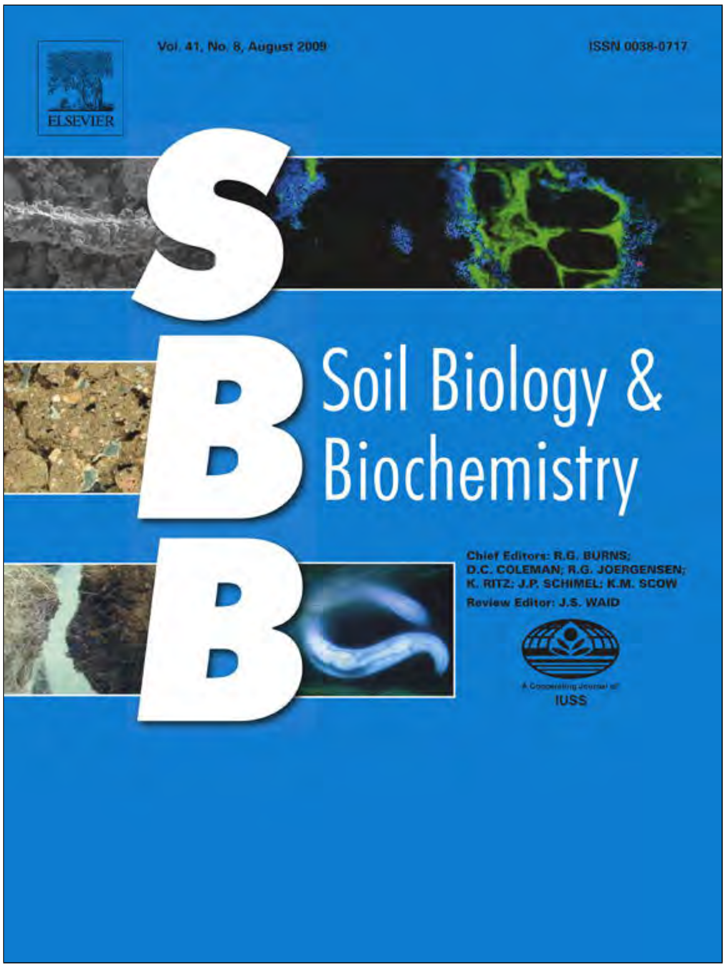
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Short communication

Relationship between total nodulation and nodulation at the root crown of peanut, soybean and common bean plants

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ABSTRACT

The objective of this study was to simplify the procedure for evaluation of legume nodulation, by examining if there is a relationship between the nodulation of the whole root system and at the crown region. Roots of peanut, soybean and common bean plants growing in soils were split in two parts (crown and bottom) and assessed for nodulation (nodule number and dry weight). In general, most nodules were concentrated at the crown, and crown nodulation was generally positively correlated with total nodulation of all three legume crops. The results are highly applicable in studies such as strain selection for inoculants and assessment of inoculation technologies, among others, and is an important contribution to help reduce the time and labor required for the evaluation of nodulation parameters.

Soybean (*Glycine max* L. Merr.), peanut (*Arachis hypogaea* L.) and common bean (*Phaseolus vulgaris* L.) are broadly grown in Brazil as protein sources and, in the case of the first and the second crops, also for oil production. These legume crops establish symbiotic associations with soil bacteria which form spherical determinate nodules, where the process of N_2 fixation takes place. Rhizobial strain selection programs and experiments aiming at evaluating the agronomic efficiency of different inoculants, strains, and inoculant technologies are based on the evaluation of several parameters related to the symbiosis, of which root nodulation represents a major component. However, despite its importance, the assessment of nodulation is very labor-intensive and time-consuming — especially when large numbers of samples are considered. Strategies have been searched, aiming at improving the procedure, one example being the determination of nodulation on the roots of plants grown in pouches using an image analysis system (Vikman and Vessey, 1993; Lira and Smith, 2000). However, there is no information concerning the comparison of partial and whole root nodulation of soil-grown legumes. The aim of this study was thus to examine if there would be a relationship between nodulation evaluated in whole plant root system and at the crown region, what would allow a considerable reduction of the time and labor involved in the evaluation.

Peanut and common bean plants were collected from greenhouse experiments, which were set up to evaluate the performance of rhizobial strains. Surface sterilized seeds were inoculated with 1.3–1.7 $\times$ 10^9 viable cells mL^{-1} of *Bradyrhizobium* spp. strains SEMIA 6144 (FEPAGRO collection, Porto Alegre, RS) and IPR-*Ah*-737 (Instituto Agronômico do Paraná - IAPAR collection) for the peanut (cultivar IAC Tatu ST) and *Rhizobium* spp. IPR-*Pv*- strains (IAPAR collection) for the common bean (cultivar IPR-Colibri).

Further samplings were undertaken with soybean plants (cultivar BRS184) from an experiment of inoculant carriers in an acidic oxisol at the Experimental Station of IAPAR in Ponta Grossa (23°13′S and 50°03′W, altitude 880 m, climate Cfb, Köppen–Geiger classification) and common bean plants (28 cultivars) from two independent field experiments performed in an oxisol at the Experimental Station of IAPAR in Londrina (23°23′ S and 50°11′ W, altitude 610 m, climate Cfa). In these field experiments

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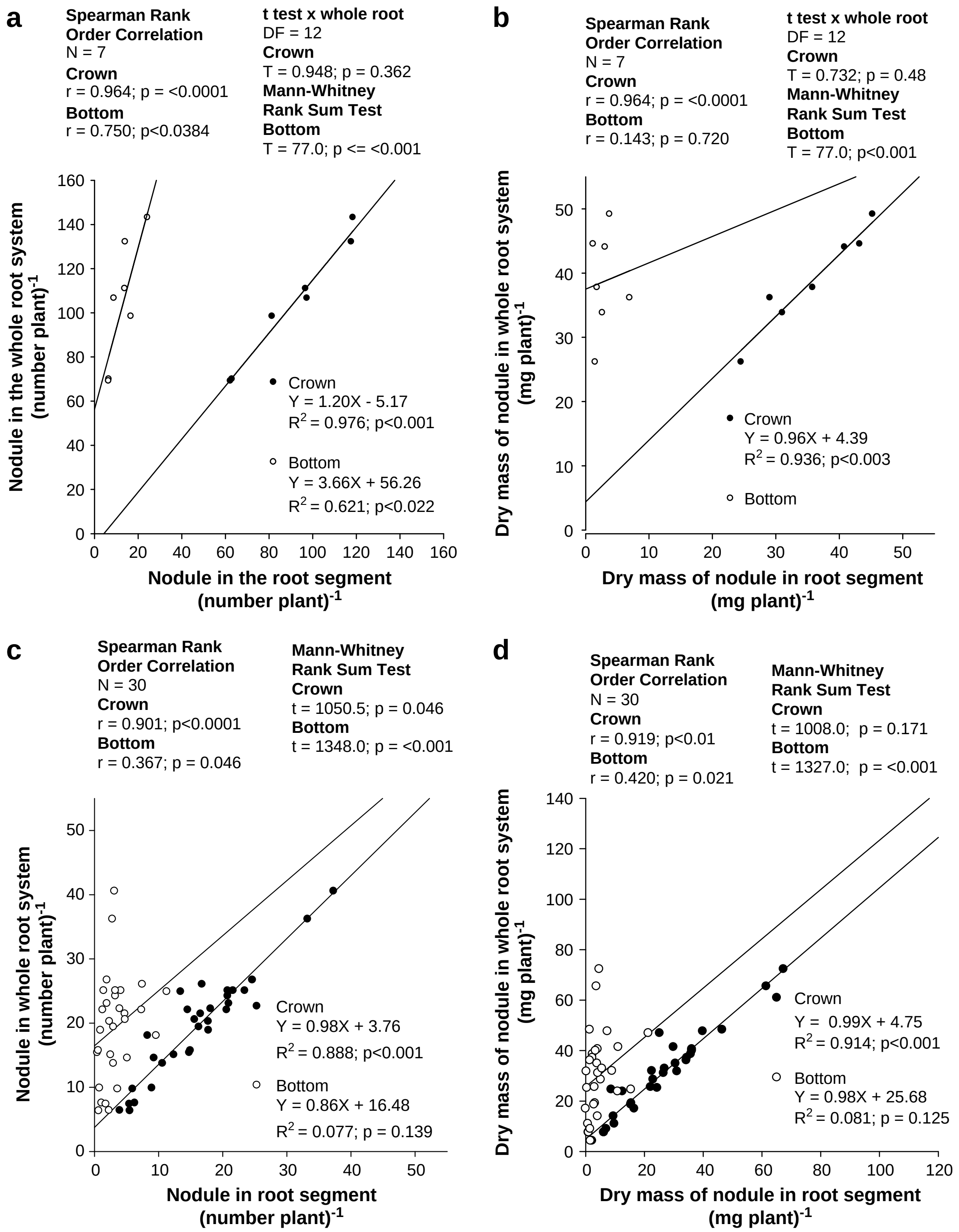

Fig. 1. Relationship between nodulation in the whole and in two segments (at crown and bottom) of the root system of peanut plants growing in pots containing soil (a and b) and of field-grown soybean plants from the experiment of inoculant carriers (c and d). N = number of samples; r = correlation coefficient; df = degrees of freedom.

approximately 10^9 viable cells g^{-1} of peat inoculant with the *Rhizobium tropici* strains SEMIA 4077 (=CIAT 899), SEMIA 4080 (=PRF 81) and SEMIA 4088 (=H 12) were used for the common bean, while soybean seeds were inoculated with *B. japonicum* strain SEMIA 5079 (=CPAC 15). Soybean was sown in early November of 2008. The first and the second common bean field experiments were sown in April of 2008 and in the end of August of 2008, respectively. At early flowering (35–42, days after emergence – DAE) common bean plants were collected from 68 to 94 pots or plots from greenhouse and field experiments, respectively. For the peanut, 21 plants from seven treatments (three replicates per treatment) were harvested at 65 DAE and for soybean 30 plots with six to seven plants from five treatments with six replicates were collected at 46 DAE.

The roots were gently pulled or removed from the soil, carefully washed over a 0.5 mm diameter sieve and then split in two parts: 1) the first, from the cotyledonal node to 7 cm below (named crown region, and including main and lateral roots, from now on referred to as crown root), and 2) the second segment included the remaining root system beyond 7 cm, referred to as the bottom part

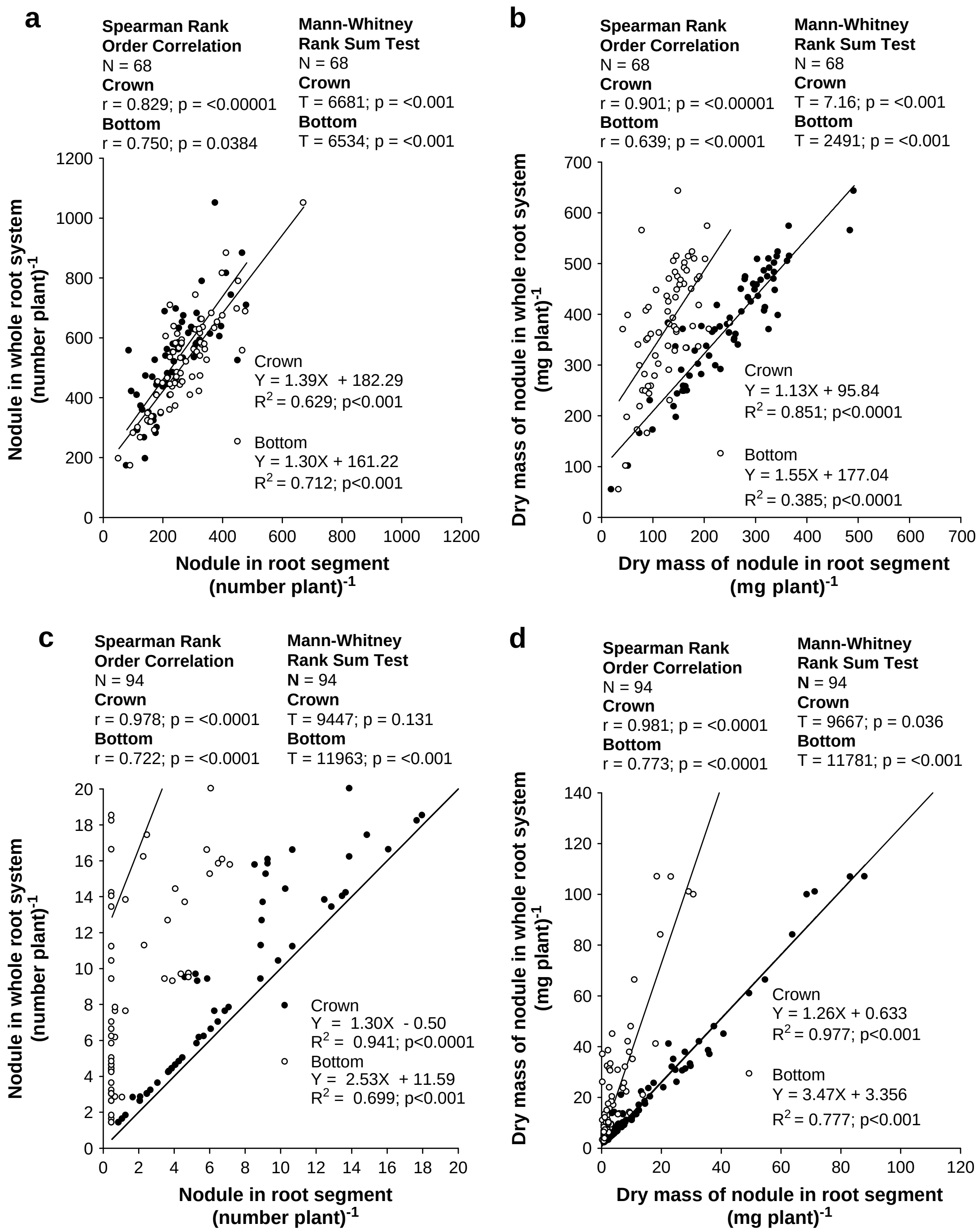

Fig. 2. Relationship between nodulation in the whole and in two segments (at crown and bottom) of the root system of common bean plants growing in pots containing soil [(a) and (b)] and in field-grown [(c) and (d)] plants from the two independent experiments. N = number of samples; r = correlation coefficient.

of the root system. Root segments were dried at 65 °C in an air forced oven till constant weight and nodules were then removed, counted and weighed. Nodulation parameters from the greenhouse experiments represent the means of three plants per pot and, in the case of field experiments (common bean and soybean), means of six to seven plants. Statistical analyses of data were performed with SAS for Windows v 9.1 software package (SAS Institute Inc., Cary, NC, USA, 1998).

For the peanut and soybean crops, most nodules were observed at the crown, representing from 82 to 92% of nodule number and dry weight, respectively. The results are in accordance to those obtained with two peanut cultivars, where most nodules were formed on the first order lateral roots (Tajima et al., 2006). In the analyses with peanut, the Spearman correlation coefficients for nodule number were significant and positively related ($r = 0.964$; $p = <0.001$ and $r = 0.750$, $p < 0.038$) for the crown and bottom regions, respectively (Fig. 1a), while for nodule dry weight a significant correlation was observed ($r = 0.964$, $p < 0.001$) only with the crown root (Fig. 1b). There were no significant differences between whole and crown root nodulation of both crops, according to the *t*-test (t value = 0.948, $p = 0.362$) (Fig. 1a and b) and (Mann–Whitney test, $p < 0.001$) (Fig. 1c and d). However, significant differences (Mann–Whitney test,

$p < 0.001$) between whole nodulation and nodulation at the bottom part were observed. Linear regressions were obtained considering the nodule number and dry weight from the whole root system and from the crown, except for nodule dry weight in the bottom part of peanut root (Fig. 1a and b).

In both greenhouse and field experiments, we have observed significant and positive relationships between nodulation (nodule number and dry weight) of the whole root system and at the crown region (Fig. 2). The mean values of nodule number and dry weight for the two segments (crown and bottom root region) were statistically different (Spearman correlation and Mann–Whitney test, $p = <0.001$) (Fig. 2a and b). Despite the higher number of nodules at the bottom root part, the dry weight of nodules did not show the same linear trend and the best fit was represented by a polynomial linear regression for both root segments (Fig. 2b). By using 28 different recommended cultivars of common bean, no significant differences (Mann–Whitney test, $p = 0.131$ and $p = 0.036$) were observed between nodulation of the whole root and at the crown region (Fig. 2c and d).

According to our study, for peanut, soybean and common bean most of the nodulation resulting from inoculation occurs at the primary crown root system and not at the secondary roots. Early studies by Kamicker and Brill (1987) have also reported that inoculant added to the seed furrow produced nodules mainly in the top region of the soybean root, indicating that the movement of the inoculated rhizobia in soil is limited. Other studies have confirmed that the vertical movement of *Bradyrhizobium* and *Rhizobium leguminosarum* bv. *trifolii* strains is very limited (Hardarson et al., 1989; Catlow et al., 1990). Also, with soybean, it appears that when there are large populations of bradyrhizobia in the soil and seeds are inoculated with selected strains, a successful inoculation results in an increased nodulation at the crown root region (Hungria and Bohrer, 2000).

The present study can highly contribute to evaluate the performance of strains, inoculants and inoculation techniques, such as cover spray inoculation (Zilli et al., 2008) in field experiments, strain selection programs, evaluation of inoculant carriers, among others. In conclusion, for soybean, peanut and common bean, the nodulation may be evaluated only at the crown region instead of the whole root system, saving time and labor in the determination of those parameters in experiments.

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