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Effect of Efficient Strains of Pink Pigmented Facultative Methylotrophs on Plant Growth Parameters of Direct Seeded Rice

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A B S T R A C T

A pot culture experiment was carried out at Department of Agricultural Microbiology, College of Agriculture Bhimarayanagudi, University of Agricultural Sciences, Raichur during 2017 to study the effect of pink pigmented facultative Methylotrophs on growth and yield of direct seeded rice. All the PPFM isolates were tested for a selective biochemical tests. All the isolates showed positive results for Oxidase test, Urease test, and Indole production. None of the isolates showed positive results for casein hydrolysis, MR and VP test, nitrate reduction test. The experiment was laid out in completely randomized block design (CRD) with three replications. The treatments comprised of (T_1-PPFM-16, T_2-PPFM-31, T_3-PSB, T_4-Reference strain, T_5-PPFM-16 + PSB, T_6- PPFM-31 + PSB, T_7-Referaece strain + PSB, T_8-Uninoculated control). The results indicated that significantly higher plant growth parameters *viz*., plant height, number of tillers/hill, number of leaves/hill, root length, root dry weight, shoot dry weight and SPAD value chlorophyll was recorded in T_6- PPFM-31 (*Methylobacterium phyllosphaerae)* + PSB (*Bacillus megatarium* var. *Phosphaticum)*, T_7-Referaece strain (*Methylobacterium extorquens)* + PSB, among that treatments T_6 significantly higher as compared to rest of the treatment combinations, individual inoculation and uninoculated control.

Introduction

The plant canopy is a host to a wide array of microorganisms that have beneficial, harmful or neutralistic effects but their importance is often not detected. One such relationship that remained undetected until Basile *et al.,* (1969) isolated a bacterium, as covert contaminant from the tissue cultures of liverwort. *Scapania nemorosa* and identified it as pink pigmented facultative methylotroph (PPFM) belonging to the genus *Methylobacterium*. PPFMs are ubiquitous in nature found in a variety of habitats. Methylotrophs have been reported to influence seed germination and seedling growth by producing plant growth regulators like zeatin and related cytokinins, auxins and to alter agronomic traits like branching, seedling vigour, rooting and heat/cold tolerance. Hence there is a possibility of

increasing the effectiveness of the conventional bioinoculants by coinoculating with PPFMs.

Rice is a staple food grown in almost all states of India accounting for more than 40 per cent of the food grain production. It is manually transplanted in standing water, destroys soil structure, is labour intensive and cumbersome. Hence, direct seeded rice without puddling is a viable alternative for its establishment as it can save water and also requires less labour. Methylotrophic bacteria are successful example that cans achieves the two purposes as PGPR with antifungal activity. Many of the microbes living on the phylloplane probably lead a saprophytic lifestyle, feeding on materials leached from the leaf. Keeping all the above points in view an experiment was conducted to study the effect of pink pigmented facultative methylotrophs on growth parameters of Direct seeded rice (pot culture condition).

Materials and Methods

The pot culture experiment was carried out to study the effect of inoculation of efficient PPFMs and PSB (*Bacillus megatarium* var. *phasphaticum)* strains on growth and yield of direct seeded rice at the College of Agriculture, Bhimarayanagudi, University of Agricultural Sciences, Raichur during 2016-17.

The medium black soil was collected from Shakhapur block, College of Agriculture, Bhimarayanagudi before initiation of the experiment. The soil was air-dried, powdered and allowed to pass through 2 mm sieve. The soil was later sterilized by autoclaving at 121°C at 15 lbs for 1 hour and filled in the surface sterilized earthen pots with 6 kg soil.

One hundred gram of lignite powder was neutralized with 5 g of $CaCO_3$ and mixed thoroughly. The properly mixed lignite powder was packed in sterilizable polyprophylene (PP) bags and sterilized in an autoclave at 121 ^{o}C for one hour. The inoculums of bioinoculants was prepared by inoculating 72 h old log phase culture of inoculants *viz*, PSB (*Bacillus megatarium* var. *phasphaticum*) in 100 ml Nutrient broth and PPFMs in 100 ml of AMS broth (Whittenbury *et al.*, 1970). The flasks were kept on shaker at 28 ± 2^{o}C for 5 days. The culture was mixed with pre-sterilized lignite powder at the rate of 30 ml per 100 g of carrier in case of single inoculation, while in case of combined inoculation 15 ml of PSB and 15 ml of PPFM isolate was added per 100 g of carrier. Seeds of Rice var. Gangavathi sona were obtained from seed unit, College of Agriculture, Raichur, University of Agricultural Sciences, Raichur

The following PPFM bacterial isolates, found efficient under *in vitro* conditions *viz*, PPFM-16 (isolated from Phyllosphere of direct seeded rice grown in Sindhanur) and PPFM-31 (isolated from Phyllosphere of direct seeded rice grown in ARS Gangavati) were used for pot culture experiment along with reference strain and PSB.

There were 08 treatment combinations, the experiment was laid out in Complete Randomized Block Design with 3 replications (T_1- PPFM-16 (Phyllosphere sample of Sindhanur), T_2-PPFM-31 (Phyllosphere sample of Gangavati ARS farm), T_3- Reference PPFM strain (*Methylobacterium extorquens*), T_4- PSB (*Bacillus megatarium* var. *phosphaticum*), T_5- PPFM-16 + PSB, T_6- PPFM-31 + PSB, T_7- Reference PPFM strain + PSB and T_8- Uninoculated control).

Phyllosphere spray with native PPFM bacterial isolates. The PPFM cultures were grown for 5 days (10^9 cfu ml^{-1}) and diluted at 1:1 ratio (Anurajan, 2003) with sterilized

distilled water and sprayed with a hand sprayer at the rate of 0.5 ml/pot on the leaves in the morning to have uniform wetting as described by Holland and Polacco (1994) at 30, 60 and 90 days after sowing. Three plants were randomly selected in each treatment for recording various observations on growth parameters at 45, 90 days after sowing (DAS) and at harvest. The total chlorophyll content was determined by using dimethyl sulfoxide (DMSO) method given by Shoef and Liun (1976).

Results and Discussion

Inoculation of *Methylobacterium* isolates in combination with PSB strain had significant influence on different plant growth parameters of DSR PPFM isolates found efficient under *in vitro* conditions were selected for pot culture studies using direct seeded rice crop. The experiment comprising of 8 treatments comparing different inoculations of PPFM were studied for their effect individually and in combination with PSB in direct seeded rice under pot culture conditions. The results showed significant influence of microbial isolates on plant growth parameters at different growth stages of direct seeded rice.

Among the different growth attributes, significant increase in the plant height (Fig. 4), number of tillers/hill (Fig. 5), number of leaves/hill (Fig. 6) and root length (Fig. 7) were noticed at different growth stages of crop (45, 90 DAS and at harvest) as influenced by inoculation of PPFM isolates either alone and in combination with PSB.

Pink pigmented facultative methylotrophic isolates and PSB significantly recorded maximum plant height, number of tillers/hill, number of leaves/hill and root length of direct seeded rice. At 45 DAS, in combined inoculation treatments, T_6 (PPFM-31 + PSB) recorded highest plant height (28.54 cm), highest number of tillers/hill (9.70), highest number of leaves/hill (25.80) and highest root length (13.10 cm) followed by T_7 (reference strain + PSB) which recorded plant height of 27.31 cm, 8.61 tillers/hill, 23.16 leaves/hill and root length of 11.86 cm. Similarly, in single inoculation treatments, T_2 (PPFM-31) recorded plant height of 24.74 cm, 7.50 tillers/hill, 17.19 leaves/hill and root length of 9.48 cm followed by T_4 (reference strain) which recorded plant height of 23.00 cm, 6.12 tillers/hill, 16.80 leaves/hill and root length of 9.22 cm. The lowest plant height (19.81 cm), number of tillers/hill (3.15), number of leaves/hill (9.97) and root length (5.07 cm) were recorded in uninoculated control (T_8). The same trends were observed at 90 DAS and at harvest. Among the individual and combined treatments, T_6 was significantly superior over all other treatments and uninoculated control.

In the present study, all the treatments significantly improved the growth parameters when compared to uninoculated control. Out of that, treatment T_6 was the best in all the parameters. It improved plant height, number of tillers, number of leaves and root length compared to uninoculated control. In fact, PPFM-31 in T_6 was better than the reference strain in T_7 where both the treatments had combined application using PSB. Incidentally, PPFM-31 produced the highest quantity of IAA (28.23 g/ml), nitrogen fixation *in vitro* (1.32 mg/g malate) and P solubilization (13 %) which might have contributed to higher growth parameters. Increased plant growth may also be due to the coordination between auxins and cytokinins allowing a balanced growth in the shoot and root system. When roots become more extensive by the action of auxins then the cytokinins gives plant signals in the shoot system to form more tillers.

These results are similar with the results of Holland (1997) on soybean and may be due to

the production of vitamin B_{12} by PPFM's as described by Basile *et al.,* (1969). The role of cytokinin is to influence the pathway of differentiation which results in increase in plant growth and hence all parameters showed increased trends than the uninoculated control. The fact that methylotrophic bacteria promote seed germination and the growth of seedlings suggests that these bacteria may synthesize not only cytokinins but also other phytohormones for instance auxins (Long, 2000) and atmospheric nitrogen fixation.

Similar improvement in growth parameters due to inoculation with *Methylobacterium* has been observed by Raja and Sundaram (2006) in cotton, Jones *et al.,* (2007) in grapevine and Radha *et al.,* (2009) in Soybean.

Legend

T_8 - Uninoculated control
T_2 - PPFM-31
T_4 - Reference strain
T_6 - PPFM-31 + PSB
T_1 - PPFM-16
T_3 - PSB
T_5 - PPFM-16 + PSB
T_7 - Reference strain + PSB

Plate 5: Influence of different treatments on growth of DSR under pot culture

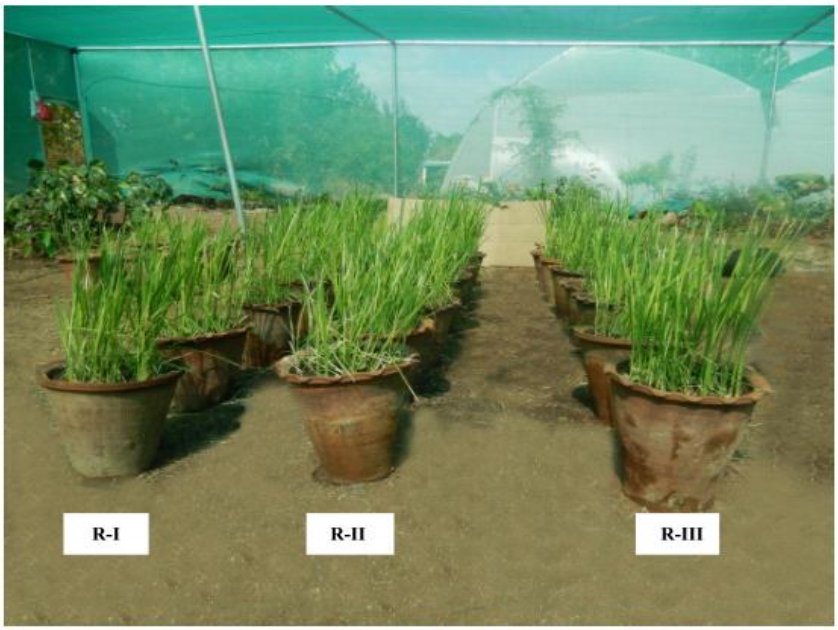

Plate 6: General view of plot culture experiment

Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on plant height, No. of tillers per hill and No. of leaves per hill of direct seeded rice

Treatment	Plant height (cm)			No. of tillers per hill			No. of leaves per hill		
	45 DAS	90 DAS	At harvest	45 DAS	90 DAS	At harvest	45 DAS	90 DAS	At harvest
T_1-PPFM-16 (*Methylobacterium mesophilicum*)	22.53^{de}	46.84^{e}	74.87^{d}	6.45^{cd}	11.20^{d}	16.50^{d}	14.20^{de}	27.36^{d}	48.30^{cd}
T_2-PPFM-31(*Methylobacterium phyllosphaerae*)	24.74^{c}	51.16^{cd}	81.16^{c}	7.50^{bc}	12.09^{cd}	17.30^{cd}	17.19^{c}	32.76^{c}	54.20^{bc}
T_3-PSB (*Bacillus megatarium* var. *phosphaticum*)	21.38^{e}	43.49^{f}	68.45^{e}	4.89^{e}	9.25^{e}	13.19^{e}	13.12^{e}	23.50^{e}	44.80^{de}
T_4-Reference strain (*Methylobacterium extorquens*)	23.00^{d}	49.23^{de}	78.91^{cd}	6.12^{d}	13.10^{c}	15.10^{de}	16.80^{cd}	31.80^{c}	51.50^{bcd}
T_5-PPFM-16 + PSB	26.99^{b}	53.42^{bc}	87.52^{b}	8.17^{b}	13.60^{bc}	19.30^{bc}	21.19^{b}	38.26^{b}	58.20^{ab}
T_6- PPFM-31 + PSB	28.54^{a}	59.94^{a}	93.15^{a}	9.70^{a}	16.96^{a}	22.50^{a}	25.80^{a}	44.17^{a}	62.77^{a}
T_7- Reference strain + PSB	27.31^{ab}	56.36^{b}	91.57^{ab}	8.61^{ab}	15.30^{ab}	21.40^{ab}	23.16^{b}	40.93^{ab}	62.44^{a}
T_8- Uninoculated control	19.81^{f}	39.83^{g}	64.19^{f}	3.15^{f}	6.40^{f}	10.03^{f}	9.97^{f}	19.30^{f}	39.30^{e}
S.Em±	0.44	1.03	1.35	0.36	0.6	0.85	0.89	1.21	2.46
CD (0.05)	1.33	3.22	4.06	1.14	1.82	2.55	2.63	3.65	7.37

Note: PPFM- 16 and PPFM-31: efficient isolates of pink pigmented facultative methylotrophs

DAS: Days after sowing; Values are mean of three replications; Means values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a> b > c.

Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on dry matter content of direct seeded rice

Treatment	Root dry weight (g hill^{-1})			Shoot dry weight (g hill^{-1})			Total dry weight (g hill^{-1})		
	45 DAS	90 DAS	At harvest	45 DAS	90 DAS	At harvest	45 DAS	90 DAS	At harvest
T_1-PPFM-16 (*Methylobacterium mesophilicum*)	0.16^{de}	1.08de	1.27^{de}	4.91^{d}	10.41^{d}	11.20^{d}	5.07^{d}	11.50^{d}	12.47^{d}
T_2-PPFM-31(*Methylobacterium phyllosphaerae*)	0.21^{d}	1.20cd	1.67^{c}	6.01^{c}	12.21^{c}	13.41^{c}	6.22^{c}	13.41^{c}	15.08^{c}
T_3-PSB (*Bacillus megatarium* var. *phosphaticum*)	0.13^{e}	0.91e	1.17^{e}	3.80^{e}	8.61^{e}	9.01^{e}	3.93^{e}	9.53^{e}	10.18^{d}
T_4-Reference strain (*Methylobacterium extorquens*)	0.34^{c}	1.33bc	1.42^{d}	5.89^{c}	13.01^{c}	14.61^{c}	6.23^{c}	14.35^{c}	16.03^{c}
T_5-PPFM-16 + PSB	0.60^{b}	1.47b	2.10^{b}	6.11^{bc}	14.82^{b}	16.82^{b}	6.71^{bc}	16.29^{b}	18.92^{b}
T_6- PPFM-31 + PSB	0.74^{a}	2.02a	2.46^{a}	7.21^{a}	16.62^{a}	19.01^{a}	7.95^{a}	18.64^{a}	21.48^{a}
T_7- Reference strain + PSB	0.63^{b}	1.82a	2.21^{b}	6.89^{ab}	15.78^{ab}	18.20^{ab}	7.52^{ab}	17.60^{ab}	20.41^{ab}
T_8- Uninoculated control	0.12^{e}	0.69f	0.77^{f}	2.71^{f}	6.61^{f}	6.81^{f}	2.83^{f}	7.30^{f}	7.85^{e}
S.Em±	0.02	0.06	0.08	0.28	0.55	0.69	0.3	0.57	0.77
CD (0.05)	0.06	0.2	0.24	0.81	1.63	2.07	0.89	1.72	2.32

Note: PPFM- 16 and PPFM-31: efficient isolates of pink pigmented facultative methylotrophs
DAS: Days after sowing; Values are mean of three replications; Means values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a> b > c.

Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on root length and chlorophyll content direct seeded rice

Treatment	Root length (cm)			Chlorophyll content (SPAD Value)		
	45 DAS	90 DAS	At harvest	45 DAS	90 DAS	At harvest
T_1-PPFM-16 (*Methylobacterium mesophilicum*)	7.97^{cd}	11.43^{d}	13.96^{d}	36.53^{cd}	44.14^{b}	42.25^{bc}
T_2-PPFM-31(*Methylobacterium phyllosphaerae*)	9.48^{c}	13.85^{bc}	16.54^{c}	38.48^{bc}	44.68^{b}	43.80^{ab}
T_3-PSB (*Bacillus megatarium* var. *phosphaticum*)	6.70^{d}	9.33^{d}	11.85^{e}	36.33^{cd}	40.86^{cd}	39.80^{c}
T_4-Reference strain (*Methylobacterium extorquens*)	9.22^{c}	13.25^{cd}	15.81^{cd}	37.51^{bcd}	44.60^{b}	43.66^{ab}
T_5-PPFM-16 + PSB	11.11^{b}	15.60^{b}	19.20^{b}	39.69^{abc}	43.27^{bc}	44.11^{ab}
T_6- PPFM-31 + PSB	13.10^{a}	17.50^{a}	23.13^{a}	42.94^{a}	47.98^{a}	45.45^{a}
T_7- Reference strain + PSB	11.86^{ab}	17.47^{a}	21.73^{a}	40.67^{ab}	46.23^{ab}	45.41^{a}
T_8- Uninoculated control	5.07^{e}	7.23^{f}	9.20^{f}	34.54^{d}	38.63^{d}	36.58^{d}
S.Em±	0.47	0.65	0.83	1.2	1.3	0.9
CD (0.05)	1.59	1.82	2.48	3.58	3.9	2.89

Note: PPFM- 16 and PPFM-31: efficient isolates of pink pigmented facultative methylotrophs
DAS: Days after sowing; Values are mean of three replications; Means values followed by the same letter are not significantly different based on Duncan's multiple range test ($p<0.05$), a> b > c.

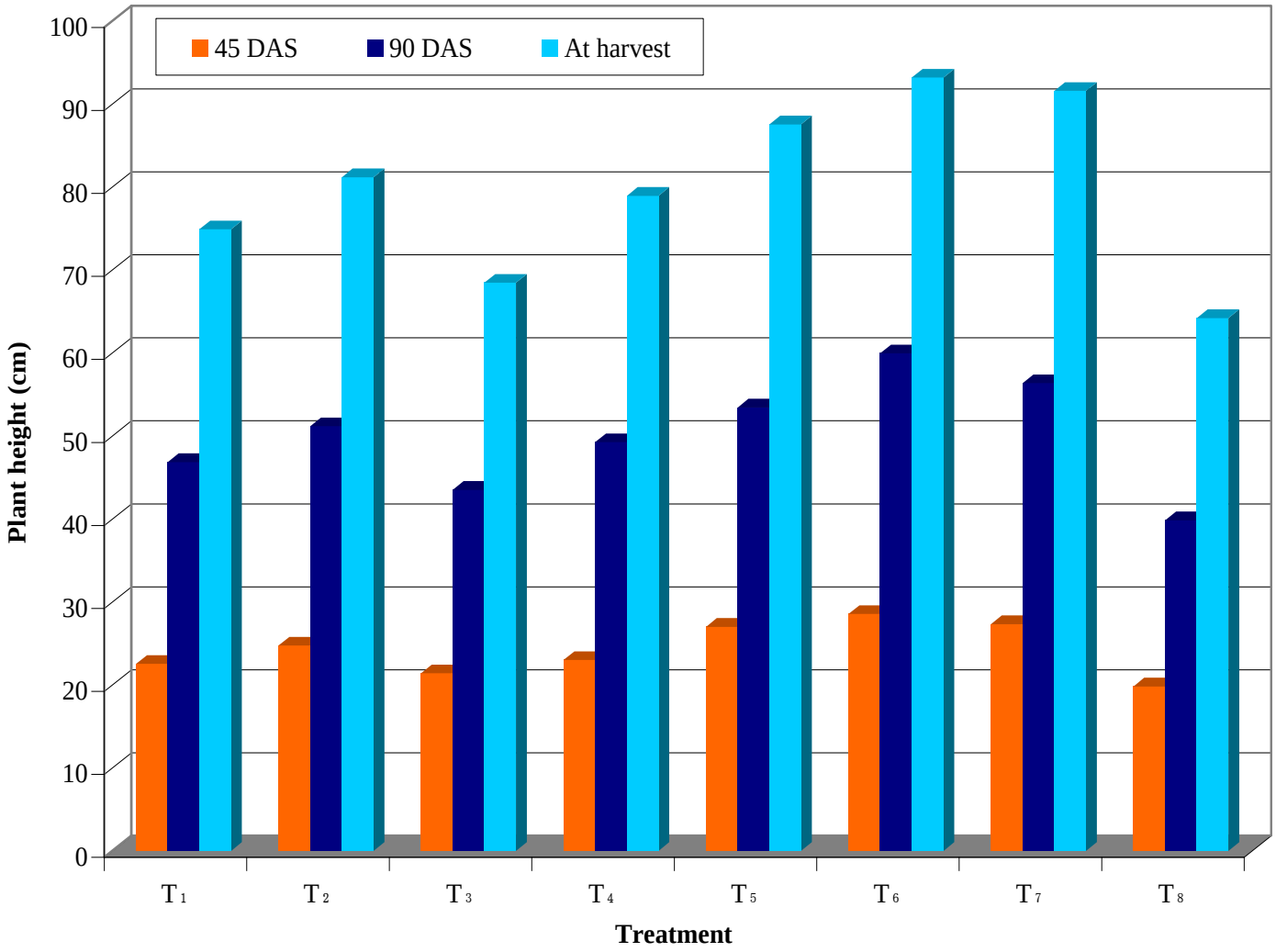

Fig. 4: Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on plant height of direct seeded rice

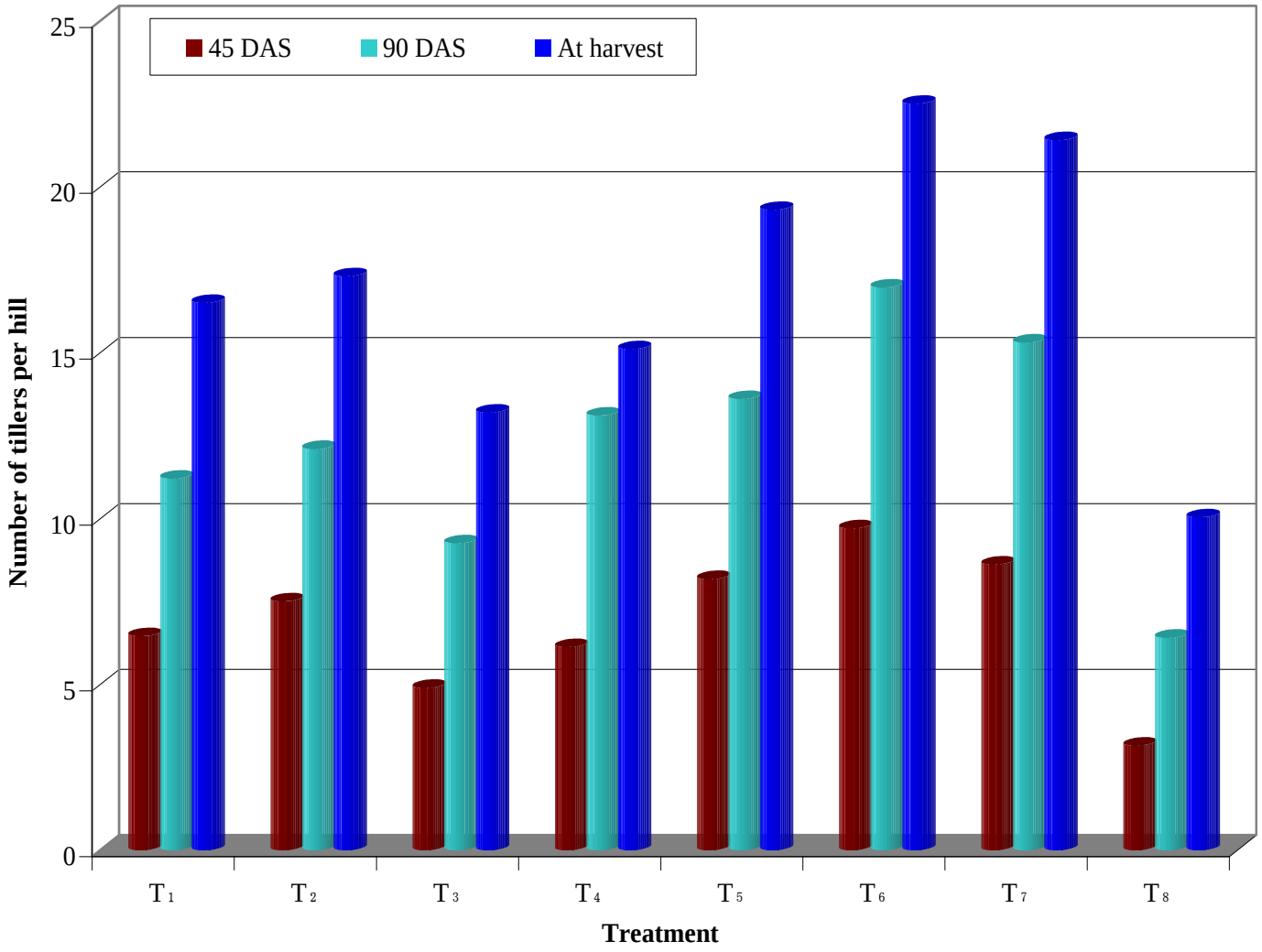

Fig. 5: Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on number of tillers of direct seeded rice

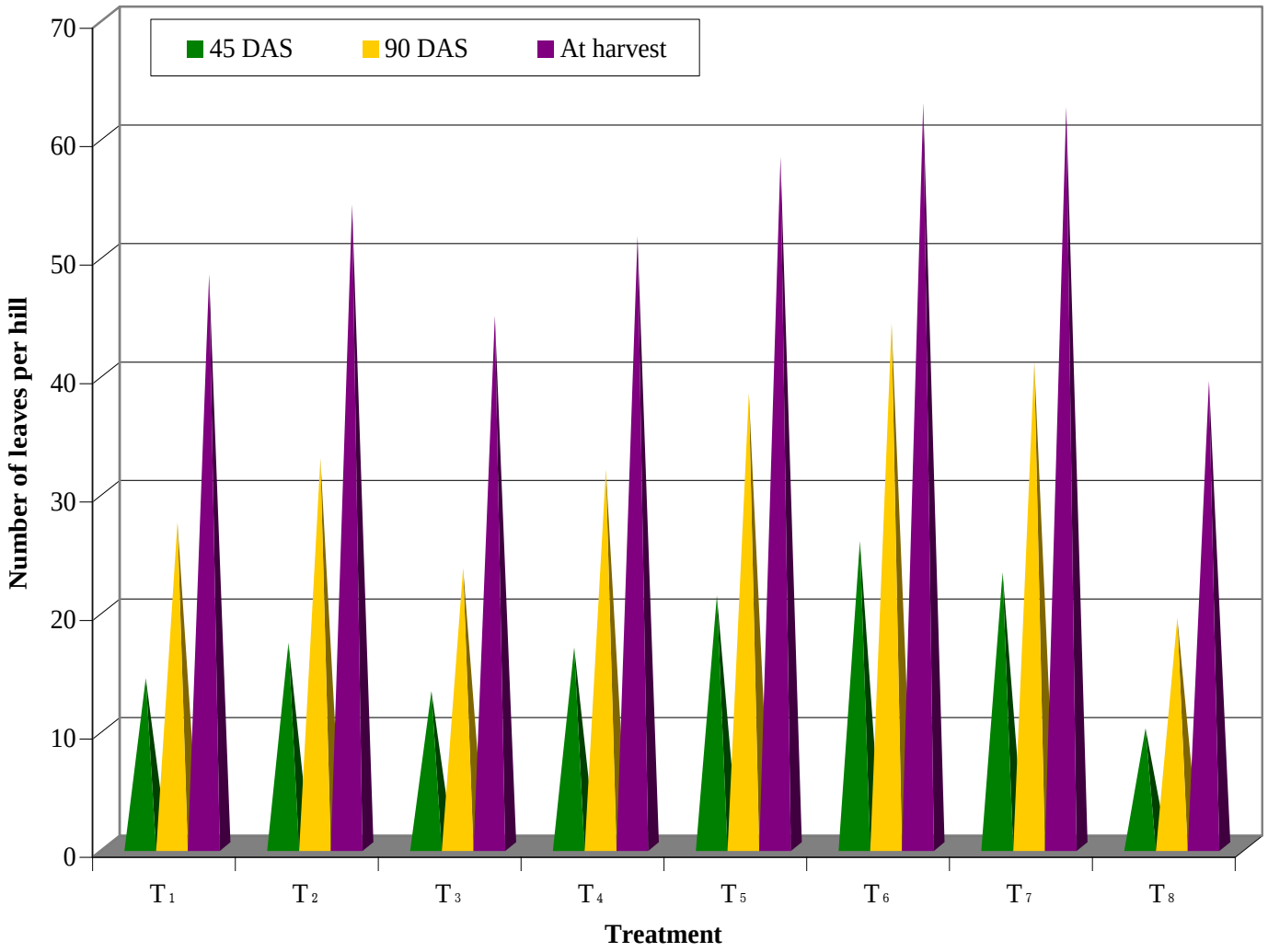

Fig. 6: Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on number of leaves of direct seeded rice

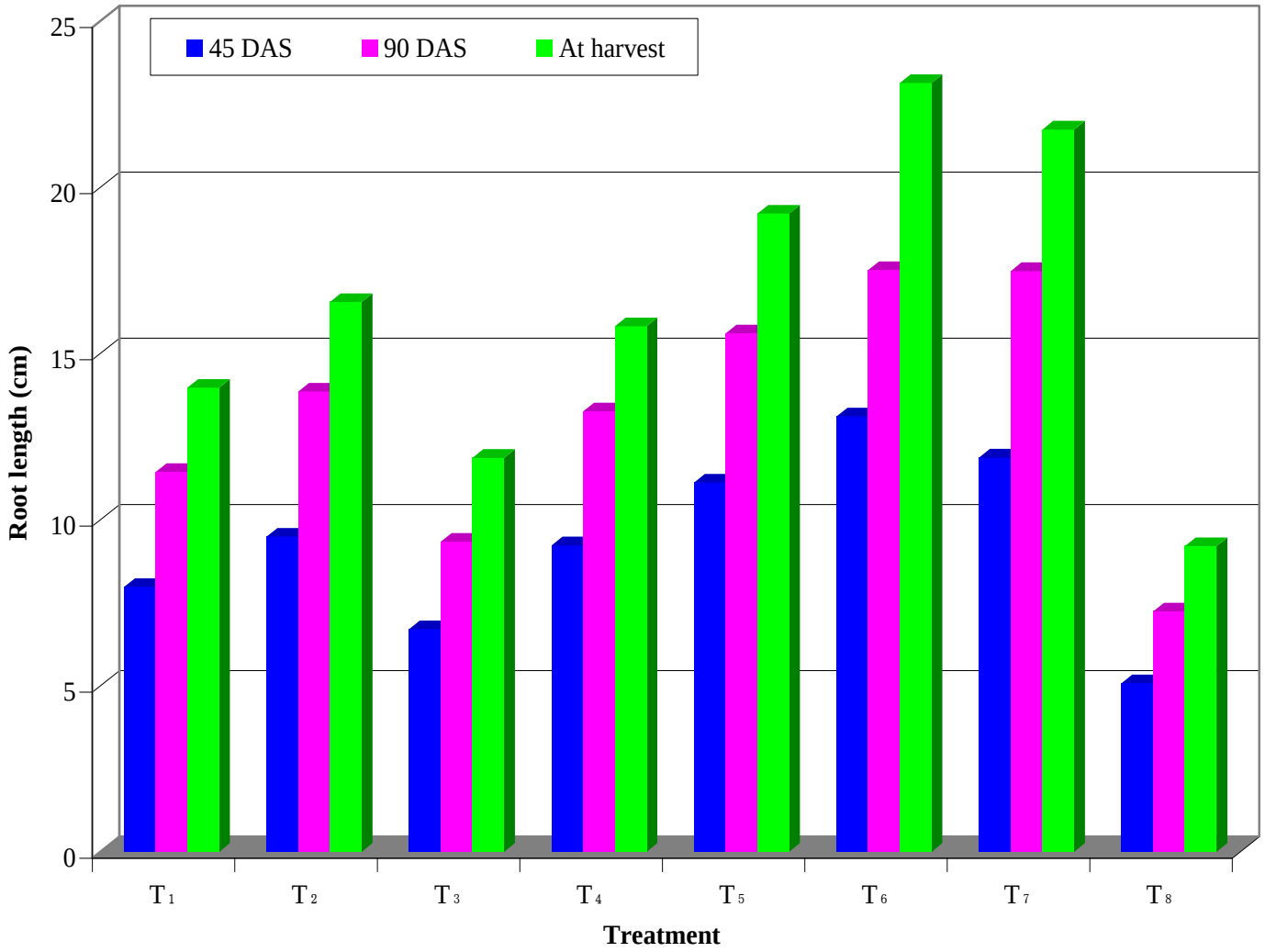

Fig. 7: Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on root length of direct seeded rice

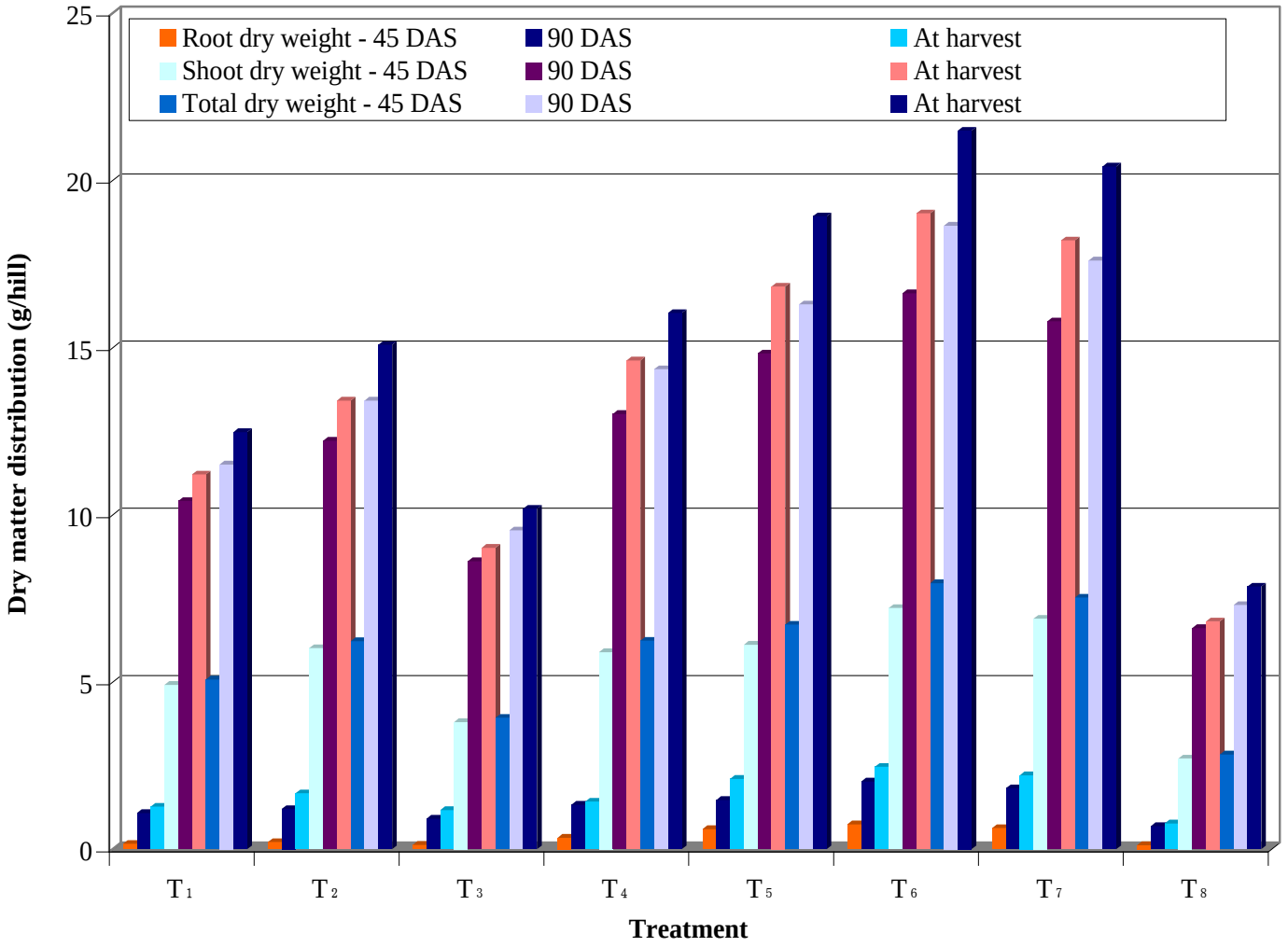

Fig. 8: Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on dry matter distribution of direct seeded rice

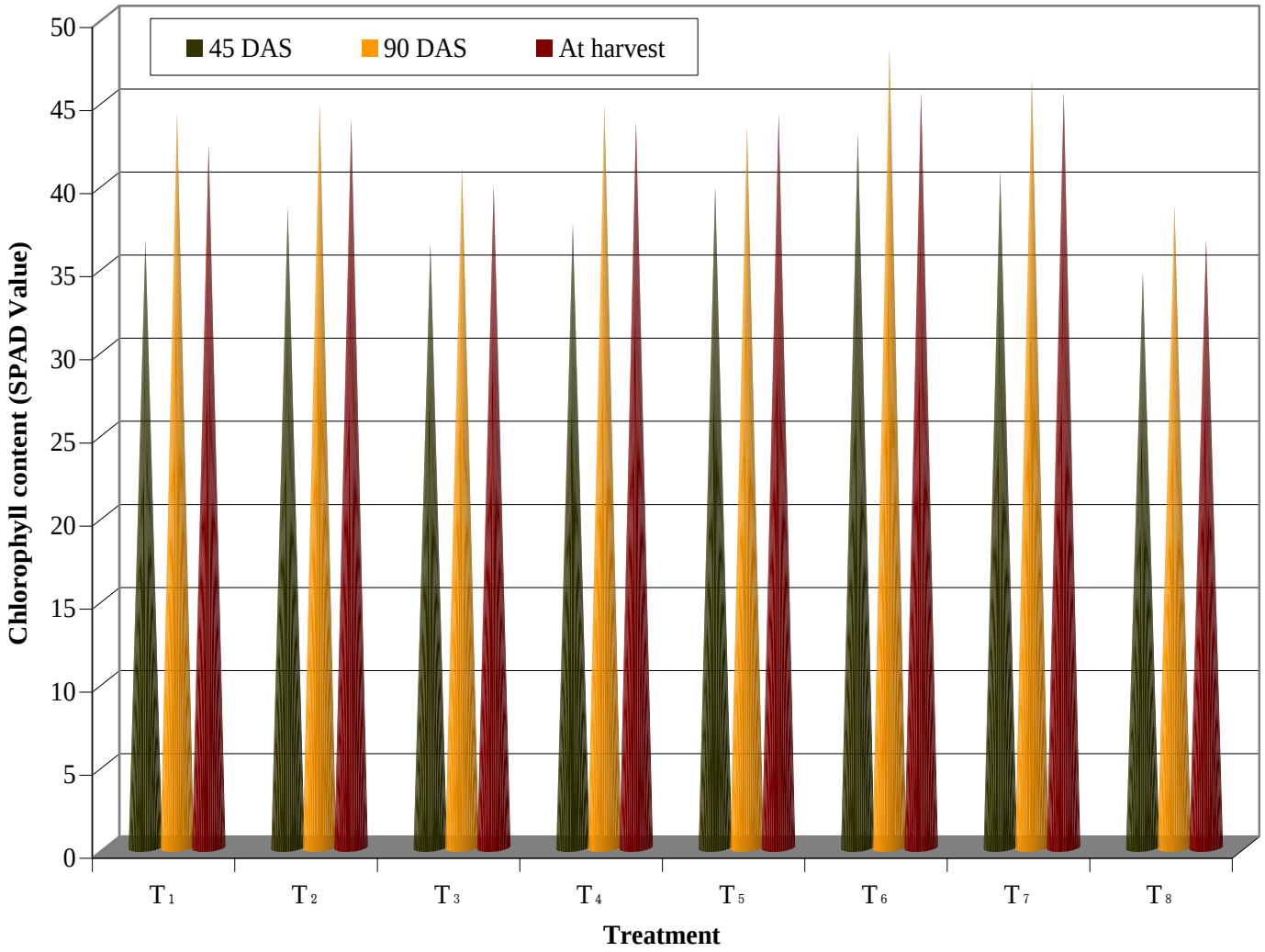

Fig. 9: Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on chlorophyll content of direct seeded rice

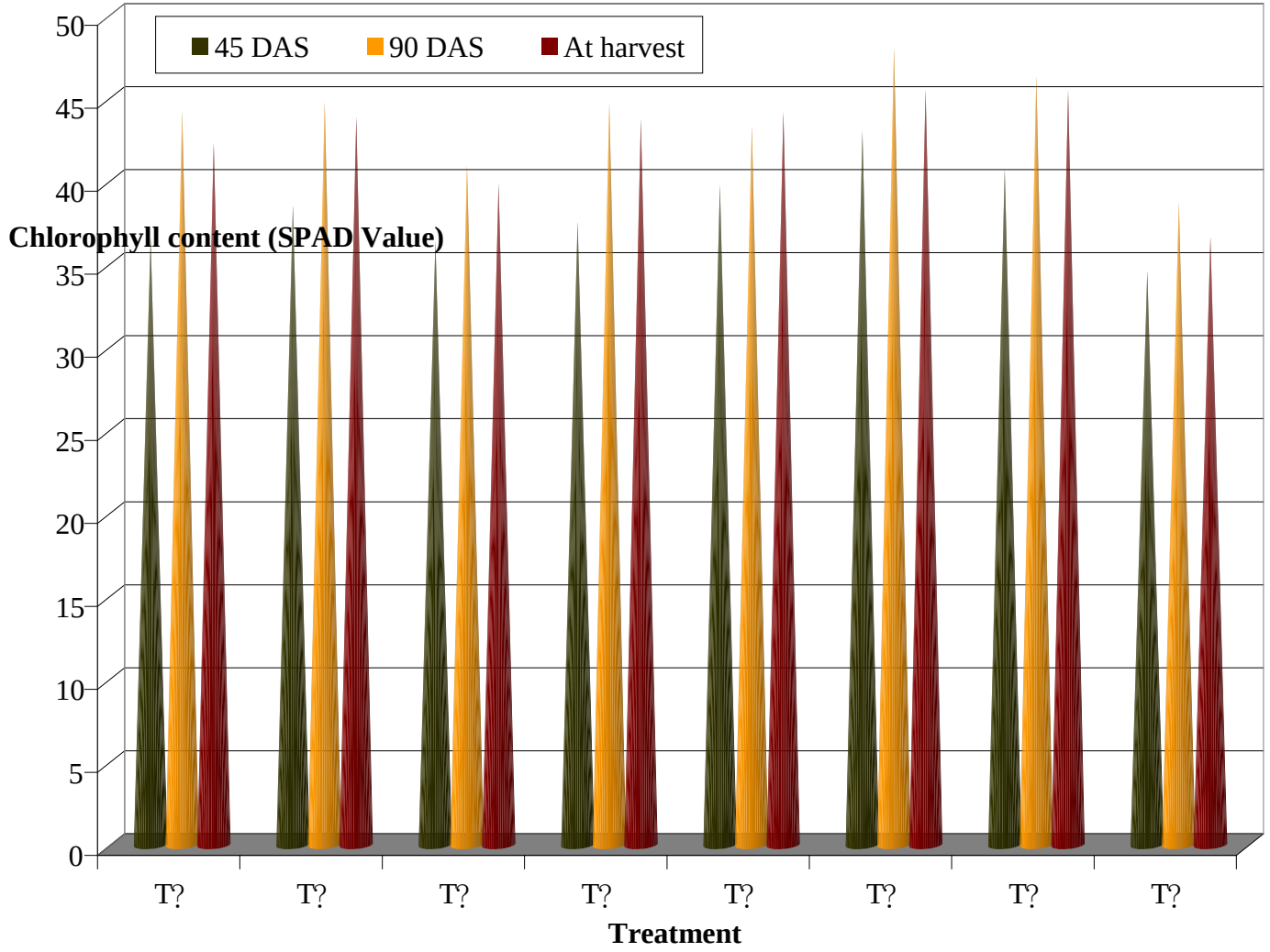

Fig. 9: Co-inoculation of efficient PPFM isolates and phosphate solubilizing bacteria on chlorophyll content of direct seeded rice

The results of the present investigation indicated in general, inoculation of efficient strains of PPFM along with PSB showed increased root and shoot dry weight as well as total dry matter production (Fig. 8) of DSR over the control at all stages of crop growth (45, 90 DAS and at harvest).

In combined inoculation, the maximum root and shoot dry weight was recorded in the treatment T_6 (PPFM-31 + PSB) showing 0.74, 20.02 and 2.46 g/hill and shoot dry weight of 7.21, 16.62 and 19.01 g /hill at 45, 90 DAS and at harvest respectively. Similarly in single inoculation treatments, T_2 (PPFM-31) recorded root dry weight of 0.21, 1.20 and 1.67 g/hill at 45, 90 DAS and at harvest respectively. Lower root dry weight of 0.12, 0.69 and 0.77 g/hill and root dry weight of 2.71, 6.61 and 6.81 g/hill were recorded at 45, 90 DAS and at harvest respectively in uninoculated control.

Similarly, in combined inoculation treatments, highest total dry matter of 7.95, 18.64 and 21.48 g /hill was noticed at 45 DAS, 90 DAS and at harvest respectively in T_6 compared to all other treatments. Lowest total dry matter production was observed in uninoculated control which showed 2.83, 7.30 and 7.85 g/hill at 45 DAS, 90 DAS and at harvest respectively.

Higher dry matter production in the above treatments might be due to higher plant height, more number of tillers/hill, more number of leaves and root length in inoculated treatments which further might have contributed for accumulation of higher dry matter in the rice crop. These results are conformity with reports of Pillai (1997), Thakur *et al.,* (1995) and Singh *et al.,* (2004).

Chlorophyll content of direct seeded rice increased significantly due to inoculation of PPFM isolates and PSB (Fig. 9).

In combined inoculation maximum chlorophyll content of 42.94, 47.98 & 45.45 SPAD value was recorded in T_6 at 45, 90 DAS and at harvest respectively. Similarly in single inoculation treatments, T_2 recorded chlorophyll content of 38.48, 44.68 and 43.80 SPAD value at 45, 90 DAS and at harvest respectively. Lowest chlorophyll content of 34.54, 38.63 and 36.58 SPAD value at 45, 90 and at harvest respectively was recorded in uninoculated control. The results have shown conformity with observations made by Madhaiyan *et al.,* (2004a) they observed that the *Methylobacterium* inoculation was found to increase the photosynthetic activity by enhancing the number of stomata, chlorophyll concentration and malic acid content of crops. Paulraj (2002) have also documented varied levels of chlorophyll content in cardamom, rubber and coffee due to PPFM bioinoculation.

The isolates selected were studied individually and in combination with PSB on endophytic association and plant growth promoting activities in direct seeded rice. Studies have shown that the endophytic diazotrophs *Methylobacterium* sp. colonizes the direct seeded rice plants and fixes atmospheric nitrogen. The effect of inoculation of PPFM isolates on plant growth parameters like plant height, number of tillers/hill, number of leaves/hill and chlorophyll content were recorded at various stages of crop growth *viz*. 45, 90 DAS and at harvest. Further, dry matter content was also recorded at 45, 90 DAS and at harvest. The results under pot culture condition clearly indicated that treatment T_6 (PPFM-31 + PSB) showed significantly maximum plant height, number of tillers/hill, number of leaves/hill, root length, total dry matter content and chlorophyll at all the stages of crop growth compared to other dual inoculation treatments, single inoculations and uninoculated control.

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