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Yield and biological nitrogen fixation of cowpea varieties in the semi-arid region of Brazil

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ABSTRACT

Cowpea is an important crop in small properties of the Brazilian semi-arid region, where it is cultivated without fertilizer application. In spite of the fundamental role played by biological Nitrogen fixation (BNF), little is known of the symbiosis between cowpea varieties and native rhizobia or recommended rhizobia strains. A field experiment was conducted aiming to estimate BNF and productivities of local varieties, in association with two previously described bradyrhizobial inoculant strains and native rhizobia (no inoculation). The plants received 20 kg ha^{-1} of enriched ^{15}N fertilizer to allow the use of the isotopic dilution method. After harvest (80 days) straw and grain biomass was determined. The varieties differed in grain and straw productivity and in N and N derived from atmosphere (%Ndfa). Corujinha had the highest grain productivity (1147 kg ha^{-1}), followed by Sempre Verde (920 kg ha^{-1}), Azul (912 kg ha^{-1}) and Cariri (889 kg ha^{-1}). Costela de Vaca had the highest straw productivity (2258 kg ha^{-1}), highest N content in the straw (28 g ha^{-1}) and highest BNF (79 %Ndfa, corresponding to 45 kg ha^{-1} of N for total aboveground biomass and 39 kg ha^{-1} for the straw), but the lowest grain productivity (381 kg ha^{-1}) and the lowest harvest index (0.14). The inoculations did not significantly alter productivities, N contents or %Ndfa but there was a tendency of lower grain productivities in the non-inoculated plants, which was reflected in lower total and biologically fixed N quantities, indicating that the native strains may be slightly less efficient.

1. Introduction

Cowpea, *Vigna unguiculata* L. (Walp), is one of the main grain crops in the semi-arid region of Northeast Brazil. It is produced mostly in small, family farms under low input agricultural systems, receiving no fertilizer application. Thus, biological nitrogen fixation (BNF) must have an important role in the plant nutrition. BNF depends on the interaction of cowpea varieties and rhizobia strains. A few selected rhizobia strains have been recommended to be inoculated in the major commercial varieties planted at national level [1]. However, they have not been tested with local varieties (landraces), which are rarely inoculated. These local varieties are preferred in small farms, where more than one are usually simultaneously planted, because their genetic diversity [2] is better suited to the erratic rainfall regime. They also provide an ample array of taste and cooking characteristics which are highly prized in these subsistence farms. Although these

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varieties nodulate abundantly, no information regarding their interaction with native strains can be found in the literature. Therefore, it is not known if they are less or more efficient than interactions with previously described bradyrhizobial inoculant strains. Native rhizobia are probably more adapted to local conditions than the previously described bradyrhizobial inoculant strains and have a long period of co-adaptation with the local cowpea varieties. Therefore, the previously described bradyrhizobial inoculant strains must not only be able to fix more nitrogen but also to compete with the local strains for symbiosis sites.

Due to their genetic diversity, good adaptation to marginal agricultural environments and to low-input systems local varieties are important in other regions and countries where cowpea is a common crop, mainly in Africa [3–7]. Efficient strains have been selected in a few of these regions [1,8–10]. However, information on the performance of local varieties with native strains is rather scarce throughout the world. BNF is a complex process and its determination poses several methodological problems. The use of ^{15}N as a tracer has been and important step that lead to the development of a better nitrogen fixation measuring procedure [11] but it has been used in very few studies of cowpea fixation [12]. Considering this scarcity of information we conducted a field experiment with seven Northeastern Brazil local cowpea varieties, inoculated or not with previously described bradyrhizobial inoculant strains, using the ^{15}N methodology.

2. Material and Methods

A field experiment was conducted from March to May 2004, at Centro Agroecológico São Miguel (7°19′ S and 33°51′ W; 635 m asl), in the municipality of Esperança, Paraíba state, Brazil. Average annual rainfall is about 800 mm, concentrated from March to August, but in 2004 it was 25% higher than the average. The soil is a Regolithic Neosol, with 830 g kg^{-1} sand, 60 g kg^{-1} clay and 110 g kg^{-1} silt, in the upper 20 cm layer [13]. The chemical characteristics, according to the methodology recommended by EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) procedure [14], are: total N = 532 mg kg^{-1}; P and K extracted by Mehlich I = 3.74 and 63.7 mg kg^{-1}, respectively; and pH (water 1: 2.5) = 6.5.

2.1. Experimental setup

The experiment was established as a split–split plot block design, with 42 treatments and three replicates. Seven local cowpea varieties were tested in the split–split plots, receiving or not nitrogen fertilization (split plots), with three inoculation treatments (main plots). The local varieties, informed by family farmers as the most planted in the state, in a poll conducted by the non-governmental organization ASP-TA, were: Cariri, Sedinha, Corujinha, Canapu, Sempre Verde, Azul and Costela de Vaca. The central holes of each fertilizer treatment received 5% enriched ^{15}N fertilizer (urea) at the rate of 20 kg ha^{-1} of nitrogen. The inoculation treatments consisted in the addition of two *Bradyrhizobium* spp strains (separately inoculated) and an uninoculated control. The strains were: BR2001 (or SEMIA 6145), isolated from *Crotalaria juncea* L., by EMBRAPA and recommended by RELARE (Rede de Laboratórios para Recomendação, Padronização e Difusão de Tecnologia de Inoculantes Microbiológicos de Interesse Agrícola); and EI6, a rhizobial strain isolated from *V. unguiculata* (L.) Walp, selected by IPA (Instituto Agronômico de Pernambuco) and recommended for crops subject to water stress conditions [15]. Peat inoculants with pure cultures were applied to the seeds just before planting in amounts sufficient to provide each seed with 10^7 cells, according to the methodology proposed by Thies [16].

The main plots were 12 × 7 m, the split plots 12 × 3.5 m and the split–split plots 1.5 × 3.5 m. In each split–split plot 25 hoe holes, spaced 0.7 × 0.3 m, were planted with two seeds each. All holes received the equivalent to 60 kg ha^{-1} of P_2O_5, as single superphosphate.

2.2. Nitrogen fixation

Biological nitrogen fixation (BNF) was estimated using the isotope dilution method. The percentage of N derived from atmosphere (%Ndfa) was calculated using the equation [17]:

$$\%\text{Ndfa} = 100\left[1 - (\%\text{A}^{15}\text{Nexcess})_{\text{fixing}} \Big/ (\%\text{A}^{15}\text{Nexcess})_{\text{control}}\right]$$

Where: $(\%A^{15}Nexcess)_{fixing}$ is the percentage excess of ^{15}N atoms in the fixing plant and $(\%A^{15}Nexcess)_{control}$ is the percentage excess of ^{15}N atoms in the control plant, preferentially a non-nodulating variety of the same fixing species. As no seeds of a non-nodulating variety of cowpea could be found, seeds of a non-nodulating variety of beans, *Phaseolus vulgaris* L., were planted in one of the ^{15}N fertilized central holes of each fertilized split–split plot. However, none of the beans seed germinated. Therefore, the $(\%A^{15}Nexcess)_{control}$ value was taken as that of the cowpea plants in the treatment with the highest average $\%A^{15}Nexcess$ value, among all 21 fertilized treatments. This procedure may result in an undefined underestimation of BNF in all treatments, as it assumes that these new control plants had no BNF, in spite of being nodulated. The relatively high BNF of most treatments (later in Results), even if underestimated, indicates that this procedure did not introduced a great distortion in the results.

2.3. Harvest, determinations and statistical analysis

The aboveground parts of the plants were harvested 80 days after seeding, when most of them had mature pods. The plants were separated into straw (including pods) and grains, which were fresh weighed, sub sampled and dried at 65–70 °C to constant mass for dry biomass determination. The material was ground and analyzed for their total N and ^{15}N concentrations (element analyzer isotope ratio mass spectrometer) at the University of Göetingen, Germany. N yield of the plants (kg ha^{-1}) were obtained multiplying concentrations by biomass. BNF quantities (kg ha^{-1}) were obtained multiplying N yield of the plants by %Ndfa values. Harvest indices were calculated dividing grain biomass by total aboveground biomass.

Data were submitted to analysis of variance and the averages compared by the Student test at 1% probability level. For the %Ndfa and BNF analysis the data of the Canapu variety were excluded, because this was the variety taken as the