MULTIPURPOSE LEGUMES OF THE TROPICAL GENUS *CROTALARIA* ARE ASSOCIATED WITH UNUSUAL RHIZOBIA

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In tropical areas, spontaneous legumes play an important role in maintenance and improvement of soil fertility, but they remain largely unexploited. Among them, the legumes belonging to the genus *Crotalaria* are actively fixing nitrogen through the nodules they form on their roots in association with rhizobia. The genus *Crotalaria* is widespread in tropical regions and includes about 550 species in Africa and Madagascar (Polhill, 1982). These annual or perennial plants can be used in farming systems as green manure crops. Furthermore, they are also grown as multipurpose legumes because some of them can control the proliferation of important plant parasites such as nematodes. Up to now, *Crotalaria* were regarded as only nodulated by *Bradyrhizobium* strains belonging to the so-called cowpea miscellany group of rhizobia. We have recently shown that several species of *Crotalaria* exhibited a strong specificity for nodulation and did not nodulate with classical bradyrhizobia (Samba et al., 1999). In this paper, we report several examples of agronomic applications by *Crotalaria* used as fertilizers in the field. Biological nitrogen fixation (BNF) of three species of *Crotalaria* native to Senegal was measured using the N15 direct isotope dilution method. We also report the characterization of the specific *Crotalaria* nodulating-bacteria which form a new branch within the alpha proteo-bacteria distinct from other known rhizobia.

Numerous species of *Crotalaria* have been used as green manure all around the world. For example, in Sri-Lanka, *Crotalaria anagyroides* has been successfully used for soil improvement in tea plantations (Sandanam, 1976). In Brazil, *Crotalaria brevidens,* used as green manure in coffee plantations, provides an efficient soil cover, suppresses undesirable weeds, prevents soil erosion, and improves soil temperature and moisture which has a beneficial effect on the coffee crop (Muzilli, 1992). In Zambia, green manure *Crotalaria zanzibarica* enhance the yield of beans (Mwambazi, 1998). Recently, in savanna regions of Ivory Coast, *Crotalaria juncea* has been planted to restore soil fertility in traditional upland rice-based cropping systems (Becker, 1999).

Another agronomic interest of *Crotalaria* is the property of some species to control nematodes populations. In 1983, Sano and Nakasano reported that the development of the larvae of *Meloidogyne incognita* inoculated to *Crotalaria spectabilis* was stopped at the3rd stage. In Brazil, experiments with *Crotalaria spectabilis* planted at 17 or 34 plants/m^2 strongly reduced population of *Meloidogyne incognita* (Huang, Tenante, 1981). In India, *Crotalaria Juncea* was recommended as safe crop for management of *Pratylenchus zeae* in sugarcane (Sundararraj, 1990). In USA, Florida, yield of eggplant and squash was reported to be enhanced after crop rotation with *Crotalaria Spectabilis* by depressing *Meloidogyne arenaria* populations (MacSorley, 1994).

However, caution is necessary when using Crotalaria because a few disadvantages can be encountered. In USA, Florida, *Crotalaria lanceolata* and *C. brevidens* became a new host plant for *Piezodorus guildini* an insect pest of soybean (Panizzi, 1985). In India, *Crotalaria juncea* can become a reservoir of entomophages, potential pests and plant diseases vectors (Sharma, 1997), and was shown to be a new alternative host of *Eurystylus oldi*, an important African pest of sorghum

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and castor plant (Maldes, 1998). A few Crotalaria, such as *Crotalaria retusa* can become an invading weed, and many species contain high level of alkaloids and are toxic for cattle, horses and other animals.

A greenhouse experiment was conducted to measure N2 fixation in three *Crotalaria* species, C. *perottetii*, C. *ochroleuca* and C. retusa, growing in Sénégal using direct isotope dilution technique. Two non-fixing plants, *Senna occidentalis* and *Senna obtusifolia* served as reference plants. Significant differences were observed between the three *Crotalaria* species. *C. ochroleuca* yielded more total nitrogen than did *C. perottetii* and *C. retusa.* The atom % 15N excess in the *Crotalaria* species was significantly lower than that of the reference plants, indicating that significant N2 fixation occurred in the three *Crotalaria* species. There was no significant difference in % Ndfa between the three Crotalaria species (47 % to 53 %). In contrast, genetic variability was observed based on total N fixed (Ndfa). *Crotalaria ochroleuca* significantly exhibited the higher amount of total N fixed, equivalent to 83 Kg of nitrogen per hectare.

117 rhizobial strains were isolated from 9 species of annual and perennial *Crotalaria* growing in Sénégal. According to their host range specificity, the strains and their *Crotalaria* hosts could be divided into two different groups:
-Group I includes fast-growing strains exclusively nodulating *C. perrottetii, C. podocarpa* and *C. glaucoides*. These fast growing rhizobia were highly specific.
-Group II includes slow-growing strains nodulating *C. comosa, C. goreensis, C. hyssopifolia, C. lathyroides, C. ochroleuca, C. retusa* and also typical cowpea type host plants such as *Macroptilium atropurpureum* and *Acacia albida.* These slow-growing strains were promiscuous and resembled typical bradyrhizobia.
Taxonomical position of all isolates was studied by SDS-PAGE. Results showed 2 main electrophoretic groups at a level of similarity of 90%. Slow-growing *Crotalaria* rhizobia formed an electrophoretic group with the wild type strain of *B. japonicum* (LMG 6138). All fast-growing *Crotalaria* strains were included in an homogenous electrophoretic group well separated from other known species of rhizobia. Analysis of 16 S rDNA sequences of two representative strains of each group confirmed that the slow-growing strains belonged to *Bradyrhizobium Japonicum*, and surprisingly that the specific fast-growing strains belonged to the *Methylobacterium* genus, thus constituting a new branch of nodulating bacteria.

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