Slow and fast-growing soybean rhizobial population, their symbiotic efficiency and soil nitrogen behavior under different cropping systems in Vertisols of Madhya Pradesh, India

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ABSTRACT
An attempt was made in this study to assess the indigenous composition (slow and fast-growers) of soybean-rhizobia, their symbiotic efficiency and nitrogen fixation in Vertisols of Madhya Pradesh under different cropping systems. The soils were collected from soybean-based (soybean-wheat and soybean-chickpea) and cereal-based (maize-wheat, rice-wheat and maize-chickpea) systems during 2013-15 from two agro climatic zones of the state. One of the sampling sites was under Long Term Fertilizer Experiment with soybean-wheat system where three treatments - absolute control, recommended dose of fertilizers (RDF) and RDF+FYM were considered. Ratio of fast-growing soybean rhizobia was more in those cropping systems where soybean was one of the crops while population of slow-growers was more with cereal-cereal or maize-chickpea cropping systems. Nodulation and N symbiotic efficiency (N content and uptake) was better with slow-growing rhizobia as compared to fast-growing. Maximum SOC was found at maximum vegetative growth stage in soybean-wheat rotation (5.7 g kg⁻¹ soil) under RDF+FYM and minimum (3.2 g kg⁻¹ soil) in absolute control. Available soil N was more in kharif season with soybean. Legume based systems and rabi season reflected better conversion of NH₄⁺ to NO₃⁻-N.

Key words: Cropping system, Nodulation, Soybean-rhizobia, Symbiotic efficiency, Vertisols.

INTRODUCTION
Vertisols are dark coloured clayey soils that shrink and swell extensively upon changing soil moisture conditions with widely variable organic matter content (1-6%). Intensive cropping and imbalanced plant nutrition has resulted in wide spread deficiency of nutrients in most of the soils. At present, the use of biological approach has become more popular as a partial substitute for chemical fertilizers for improving crop yields in an integrated plant nutrient management system. In this regard, the microorganisms that establish positive associations with plant roots play a key role in agricultural practices and are promising for their potential use in sustainable agriculture (Di Cello et al., 1997). A variety of symbiotic (Rhizobium sp.) and non-symbiotic bacteria (Azotobacter, Azospirillum, Bacillus, Klebsiella) are being used worldwide for enhancing crop productivity (Cocking, 2003) through various mechanisms.

In Madhya Pradesh legumes including soybean are usually grown in rotation with non-legumes particularly with cereals such as maize, wheat and rice. Many agricultural practices, such as cropping systems, crop rotation, continuous cropping, and tillage induce changes in microbial communities in soil (Lupwayi et al., 1998; Alvey et al., 2003) but specific microbial groups may respond differently. In order to have rhizobial inoculation to be of long-term value in tropical crop production systems, the inoculated rhizobia must survive in soil for long term, colonize, live saprophytically (outside the host) and compete with indigenous rhizobial populations present in the soils. The populations of soybean-rhizobia in tropical soil in the rhizosphere of various crops has however, not been adequately investigated. Bradyrhizobium japonicum strains that nodulate soybean, have been reported to survive well in soybean grown fields for more than 5 years, even in the absence of the host legume (Nutman and Hearne, 1980).

Bacteria of the genus Rhizobium nodulate and fix atmospheric nitrogen in symbiosis with many legumes. Various species in this genus comprise two broad groups of fast and slow-growing strains based on growth rate (Jordan and Allen, 1974). Soybean is considered to be commonly nodulated by slow-growing rhizobia but fast growing rhizobia were also isolated from soybean root nodules (Keyser et al., 1982). Rhizobium soybean symbiosis is of comparable importance to other Rhizobium-cereal associations with respect to nitrogen economy to the succeeding crop. Maize in rotation with soybean maintained high yields similar to those of sequential maize fertilized with chemical nitrogen fertilizers (Gomez, 1968).

Members of the genus Rhizobium are symbiotic N-fixing bacteria which are able to occupy and form nodules

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on the roots of legume crops. The species in this genus have been divided into two groups (fast and slow-growers) Jordan, and Allen (1974) on the basis of growth rate and effect on the pH of yeast extract-mannitol (YEM) medium under laboratory conditions. The fast-growing (media turns yellow) rhizobia have mean generation times of 2 to 4 hrs and produce a net decrease in the pH (range, 4.7-6.7) of YEM culture medium Keyser et al. (1982), whereas the rhizobia referred to as slow-growing (media turns blue) have mean generation times of 6 hrs and longer and do not lower the pH of this medium (Vincent, 1974, Jarvis et al., 1982).

MATERIALS AND METHODS

The symbiotic efficiency studies were carried out at Department of Soil Science and Agricultural Chemistry J.N. KrishiVishwaVidyalaya, Jabalpur, in collaboration with Indian Institute of Soil Science, Bhopal, ICAR, MP, India. Jabalpur is situated in the South-Eastern part of MP at 23° 09' North latitude and 79° 58' East longitude at an altitude of 411 meter from mean sea level. Jabalpur lies in the “Kymore Plateau and Satpura Hills” agro-climatic region. Seven sampling sites were selected for the study which belonged to Jabalpur and Chhindwara districts falling under Kymore Plateau and Satpura Hills and Satpura Plateau agro-climatic zones respectively with different cropping systems (Table 1). Out of these one belonged to Long Term Fertilizer Experiment with three selected treatments- absolute control, RDF and RDF+FYM (15 t ha⁻¹ annually to soybean crop) where soybean-wheat sequence is followed since 1972. Soil samples were drawn from these sites using GPS (geographical positioning system) device. Rhizosphere soil samples were drawn (0-15 cm) from each location during kharif and rabi seasons of 2013-15 at three crop growth stages (before sowing, maximum vegetative growth and after harvest). Soybean nodules were also sampled (where soybean crop was grown) for isolation of slow and fast-growing soybean-rhizobia.

Isolation of soybean-rhizobia from soybean nodules for characterization as slow and fast-growers: For isolation purpose sampled soybean nodules were used while from other cropping system (S. No. 5, 6 and 7 of Table 1) soybean plants were grown in pots using soil of each location and respective plant root nodules were used for isolation purpose. Isolation of rhizobia from root nodules was done by the method of Somasegaran and Hoben, (1994). From each sample, three healthy and pink nodules were selected randomly for isolation and washed thoroughly with sterile distilled water. After washing, nodules were surface sterilized by dipping in 0.1% mercuric chloride (HgCl₂) solution for 5 minutes followed by ethanol. Then nodules were immediately washed for 10-15 times with sterile distilled water to remove traces of HgCl₂ and ethanol. The surface-sterilized nodules were transferred to sterile tubes containing 100 ml sterile distilled water. Nodules were crushed with a
the help of sterile glass rod and were streaked with one loopful of milky suspension on congo red yeast extract mannitol agar (CRYMA) media containing 25 μg ml⁻¹ congo red and the petri plates were incubated at 28±2°C until growth appeared. The isolates were classified as slow-growing (media turns blue) or fast-growers (media turns yellow) on their reaction on YMA supplemented with bromthymol blue (BTB) using 5 ml liter⁻¹ of BTB stock solution (0.5 g 100 ml⁻¹ in ethanol). The pure cultures each of slow and fast-growing rhizobia were transferred aseptically on YMA medium slants. These slants were incubated at 28±2°C for 2-10 days and stored in refrigerator at 4°C for further studies.

**Symbiotic efficiency:** Symbiotic efficiency of isolates were determined by plant infection tests (Somasegaran and Hoben, 1994). For the purpose, paper cups were filled with 300 g of pre-sterilized sand. Healthy soybean seeds were surface sterilized with 0.1% HgCl₂ for 5 minutes followed by repeated washing with sterilized distilled water (Vincent 1970) and were allowed to germinate in sterile moist sand in a tray at 28°C for 3-5 days. Germinated seeds (3 Nos.) were planted in each cup under aseptic conditions and 1 ml of inoculated broth (3 isolates each of slow and fast-growing rhizobia were selected randomly) was poured on each germinated seed. Different cups were used for different isolates with four replications. The cups were kept in the pot house and the plants were grown for 4 weeks and the symbiotic effectiveness was determined through nodulation (number of nodules and their oven dry weight), oven dried plant biomass, nitrogen content in plant and its uptake.

**Soil characteristics:** Organic carbon content in soil was determined by oxidizing the organic matter using chromic acid and the excess of unreduced dichromate was back titrated with standard ferrous ammonium sulphate (Walkley and Black, 1934). Kjeldahl method was used to determine total nitrogen (Piper, 1967). Available nitrogen (KMnO₄ oxidizable-N) was oxidized by potassium permanganate and released ammonia was absorbed in boric acid (Subbiah and Asija, 1956). For estimation of ammonical (NH₄⁺) and nitrate (NO₃⁻) N, MgO (Magnesium oxide) and Devarda’s alloy were used respectively. Liberated ammonia was absorbed in different boric acids (2%) and both the solutions were back titrated by 0.02N H₂SO₄ (Alkaline permanganate method, Subbiah and Asija, 1956).

**RESULTS AND DISCUSSION**

**Indigenous population of slow and fast-growing soybean rhizobia:** Composition of indigenous soybean rhizobial population (slow and fast-growing) was worked out by isolating them from soybean nodules considering the fact that soybean is commonly nodulated by slow-growing rhizobia but fast-growing are also isolated from soybean root nodules (Keyser et al., 1982). Out of total rhizobia lcolonies, the ratio of fast-growing rhizobial colonies were more in...
those cropping systems where soybean was one of the crops as compared to cropping systems without soybean in both the cropping seasons. Slow-growing colonies were more with the cereal-cereal and maize-chickpea rotations. On considering the average composition, the fast-growing rhizobia with and without soybean based cropping system was 64 and 41% respectively during kharif season, while it was 36 and 59% with slow-growing rhizobia. During rabi season same trend was observed and it was 60 and 32% with fast-growing and 40 and 68% with slow-growing rhizobia (Table 2) which clearly indicate that fast-growing rhizobia are predominant under soybean cultivation for long period and slow-growers are predominant in those cropping system where soybean do not exist (Dowdle et al., 1985).

**Symbiotic efficiency:** Symbiotic efficiency of slow and fast-growing soybean-rhizobial isolates was assessed through plant infection technique and it was found that both slow and fast-growing rhizobia resulted in production of nodules (26.1 and 25.6 nODULE PLANT$^{-1}$), oven dried weight of nodules were (89.1 and 79.3 mg plant$^{-1}$), N content (1.31 and 1.26%) and total N uptake (29 and 25 mg plant$^{-1}$) respectively (Table 2) which clearly indicate that fast-growing rhizobia are predominant under soybean cultivation for long period and slow-growers are predominant in those cropping system where soybean do not exist (Dowdle et al., 1985).

**SOIL PROPERTIES**

**Soil organic carbon:** Maximum soil organic carbon (5.7 g kg$^{-1}$ soil) was found under the soybean-wheat cropping sequence with RDF+FYM while minimum (3.2 g kg$^{-1}$ soil) was recorded in absolute control (Fig. 2a). Plant biomass directly influences the soil organic carbon. Biomass production is highly dependent on the soil fertility, where the soil N availability is one of the most important factor that influence the biomass production, especially in the less fertile soils. Nitrogen is typically the most limiting nutrient for plant growth (Vitousek et al., 1997).

**Available and total soil nitrogen:** Maximum vegetative growth of crop was influence by available nitrogen and total nitrogen in soil, both during kharif and rabi seasons (Figs. 2b and 2c). Inclusion of soybean in cropping systems with wheat or chickpea was able to increase more available soil nitrogen than maize-wheat or paddy-wheat and it was 10, 44
Fig 2: Effect of different cropping systems on soil OC and N at different crop growth stages during kharif and rabi (average of 2 years)
A. absolute control, B. recommended dose of fertilizers (20:80:20 and 120:60:40 NPK kg ha\(^{-1}\) for soybean and wheat, respectively) and C. recommended dose of fertilizers + FYM (15 t ha\(^{-1}\) annually to soybean crop only); BS: before sowing; MVG: maximum vegetative growth; AH: after harvest.

and 6% respectively and was more during different crop growth stages (i.e. before sowing, maximum vegetative growth and at harvest) over cereal based cropping systems. After harvest of kharif and rabi crops the status of total soil nitrogen was found better under those situation where soybean was included as preceding crop. Under maize-chickpea sequence the soil total nitrogen was more after harvest of chickpea as compared to maize. The \(\text{N}_2\) fixed by rhizobia in legumes can also benefit subsequent associated non-legumes (Hayat et al., 2008). Under maize-chickpea system more N fixation was observed with chickpea crop as compared to maize. Chickpea could gave positive N balance,
and confirmed the rotational benefits of chickpea for improving soil fertility (Aslam et al., 2003).

Soil mineral nitrogen (NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}): A general trend reflect that both forms of nitrogen to increased in soil at maximum vegetative growth stage as compared to before sowing and it was got declined at harvest (Tables 3 and 4). NH\textsubscript{4}\textsuperscript{+}-N did not reflect any trend with cropping systems but NO\textsubscript{3}\textsuperscript{-}N did not reflect any trend with cropping systems but reflected that both forms of nitrogen to increased in soil at maximum vegetative growth stage as compared to before sowing and after harvest (Tables 3 and 4). NH\textsubscript{4}\textsuperscript{+}-N. These forms of nitrogen and application of farm yard manure further helped towards increasing the status of both forms of nitrogen and application of farm yard manure further synergized the availability of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}. These forms of nitrogen were found minimum where no fertilization was done even after incorporation of soybean under soybean-cereal system. Fertilization with inorganic fertilizers was helpful towards increasing the status of both the forms of nitrogen and application of farm yard manure further synergized the availability of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-}. These forms of nitrogen were found minimum where no fertilization was done even after incorporation of soybean under soybean-wheat system. Soil organic matter is an important source of nutrients for micro organisms and has an important role in soil N mineralization (Besnard et al., 1996). Due to more microbial activity at maximum vegetative growth stage of the crops it was noticed that mineralization rate was higher at this stage as compared to before sowing and after harvest of crops. The status of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3}\textsuperscript{-} depends on soil conditions, particularly aeration. Under aerobic conditions, nitrate is the dominant end-product of organic N mineralization, whereas under waterlogged (anaerobic) conditions, NH\textsubscript{4}\textsuperscript{+}-N is the only predominant end-product. Results clearly indicate that soil aeration supported conversion of NH\textsubscript{4}\textsuperscript{+} to NO\textsubscript{3}\textsuperscript{-} during the rabi season as compared to kharif where paddy crop was under the cropping system. Among cropping systems, soils under legume-based (soybean or chickpea) showed greater mineralizable N (ammonical and nitrate) as compared to cereal-cereal systems (rice-wheat or maize-wheat). Fosu et al. (2007) suggested that legume have low C/N ratio and low lignin content hence, immobilize soil mineral N to a less extent and enter the net mineralization phase faster than cereal residues.

CONCLUSION

Inclusion of any of the leguminous crop (oil seed or pulses) in different cropping systems is beneficial towards building up of soil fertility. Slow-growing rhizobia gave more stable results towards nodulation, plant nitrogen content and total N uptake by plant as compared to fast-growing *Rhizobium*. More rhizobial population in soil minimize the cost of inputs (i.e. chemical fertilizers) and building

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Table 3: Soil NH\textsubscript{4}\textsuperscript{+}-N at different crop growth stages under different cropping systems.

<table>
<thead>
<tr>
<th>Crops</th>
<th>Kharif</th>
<th>Rabi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BS</td>
<td>MVG</td>
</tr>
<tr>
<td>Soybean</td>
<td>Wheat\textsuperscript{a}</td>
<td>27</td>
</tr>
<tr>
<td>Soybean</td>
<td>Wheat\textsuperscript{b}</td>
<td>34</td>
</tr>
<tr>
<td>Soybean</td>
<td>Wheat\textsuperscript{c}</td>
<td>41</td>
</tr>
<tr>
<td>Soybean</td>
<td>Chickpea</td>
<td>34</td>
</tr>
<tr>
<td>Maize</td>
<td>Wheat</td>
<td>31</td>
</tr>
<tr>
<td>Rice</td>
<td>Wheat</td>
<td>37</td>
</tr>
<tr>
<td>Maize</td>
<td>Chickpea</td>
<td>34</td>
</tr>
</tbody>
</table>

| CD (p= 0.05) | 5.15 | 14.58 | 6.82 | 6.98 | 9.71 |

A: absolute control; B: recommended dose of fertilizers (20:80:20 and 120:60:40 NPK kg ha\textsuperscript{-1} for soybean and wheat, respectively); C: recommended dose of fertilizers + FYM (15 t ha\textsuperscript{-1} annually to soybean crop) under AICRP on LTFE; BS: before sowing; MVG: maximum vegetative growth; AH: after harvest.

Table 4: Soil NO\textsubscript{3}\textsuperscript{-}N at different crop growth stages under different cropping systems.

<table>
<thead>
<tr>
<th>Crops</th>
<th>Kharif</th>
<th>Rabi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BS</td>
<td>MVG</td>
</tr>
<tr>
<td>Soybean</td>
<td>Wheat\textsuperscript{a}</td>
<td>36</td>
</tr>
<tr>
<td>Soybean</td>
<td>Wheat\textsuperscript{b}</td>
<td>42</td>
</tr>
<tr>
<td>Soybean</td>
<td>Wheat\textsuperscript{c}</td>
<td>51</td>
</tr>
<tr>
<td>Soybean</td>
<td>Chickpea</td>
<td>61</td>
</tr>
<tr>
<td>Maize</td>
<td>Wheat</td>
<td>48</td>
</tr>
<tr>
<td>Rice</td>
<td>Wheat</td>
<td>27</td>
</tr>
<tr>
<td>Maize</td>
<td>Chickpea</td>
<td>57</td>
</tr>
<tr>
<td>CD (p= 0.05)</td>
<td>4.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

|        | 13.9 | 17.4 | 12.9 | 12.6 | 19.5 |

A: absolute control; B: recommended dose of fertilizers (20:80:20 and 120:60:40 NPK kg ha\textsuperscript{-1} for soybean and wheat, respectively); C: recommended dose of fertilizers + FYM (15 t ha\textsuperscript{-1} annually to soybean crop) under AICRP on LTFE; BS: before sowing; MVG: maximum vegetative growth; AH: after harvest.
up higher N stock in soil to maximize production of field crops in eco-friendly manner.

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