



Response of photosynthesis, chlorophyll fluorescence and yield of finger millet (*Eleusine coracana*) influenced by bio-chemical fertilizers

RAMWANT GUPTA¹, S K PANDEY², A K SINGH³ and MUNNA SINGH⁴

G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145

Received: 13 October 2010 Revised accepted: 17 March 2011

ABSTRACT

Finger millet (*Eleusine coracana*) is the main food grain for many people, especially in dry areas of India and Sri Lanka. The grains contain higher protein, fat and minerals than rice, corn, and sorghum. It provides a sustaining diet, especially for people associated with hard work. The effect of biofertilizer (*Trichoderma*) (TRI) along with farmyard manure (FYM) and chemical fertilizer (urea) with different doses applied to study their effects on photosynthetic characteristics, chlorophyll fluorescence (Fv/Fm), and yield of three finger millet varieties ('PRM 1', 'PRM 701' and 'PRM 801'). The yield attributes, viz grain yield; harvest index and 1 000 grain weight were found enhanced under the influence of FYM+TRI. The varieties 'PRM 1' and 'PRM 701' have responded to improved seed yield (ca. 60–66%) compared to 'PRM 801' (40%). Low nitrogen (12 mg/kg soil) and *Trichoderma* (0.5 mg/kg soil) have insignificantly favoured seed yield. Normal (24 mg/kg soil) and high nitrogen (36 mg/kg soil) both have up-regulated yield significantly. Optimal Fv/Fm values were observed in all three varieties as influenced by *Trichoderma* with FYM, with improved chlorophyll content in the leaves, better plant growth and biomass. The higher value for photosynthetic CO₂ assimilation, transpiration and stomatal conductance were influenced by FYM+TRI.

Key words: Chlorophyll fluorescence, Net photosynthetic rate, SPAD, Stomatal conductance, Transpiration rate

Finger millet (*Eleusine coracana*), commonly known as *ragi* is the main food grain for many people, especially in dry areas of India and Sri Lanka. The grains contain higher protein, fat and minerals than rice, corn, and sorghum (Maleshi 2001) converted into flour for making cakes, and puddings to provide a diet, especially to the people associated with hard work. The wide adaptability of the crop can be attributed to its C₄ nature. The Nitrogen (N) application often increases cell wall rigidity and osmotic adjustment (Saneoka *et al.* 2004). Photosynthetic responses of plants and N availability both have significant and positive correlation between photosynthetic capacity and leaf N content. The nitrogen is used for the bio-synthesis of components essential

for dynamics of photosynthetic apparatus (Alpha *et al.* 2009). It also improves photosynthetic capacity or stomatal control in water and N deficit conditions (Lima *et al.* 1999). Photo system-II (PSII) photochemistry has also demonstrated that N-deficiency has no effect on the quantum yield of PSII (Mauromicale *et al.* 2006). The N-deficiency reduces the maximal efficiency of PSII (Fv/Fm) and quantum yield of PSII in spinach (Huang *et al.* 2004). Biofertilizers are living microorganisms, able to fix atmospheric nitrogen either by living freely in the soil or being associated symbiotically with plants. *Trichoderma* spp. is free-living fungi or a virulent plant symbiont commonly found in soil *i.e.*, root ecosystems (Vinale *et al.* 2008). The effect of biofertilizer (*Trichoderma*) and chemical fertilizer (urea) were studied in isolation, and in combination to analyze the photosynthesis, chlorophyll fluorescence, and yield characteristics in *Eleusine coracana*.

MATERIALS AND METHODS

Two subsequent experiments were done at Department of Plant Physiology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during rainy (*kharif*) season of 2007 and 2008. The experiments were planned with completely randomized design (CRD) having seven treatments with three replications. Nursery of 'PRM 1', 'PRM

¹Post Doctoral Fellow (e mail: ramwantgupta@yahoo.com), Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi 110 012

¹ Ph.D. Scholar Department of Plant Physiology, CBSH

²Senior Research Fellow (e mail: sunilbhu123@gmail.com), Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110 012

³Senior Scientist (e mail: singhabr1972@yahoo.com), CPCT, Indian Agricultural Research Institute, New Delhi 110 012

⁴ Professor (email: drmunnaSingh@yahoo.com), Botany Department, Lucknow University, Lucknow, Uttar Pradesh 226 007

701' and 'PRM 801' were grown in trays (45 cm×30 cm×5 cm) filled with sterilized soil (5 kg soil in autoclave bag), after 21 days seedlings were transplanted in to plastic pots (4 plants/pot) filled with the sterilized soil (5 kg) autoclaved soil was treated with *Trichoderma harzianum* powder (TH-38) @ 2.5 mg/5 kg soil, and with urea, normal (NN) @ 120 mg, low (LN) @ 60 mg and high (HN) @ 180 mg/5 kg soil Nitrogen. The farmyard manure (FYM) mixed with of soil in the ratio of 1: 1 (2.5 kg FYM: 2.5 kg soil). Two subsequent doses of fertilizers were applied at 10 and 60 days after transplanting. Photosynthetic rate, P_N ($m \text{ mol CO}_2/m^2/s^1$), was measured with the help of portable CO_2 gas analyzer (Open system, CID Inc. USA). The observations were recorded in the forenoon (11 am) using light ($\sim 1500\text{--}1800 \text{ m mol/m}^2/s^1$). Net Photosynthetic rate, Transpiration rate, and stomatal conductance were assessed on intact leaves.

Chlorophyll fluorescence emitted by green plants reflects photosynthetic ability of PS-II. A handy plant efficiency analyzer (Handy PEA, Hansatech, UK) was used to monitor (Fv/Fmax). Measurements were recorded in the forenoon hours (10–11 am) to avoid photo inhibition. The minimal fluorescence level (F_0) with PS II reaction centre was determined by measuring under modulated light, not to induce any significant variable fluorescence. The maximum fluorescence level (F_m) of closed PS-II centre was determined by providing 1.5 s. saturating pulse at $3000 \text{ m mol/m}^2/s^1$ on dark adopted leaves by using leaf clips attached to mid portion of each leaf for 10 minutes. The fluorometer probe connected to leaf clip holder and Fv/Fmax ratio recorded. Leaf chlorophyll contents were checked in three leaflets, utilized in obtaining net photosynthetic rate (P_N) measurements by using portable chlorophyll meter (SPAD-502, Minolta Camera Co. Ooka, Japan) based on the comparison of leaf transmittance at 650 and 940 nm.

The seed yield was calculated after harvesting plants grown in the pots. The calculations made as per the yields on hectare basis. Calculated total number of plants in one hectare, one pot contain 4 plants (Area of pot= $\delta r^2 \text{ (m}^2\text{)}$, 1 hectare= $10,000 \text{ m}^2$, and multiply the weight of grain of one plant into total number of plants.

RESULTS AND DISCUSSION

Application of high nitrogen, FYM and combination of FYM+TRI improved SPAD values and total chlorophyll content matches well with the level of green appearances of the leaves, treated differentially (Fig 1). *Trichoderma* in isolation have not responded, low and normal nitrogen doses slightly enhanced SPAD values and total chlorophyll content. Chlorophyll fluorescence (Fv/Fm) correlates quantum yield of PS II. The chlorophyll fluorescence (Fv/Fm) value was up-regulated by high nitrogen, FYM and FYM+TRI. *Trichoderma* alone have not positively influenced Fv/Fm values (Fig 2). The values were found superior during flowering as compared to the vegetative stage irrespective

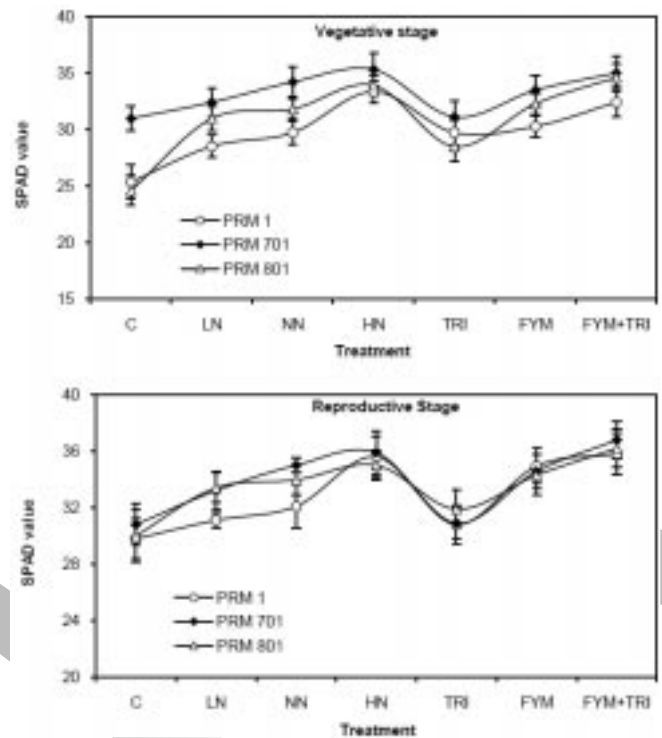


Fig 1 Effect of chemical/biofertilizer on SPAD value of finger millet varieties

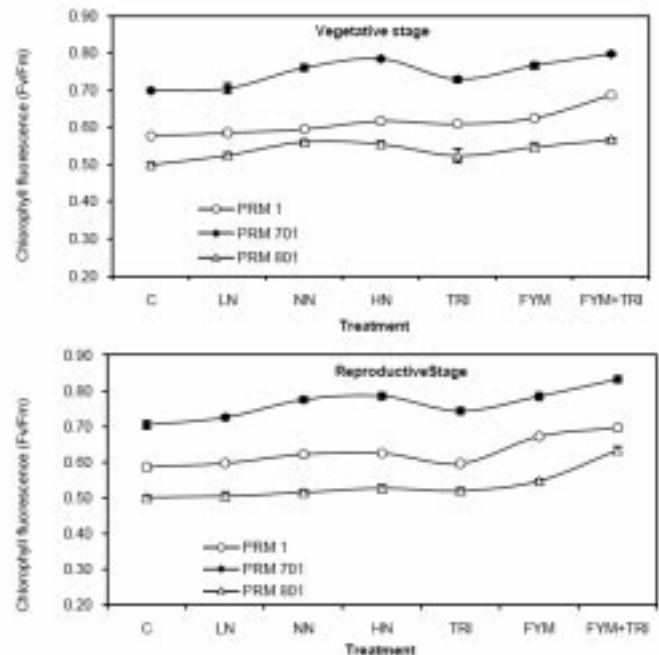


Fig 2 Effect of chemical/biofertilizer on chlorophyll fluorescence (Fv/Fm) of finger millet varieties

of control and various treatments. The application of HN, FYM and combination of FYM+TRI helped in acquiring higher photosynthetic CO_2 assimilation rate. The CO_2 assimilatory rates were found ca. $12.6 \mu\text{mol/m}^2/s^1$ and 7.9

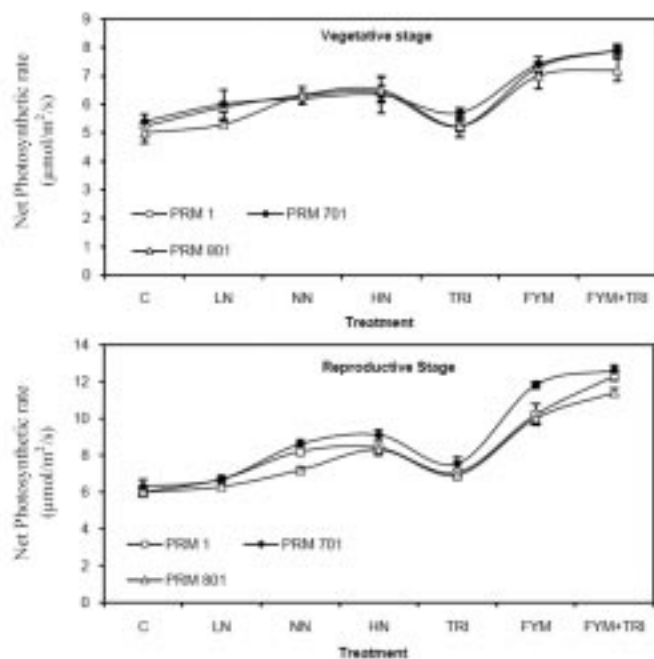


Fig 3 Effect of chemical/biofertilizer on net photosynthetic rate (P_N) of finger millet varieties

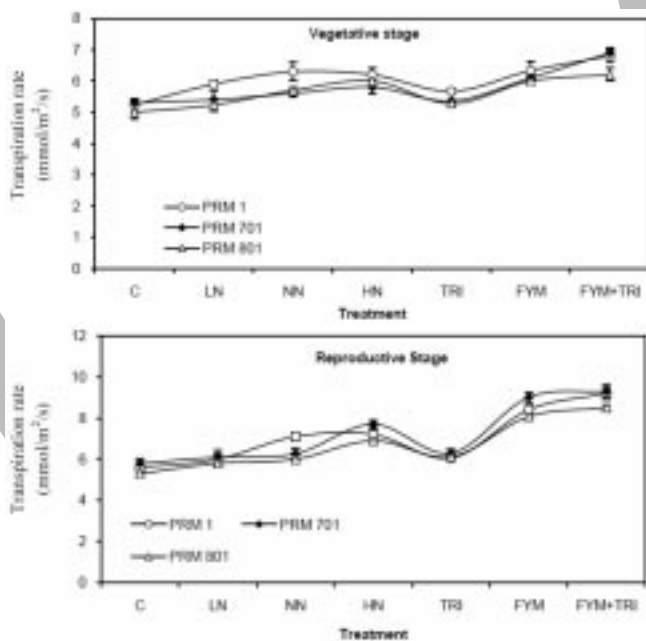


Fig 4 Effect of chemical/biofertilizer on transpiration rate of finger millet varieties

$\mu\text{mol}/\text{m}^2/\text{s}^1$ during reproductive and vegetative stages (Fig 3) while trend transpiration rate were found more or less similar (Fig 4). The HN, FYM and FYM with TRI promoted stomatal conductance (Fig 5). The SPAD values retention of functional chlorophyll/chloroplast were found up-regulated by the availability of adequate nitrogen. The photosynthetic capacity of leaves is related to the nitrogen content primarily because of proteins of the Calvin cycle, and thylakoids which

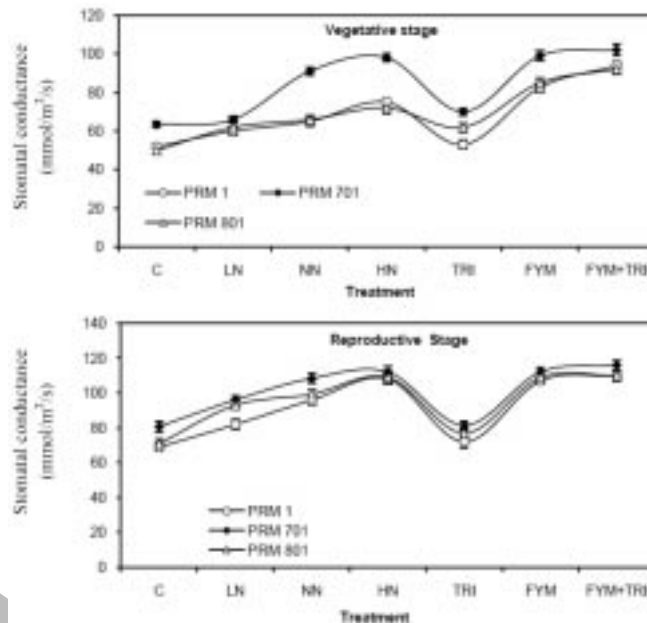


Fig 5 Effect of chemical/biofertilizer on stomatal conductance of finger millet varieties

represent the majority of leaf nitrogen associated with chlorophyll (Li *et al.* 2007, An and Shangguan 2008, Wu *et al.* 2008 and Hgaza *et al.* 2009). FYM+TRI might have extended fertile rhizospheric surroundings around the root system for improved plant fitness and to facilitate the acquisition of mineral nutrients from the soil (Lopez-Bucio *et al.* 2005). An imbalance in these characteristics may down-regulate ca. 40% of photosynthesis product, can be lost from the roots (Bais *et al.* 2006). The chlorophyll fluorescence was found to increase with increasing N levels, could also be seen by the increase pool size of electron acceptors of PSII due to direct response of chlorophyll content (Mauromicale *et al.* 2006). The value of net photosynthesis, transpiration and stomatal conductance seemed to be influenced by higher nitrogen availability (Wu *et al.* 2008) because photosynthesis involve in reducing atmospheric CO_2 (Schmidhuber and Tubiello 2007). Hence increased photosynthesis is associated with improved carboxylation efficiency linked with nitrogen availability in all finger millet varieties to enhance photosynthetic capacity, which eventually conferred improved plant productivity/seed yield (An and Shangguan 2008 and Wu *et al.* 2008). The value of grain yield, harvest index and 1000-grain weight were found higher with increasing N levels in all three ragi due to the enhanced availability of nitrogen to the roots. Consequently, it favored higher leaf area which resulted in higher intercepting light energy for its phototransformation to the level of biomolecules/photosynthates. This has sustained higher grain yield and dry matter accumulation ultimately. Hence nitrogen fertilizer improved plant growth, photosynthetic CO_2 assimilation, translocation of assimilates

Table 1 Effect of chemical/biofertilizer on harvest index, 1000 grain weight and yield of finger millet varieties.

Varieties	Treatments	HI (%)	1000 grain weight (g)	Yield(q/ha)
PRM-1	C	37.3±1.11	2.3±0.079	21.6±1.05
	LN	38.2±1.27	2.4±0.079	23.0±2.62
	NN	40.1±1.25	2.5±0.079	30.4±1.43
	HN	41.1±1.32	3.0±0.081	32.2±1.24
	TRI	38.0±1.27	2.3±0.079	23.0±2.13
	FYM	42.6±1.15	3.1±0.079	34.0±1.85
	FYM+TRI	43.6±1.22	3.1±0.076	35.5±1.51
	PRM-701	C	33.6±1.07	2.3±0.079
LN		33.9±1.26	2.3±0.078	23.1±1.14
NN		39.3±1.24	2.5±0.079	27.2±1.11
HN		41.4±1.28	3.1±0.079	32.0±1.12
TRI		35.7±1.24	2.3±0.080	23.1±1.11
FYM		42.1±1.26	3.2±0.079	33.8±1.12
FYM+TRI		43.9±1.25	3.4±0.079	35.6±1.10
PRM-801		C	31.2±1.28	2.2±0.079
	LN	33.1±1.28	2.3±0.080	19.6±1.03
	NN	37.3±1.24	2.3±0.079	20.7±1.12
	HN	38.6±1.28	2.6±0.079	22.4±1.11
	TRI	31.5±1.24	2.3±0.080	18.9±1.07
	FYM	40.4±1.25	2.7±0.079	25.9±1.14
	FYM+TRI	41.6±1.26	2.8±0.079	26.4±1.15

and yield attributes associated with grain yields (Manzoor *et al.* 2006 and Rehman *et al.* 2010). The application of chemical and biological fertilizers up regulated value of harvest index, 1000 grain weight and seed yield (q/ha). The low nitrogen and *Trichoderma* alone have insignificantly enhanced seed yield characteristics i.e. ca. 5–6%. The NN and HN both have improved to acquire higher seed yield ca. 40%. FYM and FYM+TRI increased the yield ca. 2 fold. The ragi varieties, viz ‘PRM 701’ and PRM-1 both have responded to enhance seed yield (ca.60–66%) compared to PRM-801 (ca. 40%). The *Trichoderma* per se could not regulate seed yield (Table 1). FYM and FYM with *Trichoderma* significantly affected the grain yield, harvest index as well as 1000 grain weight due to increased root growth response for the better uptake of macro-micronutrients from the conducive rhizosphere (Yedidia *et al.* 2001). The increased growth response may subsequently result in enhanced yield, as found in other crops (Bal Altintas 2008 and Hasanloo *et al.* 2010).

REFERENCES

- An H and Shangguan, Z P. 2008. Specific leaf area, leaf nitrogen content, and photosynthetic acclimation of *Trifolium repens* seedlings grown at different irradiances and nitrogen concentrations. *Photosynthetica* **46** (1): 143–7.
- Alpha J M, Chen J and Zhang G. 2009. Effect of nitrogen fertilizer forms on growth, photosynthesis, and yield of rice under cadmium stress. *Journal of Plant Nutrition* **32**: 306–17.
- Bais H P, Weir T L, Perry L, Gilroy S and Vivanco J M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology* **57**: 233–66.
- Bal U and Altintas S. 2008. Effects of *Trichoderma harzianum* on lettuce in protected cultivation. *Journal of Central European Agriculture* **9** (1): 63–0.
- Hasanloo T, Kowsarib M, Naraghia M S and Bagheri O. 2010. Study of different *Trichoderma* strains on growth characteristics and silymarin accumulation of milk thistle plant. *Journal of Plant Interactions* **5** (1): 45–9.
- Hgaza V K, Diby L N, Akéa S and Frossard E. 2009. Leaf growth and photosynthetic capacity as affected by leaf position, plant nutritional status and growth stage in *Dioscorea alata*. *Journal of Animal and Plant Sciences* **5** (2): 483–93.
- Huang Z A, Jiang D A, Yang Y, Sun, J W and Jin S H. 2004. Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. *Photosynthetica* **42** (3): 357–64.
- Li P, Caib R, Gaoa H, Penga T and Wang Z. 2007. Partitioning of excitation energy in two wheat cultivars with different grain protein contents grown under three nitrogen applications in the field. *Physiologia Plantarum* **129**: 822–29.
- Lima J D, Mosquim P R, Da Matta F M 1999. Leaf gas exchange and chlorophyll fluorescence parameters in *Phaseolus vulgaris* as affected by nitrogen and phosphorus deficiency. *Photosynthetica* **37**: 113–21.
- Lopez-Bucio J, Cruz-Ramírez A, Perez-Torres A, Ramírez-Pimentel J G, Sanchez-Calderon L, and Herrera-Estrella L. 2005. Root Architecture. *Plant Architecture and Its Manipulation*, pp 181–06. Turnbull C (Eds). Blackwell Scientific, Oxford.
- Malleshi N G. 2001. Nutritional qualities of millets and pseudo cereals. Course material of *short-term Course on Processing and Value-addition to Millet and Pseudo-cereals*, pp 17–6. 19–23 November. CFTRI, Mysore.
- Manzoor Z, Ali R I, Awan T H, Khalid N and Ahmad M. 2006. Appropriate time of nitrogen application to five rice (*Oryza sativa*). *Journal of Agricultural Research* **44**: 261–7.
- Mauromicale G, Ierna A and Marchese M. 2006. Chlorophyll

- fluorescence and chlorophyll content in field-grown potato as affected by nitrogen supply, genotype, and plant age. *Photosynthetica* **44** (1): 76–2.
- Rehman H, Ali A, Waseem M, Tanveer A, Tahir M, Nadeem M A and Zamir M S I. 2010. Impact of nitrogen application on growth and yield of maize (*Zea mays*) grown alone and in combination with cowpea (*Vigna unguiculata*). *American Eurasian Journal of Agriculture and Environmental Science* **7** (1): 43–7.
- Saneoka H, Moghaieb R E A, Premachandra G S, Fujita K. 2004. Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostis palustris* Huds. *Environmental and Experimental Botany* **52**: 131–38.
- Schmidhuber J and Tubiello F N. 2007. Global food security under climate change. *Proceedings of National Academy of Sciences* **104** (50): 19703–8.
- Vinale F, Sivasithamparamb K, Ghisalbertic E L, Marraa R, Wooa S L and Loritoa M. 2008. *Trichoderma* plant pathogen interactions. *Soil Biology and Biochemistry* **40**: 1–0.
- Wu F Z, Bao W K, Li F L and Wu N. 2008. Effects of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings. *Photosynthetica* **46** (1): 40–8.
- Yedidia I, Srivastva A K, Kapulnik Y and Chet I. 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant and Soil* **235** (2): 235–42.

ICAR