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EFFECT OF ALGAL BIOFERTILIZERS ON GROWTH AND YIELD OF *ORYZA SATIVA* L. (var. jaya) IN COASTAL REGION OF GOA

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Pot experiments were conducted in rabi season to study the influence of three algal biofertilizers *viz., Anabaena oryzae, Calothrix membranacea* and *Nostoc rivulare* on growth and yield of Paddy (*Oryza sativa* var. jaya). The most abundant indigenous heterocystous cyanobacteria were isolated from the local paddy fields, cultured in BG-11 medium and used for the study. The study revealed that the combination of all the three cyanobacterial species significantly improved yield and could effectively reduce the use of inorganic fertilizers.

INTRODUCTION

Blue green algae (BGA) or Cyanophytes or Cyanobacteria are a group of prokaryotes that have existed almost from the origin of life on Earth and have been recorded as Precambrian microfossils (Schopf, 1970) and tolerant to dessication, extremes of temperatures, pH, salinity, light intensity and nutrients (Whitton, 2000). The group is known to occur abundantly in rice fields of several countries *viz.*, Japan, Thailand, China, Philippines, Bangladesh and India (Roger and Kulasooriya, 1989; Venkataraman, 1981). Fritsch (1907) for the first time observed the role of BGA in increasing fertility of rice fields, and De (1939) emphasized the importance in maintaining the fertility of rice fields through biological nitrogen fixation which was later confirmed by Singh (1950, 1961), Holm-Hanson (1968) and Alexander (1975). Experimental evidences showed enhanced grain and straw yield (De and Mandal, 1956).

Most BGA are known to fix atmospheric nitrogen, their occurrence in rice fields is known to maintain nitrogen levels in the soil (Venkataraman, 1993). Heterocysts have been demonstrated experimentally to be specialized cells capable of fixing nitrogen (Fogg, 1949). Considerable amount of research on heterocystous forms has become a major attraction (Stewart, 1980; Adams and Duggan, 1999), with immunolabelling studies proving heterocysts as sites of nitrogenase (Flemming and Haselkorn, 1973). The prospects of BGA as biofertilizers has triggered a considerable amount of research in evolving methods and means to effectively utilize these organisms (Brouers *et al.*, 1987; Shi *et al.*, 1987; Shi and Hall, 1988; Prasanna and Kaushik, 1994; Anand, 1998; Vaishampayan *et al.*. 2000). BGA are

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more prevalent in Tropical and Sub-tropical regions as compared to Temperate belts (Vaishampayan *et al.*, 2001). They find a highly favourable environment in the water-logged conditions of rice fields where they provide nitrogen to plants, improving crop yields by making soil fertile, vital and productive. BGA inoculation popularly known as "Algalization" helps to provide an environmentally safe agro-ecosystem contributing to economic viability in rice cultivation, reducing cost and energy inputs (Sunil Pabbi, 2008).

Rice is the staple food of over 40% of the World's population and the most important food crop (Yadav *et al.*, 2000). The cultivated area of rice in Asia is about 90% of the global total and supports more than 60% of the World's population. In developing countries, though the use of nitrogenous fertilizers is much less, nitrogen fixing BGA contribute significantly to the nutritional requirements of rice (Roger and Kulasooriya, 1980). The nitrogen requirement is a factor which determines the yield of the crop far beyond the natural capacity of any soil type (Ahlawat *et al.*, 1998). Thus large amounts of chemical fertilizers must be added to the rice fields to improve yield. This excessive use of nitrogenous fertilizers can lead to high soil nitrate concentration after crop harvest (Jokela and Randall, 1989; Roth and Fox, 1992; Gordon *et al.*, 1993), a situation which can lead to increased nitrate contamination of portable water (Singh *et al.*, 1995). Nitrogen fixation carried out by rhizosphere bacteria contributes significantly to soil fertility (Vlassak *et al.*, 1992), but the less efficient use of this fixed nitrogen by plants in incorporating it into grain protein could be a limiting factor (Vlassak *et al.*, 1992). Thus studies could be oriented more toward an alternative source of nitrogenous fertilizers, such as BGA, which act as biofertilizers.

The present study was carried out to assess the effect of biofertilization by BGA on rice crop in Goa. Goa is a coastal region with a hot, humid and tropical climate with about 44.66 thousand hectares of land under rice cultivation, which is the staple food of Goans (Sakshena, 2003). With increasing population and risk of contamination of ground water by excess leaching of nitrates from the surrounding fields, the application of dominant nitrogen fixing cyanobacteria found in local paddy fields would help improve yield of paddy and be cost effective. Since the local ecological conditions differ with different agro climatic regions, it becomes very difficult for general BGA formulations to establish itself. Thus, there is a need to develop algal inoculants suitable for different agro climatic regions by isolating the indigenous strains from that particular area which can grow fast and efficiently fix nitrogen. Hence the present study was carried out to identify the efficient heterocystous nitrogen fixing cyanobacteria that are part of the indigenous algal flora in Goan rice fields.

MATERIAL AND METHODS

Algal samples collected from rice fields were screened to obtain dominant heterocystous forms of BGA and were identified using standard keys (Desikachary, 1959).

Anabaena oryzae Frithsch, *Calothrix membranacea* Schmidle and *Nostoc rivulare* KÜtzing ex Born et Flah, were isolated from rice grown soils of Goa. Clonal cultures were obtained from a single filament grown in BG-11 medium (Stainer *et al.*, 1971). The cultures were maintained by repeated sub culturing. Pure cultures of the algal species were employed for mass culture and algalization studies. Isolated strains were mass cultured in 2L flasks with simple liquid nutrient medium composed of sterilized tap water supplemented with super phosphate and ash (Bongale, 1986). These flasks were maintained at 27°-30°C. Flasks were manually shaken twice a day. Soil collected from local paddy field was sterilized in an autoclave for 1 hour at 121°C (15psi). Seeds of *Oryza sativa* (var. jaya) were obtained from the local Agriculture Department. Six rice seedlings (10 days old) transplanted in each pot were thinned to four per pot in the later stages (before flowering). 15 day old seedlings were inoculated using 10 ml of exponentially growing fresh culture per pot. There were three replicates for each treatment. The following algal treatments were prepared and administered.

1. *Un-inoculated control*
2. *Anabaena oryzae* (Ao)
3. *Calothrix membranacea* (Cm)
4. *Nostoc rivulare* (Nr)
5. *Anabaena oryzae* + *Calothrix membranacea* (Ao + Cm)
6. *Anabaena oryzae* + *Nostoc rivulare* (Ao+ Nr)
7. *Calothrix membranacea* + *Nostoc rivulare* (Cm + Nr)
8. *Anabaena oryzae* + *Calothrix membranacea* + *Nostoc rivulare* (Ao + Cm + Nr)

The experiments were conducted in the Rabi season (December-March 2009) and harvested after 125 days after transplanting. Various growth parameters *viz,* plant height, plant weight, leaf length, spike length, total root length, number of tillers per hill and leaf area index was recorded at regular intervals until flowering stage. Yield parameters, the number of panicles per hill, length of panicles, number of spikelets per panicles, straw yield per plant and grain yield per plant were recorded at harvesting.

Statistical analysis

Statistical analysis was performed by SPSS version 16 statistical package and MINITA B version 13. A p-value of 0.05 or less was considered statistically significant. One way ANOVA was used for multiple group comparisons followed by Post-hoc test for group wise comparisons. Pearson's correlation coefficient (r) analysis was performed to assess the interrelationships and predictions of the effects.

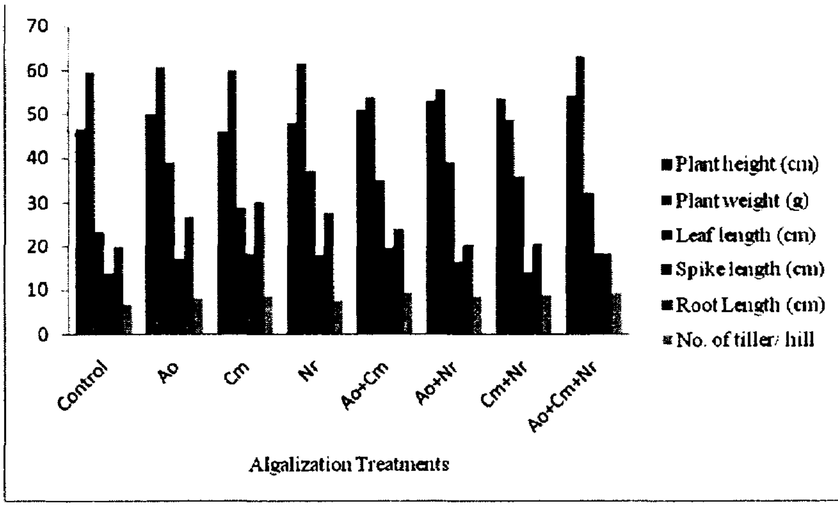
RESULTS

BGA inoculation had significant effects on growth and yield of *Oryza sativa* (var. jaya) (Table 1 and 2; Fig. 1 & 2). The combination treatment of local BGA (Ao + Cm + Nr) resulted in maximum increase in plant height, plant weight, spike length, number of tillers per hill, length of panicle and grain yield compared to control.

Effects of different BGA combinations on plant growth parameters and grain yield were studied and their comparative results analyzed by one way ANOVA, followed by Post-hoc test for comparison with control (Table 3).

The action of BGA biofertilizers on *O. sativa* (var. jaya) indicates blue green algae individually or in combination with other blue greens produce significant results in most of the growth and yield parameters (Table 1 & 2). A significant increase in plant height was seen in plants treated with combination treatments *Ao+Nr, Cm+Nr, Ao+Cm+Nr* where the percentage increase over control was 13%, 14% and16% respectively. Plant weight showed little significant increase in all treatments except *Ao+Cm+Nr* where the percentage increase

Figure 1: Effect og BGA on growth parameters of *Oryza sativa* L. var. Jaya



Legend: *Ao - Anabaena oryzae*; *Cm - Calothrix membranacea; Nr - Nostoc rivulare*

Figure 2: Effect of BGA on yield parameters of *Oryza sativa* L. var. Jaya

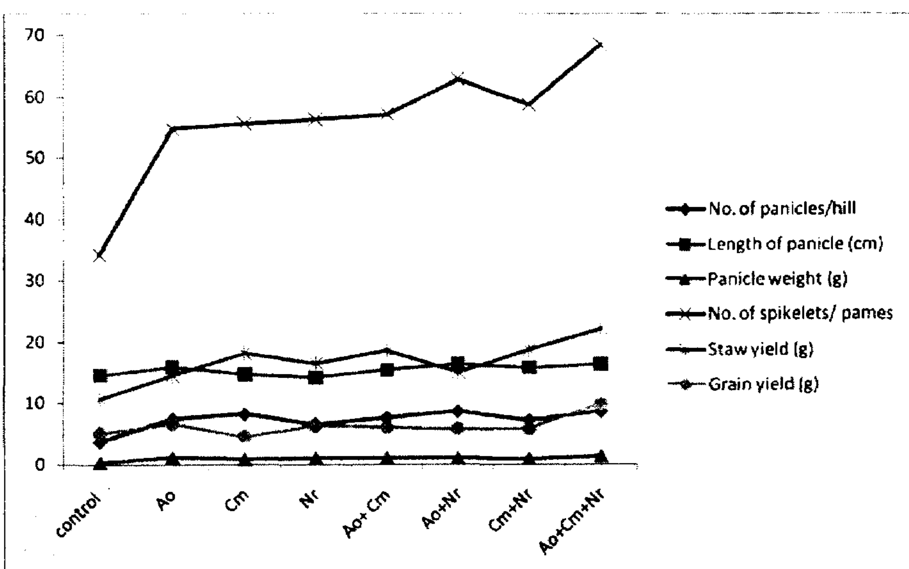


Table 1: Effect of BGA isolates on growth parameters of *Oryza sativa* L. var. Jaya

Sr. No.	Treatment	Plant height(cm)	Plant weight(g)	Leaf length(cm)	Spike length(cm)	Root length(cm)	No. of Tiller/hill
1	Control	46.64 ±0.21	59.7±0.35	23.42±0.04	13.6±0.2	20.02±0.19	6.8±0.45
2	*Ao*	50.0±3.47	60.68±0.4	38.94±0.47	16.98±0.08	26.68±0.27	8.0±0.70
3	*Cm*	45.8+5.02	59.92+0.26	28.72+1.03	18.16+0.11	30.12+0.43	8.6+1.14
4	*Nr*	48.02 ±0.44	61.36±0.30	37.12±1.27	18.0±0.07	27.58±13.38	7.6±1.14
5	*Ao + Cm*	50.78±1.07	53.82±1.09	35.0 ±0.07	19.42±0.33	24.0±0.07	9.4±1.14
6	*Ao + Nr*	52.88±1.62	55.68±1.11	38.8±0.29	16.34±0.11	20.38±0.52	8.6±1.14
7	*Cm + Nr*	53.3+1.00	48.56+3.75	35.64+0.23	13.88+0.19	20.62+0.54	8.8+0.84
8	*Ao + Cm + Nr*	54.08 ±0.69	63.0±0.07	32.02±0.08	18.32±0.11	18.44±0.09	9.4±1.14

Legend: *Ao - Anabaena oryzae, Cm - Calothrix membranacea, Nr - Nostoc rivulare*

Table 2: Effect of BGA isolates on yield of *Oryza sativa* L. var. Jaya

Sr. No.	Treatment	No. of panicle/hill	Length of panicle (cm)	Panicle weight (g)	No. of spikelets/ pames	Straw yield (g)	Grain yield (g)
1	**Control**	3.80±0.84	14.62±0.04	0.38±0.08	34.20±0.84	10.68±1.20	5.20±0.16
2	***Ao***	7.40±0.55	15.88±0.25	1.22±0.13	54.80±3.63	14.60±1.67	6.66±1.20
3	***Cm***	8.20±0.84	14.74±0.27	0.90±0.19	55.60±7.64	18.22±1.88	4.62±0.24
4	***Nr***	6.60±0.55	14.2±0.84	1.10±0.07	56.40±2.07	16.60±1.14	6.34±0.34
5	***Ao + Cm***	7.60±1.14	15.42±0.54	1.08±0.24	57.20±1.92	18.60±2.07	6.12±0.32
6	***Ao + Nr***	8.60±055	16.42±0.43	1.06±0.11	62.80±2.28	15.00±3.08	5.82±0.19
7	***Cm + Nr***	7.20±0.84	15.84±0.15	0.98±0.13	58.80±0.84	18.8±1.92	5.86±0.27
8	***Ao + Cm + Nr***	8.60±0.55	16.42±0.45	1.44±0.09	68.40±1.52	22.20±1.48	9.80±0.97

Legend: *Ao - Anabaena oryzae*; *Cm - Calothrix membranacea*; *Nr - Nostoc rivulare*

Table 3: Comparison of growth parameters and grain yield between various treatments of biofertilizations and their combinations.

Sr. No.	Treatments	Plant height (cm)	Plant weight (g)	Spike length (cm)	No. of tiller/hill	Length of panicle (cm)	No. of spikelets/ pames	Grain yield/plant (g)
1	Control	46.6±0.2	59.7±0.4	13.6±0.2	6.8±0.5	14.6±0.1	34.2±0.8	5.20±0.16
2	***Ao***	50.0±3.5	60.7±0.4	17.0*±0.1	8.0±0.7	15.9*±0.3	54.8*±3.6	6.66*±0.20
3	***Cm***	45.8±5.0	59.9±0.3	18.2*±0.1	8.6*±1.1	14.7±0.3	55.6*±7.6	4.62±0.24
4	***Nr***	48.0±0.4	61.4±0.3	18.0*±0.1	7.6±1.1	14.2±0.8	56.4*±2.1	6.34*±0.34
5	***Ao + Cm***	50.8±1.1	53.8*±1.1	19.4*±0.3	9.4*±1.1	15.4±0.5	57.2*±1.9	6.12±0.32
6	***Ao + Nr***	52.9*±1.7	55.7*±1.1	16.3*±0.1	8.6*±1.2	16.2*±0.4	62.8*±2.3	5.82±0.19
7	***Cm + Nr***	53.3*±0.7	48.6*±3.7	13.9±0.2	8.8*±0.8	15.8*±0.2	58.8*±0.8	5.86±0.27
8	***Ao+Cm+Nr***	54.1*±1.0	63.0*±0.1	18.3*±0.1	9.4* ±1.2	16.4*±0.5	68.4*±1.5	9.80*±0.97
9	ANOVA F P	9.16<0.01	54.7<0.01	752.2<0.01	4.05<0.05	18.51<0.01	44.36<0.01	34.55<0.01

Significant at P<0.05 and P<0.01

Legend: *Ao - Anabaena oryzae*; *Cm - Calothrix membranacea; Nr - Nostoc rivulare*

Table 4: Correlation analysis between overall plant characters and grain yield/plant (g).

Sr. No.	Characters	1	2	3	4	5	6	7
1	Plant height	1	-0.32 *	0.01	0.36 *	0.63 **	0.44 **	0.02
2	Plant weight	-	1	0.36 *	-0.21	-0.18	-0.07	0.24
3	Spike length	-	-	1	0.38 *	0.01	0.51 **	0.27
4	No. of tiller/hill	-	-	-	1	0.43 **	0.58 **	-0.16
5	Length of panicle	-	-	-	-	1	0.59 **	-0.25
6	No. of spikelet's	-	-	-	-	-	1	0.18
7	Grain yield/plant(g)	-	-	-	-	-	-	1

Pearson's correlation coefficient * $P<0.05$ ** $P<0.01$
Legend: *Ao - Anabaena oryzae*; *Cm - Calothrix membranacea*; *Nr - Nostoc rivulare*

over control was 5%. A marked increase in spike length was observed over control ranging from 19% to 42%. *Ao* recorded 25% increase, *Cm* 33% and *Nr* 32%. Among the combination treatments, *Ao+Cm* recorded 42% increase, *Ao+Nr* recorded 19% increase and *Ao+Cm+Nr* recorded 34% over control. Increase in number of tillers per hill ranged from 26 to 38% over control. Plants treated with *Cm* and *Ao+Cm* showed an increase of 26% over control and plants treated with a combination treatment *Cm+Nr* showed 29% increase. Maximum increase (38%) was seen in combination treatment *Ao+Cm+Nr* and *Ao+Cm*. Similarly, panicle length showed a significant increase in individual treatments and combination treatments ranging from 8 to 12%. Individual treatment *Ao* and combination treatment *Cm+Nr* both showed 8% increase, *Ao+Nr* 10% increase and a maximum of 12% increase was shown by plants treated with the triple combination of BGA. Number of spikelets per panicles showed a significant increase in all individual treatments as well as combinations, ranging from 60% to 100% increase over control, the effect being more significant in combination algal treatments. Grain yield showed significantly higher yield in the majority of the singular biofertilization treatments and combinations compared to control. Only *Cm* treatment showed reduced grain yield. The percentage increase over control in individual treatments Ao and Nr was 28% and 21% respectively and combination *Ao+Cm+Nr* showed maximum increase of 88%.

Correlation analysis (Table 4) showed that plant height is significantly correlated to plant weight, number of tillers per hill, length of panicle and number of spikelets per panicle, but grain yield did not show any relationship with plant height. Plant weight showed positive correlation with spike length but is negatively correlated with number of tillers per hill, length of panicle and number of spikelets per panicle. Grain yield did not show significant correlation to plant weight. Spike length is significantly correlated with number of tillers per hill and number of spikelets per panicle but not correlated with grain yield. Number of tillers per hill shows significant correlation with length of panicle, number of spikelets per panicle and shows a negative correlation with grain yield. Length of panicle is significantly correlated with number of spikelets per panicle and shows a negative correlation to grain yield. Number of spikelets per panicle is positively correlated with grain yield. Grain yield shows a positive correlation with plant height, plant weight, spike length and number of spikelets per panicle and a negative correlation with number of tillers per hill and length of panicle.

DISCUSSION

Earlier studies have indicated that application of BGA biofertilizers increased the availability of nitrogen resulting in increased leaf area, light intercepting photosynthesis and greater accumulation of dry matter (Rekhi *et al.*, 2000). Cyanobacteria introduced as a result of algalization can establish themselves permanently if inoculation is done consecutively for three to four cropping seasons (Upassana and Pabbi, 2004). Earlier studies have reported increase in plant height, tiller number and grain weight in paddy due to algalization with BGA (Jagannathan and Kannaiyan, 1977; Pantastico and Gonzalves, 1976; Kannaiyan, 1978b; Kannaiyan, 1979).

In the present study it was observed that BGA treatment lead to an increase in the height of the plant, plant weight, length of leaf, length of panicle and grain yield. Giriyappanaver, (1989) reported a similar marked increase in plant weight, leaf breadth, spike length and grain yield in *O. sativa* when treated with a combination treatment of BGA biofertilizer consisting of indigenous species of *Anabaena oryzae, Nostoc rivulare* and *Nostoc entophytum* from north Karnataka.

Cyanobacterial inoculation increased the yield maximum and supplemented more than 20% nitrogen. It resulted in significant increase in plant height, number of grains/panicle, panicle length and straw yield but however weight of 1000 grain, number of tillers/hill did not show significant increase (Aziz and Hashem, 2004). The inoculation studies of soils with BGA or algalization carried out in India and abroad have shown beneficial effects on grain yield and nitrogen savings (Venkataraman, 1972; Singh, 1978). Effects of algalization on grain yield under field conditions has been reported from China (Ley, 1959), Egypt (El-Nawamy and Hamdi, 1975), Japan (Watanabe, 1965) and India (Jha *et al.*, 1965; Venkataraman, 1979; Singh, 1988). An average increase of 14% in rice yield over control was reported due to BGA inoculation (Roger and Kulasooriya, 1980) which was observed in our studies also. Studies on the indigenous BGA from Chilean rice field soils has proved to be good option for the formulation of a biofertilizer, allowing 50% decrease in the use of synthetic nitrogen fertilizers with similar results with respect to grain yield in comparison with control (Pereira *et al.*, 2009). A multani-mitti (fullers earth) based carrier BGA biofertilizer was shown to increase the grain yield of 'PNR 381' variety of rice by 48.83 q/ha upon combination with low amounts of chemical fertilizers (Dhar *et al.*, 2007). Rice variety Pathumthani 1 when grown in two crops at different times of the year and inoculated with vegetative cells and akinites of *Nostoc* species separately, showed an increase in grain yield by 7.29% and 6.25% respectively (Innok *et al.*, 2009). An increase in grain yield of 15-23% by BGA inoculation was also reported by Singh and Singh, (1987). Pot experiments with unialgal and composite cultures of BGA increased rice yield considerably (Dhaliwal *et al.*, 1995). Whereas in the present study the combination treatment of *Anabaena oryzae, Calothrix membrancea* & *Nostoc rivulare* has resulted in 88% increase in the grain yield.

The results of the present study are in accordance to those reported earlier by researchers who have shown enhancement in grain yield due to algalization in rice fields (Venkataraman and Goyal, 1968; Singh, 1985 & 1988; Kannaiyan, 1985). In a study conducted in Madhya Pradesh, India, 56 trials showed an increase of 15-20% in grain yield of rice through algal application alone without any nitrogenous fertilizers. Field studies at

CRRI, Cuttack reported that BGA enhanced grain yield of rice by 6 to 35 % (Singh, 1988). The present study showed that biofertilization significantly increased growth and yield parameters in *O. sativa* (var. jaya). In addition to nitrogen fixation, BGA is also known to produce growth promoting substances like vitamins and hormones which may foster better crop growth (Venkataraman and Neelkantan, 1967; Mishra and Kaushik, 1989a, 1989b) and are also known to add a substantial amount of organic matter to the soil (Goyal, 2002). An increase of 5-32% of soil organic carbon under field conditions was reported due to algal inoculation (Singh and Bisoyi, 1989). BGA is also reported to have phosphate solubilizing ability (Bisoyi and Singh, 1988) and improve the physical properties of soil (Bertocchi *et al.*, 1990; Roger and Burns, 1994). In pot culture treatments of cyanobacteria to rice showed 53% increase in plant height, 66% increase in plant roots length and 69% increase in plant fresh weight. Rice inoculation with heterocystous cyanobacteria isolated from Iran rice fields showed that algae have positive effects on rice planted in vitro and they modify physical and biological population of soils in ways which are beneficial to rice and soil (Sadatnia *et al.*, 2009). In the present study, a significant response to indigenous cyanobacterial strains was encouraging and proved to be an efficient biofertilizer for soils. It may be thus concluded that algalization of jaya variety of rice by these selected combination of indigenous cyanobacterial strains showed significant results in both its growth and yield parameters proving to be an efficient biofertilizer.

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