Economic benefits of *Bradyrhizobium japonicum* inoculation and phosphorus supplementation in cowpea (*Vigna unguiculata* (L) Walp) grown in northern Tanzania

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Abstract

The field and screen-house experiments were conducted at Tanzania Coffee Research Institute (TaCRI) and Seliani Agricultural Research Institute (SARI) respectively to assess the yield and economic benefit of using *Bradyrhizobium japonicum* and phosphorus on cowpea production. The experiment was laid down in split plot design whereby main plots comprised of two inoculation treatments (with and without inoculation of rhizobia) and sub plots included application of phosphorus at four different levels (0, 20, 40, and 80 kgP/ha) and both experiments were replicated four times. The results showed that *B. japonicum* inoculation and supplementation of phosphorus significantly improved grain yield and yield components of cowpea. The economic analysis showed that the use of *B. japonicum* inoculants and phosphorus resulted into improved grain yield which is translated into improved economic benefit that farmers could attain. Therefore, the marginal rate of return of farmers can be improved by using less expensive biofertilizers such as *B. japonicum* and phosphorus in the production of cowpea.

***Keywords*.** Yield, number of pods, seed per pod, 100-seed weight, nodulation, BNF

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Introduction

Cowpea (*Vigna unguiculata* (L) Walp) is an important vegetable and grain legume crop, mostly grown by subsistence farmers in west and central sub-Saharan Africa. Its grain and stover are highly valued for food and forage (Timko et al., 2008). Cowpea is tolerant to moisture stress making it suitable for cultivation in semiarid areas. In Tanzania, cowpea is grown in almost all the areas below 1500m above sea level (Price et al., 1982). Normally, it is intercropped with cereals or other crops, though it is sometimes grown as a monocrop.

Phosphorus and nitrogen are the most important macronutrients for any plant growth and crop production. However, most soils of sub-Saharan Africa Tanzania inclusive, are deficient in these important macronutrients. Phosphorus deficiency is the most limiting soil fertility factor for cowpea production (Singh et al., 2011). Phosphorus is responsible for nodulation in legume, and thus higher nodulation results in higher nitrogen fixation by rhizobia and eventually the number of pods and seeds per plant (Singh et al., 2011). Phosphorus is required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid. It also aids in flower initiation, seed and fruit development (Ndakidemi and Dakora, 2007). A number of studies (Ndakidemi et al., 1998; Ndakidemi et al., 2006; Owolade et al., 2006; Rahman et al., 2008; Hussain et al., 2012; Ndor et al., 2012) have shown that grain yield and stover yield of legumes ware significantly recorded higher in plots supplied with different levels of phosphorus. Phosphorus application is also reported to significantly enhance number of nodules, nodules dry weight, nitrogen and phosphorus uptake of the cowpea (Stamford et al., 2006). Singh et al., (2011), also reported the significant effect of phosphorus on yield of legumes and concluded that 60 kg P_2O_5 ha^{-1} may not be the optimum as further application of phosphorus may or may not increase the yield of cowpea. Apart from influencing yield and yield attributes, phosphorus also enhances symbiotic association between rhizobia and the roots of host plant. Different researchers (Giller et al. 1998; Ssali and Keya 1986; Rotaru, 2010) have shown that symbiotic performance can be improved with adequate phosphorus application, resulting to high grain yield.

Nitrogen is also an important macronutrient for legume production. However, most legumes have potential of fixing their own nitrogen when they come in symbiotic association with nitrogen fixing bacteria such as rhizobia. Rhizobia are special bacteria that can live in the soil or in nodules formed on the roots of legumes. In root nodules, they form a symbiotic association with the legume, obtaining nutrients from the plant and producing nitrogen in a process called biological nitrogen fixation (BNF) (Uchida, 2000). Nitrogen fixation is one of the ways through which soil fertility can be improved (Mclaughlin, et al.,1990). The augmentation of rhizobial population through inoculation increased grain yields by 22.5% in treatments with Triple Superphosphate (TSP) and by 6.8% in non-TSP fertilized plots as reported by Onduru et al. (2008). Other workers reported that *Rhizobium* inoculation significantly improved the yield and all the other yield components such as number of pods.plant^{-1}, number of seeds.plant^{-1}, 100-seed weight, and seed yield compared with control (Bambara and Ndakidemi 2010). Other research findings demonstrate that *Rhizobium* inoculated plants gave significantly higher seed yield compared with un-inoculated control (Bhuiyan et al., 2009). The increase in seed yield could be due to high nodulation enhanced by phosphorus which results in high N_2 fixation and hence higher stover yield and seed yield. Rhizobia inoculation also increased nodules number in the field experiment conducted to investigate the performance of three soybean cultivars with five foreign *bradyrhizobia* strains in different regions in Nigeria (Okereke et al., 2001).

The combination of rhizobia inoculation and phosphorus supplementation in legume production may improve production (Ndakidemi et al., 2006) and reduce the problem of hunger, poverty and poor health of most rural population of sub-Saharan Africa (SSA) who cannot afford high price of inorganic nitrogenous fertilizers. Rhizobium inoculation of cowpeas (*Vigna unguiculata* (L.) Walp.) and application of phosphorus is reported to contribute in arresting declining soil fertility and improving farm agro-economic performance in terms of major economic indicators (gross margins, net cash income and return to labour) (Onduru et al., 2008). The results were in the order Rhizobium + TSP > TSP >Rhizobium > control. Other report by Ndakidemi et al., (2006), shows that the combined application of bacterial inoculants and phosphorus fertilizer to field legume plants of soybean and common bean significantly increased grain yield compared with the single use of nitrogen and phosphorus or *(brady)rhizobial* strains. The increased grain yield with inoculation was then translated into a significantly higher marginal rate of return and dollar profit for soybean and common bean farmers in northern Tanzania.

Thus, basing on the fact that inoculation of rhizobia and supplementation of phosphorus increases nitrogen and phosphorus levels in the soil, their application may play key roles on yield and economic benefits of cowpea grown in poorly depleted soils in Northern Tanzania. Therefore, their influence on the yield and economic benefits and the optimum phosphorus application rate per unit area needs to be investigated and documented in northern Tanzania.

MATERIAL AND METHODS

Description of Location

Field and pot experiments were conducted at two different locations from mid March to late July 2013. A screen house experiments was conducted at Seliani Agricultural Research Institute (SARI) which is situated in an area which is 1390m above the sea level in Arusha, Tanzania of latitude 3°21’50.08’’S and longitude 36°38’06.29’’E. The field experiment was conducted at the Tanzania Coffee Research Institute which is situated in an area which is 1390m above the sea level in Kilimanjaro region, Tanzania of latitude (3˚14’44’’)S and longitude (37˚14’48)E. The field experiment was conducted in an area with bimodal rainfall pattern and mean annual rainfall of 1200mm.

Experimental design

The experiment was laid out in a split plot design. The main plots comprised two inoculation treatments viz. i) with inoculation of *B. japonicum* and ii) without inoculation of *B. japonicum.* Sub plots contained application of phosphorus (TSP) at four different levels (ie. 0, 20, 40, and 80 kgP.ha^{-1}). Both screen house and field experiments were replicated four times. The *B. japonicum* inoculants were purchased from MEA Fertilizer Company in Nairobi – Kenya. The inoculants packets were supplied with gum Arabic for sticking as many cells as possible into the cowpea seeds.

Field and Screen house experiment

The crop plant used for this experiment was Cowpea (*Vigna unguiculata* (L) Walp) supplied by the breeder from Sokoine University of Agriculture, Morogoro, Tanzania. Three (3) gram of gum Arabic was added to two tablespoonful of water and mixed to form a solution. 1 kg of cowpea seeds was weighed and 2 tablespoonful of gum Arabic solution was added and mixed well. 10 gm of legume inoculants was added and mixed well so that all seeds are coated. The inoculated seeds were put under shade to dry and the seeds were then sown immediately in a wet moist soil. The soil for screen house experiment was collected from the site where field experiment was conducted. The soil was packed into 4 kg Pots where four seeds were germinated in each pot, and later thinned to two after germination and uniform established. The field was ploughed and harrowed by using tractor before planting. The crop was seeded at a spacing of 50cm by 20cm, where the plot size was 4m by 3m. In the field trial, three seeds were seeded per hill and then thinned to two plants. The plots were weeded twice where the first weeding was done two weeks after emergence and the second weeding was done just before flowering. Each plot comprised of six rows. Data were collected from the four middle rows. Both screen house and field experiment were planted at the mid of March 2013, and closely monitored from this point until physiological maturity for field, and pod formation for screen house experiment.

Yield and yield component data

Yield and yield components of cowpea measured were: number of branches per plant, seed yield per ha, number of pods per plant, number of seeds per pod and mean pod weight. At 50% pod formation, plants were randomly sampled from two rows near border and middle rows of each plot for assessment of number of nodules. At physiological maturity, the plants in the two central rows of each plot were counted and harvested for assessing grain yield. The border plants within each row were excluded. For yield components, 10 plants were sub-sampled from each plot to determine the number of pod per plant and number of seeds per pod. All pods were manually threshed and allowed to dry to 13% moisture content. Grain yield was determined for each plot and 100-seed weight recorded.

Estimation of economic benefits

To compare the profit of treatments used, the simple economic analyses were carried out in which the profit or marginal net return (MNR) was computed for each treatment as shown bellow.

MNR = $Y \times P$ –TVC,

Where Y is grain yield of cowpea crop (kg/ha), P is selling price of crop at harvest (USD/kg), and TVC is the total variable cost or cost of inputs related to the treatment (e.g. fertilizer, seeds, labour, etc. in USD/ha). The cost of inputs and labour charges of harvested cowpea crops used in calculating MNR and MRR are shown in (Tables 1). The selling price of the cowpea grain at harvest was USD 1.6/kg. The marginal rate of return (MRR) for each treatment was calculated using the formula:

MRR = MNR/TVC

Table 1. Variable costs or cost of inputs and labour charges for various farm operations used in the calculation of profits

Cost of Inputs				Labour Charges.	
Input	**Amount/ha**	**Unit price (USD)**	**Total cost (USD /ha)**	**Activity**	**Cost or charges (USD/ha)**
Seeds	5 kg	1.6	8	Land Preparation	93.8
Inoculants	5 Packet	0.94	4.7	Planting and Fertilizer Application	46.9
Pesticides	2 Litre	12.5	25	Weeding	62.5
Phosphorus	0 kgP/ha	2.2	0	Pesticides Application	31.25
	20 kgP/ha	2.2	44	Harvesting	31.25
	40 kgP/ha	2.2	88		
	80 kgP/ha	2.2	176		

STATISTICAL ANALYSIS

The statistical analysis was performed using the 2-way analysis of variance (ANOVA) in factorial arrangement, with the computations being performed with the software program STATISTICA. The fisher's least significance difference (L.S.D.) was used to compare treatment means at p=0.05 level of significance (Steel and Torrie, 1980).

RESULTS

Effects of *B. japonicum* inoculation on number of nodules, yield and yield components of cowpea

Table 2 shows that *B. japonicum* inoculation had significant effect on the nodule count per plant and number of branches per plant over un-inoculated control in both screen house and field experiment. For example, *B. japonicum* inoculation significantly improved the number of branches per plant by 37.2% for screen house experiment and 28.8% for the field experiment relative to control treatment. *B. japonicum* inoculation also increased the nodule count by 26.5% in the screen house and 19.8% for the field experiment compared with control treatments (Table 2). In addition, *B. japonicum* inoculation significantly improved all the yield and yield components measured in this study. For example, *B. japonicum* inoculation statistically increased the number of pod per plant by 13.7%, number of seeds per pod (11.6%), mean pod weight (24.6%), 100 seed weight (8.5%), grain yield in kg per plot by 26.7% and grain yield in kg per hector by 12.44% in the field experiment over control (Table 2).

Effects of phosphorus supplementation on number of nodules, yield and yield components of cowpea

The findings of this study (Table 2) shows that phosphorus supplementation had significant positive effect on all yield and yield component of cowpea grown under screen house and field condition relative to control. It is well indicated in Table 2 that phosphorus supplementation significantly improved the grain yield per plot, grain yield per hector, number of pods per plant, number of seeds per pod, mean pod weight and 100 seed weight in the field experiment compared with control. Furthermore, phosphorus supplementation significantly increased the number of branches per plant, and number of nodules per plant in both the screen house and field experiment relative to control. For example, for every parameter measured, supplementation of phosphorus at 40 and 80 kgP/ha produced greater values of the above mentioned yield components over other treatments but numerically 80 kgP/ha produced greater values of all parameters measured relative to other treatments (Table 2).

Table 2: Effects of rhizobia and phosphorus on nodule formation, grain yield and yield components of cowpea (*Vigna unguiculata* (L) Walp) grown under field and screen house condition

Treatments	Screen House		Field							
	Branches/ Plant	Nodule Count/Plant	Branches/Plant	Nodule Count/Plant	Pods/Plant	Seed/Pod	Mean Pod Weight (gm)	100 seed weight (gm)	Grain Yield (Kg/Plot)	Grain Yield (Kg/Ha)
Rhizobium										
-R	2.69±0.0b	27.19±1.8b	5.0±0.0b	14.39±1.4b	9.44±0.4b	9.99±0.3b	0.6±0.02b	8.83±0.2b	0.543±0.03b	490.86±18.0b
+R	3.69±0.0a	34.41±2.8a	6.4±0.0a	17.24±1.1a	10.7±0.5a	11.2±0.3a	0.7±0.05a	9.58±0.2a	0.688±0.03a	551.94±24.2a
P Levels Kg.ha^{-1}										
0	2.25±0.0c	21.06±1.0c	3.5±0.0c	11.50±1.7b	8.53±0.5b	9.43±0.2c	0.5±0.03c	8.42±0.3b	0.452±0.02d	441.43±23.2b
20	3.13±0.0b	28.94±2.5b	5.6±0.0b	14.75±1.7ab	9.9±0.7ab	10.3±0.3b	0.6±0.04bc	9.2±0.2ab	0.570±0.03c	514.7±29.3ab
40	3.50±0.0ab	32.44±3.8b	6.8±0.0a	18.35±1.1a	10.8±0.7a	10.6±0.4b	0.7±0.07ab	9.58±0.3a	0.693±0.03b	546.05±23.1a
80	3.88±0.0a	40.75±2.1a	7.0±0.0a	18.66±1.7a	11.1±0.7a	11.9±0.4a	0.7±0.06a	9.63±0.3a	0.747±0.04a	583.45±30.8a
2 – Way ANOVA (F-Statistics)										
R	21.33***	12.03**	58.78***	4.47*	4.27*	15.41***	10.40**	8.25**	87.24***	5.47*
P	10.33***	15.39***	72.41***	6.27**	3.47*	11.58***	5.17**	4.55*	72.21***	5.32**
R*P	2.00ns	2.07ns	0.11ns	3.28*	0.09ns	0.47ns	0.82ns	0.02ns	2.02ns	0.02ns

+R: With rhizobia; **-R:** Without rhizobia; **R:** rhizobia; **P:** Phosphorus; Values presented are means ± SE; *;**;***; significant at $p \le 0.05$, $p \le 0.01$, $p \le 0.001$ respectively, **ns** = not significant, **SE** = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at $p=0.05$ according to Fischer least significance difference (LSD)

Economic benefits of using *B. japonicum* inoculants and phosphorus in the production of cowpea.

The economic analysis was done and the results presented in Table 3, showed that *B. japonicum* inoculation and phosphorus supplementation on cowpea grown under field conditions resulted into greater economic benefits relative to control treatments. For example, *B. japonicum* inoculation on cowpea have resulted into greater profit of 495.2 US$ relative to un-inoculated treatments which gave 307.4US$ and ultimately resulted into higher percentage increase over control and marginal rate of return. Supplementation of phosphorus (40 kgP/ha) resulted into greater profitability of cowpea over all other treatments. The same results were also observed in percentage increase over control. However, marginal rate of return was significantly higher in plots supplied with 20 kgP/ha over other treatments (Table 3).

Interactive effect between *B. japonicum* inoculation and Phosphorus on the number of nodules

Fugure 1 shows the interactive effect between *B. japonicum* and phosphorus on the number of nodules counted at 50% pod formation in the field experiment. From results presented in Figure 1, it is clear that phosphorus supplementation without *B. japonicum* produced greater number of nodules at 40 and 80 kgP/ha relative to other treatments. Supplementation of phosphorus with *B. japonicum* significantly resulted in improved number of nodules relative to phosphorus supplementation without *B. japonicum*. Most importantly, the plots that were not supplied with phosphorus (control treatments) whether inoculated or not produced less number of nodules over other phosphorus treatments. However, 0 kgP/ha with *B. japonicum* gave greater number of nodules compared with 0 kgP/ha without *B. japonicum* (Figure 1).

Table 3. Economic profit (MNR), total variable costs(TVC) and marginal rate of return (MRR) of cowpea under different treatments

Treatment	Profit /MNR (USD/ha)	Increase over control (%) (USD/ha)	TVC (USD/ha)	MRR
Rhizobia				
-R	307.4±20.7b	–	424.4	0.8±0.06b
+R	495.2±24.8a	68.2±9.8a	419.7	1.2±0.07a
P Levels Kg.ha^{-1}				
0	303.1±30.1c	–	301.05	1.0±0.09b
20	415.6±42.5b	43.7±15.2ab	345.05	1.2±0.12a
40	493.8±38.8a	69.8±13.0a	433.05	1.1±0.09ab
80	392.9±51.4b	29.5±9.4bc	609.05	0.6±0.84c

+R: With rhizobia; **-R:** Without rhizobia; **R:** rhizobia; **P:** Phosphorus; **SE** = standard error; Values presented are means (± SE); Means followed by dissimilar letter(s) in a column are significantly different from each other at *p*=0.05 according to Fischer least significance difference (LSD)

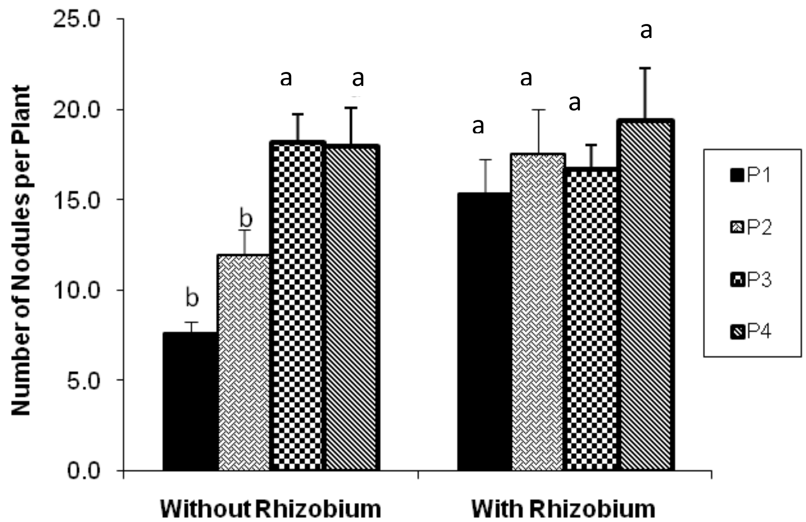

Figure 1. Interactive effects of Rhizobia and Phosphorus (P) on the number of nodules per plant counted at 50% pod formation. P1 = Control, P2 = 20 KgP.ha^{-1}, P3 = 40 KgP.ha^{-1}, P4 = 80 KgP.ha^{-1}. Bars followed by similar letter(s) are not significantly different.

DISCUSSION

The field and screen house experiment was carried out in 2013 at northern Tanzania to determine the yield and economic benefits of using phosphorus (TSP) and *B. japonium* inoculation in the production of cowpeas (*Vigna unguiculata* (L) Walp). The results from this study revealed that *Rhizobium* inoculation had significant effect on different yield and yield components of cowpeas. The significant improvement in the yield components in rhizobial inoculated treatments is an indication that the *B. japonicum* strain used in this study was efficient in fixing nitrogen and provided cowpea with nitrogen the same as nitrogenous fertilizer would provide and resulted in improved yield and yield components over the control. Similar to our findings, other studies (Chowdhury et al., 1983; Peoples et al., 1995; Ndakidemi et al., 1998 and 2006; Teymur et al., 2012;

Gicharu et al., 2013) have reported the significant increase in grain yield, number of nodules, and nodule fresh weight in legumes following inoculation with rhizobium. Furthermore, other workers (Bambara and Ndakidemi, 2010) have reported the significant increase on yield and all other yield components such as number of pods per plant, number of seeds per plant, 100-seed weight, and seed yield following rhizobia inoculation. However, in the current study number nodules were observed in the control treatments which signify that there was native rhizobia in the soil which nodulated the cowpea. This also signifies that the cowpea were promiscuous variety which can be nodulated by any indigenous rhizobia in the soil. Gwata et al. (2004) have shown that promiscuous genotype of soybean produced heavy nodule dry matter than non promiscuous. Other workers (Ndakidemi et al. 1998) reported significant improvement of common bean yields in un-inoculated treatments following phosphorus supplementation, and attributed it to the presence of efficient indigenous rhizobia in the soil.

Phosphorus supplementation also had significant effect on the number of branches per plant, number of nodules, number of pods, number of seeds per pod, mean pod weight, 100 seed weight and grain yield in the phosphorus treated cowpeas over the control. The improved components of cowpea yield over control may be attributed to the availability of adequate phosphorus which is essential for nodulation, photosynthesis, pod development and grain filling in leguminous crops and improves seed quality (Okeleye and Okelana, 1997; Mokwunye and Bationo, 2002). Therefore, the higher nodulation resulted in higher nitrogen fixation and ultimately the yield and yield components of cowpea. Similar to this study, other studies (Ndakidemi et al., 2006; Zafar et al., 2011) have shown significant improvements on the yield and yield components of common bean such as number of pods per plant, grain yield following phosphorus supplementation over the control treatment.

Increased yield parameters in the current study agrees with other recent studies (Ankomah et al. 1995; Bolland et al. 2001; Magani and Kuchinda, 2009; Ndor et al. 2012) who reported that phosphorus fertilizer significantly affected the entire yield parameters such as number of pod per plant, pods weight per plant, and seed weight per hector over control treatment. This creates an attention that phosphorus is still an important macro element for improving yield of legumes in Tanzania and Africa at large.

The increased grain yield due to inoculation and phosphorus supplementation are reflected in the economic benefits and marginal rate of return. The economic analysis showed that *B. japonicum* inoculation on cowpea is very profitable and the technology is feasible to farmers who may adopt it. With regard to phosphorus supplementation, also there was a significantly higher marginal net return and as a result the percentage increase over control was also improved. The results of current study showed that at 40 kgP/ha farmers may get higher profit relative to all other rates. The percentage increase of net profit (US$) over control increased from 43.7 (20 kgP/ha) to 69.8 (40 kgP/ha) and then declined to 29.5 (80 kgP/ha). This decline may be due to high total variable costs incurred in plots supplied with 80 kgP/ha. The findings of this study agree with Ndakidemi et al., (2006) who reported that both inoculation and phosphorus supplementation resulted in high dollar profit for common bean and soybean farmers in northern Tanzania.

The current study also recorded a significant interaction between *B. japonicum* and phosphorus on the number of nodules per plant (Figure 1). The results in Figure 1 indicated that phosphorus supplementation without *B. japonicum,* produced greater number of nodules at 40 and 80 kgP/ha relative to other treatments. This shows that phosphorus is important in enhancing nodulation, symbiotic association between rhizobia and host plant and consequently improved N_2 fixation. Supplying phosphorus with *B. japonicum* significantly resulted in improved number of nodules compared with supplying phosphorus without *B. japonicum*. Most interestingly, the control treatment (0 kgP/ha) with or without *B. japonicum* produced fewer number of nodules over other phosphorus treatments which still stress the importance of phosphorus in legumes production. However, inoculated plots without phosphorus fertilization (0 kgP/ha) gave greater number of nodules compared with un-inoculated and un-fertilized plots (Figure 1).

In conclusion, inoculation of cowpea with *B. japonicum* and supplementation with phosphorus resulted into increased number of nodules, grain yield and yield components. The increased grain yield is translated into economic benefits to farmers. The economic analysis indicated that *B. japonicum* inoculated plots significantly ($p \leq 0.05$) increased the marginal net return (net profit) over un-inoculated treatments. The same results were observed in phosphorus fertilized plot, where 40 kgP/ha produced greater marginal net return over all other treatments. From this study we can therefore conclude that for maximum profit of cowpea in northern Tanzania, inoculation of cowpea seeds with *B. japonicum* and supplementation of phosphorus at the rate of 40 kgP/ha is significant.

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