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Comparative yield analysis of two varieties of *Phaseolus vulgaris* L. in *Rhizobium* inoculated post-solarized soil

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ABSTRACT

To exploit the maximum benefits of an agronomical technique, it is imperative to ascertain with some degree of certainty how varieties of plant will respond to its application. Consequently, the influence of *Rhizobium* inoculation on the yield of two varieties of *P.vulgaris* (white and brown) in post-solarized soil were investigated by subjecting soil to these treatments: unsolarized uninoculated (control), unsolarized inoculated, solarized uninoculated and solarized inoculated with *Rhizobium*. Thereafter planting of viable seeds of *P. vulgaris* commenced 2 weeks after treatments and observed for a period of 4 months to determine the effect of treatments on germination, plant heights, root lengths, fresh weights, nodule and pod numbers as well as pod lengths in both varieties. Solarization temperature ranged between 29.0 and 53.0 ^{0}C. Treatments had no significant effects on plant germination in the two varieties. However, Fresh weights, nodule and pod numbers differ significantly with treatment: solarized inoculated > unsolarized inoculated > unsolarized uninoculated > solarized uninoculated in both varieties ($P < 0.05$). The longest pod lengths (11.5 and 11.0 cm) and plant heights (142.5 and 141.7cm) were recorded in solarized inoculated, while the shortest pod lengths (8.0 and 7.5 cm) and heights (124.0 and 123.3 cm) in solarized uninoculated soils for white and brown varieties respectively. Solarized uninoculated soils also recorded the shortest root lengths. While response of the two varieties of *P. vulgaris* to each of the treatments did not differ significantly ($P < 0.05$), their yield were enhanced by *Rhizobium* inoculation in the post-solarized soil.

INTRODUCTION

Phaseolus vulgaris is an important member of the legume family, which provides significant alternative dietary protein to man and animals in the absence of meat (Grandawa 2014). The white and brown varieties of *P. vulgaris* are among the most propagated beans in Nigeria. However, with the ever increasing human population and food demand, prohibitive cost of inorganic fertilizers and their accompany surface and groundwater pollution through leaching and run-off arising from excessive fertilization; there is need to proactively search for cheap and environmentally friendly way of improving crop growth and yield. Furthermore, to exploit the maximum benefits of an agronomical technique, it is imperative to ascertain with some degree certainty how different varieties of plant will respond to its application.

Soil solarization has been reported to improve plant overall growth (Chauhan et al. 1988; Elmore et al. 1997; Pokharel 2011). However, this economical and environmentally friendly non-pesticidal method of managing soil-borne plant diseases and weeds has been reported to affect the population of *Rhizobium* in soil. This important group of nitrogen fixers is highly sensitive to elevated temperatures which decimate their population (Chauhan et al. 1988; Linke et al. 1991; Pokharel 2011) thus, reducing nodulation and the ability to efficiently fix nitrogen critical for robust growth and yield of legumes.

While there are enormous body of evidence in literature that *Rhizobium* inoculation in soil brings about improve legume yield; microbial soil inoculants are confronted with the challenge of competing favorably with well adapted native soil microflora in addition to resisting protozoan's

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predation Bashan (1998). Post-solarization inoculation may therefore provide an avenue to overcoming this challenge. It is on this basis that the influence of *Rhizobium* inoculation on the yield of two varieties of *P.vulgaris* (white and brown) in post-solarized soil was investigated.

MATERIALS AND METHODS

Physicochemical Analysis of Experimental Soil

Experimental soil was first analyzed for baseline physicochemical properties before the commencement of treatments. The percentage content of nitrogen (N) in soil was analyzed in adherence to the procedure of micro-Kjeldahl (van Reeuwijk 2002). Hydrometer method (Aliyu and Oyeyiola 2011) was employed in textural component analysis of soil. Soil was also analyzed for porosity, using the method of Ezzati et al. (2012); while pH, was analyzed using the method of Hendershot et al. (2006). Furthermore, phosphorus was analyzed through the method of Skjemstad et al. (2006); and total organic carbon using the method of van Reeuwijk, (2002).

Preparation of Soil for Plant Propagation

Soil meant for plant propagation was taken within the depth of 0 to 15 cm of soil vertical profile. In order to cultivate each of the varieties of *P. vulgaris*, 4.0 kg of soil was put in 12 different plastic bowls (with a dimension of 20 cm depth and 22 cm width). Solarization of soil in six of the bowls was carried out for 16 days, while soil in the remaining six bowls was left unsolarized.

Solarization of soil was done between the months of late November and early December by covering moisturized soil with sheets of transparent polyethylene of 0.025 mm thickness and made to stay in open glare of the sun. Soil was completely covered preventing any form of heat escape with the transparent sheets making direct contact with soil. Temperature of soil was taken at constant intervals in the morning and evening within the period of solarization with the help of an inserted thermometer to the depth of 10 cm.

Isolation of *Rhizobium* from Nodules of *P. vulgaris*

Rhizobium intended for scale-up operation and inoculation of soil were first isolated from root nodules of the two varieties of *P. vulgaris*. Adapting the methods of (Deka and Azad 2006) and (Ben-Gweirif et al. 2005) well developed nodules (big and pinkish) were detached from roots and washed thoroughly in tap water before sterilizing surface with 70 % ethanol for 2.0 minutes. Nodules were subsequently rinsed in distilled sterile water. Sodium Hypochlorite (3.5 % w/v) was then employed for further sterilization of nodule surface for 2.0 minutes followed by sequential rinsing with sterile distilled water (thrice), after which nodules were crushed in few drops of sterile water in Mac Cartney bottle.

Loop-full of crushed nodules were collected and streaked on yeast extract manitol agar (YEMA) to which congo red was incorporated in petri dishes and incubated for 72 hours at30 ± 2 ^{0}C (room temperature). Smooth, viscous, circular edge, musky odour, raised and convex colonies which take up incorporated dye weakly were regarded as colonies of *Rhizobium*. These were picked up from plates, purified, identified and stored in YEMA slant as stock culture for scale-up operation.

Characterization of Isolated *Rhizobium*

Isolated *Rhizobium* from root nodules of the two varieties of *P. vulgaris* were characterized based on cultural, morphological, and biochemical features in line with Bergey's Manual of Determinative Bacteriology John et al. 1994).

Scaling up *Rhizobium* Population and Inoculation of Solarized and Unsolarized Soil

Twenty four hours after solarization, each of the three solarized bowls and three of unsolarized bowls of soil were inoculated with *Rhizobium* previously isolated from the root nodules of *P. vulgaris*.

Pure culture of isolated *Rhizobium* was first scaled-up in a 2 L yeast extract manitol broth (YEMB) in a 4 L jerry can (previously sterilized using 70 % ethanol) following transfer from a 200 ml YEMB culture in a 500 ml conical flask. Cells were harvested through centrifugation of culture aliquot in test tubes at the speed of 4000 rev/min. for 5 minutes. Harvested *Rhizobium* cells were collected in a sterile 4 L jerry can and made up to 3 L mark using normal physiological saline at the rate of 8.5 x 10^8 cfu/ml. Each of the respective soil- containing bowls was then inoculated with *Rhizobium* in a 500 ml of physiological saline at the above stated rate. Inoculum was then properly worked into to soil using a sterile hand trowel.

Selection of Healthy Seeds

Healthy seeds of *P. vulgaris* from white and brown varieties obtained from a local market in Nigeria were selected before propagation. Healthy seeds were selected using floatation method by soaking seeds in lukewarm water for 12 hours. Thereafter submerged seeds were employed for propagation and floating seeds regarded as not healthy were discarded.

Soil Treatments and Plant Propagation

Soil was subjected to the following treatments in triplicates: Unsolarized uninoculated (control), Unsolarized inoculated, Solarized uninoculated and Solarized inoculated soil with *Rhizobium* in two sets (one set for white and the other for brown varieties of *P. vulgaris*). Inoculated soil was regularly watered before planting.

Propagation of legume was carried out two weeks after inoculating soil with *Rhizobium*. Five viable seeds were planted in each bowl maintaining a depth of 2.0 cm. After germination thinning was done ensuring the best growth stay (two stands per bowl). Propagated plants were observed for 120 days. Within this period, plants were regularly watered; ensuring soil was not flooded during watering process.

Determining the Effect of Treatments on Germination in Two Varieties of *P. vulgaris*

The effect of treatments on germination time was evaluated by taking note of the earliest time for the appearance of shoots in the respective treated soils. On the other hand percentage germination was assessed using the formula:

$$\text{Percentage germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of propagated seeds}} x\ 100$$

Determining the Influence of Treatments on Nodule and Pod Formation in Two Varieties of *P. vulgaris*

Treatments effects on nodule and pod numbers per plant as well as pod lengths in the two varieties of *P. vulgaris* were assessed after 4 months of planting. Slow running tap water was employed in washing the roots of uprooted legume ensuring no damage to roots. The number of nodules per plant in the two varieties was determined by counting after air-drying roots for 30 minutes. Similarly, pod numbers per plant determined by counting, while that of pod length by measuring with graduated meter rule.

Determining the Influence of Treatments on the Fresh Weight, Height and Root Length Growth in the Two Varieties of *P. vulgaris*

The influence of treatments on the fresh weights, height and root lengths in the two varieties of *P. vulgaris* was evaluated at day-120 of legume propagation. In order to assess legume weights, plant was uprooted, roots washed with slow running tap water to free roots of any adhering soils, allowed to air-dry and weight taken using an electronic weighing balance.

Furthermore, the influence of treatments on growth with regards to height and root lengths were assessed by taking measurement with a calibrated meter rule from base of plant to the tallest leaf tip and from the tip of the longest root to base of the plant for height and root length growth respectively. To prevent root damage, roots were dislodged from soil by first soaking roots together with soil in water to loosen roots from soil. Thereafter, the remaining soil adhering to roots were removed by flushing gently in a slow running tap water.

Data Evaluation

Numerical data gathered from this investigation were analyzed deploying statistical tools such measure of central tendency and dispersion, Student's *t*-test and analysis of variance ($P < 0.05$).

RESULTS

Baseline Physicochemical Properties of Experimental Soil

Reference data on the physicochemical characteristics of the soil engaged in this study indicated the soil possess a reasonable nitrogen content and porosity suitable for legume cultivation. The soil may also be classified as loamy sand with neutral pH (Table 1).

Table 1. Baseline physicochemical properties of experimental soil

Soil Properties	Values
Texture	
Sand (%)	76.0
Silt (%)	16.0
Clay (%)	8.0
pH	7.1
Water Holding Capacity (%)	69.0
Nitrogen (%)	0.09
Phosphorus (mg/kg)	0.8
Organic Carbon (%)	1.4
Organic Matter (%)	1.6

Solarized Soil Temperature

Soil temperature in this study ranged between 29.0 and 53.0 ^{0}C during the 16 days period of solarization with a clear-cut higher temperature readings in the evening hours than that of the morning hours (Figure 1).

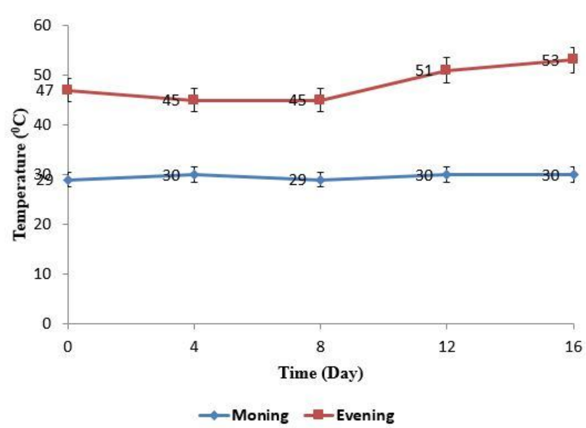

Figure 1. Temperature fluctuation within the period of soil solarization

Effect of Treatments on Germination in Two Varieties of *P. vulgaris*

Germination time in the two varieties of *P. vulgaris* was not affected by the various treatments applied (P < 0.05). Recorded time of germination ranged from 3.0 to 3.7 and 3.0 to 3.3 days for white and brown varieties respectively for all the treatments applied (Table 2). In a similar trend, percentage germination in the two varieties was not significantly affected by the various treatments applied (P < 0.05). The percentage germination was in the range of 86.7 to 93.3 and 80.0 to 93.3 % for white and brown varieties respectively for all the treatments applied (Table 3).

Table 2. Effect of treatments on germination time in different varieties of *P. vulgaris*

Treatment	Germination Time (Days)	
	Variety	
	White	Brown
Unsolarized uninoculated	3.7 ± 0.6^a	3.3 ± 0.6^a
Unsolarized inoculated	3.3 ± 0.6^a	3.0 ± 0.0^a
Solarized uninoculated	3.3 ± 0.6^a	3.3 ± 0.6^a
Solarized inoculated	3.0 ± 0.0^a	3.3 ± 0.6^a

Values with the same superscript alphabet in the same column did not differ significantly. (P < 0.05)

Table 3. Influence of different treatments on percentage germination in different varieties of *P. vulgaris*

Treatment	% Germination	
	Variety	
	White	Brown
Unsolarized uninoculated	86.7 ± 11.5^a	80.0 ± 0.0^a
Unsolarized inoculated	93.3 ± 11.5^a	86.7 ± 11.5^a
Solarized uninoculated	86.7 ± 11.5^a	86.7 ± 11.5^a
Solarized inoculated	93.3 ± 11.5^a	93.3 ± 11.5^a

Values with the same superscript alphabet in the same column did not differ significantly. (P < 0.05)

Influence of Treatments on Nodule and Pod Formation in Two Varieties of *P. vulgaris*

In both white and brown varieties of *P. vulgaris*, nodule numbers varied significantly with the treatment applied (P < 0.05). The lowest nodule numbers was recorded in Solarized uninoculated (8.0 and 7.3 per plant), while the highest nodule numbers in the Solarized inoculated soil (29.7 and 24.3 per plant for white and brown variety respectively (Table 4).

In the same vein, pod formation varied significantly with the treatment applied (P < 0.05). The lowest pod numbers was recorded in Solarized uninoculated (2.5 and 2.0 per plant), while the highest pod numbers in the Solarized inoculated soil (8.3 and 7.4 per plant for white and brown variety respectively (Table 5).

Table 4. Influence of treatments on nodule formation in different varieties of *P. vulgaris*

Treatment	Nodules/plant	
	Variety	
	White	Brown
Unsolarized uninoculated	16.0 ± 3.6^a	10.0 ± 1.2^a
Unsolarized inoculated	24.7 ± 5.5^b	20.3 ± 3.5^b
Solarized uninoculated	8.0 ± 1.0^c	7.3 ± 0.6^c
Solarized inoculated	29.7 ± 4.6^d	24.3 ± 3.5^d

Values with the same superscript alphabet in the same row and column did not differ significantly. (P < 0.05)

Table 5. Influence of treatments on pod formation in different varieties of *P. vulgaris*

Treatment	Pods/plant	
	Variety	
	White	Brown
Unsolarized uninoculated	5.6 ± 1.4a	4.7 ± 2.1^a
Unsolarized inoculated	6.3 ± 1.4^{ab}	5.3 ± 2.1^{ab}
Solarized uninoculated	2.5 ± 0.8^c	2.0 ± 1.0^c
Solarized inoculated	8.3 ± 0.8^{bd}	7.4 ± 1.6^{bd}

Values with the same superscript alphabet in the same row and column did not differ significantly. (P < 0.05)

While response of the two varieties of *P. vulgaris* to each of the treatments was the same, overall, treatments affected pod lengths (P < 0.05). The longest pod lengths (11.5 and 11.0 cm) and plant heights (142.5 and 141.7cm) were recorded in Solarized inoculated, while the shortest pod lengths (8.0 and 7.5 cm) and heights (124.0 and 123.3 cm) in Solarized uninoculated soils for white and brown varieties respectively (Figure 2).

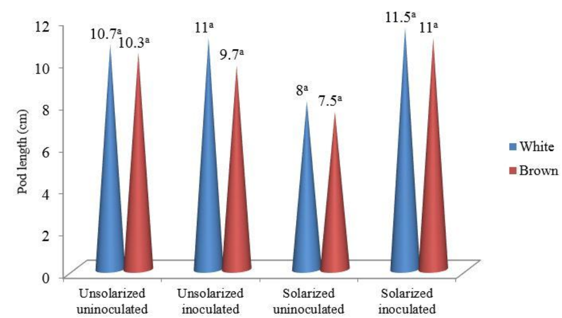

Figure 2. Influence of treatments on pod length in different varieties of *P. vulgaris*.
Values with the same superscript alphabet for the same treatment did not differ significantly (P < 0.05)

Influence of Treatments on the Fresh Weight, Height and Root Length Growth in the Two Varieties of *P. vulgaris*

Notwithstanding the fact that the fresh weight and height measurements in this study indicate that response of the two varieties to each of the treatments were virtually the same, these growth parameters differ significantly with the different treatments applied (P < 0.05). The highest fresh weight and height (23.1 and 22.0 g) and (142.5 and 141.7 cm), and the lowest (18.6 and 18.5 g) and (124.0 and 123.3 cm) for white and brown varieties were recorded in Solarized inoculated and

Solarized uninoculated varieties respectively. Furthermore, Solarized uninoculated soils also recorded the shortest root length measurements (27.5 and 26.0 cm) for white and brown varieties respectively as against that of Solarized inoculated (31.0 and 29.7 cm).

Table 6. Influence of treatments on fresh weights of *P. vulgaris*

Treatment	Fresh weight (g)	
	Variety	
	White	Brown
Unsolarized uninoculated	20.7 ± 2.1[a]	19.3 ± 1.6[a]
Unsolarized inoculated	20.4 ± 1.5[a]	19.6 ± 1.0[a]
Solarized uninoculated	18.6 ± 1.0[b]	18.5 ± 0.6[b]
Solarized inoculated	23.1 ± 1.2[c]	22.0 ± 1.5[c]

Values with the same superscript alphabet in the same row and column did not differ significantly. ($P < 0.05$)

DISCUSSION

Baseline physicochemical data showed that the soil engaged in this study is moderately porous and may be classified as loamy sand with neutral pH. The survival and proliferation of *Rhizobium* in soil is dependent on its hydrogen ion activity (Vincent 1965). Nodulation in legumes which ultimately affect their growth and yield have been reported to be inhibited specifically by low pH (Munns 1968; Munns 1970). Premise on the reports of these workers, the baseline pH of 7.1 in the experimental soil indicates the soil is ideal for the propagation of the two varieties of the legume under investigation.

Within the depth of 10 cm, temperature of solarized soil undulated between 29.0 and 53.0 ^{0}C attaining higher peaks in the evening (16.00 hours) and lower peaks in the morning (10.00 hours). The findings here reported are in consonant with the previous report (Chauhan et al. 1988), that solarized soil witnessed temperature fluctuations, and diminishing with increasing soil depth, with comparatively higher temperatures in the evening than morning hours. Furthermore, it was reported that solarization temperature of 53.0 ^{0}C attained at soil depth of 10 cm in greenhouse were enough to destroy soil microbes within a space of 14.0 days (Elmore et al. 1997). The 29.0 to 53.0 ^{0}C temperatures accomplished within 16 days in this study is sufficient to achieve the desired soil solarization effect.

The applied treatments in this study as compared to the control did not significantly affect the time and percentage germination of the two varieties of *A. hypogaea*. Although, it had been reported that plant germination are usually enhanced by soil solarization (Stapleton and Devay 1986; Jat et al. 2014), the results of this study is at variance with that assertion. The findings here however correlate with that of Argaw (2012) and Ndlovu (2015), who reported that germination of legumes was never enhanced by the inoculation of *Rhizobium* species. While investigating the effect of *Rhizobium phaseoli* inoculation on the germination time of small white haricot and red speckled bean (cultivars of dry bean), Ndlovu (2015), reported that there was no difference in germination time in inoculated and un-inoculated plants irrespective of the variation in their seed sizes. Similarly, Argaw (2012) while studying the influence of *Bradyrhizobium japonicum* on soybean germination reported that germination time in the legume were not accelerated by *Rhizobium* inoculation. The failure of *Rhizobium* inoculation to advance legume germination is based on the fact that its inoculation has no relevance to the germination process Ndlovu (2015), as N_2-fixation commences only about 14 to 35 days after *Rhizobium* infection (Dupont et al. 2012).

There were no differences in the response of the two varieties of *P. vulgaris* to each of the applied treatments as per nodulation and pod formation. Nevertheless, nodule and pod numbers as well as pod length were significantly impacted by the various treatments with solarized inoculated soil recording the highest improved yield. This is in concordance with the findings of Megueni et al. (2006) who reported that solarized inoculated soil with *Rhizobium*, yielded higher numbers of nodules per plant and tones of seeds per hectare in soybean than the other treatments (unsolarized uninoculated, solarized uninoculated and unsolarized inoculated soil with *Rhizobium)*. He however reported differential yield responses in nodules and seeds in the two cultivars of soybean investigated; whereas there was 90 % yield increase in nodulation in one cultivar, the other recorded 166 % increase. Similarly, Habete and Buraka (2016), reported differential responses of two varieties of *P. vulgaris* (Om-95 and Red Wolaita) to *Rhizobium* inoculation while studying the influence of nitrogen fertilization and *Rhizobium* inoculation on nodulation and yield response of *P. vulgaries*. Furthermore, their findings revealed that while *Rhizobium* inoculation increased nodule and pod numbers in both varieties, inorganic nitrogen fertilization reduced nodulation but enhanced pod yield. In this study, experimental soil pH was within neutrality, while *Rhizobium* employed for soil inoculation has inoculum size of 8.5 x 10^{8}cfu/ml. *Rhizobium* and legume response to acidic soil pH varies with species (Vincent, 1965; Rice et al. 1977), *R. trfulii* for instance has a minimum growth pH that is lower than *R. meliloti* (Jensen, 1942). In a study to determine the influence of soil acidity on Rhizobia population, nodule formation and nitrogen fixation by red clover and alfalfa (Rice et al. 1977), reported that while the yield of alfalfa rose remarkably with a rise in soil pH from pH 4.5 to 6.0, there were no noticeable change in the yield of red clover as soil pH rose from 4.5 to 7.2. Furthermore, increasing inoculum size of *Rhizobium* from 3 x 10^2 to 3 x 10^7 per seed has been reported to raise the yield of alfalfa propagated at soil pH of 5.4 (Rice 1975). Thus, the non-differential response of the two varieties of *P. vulgaris* to *Rhizobium* inoculation in this study in contrast to the differential responses of legume varieties and cultivars to *Rhizobium* inoculation reported by other workers may be ascribed to variation in *Rhizobium* species, inoculum size, and soil pH.

The responses of the two varieties of *P. vulgaris* to each of the applied treatments were the same in terms of weight, height and root length increases. However, these parameters of growth differed significantly with the applied treatments as compared to the control. Solarized inoculated soil yielded the best growth, while solarized uninoculated, yielded the weakest growth. In a similar study, Megueni et al. (2006) reported differential responses in terms of biomass yield between two cultivars (C_1 and C_5) of soybean with C_1 producing higher biomass than C_5 and that solarized uninoculated and solarized inoculated soils (with the same yield) gave the best result, while on the other hand, solarized uninoculated gave the highest biomass yield and solarised inoculated the lowest in for cultivar C_5. Ahmed et al. (2006), investigated the influence of *Rhizobium* inoculation and nitrogen on mungbean (*Vigna radiata* L.) growth performance and reported that *Rhizobium* inoculation of soil did not produce any significant root length, height and weight increases over uninoculated control. In this study, the two varieties of *P. vulgaris* yielded the highest root length growth in solarised inoculated soil while unsolarized uninoculated yielded the lowest. While the findings here are in agreement with the report of these workers at some points, it is at variance at other points. Notwithstanding, the recorded higher growth in plant weight and height in the solarized inoculated soil in this study is worthy of note as it solidly confirm earlier assertion that soil solarisation promote shoot and root growth (Chauhan et al. 1988; Elmore et al. 1997; Pokharel 2011) in conjunction with the fact that inoculated *Rhizobium* (a plant growth promoting rhizobacteria (PGPB) (Ahemad and Khan 2010), enhances plant hieigh and leaf area growth, speed up photosynthesis and increase dry matter yield Tharkur and Panwar (1995).

CONCLUSIONS

The results of this study showed that the inoculation of Rhizobium in post solarized soil enhanced nodulation, pod formation and fresh weight in the two varieties of P. vulgaris in comparison to the other treatments (unsolarized inoculated, solarized uninoculated and unsolarized uninoculated soils). Thus, this agronomical technique can be employed for P. vulgaris yield improvement.

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