culturing was done for 15 days and stored in refrigerator at 4° C in aerobic conditons. Further, P was applied as basal dose before sowing as per the treatments.In addition, 15 kg N ha⁻¹ in the form of urea was applied in all the treatments at sowing time. The periodic data of growth attributes were taken at 30, 60, 90, 120 DAS and at harvest.

Plant height: The height of five randomly selected plants from each treatment was measured from the ground surface up to the top of the main stem of plant. The initial observation was recorded at 30 DAS and then subsequently at 30 days interval till harvesting.

Number of branches: Five representative plants from each treatment were selected randomly and number of primary as well as secondary branches were counted, and then presented on plant⁻¹ basis. These were recorded periodically at 30 days interval, starting from 60 DAS and at harvesting.

Shoot dry matter accumulation: For shoot dry matter accumulation, the plants from half metre row length of one row from each treatment were harvested and sun dried and then dried in the oven at 60° C to a constant weight. The initial observation was recorded at 30 DAS and subsequently at 30 days interval till harvesting and represented in kg ha⁻¹.

Root dry matter accumulation: For root dry matter accumulation, the roots from half metre row length of one row from each treatment were taken and washed under running tap water using a sieve and afterwards sun dried. Then these were dried in the oven at 60°C to a constant weight. The initial observation was recorded at 30 DAS and subsequently at 30 days interval till harvesting and represented in kg ha⁻¹.

Growth indices: Crop growth rate (CGR) and relative growth rate (RGR) for each specified stage were calculated using the standard equations given by Radford (1967) given below:

CGR (g m⁻² day⁻¹) =
$$\frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{A}$$
 (1)
Where,

 $W_2 = Dry$ weight of crop plant at the time interval $T_2(g)$

 $W_1 = Dry$ weight of crop plant at the time interval $T_1(g)$

A = Ground area (m^2)

$$\operatorname{RGR}\left(\operatorname{g}\operatorname{g}^{-1}\operatorname{day}^{-1}\right) = \frac{Log_e W_2 - Log_e W_1}{T_2 - T_1}$$

Where,

(2)

 $W_2 = Dry$ weight of crop plant at the time interval T_2 (g)

 $W_1 = Dry$ weight of crop plant at the time interval T_1 (g)

Treatment	Plant height (cm)					
	30 DAS	60 DAS	90 DAS	120 DAS	At harvest	
Biofertilizers						
Uninoculated	15.9	25.1	50.0	59.8	61.0	
Mesorhizobium	17.5	28.5	54.3	62.7	65.2	
Mesorhizobium + RB-1	19.6	33.1	59.6	65.5	67.5	
Mesorhizobium + RB-2	18.9	32.9	58.7	64.7	66.6	
CD (p=0.05)	1.3	2.3	4.3	2.3	2.2	
$P_2O_5(kg ha^{-1})$						
0	16.0	25.4	49.5	56.9	57.7	
15	17.7	29.2	54.4	61.9	63.6	
20	19.0	32.1	58.7	66.0	68.6	
25	19.2	32.9	60.0	67.9	70.4	
CD (p=0.05)	1.3	2.3	4.3	2.3	2.2	

Table 1. Effect of biofertilizers and phosphorus on plant height of chickpea.

Table 2. Effect of biofertilizers and phosphorus on primary branches of chickpea.

Treatment	Number of primary branches plant ⁻¹					
	60 DAS	90 DAS	120 DAS	At harvest		
Biofertilizers						
Uninoculated	3.1	4.2	4.4	4.4		
Mesorhizobium	3.4	4.7	4.8	4.8		
Mesorhizobium + RB-1	3.7	5.4	5.6	5.6		
Mesorhizobium + RB-2	3.6	5.2	5.4	5.4		
CD (p=0.05)	0.2	0.4	0.3	0.3		
$P_2O_5(kg ha^{-1})$						
0	3.1	4.2	4.5	4.5		
15	3.4	4.7	5.0	5.0		
20	3.6	5.3	5.3	5.3		
25	3.7	5.3	5.3	5.3		
CD (p=0.05)	0.2	0.4	0.3	0.3		

Table 3. Effect of biofertilizers and phosphorus on secondary branches of chickpea.

Treatment	Number of secondary branches plant ⁻¹				
	60 DAS	90 DAS	120 DAS	At harvest	
Biofertilizers					
Uninoculated	8.7	20.2	22.6	23.4	
Mesorhizobium	9.5	21.4	25.1	26.0	
Mesorhizobium + RB-1	10.2	22.8	26.9	27.8	
Mesorhizobium + RB-2	10.2	22.2	26.3	27.1	
CD (p=0.05)	0.6	0.9	1.8	1.8	
$P_2O_5(kg ha^{-1})$					
0	8.6	19.5	22.1	22.9	
15	9.5	21.4	24.8	25.7	
20	10.2	22.6	26.8	27.6	
25	10.3	23.1	27.2	28.1	
CD (p=0.05)	0.6	0.9	1.8	1.8	

Table 4. Effect of biofertilizers and phosphorus on shoot dry matter accumulation of chickpea.

Treatment	Shoot dry matter accumulation (kg ha ⁻¹)					
	30 DAS	60 DAS	90 DAS	120 DAS	At harvest	
Biofertilizers						
Uninoculated	198	608	3239	4506	5667	
Mesorhizobium	251	716	3536	4872	6011	
Mesorhizobium + RB-1	288	858	3819	5267	6256	
Mesorhizobium + RB-2	284	813	3789	5131	6173	
CD (p=0.05)	23	58	244	362	313	
$P_2O_5(kg ha^{-1})$						
0	200	591	3008	4466	5630	
15	245	719	3525	4843	5936	
20	285	816	3850	5203	6208	
25	291	869	4000	5264	6333	
CD (p=0.05)	23	58	244	362	313	

Zorawar Singh <i>et al.</i>	J. Appl. & Nat.	Sci. 9 (3): 1422	2 -1428 (2017)
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Treatment	Root dry matter accumulation (kg ha ⁻¹)					
	30 DAS	60 DAS	90 DAS	120 DAS	At harvest	
Biofertilizers						
Uninoculated	99	182	305	272	212	
Mesorhizobium	118	205	329	301	236	
Mesorhizobium + RB-1	148	231	355	335	262	
Mesorhizobium + RB-2	144	227	350	331	257	
CD (p=0.05)	11	20	21	28	26	
$P_2O_5(kg ha^{-1})$						
0	107	181	304	276	211	
15	124	206	330	305	237	
20	137	228	351	328	258	
25	141	230	354	330	261	
CD (p=0.05)	11	20	21	28	26	

Table 5. Effect of biofertilizers and phosphorus on root	dry matter accumulation of chickpea.
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Table 6. Effect of biofertilizers and phosphorus on CGR of chickpea.

Treatment	$CGR (g m^{-2} day^{-1})$				
	30-60 DAS	60-90 DAS	90-120 DAS	120 DAS-Harvest	
Biofertilizers					
Uninoculated	1.37	8.76	4.18	3.91	
Mesorhizobium	1.55	9.39	4.77	3.47	
Mesorhizobium + RB-1	1.90	9.87	4.96	3.16	
Mesorhizobium + RB-2	1.76	9.91	4.04	3.54	
CD (p=0.05)	0.19	0.81	NS	NS	
P_2O_5 (kg ha ⁻¹)					
0	1.30	8.06	5.02	3.72	
15	1.58	9.35	4.69	3.34	
20	1.77	10.11	4.44	3.42	
25	1.93	10.43	4.17	3.59	
CD (p=0.05)	0.19	0.81	NS	NS	

Table 7. Effect of biofertilizers and phosphorus on RGR of chickpea.

Treatment	RGR (g g ⁻¹ day ⁻¹)					
	30-60 DAS	60-90 DAS	90-120 DAS	120 DAS- harvest		
Biofertilizers						
Uninoculated	0.016	0.024	0.005	0.003		
Mesorhizobium	0.015	0.023	0.005	0.003		
Mesorhizobium + RB-1	0.016	0.022	0.005	0.002		
Mesorhizobium + RB-2	0.015	0.022	0.004	0.003		
CD (p=0.05)	NS	0.001	NS	NS		
$P_2O_5(kg ha^{-1})$						
0	0.016	0.024	0.006	0.003		
15	0.016	0.023	0.005	0.003		
20	0.015	0.023	0.004	0.003		
25	0.016	0.022	0.004	0.003		
CD (p=0.05)	NS	NS	NS	NS		

The data were subjected to analysis of variance as per CPCS 1 software developed by Department of Statistics, Punjab Agricultural University, Ludhiana (Cheema and Singh, 1991) and the mean values were compared at 0.05 probability level.

RESULTS AND DISCUSSION

Plant biometery:

Plant height: The plant height was significantly affected by both microbial treatments as well as P levels (Table 1). At 30 DAS, the highest plant height was observed with *Mesorhizobium* + RB-1 (19.6 cm) which was statistically at par with *Mesorhizobium* +

RB-2 (18.9 cm) and significantly higher than control (15.9 cm) and sole treatment of *Mesorhizobium* (17.5 cm). This improvement in plant height may be attributed to phytohormone IAA production by PGPR strains (*Pseudomonas* sp. and *Bacillus* sp.) which plays a major role in regulation of plant development. Kaur *et al.* (2015) also reported improvement in plant height in consortium treatments as compared to sole treatment and control. Similar trend was observed at 60 and 90 DAS. Further, at all the sampling stages, the periodic plant height was found to be highest with 25 kg P₂O₅ ha⁻¹ which was statistically at par with 20 kg P₂O₅ ha⁻¹.

The increase in the height at decreasing rate was found after 90 DAS. Increment in plant height might be due to improved biological activities in the presence of balanced supply of phosphorus. These results are in agreement with the findings of other researchers (Basir *et al.*, 2008, Gulpadiya *et al.*, 2014).

Number of branches: At different intervals, the coinoculation with Mesorhizobium and RB-1 produced highest number of primary branches plant⁻¹ which was statistically at par with Mesorhizobium + RB-2 but significantly higher than individual inoculation of Mesorhizobium and control (Table 2). Similar trend was observed in number of secondary branches plant⁻¹ except at 120 DAS and at harvest where number of secondary branches with Mesorhizobium + RB-1 was at par with sole inoculation of Mesorhizobium (Table 3). Such improvement in number of branches plant⁻¹ might be related to the microbial production of phytohormones which cause changes in root morphology and physiology, resulting in increased nutrient uptake from soil which results in better growth and development of plants (Sharma et al., 2013). As compared to unfertilized control, differential levels of phosphorus dose produced higher number of primary and secondary branches plant⁻¹ at different periods of observation. At 60 and 90 DAS, application of 25 kg P_2O_5 ha⁻¹ gave significantly higher number of primary branches plant ¹ than 0 and 15 kg P_2O_5 ha⁻¹ while it was at par with 20 kg P_2O_5 ha⁻¹. At 120 DAS and at harvest, 15, 20 and 25 kg P_2O_5 ha⁻¹ were at par in primary branches plant⁻¹. Furthermore, no increment in the number of primary branches was observed after 120 days of sowing. At all the periods of observations, application of 25 kg P₂O₅ ha⁻¹ recorded significantly higher number of secondary branches plant⁻¹ than 0 and 15 kg P₂O₅ ha⁻¹ but at par with 20 kg P_2O_5 ha⁻¹ (Table 3). The progressive increase in number of branches might be attributed to the role of phosphorus in better root development and proliferation, nodules formation and N₂ fixation by supplying assimilates to the roots. These results are in agreement with those of Das et al., (2013) who also reported improvement in number of branches plant⁻¹ with incremental doses of phosphorus from 15 to 45 kg P_2O_5 ha⁻¹ in chickpea.

Shoot dry matter accumulation: At all sampling stages, the lowest shoot dry matter accumulation (SDMA) was recorded in control (Table 4). At 30 DAS, coinoculation of *Mesorhizobium*+ RB-1 gave maximum SDMA which was found to be significantly higher than *Mesorhizobium* only and control. Similar trend was observed at 60, 90 and 120 DAS but not at harvest where *Mesorhizobium*+ RB-1 and *Mesorhizobium* were statistically at par with each other. However, dual inoculation of *Mesorhizobium*+ RB-2 produced statistically similar shoot dry weight as with *Mesorhizobium*+ RB-1 at all the growth stages. The higher SDMA with dual inoculations may be attribut-

ed to the favourable synergistic effect of Mesorhizobium and PGPRs on plant growth through phosphate solubilisation and IAA and siderophore production. These results are in close proximity with those of Verma et al. (2013). Further, application of phosphorus showed a significant increase in SDMA at different periods of observations. At 30 DAS, highest SDMA was recorded with 25 kg P2O5 ha-1 which was significantly higher than 0 and 15 kg P_2O_5 ha⁻¹ and at par with 20 kg P_2O_5 ha⁻¹. The data regarding SDMA followed the similar trend at 60, 90 and 120 DAS and at harvest. Similar results were reported by Gulpadiya and Chhonkar (2014) who revealed that growth parameters of chickpea viz. plant height (45.85 cm), dry matter $(15.28 \text{ g plant}^{-1})$ and number of branches plant⁻¹ (4.4) increased significantly with incremental levels of phosphorus from 0 to 90 kg P ha⁻¹.

Root dry matter accumulation (RDMA): Dual inoculation of Mesorhizobium + RB-1 and Mesorhizobium + RB-2 produced statistically similar root dry matter and both were significantly better than Mesorhizobium only and control at all the sampling stages i.e. 30, 60, 90 and 120 DAS except at harvest where Mesorhizobium only produced statistically similar root dry weight as compared to Mesorhizobium + RB-2. These results cognate with those of Verma et al. (2013) who also reported that dual inoculation of Rhizobium sp. and Pseudomonas fluorescens as well as Rhizobium sp. and Bacillus megaterium gave significantly higher root dry weight in contrast to sole inoculation and control. The improvement in the RDMA might be attributed to synergistic effect of microbial inoculants on the root proliferation. At 30 DAS, application of 25 kg P_2O_5 ha⁻¹ gave highest root dry weight which was significantly higher than 0 and 15 kg P_2O_5 ha⁻¹ and at par with 20 kg P_2O_5 ha⁻¹ (Table 5). Similar trend was observed at 60 and 90 DAS. However, at 120 DAS and at harvest, 15, 20 and 25 kg P_2O_5 ha⁻¹ were at par in RDMA. The improvement in RDMA with phosphorus application may be attributed to essential role of phosphorus in the root development. Gulpadiya and Chhonkar (2014) also reported significant improvement in root weight with incremental dose of phosphorus from 0 to 60 kg P ha⁻¹.

Growth indices

Crop growth rate: Starting from the lower value, CGR reached a certain peak and then declined at the later stages; highest value of CGR was observed at 60-90 DAS (Table 6). The lower value of the CGR during the initial stages of growth might be due to slower growth of chickpea during the early stage and sudden increase in the growth during the period of 60-90 DAS resulted in higher CGR value, which might be due to conducive environmental conditions for chickpea growth. There was a variable trend in its values at different periods of observation in all the treatments and

significant changes with respect to biofertilizer treatments as well as phosphorus were observed at 30-60 and 60-90 DAS. The lowest value of CGR was observed in uninoculated and unfertilized control. During 30-60 DAS, the peak value of CGR was observed with Mesorhizobium + RB-1 which was significantly higher than control and Mesorhizobium only but at par with Mesorhizobium + RB-2 which might be attributed to better root and shoot development in the respective treatments (Table 4 and 5). However, during 60-90 DAS highest value of CGR was realized with Mesorhizobium + RB-1 which was found to be at par with Mesorhizobium only and Mesorhizobium + RB-2 but significantly higher than control. After that a sudden decline in the CGR was observed during the subsequent intervals of growth. As per the P levels, during 30-60 and 60-90 DAS, the peak value of CGR was observed with 25 kg P_2O_5 ha⁻¹ which was significantly higher than 0 and 15 kg P_2O_5 ha⁻¹ but at par with 20 kg P_2O_5 ha⁻¹ which might be attributed to better growth and development of plants with incremental levels of phosphorus. However, decline in the CGR values was observed after 90 DAS and treatments showed nonsignificant effect.

Relative growth rate: The relative growth rate showed variable trend in its values at different periods of observation in all the treatments and significant changes with respect to biofertilizer treatments were observed at 60-90 DAS (Table 7). During the initial stages of the crop growth the ratio between alive and dead tissues is high and almost the entire cells of productive organs are actively engaged in vegetative matter production. The results exhibited declining trend in RGR values with the advancement of the crop age after 90 DAS. Similar results were reported by Namvar et al., (2011) who noticed decline in the RGR value as the crop ages in chickpea which may be due to the fact that, with the ageing of the crop, the metabolic activity of tissues decreases and hence these tissues cannot contribute to the growth of the crop. Kour et al., (2016) also reported decline in the RGR value which may be attributed to increase in the dead and woody tissue as compared to alive tissue towards the maturity of crop.

Conclusion

The findings of this study indicate that application of microbial inoculants and phosphorus levels have pronounced effect on the growth attributes *viz.* plant height, number of primary and secondary branches, root and shoot dry matter accumulation as well as on growth indice sat different growth stages. Application of 20 kg P_2O_5 ha⁻¹ was found to be the appropriate dose and *Mesorhizobium* + RB-1 was superior among the biofertilizer treatments. The dual inoculation with PGPR strains along with phosphorus

application has a supplementary effect on the growth and development of chickpea.

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