Growth and yield response of cowpea to multi-action bioinoculants

Jidhu Vaishnavi, S.*, and Jeyakumar, P.

Department of Crop Physiology, Tamil Nadu Agriculture University, Coimbatore-641 003, India.
Received: 10-03-2015 Accepted: 19-10-2015

ABSTRACT
Studies were taken up to estimate the morpho-physiological changes in cowpea due to a multi-action bioinoculants (TagTeam) as seed treatment. Seed treatment with TagTeam @ 8.1g/kg showed significant increase in plant height, root length and number of nodules as compared to control. Physiological parameters such as leaf area, total dry matter accumulation, photosynthetic rate and chlorophyll index were also found higher in seed treatment with TagTeam @ 8.1g/kg. Co-inoculation of Rhizobium and Penicillium (TagTeam) @ 8.1g/kg enhanced the uptake of NPK in cowpea. Available NPK was found non significant with initial soil sample and found maximum with control and lower content in TagTeam seed treatment @ 8.1g/kg. Seed treatment with TagTeam @ 8.1g/kg increased the yield by 13.36 per cent over control and improved yield components and seed protein content in cowpea.

Key words: Cowpea, Hormones, Nutrients, Penicillium, Rhizobium, TagTeam, Yield.

INTRODUCTION
Leguminous plants are one of the prime source of proteins for human being especially vegetarians. Cowpea is one amongst them, which is well adapted to tropical regions. In India, cowpea is cultivated in an area of 0.5 million ha with a productivity of around 600-750 kg grains ha⁻¹ (www.dacnet.nic.in.). In Tamil Nadu, cowpea is grown in 1.2 lakh ha with a production of 0.25 lakh tonnes and the productivity is very low with 205 kg ha⁻¹. Cowpea has the ability of fixing atmospheric nitrogen in root nodules through symbiosis with Rhizobium. This attribute ensures that, it will enhance yield in an era of increasing food demand and concern for sustainable agricultural production system. Rhizobium legume symbiosis is the primary source of fixed nitrogen that definitely increases the biological nitrogen fixation into soil ecosystem through nodule formation. The benefit of nitrogen uptake by Rhizobia includes production of phytohormones and siderophores which can solubilize sparingly soluble organic and inorganic phosphates.

Biological N₂ fixation requires high P because the process requires more energy and energy generating metabolism is associated with availability of P (Plaxton, 2004). Several fungal species like Penicillium and Mycorrhiza stimulates phosphorus uptake by altering root architecture of the plant. The addition of Penicillium can solubilize rock phosphate and organically bound soil P (which constitutes 95 - 99% of the total phosphate in soils). It improves nutrient uptake of the plant through root hair enrichment from sparingly available to well-developed one. There are many works on growth and yield of cowpea in response to Rhizobia (Ahmed and Kibret, 2014). Similarly, effect of Penicillium on growth and yield has been reported by few scientists (Reyes et al., 1999; Prasanna et al., 2012 and Jeyakumar, 2014). Dual inoculation of Rhizobium and fungi helps in increasing nutrient uptake by plant thereby supports in growth and development which ultimately leads to increase in yield. However, the effect of dual inoculation of bacteria and fungi on physiology of crops is not well documented.

The aim of the present study was to assess the effect of TagTeam, a bioinoculant, on morphological, physiological characters and yield in vegetable cowpea. TagTeam is a multi-action legume fertility inoculant, a combination of the naturally occurring soil fungus Penicillium bilaii (1.3 x 10⁶ cfu) and the bacterial strain Rhizobium leguminosarum (1.3 x 10⁶ cfu). It makes better use of phosphate and provides more fixed nitrogen to the plant. The fungus, Penicillium forms a symbiotic relationship with plant roots that makes more phosphate available to the plants, while Rhizobium provides better nodule development for more nitrogen fixation. Cell-Tech contains Rhizobium leguminosarum which works in symbiotic relationship with the plant to fix nitrogen. The improvement in the plant nutrient status and resultant physiological efficiency of the crop favours higher yield and quality.

MATERIALS AND METHODS

The field experiment was conducted in cowpea (var. VBN2) at Tamil Nadu Agricultural University, Coimbatore during Kharif season with four replications in Randomized Block Design using TagTeam and CellTech as treatments. The treatments include T₁: untreated control, T₂: Seed

*Corresponding author’s e-mail: sjvaishnavi@gmail.com
treatment with *Rhizobium* @ 30g/kg, T5; Seed treatment with *TagTeam* @ 2.7g/kg, T6; Seed treatment with *TagTeam* @ 5.4g/kg, T7; Seed treatment with *TagTeam* @ 8.1g/kg, T8; Seed treatment with *TagTeam* @ 10.8g/kg and T9; Seed treatment with *CellTech* @ 2.8ml/kg.

Morphological observations like plant height (cm plant	extsuperscript{1}), root length (cm plant	extsuperscript{1}), number of nodules, leaf area and total dry matter production (g plant	extsuperscript{1}) were recorded in five plants per replication. Leaf area for the whole sampling unit was measured by using Leaf Area Meter (LI-3100, Licor Inc., Nebraska, USA) and expressed as cm	extsuperscript{2} plant	extsuperscript{1}. Photosynthetic rate ($P_o$) was recorded using an advanced portable CO$_2$ gas analyzer (LI-6400 XT, Licor Inc, Nebraska, USA) in open type principle. Chlorophyll meter from Minolta (model 502 of Minolta, Japan) was used to measure chlorophyll index.

The soil samples and dried plant samples were collected and determined for nitrogen, phosphorus and potassium content from triple acid extract and soil extract respectively (Piper, 1966). The total nitrogen content in plant samples were estimated by Microkjeldhal method as proposed by Humphries (1956), phosphorus by colorimetric method (Piper, 1966) and potassium by flame photometer (Piper, 1966). The total NPK uptake at different stages were estimated by Microkjeldhal method as proposed by Humphries (1956), phosphorus by colorimetric method (Piper, 1966) and potassium by flame photometer (Piper, 1966). The total NPK uptake at different stages were worked out by summing up the uptake in different parts of the plant after multiplying the total dry matter of the plant parts with the corresponding NPK content and expressed as kg ha	extsuperscript{-1}.

The important components contributing to the yield potential of the crop were recorded from the commencement of flowering to the harvest of the crop. Number of days from sowing to the commencement of flowering in 50 per cent of the population was counted in each treatment of all the four replications and the mean values expressed as days to 50 per cent flowering. The total number of flowers produced from the commencement to the end of the flowering period was recorded in five plants in each treatment for each replication and the mean values were worked out. The fertility co-efficient was arrived from relationship between the number of flowers produced per plant and the number of pods produced per plant and expressed in per cent. The number of pods produced in each plant was counted from the five plant samples in each treatment and replication at the harvest stage and the average was calculated and expressed in number. Grain yield per hectare was calculated and expressed in tonnes ha	extsuperscript{-1}. The protein content of the seed was estimated by following Bradford (1971). The data collected were subjected to statistical analysis in randomized block design following the method of Panse and Sukthme (1961).

**RESULTS AND DISCUSSION**

**Plant height:** Seed treatment with bioinoculants like *Rhizobium*, *TagTeam* and *CellTech* showed significant influence on plant height. In cowpea, maximum plant height was observed in T7 (46.43 cm) which was on par with T5 (46.40 cm). The lowest plant was observed in control (44.67 cm). This increase in plant height was due to the effect of both *Rhizobium* and *Penicillium bilaii* in the product (*TagTeam*) compared to control and *Rhizobium* treatment (Fig. 1). *Rhizobium* inoculation play a major role in synthesis of plant growth promoting hormones like auxin, cytokinin and gibberellins (Madhavan et al., 2012). Plant growth promoting fungi (PGPF) improve plant growth indirectly by altering the structure of soil rhizosphere, producing certain metabolites and providing minerals to plants (Murali et al., 2012). *Penicillium* plays a major role in enhanced nutrient uptake leads to increase in cell division, elongation and the improvement in metabolite activity which leads to increased vegetative growth of plant. The increase in plant height with dual inoculation was attributed to increase in nitrogen and phosphorous uptake by the plant (Geeta et al., 2013), plant growth promoting substances and also nutrient requirement for their growth.

**Root length:** Seasonal influence on root length was observed. Maximum root length (Fig. 1) was observed in T7 (16.07 cm) followed by T5 (15.14 cm) and T4 (15.09 cm) and minimum root length was in control (17.34 cm). The increase in root length was due to the production of auxin and mineralization of nutrients by PGPR (Steenhoudt and Vanderleyden, 2000). *Rhizobium* helps in auxin synthesis which has direct role in root development (Bhattacharyya and Pati, 2000). *P. bilaii* inoculation helps in enhancing phosphorous uptake by the plant which has direct role in root development (Prasanna et al., 2012). The dual inoculation of bacteria and fungi enhances the length by producing plant growth promoting substances and also nutrient requirement for their growth.

**Number of nodules:** On comparing the number of nodules in different treatments, T7 (23.00) excelled other treatments (Fig. 1). The lowest number was observed in T1 (11.80). The increase in nodule number with *Rhizobium* treatment was due to their multiplication in the roots to form nodules (Fontenele et al., 2014). *P. bilaii* indirectly enhances the nodule number by providing more P available to the plants. P is required for energy production. Thus energy required for nodule formation was supported by *P. bilaii* (Heisinger, 1998). More number of nodules in seed treatment with *TagTeam* than *CellTech* and *Rhizobium* treatment is due to the presence of both *Rhizobium* and *Penicillium* in *TagTeam* which causes higher energy production from *Penicillium* and are effectively utilized by the *Rhizobium* for their multiplication to obtain more number of nodules in the roots (Guo et al., 2012).

**Total dry matter production:** It has direct relationship with photosynthesis and yield. Seed treatment with *TagTeam* @ 8.1g/kg has enhanced total dry matter production (24.55g
Fig 1: Effect of seed treatment with multiaction bioinoculants on growth characters of vegetable cowpea at flowering stage

plant$^1$) compared to all other treatments (Fig. 1) followed by seed treatment with TagTeam @ 5.4g/kg (24.33g plant$^{-1}$). The lowest total dry matter production was observed in T$_1$ (21.97g plant$^{-1}$). *Rhizobium* present in TagTeam applied as seed treatment enhances the nitrogen uptake and directly correlates with improved vegetative growth. Most of the legumes, being source limited crop, improvement in vegetative growth reduce their limitation and enrich the dry matter production (Yadegari and Ashadi, 2010; Cigdem, 2011). *Penicillium* present in TagTeam enhances the nutrient uptake in the plants, especially phosphorous. The increase can be correlated with increase in metabolite activity and adequate nutrient supply (Vessey and Hasinger, 2001). The increased nutrient supply enhanced the assimilation of carbon, nitrogen, phosphorous and sulphur. The increased assimilate production; translocation and accumulation in the different parts of the cowpea $viz.$, shoot, root and pod enhanced the total dry matter content (Dashti et al., 1997). The co-inoculation of *Rhizobium* and *Penicillium* enriches the nutrient uptake and enhance photosynthetic efficiency, chloroplast content, leaf area, vegetative growth of plant and dry matter accumulation. Similar increase was observed with dual inoculation of *Rhizobium* and *Trichoderma* and *Pseudomonas* on mungbean (Gangwar et al., 2013; Geeta et al., 2013).

**Leaf Area:** Maximum leaf area was observed in seed treatment with TagTeam @ 8.1g/kg (558.42) followed by seed treatment with TagTeam @ 5.4g/kg (557.74). Minimum leaf area was observed in control (513.85). The increase in leaf area is due to *Rhizobium* inoculation which also improves the chlorophyll content in the leaves (Anjum et al., 2006). Phosphorous uptake by *Penicillium* serves many functions like sugar-starch utilization, cell division and organization, photosynthetic use efficiency and formation of green pigments in plants which finally improve leaf area in the plant (Fatima et al., 2006). Large leaf area helps the plants to intercept large amount of solar radiation which indirectly increases the dry matter production (Shibles and Weber, 1966). The leaf area with high photosynthetic activity has a direct relationship with seed yield of crop plants (Thandapani, 1985 and Chandrababu, 1990). An increased ability for nutrient uptake caused an increase in growth and photosynthesis, and therefore increased crop leaf area. These results are in agreement with those obtained by Beauchamp (1986) and Shah and Ahmad (2006).

**Photosynthetic rate:** Maximum photosynthetic rate was observed in T$_5$ (40.37µmol CO$_2$ m$^{-2}$ s$^{-1}$) and minimum photosynthetic rate was observed in T$_1$ (23.48µmol CO$_2$ m$^{-2}$ s$^{-1}$). *Rhizobium* treatment enhances the photosynthetic rate with increase in uptake of nitrogen which has direct role in enhancing the chlorophyll content and photosynthetic activity of the plant. The increase in phosphorous content and their role on enhancement in photosynthetic rate was observed with *Penicillium bilaii* seed treatment in maize (Muthaiya,
The combination of *Rhizobium* and *Penicillium bilaii* provides nutrients (N and P) directly to the plant for photosynthetic process to occur. The concentration of P in the leaf is thought to affect the photosynthetic rate via the P translocator, an antiporter that facilitates the export of triose phosphate from the stroma to the cytosol in exchange for P (Stitt and Quick, 1989). Large amount of P had to be regenerated and retranslocated to the stroma to maintain carbon fixation, as one molecule of P₃ is required for every three molecules of CO₂ fixed as triose phosphate. Low cytosol P concentration may therefore have negative effect on the Calvin cycle either altering levels of phosphorylated intermediates or by affecting the required enzymes through level of activation (Biari et al., 2008).

**Chlorophyll index:** Maximum chlorophyll index was observed in T₁ (63.79) and minimum in T₄ (62.23). The increase in chlorophyll content with *Rhizobium* treatment might be due to an increase in stomatal conductance, photosynthesis, transpiration and enhanced plant growth (Sampath and Ganesh, 2003; Rajasekaran et al., 2006) and also due to the presence of large and more numerous bundle sheath chloroplasts in the inoculated leaves (Krishna and Bagayaraj, 1984). Lebot et al. (1994) reported higher photosynthetic ability of plants inoculated with PSMs due to increased availability of phosphorus in the rhizosphere. The chlorophyll concentration has been shown to have direct connection with P and with net photosynthetic rate in crop plants (Ameresh and Bhatt, 1999). Plants inoculated with AM fungi, either alone or in combination with *Rhizobium*, brought about significant changes in total chlorophyll content (Hayman, 1983) and the results of the present study are in line with this.

**Nitrogen uptake:** The nitrogen content enhanced the activity of several physiological process in the plants (Fig. 2). The effect of bioinoculant (*TagTeam, CellTech* and *Rhizobium*) on nitrogen uptake was higher in seed treatment with *TagTeam* @ 8.1g/kg compared to control (11.46 kg ha⁻¹). The increase in nitrogen uptake is due to biological nitrogen fixation process of converting atmospheric nitrogen to ammonia and their translocation in the plant as uride or amide form carried out by *Rhizobium* sp. The enhancement of total nitrogen uptake by *Rhizobium* might be assigned to more availability of nitrogen for uptake and enhanced cation exchange capacity of roots due to better root proliferation besides higher dry matter accumulation and grain yield (Utpal and Bandopadhyay, 2004). Fungal species also helps in enhancing nitrogen uptake by scavenging or solubilizing large volume of soil with hypheae or organic acids. Thus, the combination of bacteria (*Rhizobium*) and fungi (*Penicillium*) helps in enhancing the nitrogen uptake by biological nitrogen fixation and solubilizing unavailable form of phosphorous to available form for plant uptake (Guo et al., 2012).

**Phosphorous uptake:** The phosphorous content plays a major role in energy production, root development and their utilization (Fig. 2). Among the treatments, T₁ enhanced phosphorous uptake by the plants (4.13 kg ha⁻¹). This increase was due to the ability of these bacteria to produce organic, inorganic acids and CO₂ which lead to an increase in soil acidity and consequently convert the insoluble forms of phosphorus into soluble ones (Park et al., 2003). Bacteria may also support the AMF symbiosis by increasing bioavailable phosphate. *Penicillium* is considered as one of the key groups in soil microflora involved in phosphorus cycling apart from other properties such as biocontrol and biodegradation. Production of organic acids like citric, gluconic and oxalic acids have been recognized for phosphate solubilization by several species of *Penicillium* namely, *P. bilaii, P. radicum* and *P. rugulosum* (Pandey et al., 2008). Omar (1998) reported that filamentous fungi are widely used to solubilize phosphates and they have ability to release organic acid by decreasing the pH of the soil and increases the availability of phosphorus. The combined inoculation of bacteria (*Rhizobium*) and fungi (*Penicillium*) enhanced P uptake by solubilisation process.

**Potassium uptake:** The potassium content plays a major role in stomatal conductance, membrane changes and their integrity (Fig.2). Maximum potassium uptake was observed with seed treatment with *TagTeam* @ 8.1g/kg (31.76 kg ha⁻¹) and minimum was observed in control (20.71 kg ha⁻¹). The increase in K nutrient uptake might be due to the synergistic effect of higher N uptake (Srinivasan Rao et al., 1997). The preferential potassium accumulation in the leaves as a result of bacterial inoculation might be also related to changes in membrane activity and subsequently proton efflux in roots (Bashan et al., 1991).

**Available NPK:** The available NPK in the postharvest soil, revealed the highest content in control (74.37, 9.60 and 152.68 kg ha⁻¹) and lowest in seed treatment with *TagTeam* @ 8.1g/kg (55.22, 5.16, 95.71 kg ha⁻¹). This was due to effective utilization of nutrients present in the soil mainly by converting the unavailable form to available form by *Rhizobium* and *Penicillium* species in *TagTeam* (Table 1). *Rhizobium* inoculation alone could enhance the uptake through atmospheric nitrogen and the uptake of other nutrients from the soil. Jeyakumar et al. (2014) observed that seed treatment with *P. bilaii* in maize could compensate 50 per cent reduction in soil applied P and improved the grain yield. *Penicillium* are considered to be a key component of the mycoflora involved in the phosphorus cycling in soils (Wakelin et al., 2007).

**Yield components, yield and seed protein content:** The data collected on yield and yield components are presented in Table 2. More number of flowers (61.00) was recorded in plants treated with *TagTeam* @ 8.1g/kg which was significantly superior over control (45.05). Increase in
number of flowers has been related to increase in plant hormones that cause a positive effect on the efficient utilization of NO₃, resulting in an increased fruit yield in chilli (Ruiz et al., 2000).

The number of pods per plant was significantly influenced by multiaction bioinoculants (TagTeam). More number of pods (41.40) was recorded in the treatment, T₅ (Seed treatment with TagTeam @ 8.1g/kg) while the least number of pods (27.50) was recorded in the control plants. Fertility coefficient was found maximum with T₅ (67.86) and the minimum was observed in T₁ (61.04). The total sugar accumulation in plants could increase the osmotic concentration and therefore had the capability to absorb water and maintain turgidity, thereby inhibiting flower abscission (Upadhayay et al., 1993).

The vegetable yield of cowpea as influenced by multiaction bioinoculants (TagTeam) treatments is presented in Table 2. Significantly higher vegetable pod yield (7.04t ha⁻¹) was recorded in T₅, followed by T₄ (6.93) which was on par with T₂ (6.84). Control recorded lower yield of 6.21t ha⁻¹ compared to other treatments. This could be due to the synergistic effect between the microbial rhizosphere (bacteria) and biocontrol agents (fungi). They have the potentialities to produce plant growth promoting substances which might create favourable conditions for improving mineral uptake by plants. Improved nutrient and water availability and high yield due to mycorrhizal association

Table 1: Effect of seed treatment with multiaction bioinoculants on available nutrients in soil sample

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Available N (kg ha⁻¹)</th>
<th>Available P (kg ha⁻¹)</th>
<th>Available K (kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>final</td>
<td>Initial</td>
</tr>
<tr>
<td>T₁: Control</td>
<td>125.59</td>
<td>74.37</td>
<td>14.51</td>
</tr>
<tr>
<td>T₂: Rhizobium @ 30g/kg</td>
<td>122.86</td>
<td>58.93</td>
<td>14.41</td>
</tr>
<tr>
<td>T₃: TagTeam @ 2.7g/kg</td>
<td>123.56</td>
<td>65.39</td>
<td>14.40</td>
</tr>
<tr>
<td>T₄: TagTeam @ 5.4g/kg</td>
<td>124.17</td>
<td>57.29</td>
<td>14.63</td>
</tr>
<tr>
<td>T₅: TagTeam @ 8.1g/kg</td>
<td>125.26</td>
<td>55.22</td>
<td>14.59</td>
</tr>
<tr>
<td>T₆: TagTeam @ 10.8g/kg</td>
<td>124.77</td>
<td>61.39</td>
<td>14.57</td>
</tr>
<tr>
<td>T₇: CellTech @ 2.8ml/kg</td>
<td>122.21</td>
<td>69.57</td>
<td>14.30</td>
</tr>
<tr>
<td>Mean</td>
<td>124.06</td>
<td>63.16</td>
<td>14.49</td>
</tr>
<tr>
<td>SEd</td>
<td>3.04</td>
<td>0.70</td>
<td>0.19</td>
</tr>
<tr>
<td>CD (P=0.05)</td>
<td>NS</td>
<td>1.48</td>
<td>NS</td>
</tr>
</tbody>
</table>

Fig 2: Effect of seed treatment with multiaction bioinoculants on nutrient uptake of vegetable cowpea at flowering stage
have been reported (Sampath and Ganesh, 2003). Christy Kala (2011) found that seed inoculation significantly increased biological yield and biochemical constituents as compared to control. Pandey et al. (1981) stated that senescence and abscission of the older leaves might cause the depletion of LAI at the later stages of growth. Delaying of abscission by enhancing LAD reflects their increase in translocation from source to sink leads to increase in yield. Better effects of co-inoculation with Rhizobium and Vesicular–Arbuscular Mycorrhizal fungi on pod yield of Cowpea were shown by Thiagarajan et al., (1992).

Seed treatment with Tag Team @ 8.1g/kg showed higher seed protein content (22.10) followed by seed treatment with Tag team @ 5.4g/Kg (21.36). The lowest value was observed in control (18.24). The increase in amide production due to Rhizobium inoculation enhanced the amino acid content and further an increase in protein production and their translocation into pods for quality improvement. Similar results were reported by Ahmed et al. (2007) who found that the effect of Rhizobium inoculant on protein content of both green and mature seeds of pea was significant. Solaiman and Rabbani (2005) found that the performance of Rhizobium inoculant alone was superior to uninoculated control in protein content in green and mature seeds of pea.

**CONCLUSION**

It was observed from the study, that seed treatment with TagTeam @ 8.1g/kg was effective in improving vegetable pod yield than other treatments. This increase was due to improvement in physiological parameters like photosynthetic rate, leaf area and total dry matter accumulation by inoculation with Rhizobium and Penicillium present in the TagTeam. The increase in nodules enhances nodulation and nutrient uptake, improves the physiological parameters.

**REFERENCES**


