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Effect of root temperature on nodule development of bean, lentil and pea

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Abstract

The rhizobia–legume symbiosis is the main source of fixed nitrogen for many agricultural systems. However, it is inhibited by low soil temperature. To date, research on nodulation has involved either qualitative or destructive analyses. The use of computer-based image analysis potentially allows nodules to be followed during the course of development. Seedlings of bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medik.) and pea (*Pisum sativum* L.) were transplanted into plastic growth pouches suspended in water baths maintained at 10, 15, 20 or 25 °C. Two days after transplanting, all plants were inoculated with appropriate rhizobial strains. Seven days after inoculation, plant roots were scanned; this was repeated weekly for 7 weeks. Data on nodule length were collected through image analysis. Nodule length was correlated with nodule size and development. There were increases in the precision of estimates of environmental effects through observation of individual nodule development, as opposed to averages for populations of nodules. The effects of root temperature on nodulation and nodule development were observed both in the delayed onset of nodulation and in reduced subsequent nodule growth rate, resulting in effects on final nodule size.

Keywords: Nodulation; Rhizobia; Environmental factors; Image analysis

1. Introduction

The symbiosis between legumes and rhizobia is the main source of biologically fixed nitrogen for agricultural systems. However, it is sensitive to environmental constraints (Hungria and Bohrer, 2000). To better understand these effects, there is a need to study the development of the root nodules in which N_2 fixation takes place (Hungria and Bohrer, 2000). All previous studies of nodule development have involved either qualitative or destructive methods. The use of hydroponic systems and computer-based image analysis could allow us to follow the development of nodules through time. It has previously been shown (Lira Junior and Smith, 2000) that image analysis can be used to accurately measure individual nodules.

One very important environmental constraint to nodule development for legumes growing in the temperate zone is low soil temperature, prevalent during early spring (Zhang and Smith, 1994). The growth of plants dependent on nitrogen fixation is more hampered by low soil temperature than plants receiving mineral nitrogen (Abberton et al., 1998). One of the effects of low soil temperature is a delay in nodule initiation (Pan and Smith, 1998).

The objective of the research described in this paper was to study the effects of low root temperatures on nodule formation and development, and compare these effects among three pulse crops (bean, lentil and pea) using a hydroponic system and computer-based image analysis.

2. Material and methods

Seeds of bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medik.) and pea (*Pisum sativum* L.) were surface sterilized, put to germinate in sterile vermiculite and transplanted, at 5-days-old, into plastic pouches suspended in water baths maintained at 10, 15, 20 or 25 °C, following

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Pan and Smith (1998). Air temperature was maintained at 25/16 °C (under a day/night cycle of 16/8 h). The pouches received 500 ml of nitrogen-free Hoagland nutrient solution (Hoagland and Arnon, 1950), which was replaced weekly.

Two days after transplanting, bean seedlings were inoculated with *Rhizobium leguminosarum* bv. *phaseoli* strain 127K105, while lentil and pea seedlings were inoculated with *R. leguminosarum* bv. *viciae* strain 175G106 (both from Liphatech, Milwaukee, WI, USA). Inoculation was carried out by adding 1 ml of bacterial culture with 10^8 cells/ml to each pouch.

Beginning at seven days after inoculation, the root systems were scanned weekly for 7 weeks with a Plustek OpticPro 4800P© scanner, at 24-bit color, and 100 dpi resolution. Before scanning, the pouches were emptied of nutrient solution, placed on the scanner, and the scanner lid was closed. The root system was considered to have only two dimensions. The data thus obtained were stored as TIFF uncompressed files.

For each plant, at each sampling, all indentified individual nodules (visible from first to the last sampling) were selected and measured each time, following Lira Junior and Smith (2000).

Nodule length data was initially analyzed with Guided Data Analysis of SAS (SAS Institute, 1999) to identify outliers or data transformation requirements (square root, $\log_{10}$ or best power). The Guided Analysis procedure allows the verification of assumptions made during data analysis, including presence of outliers and recommendation of data transformation due to constant variance or lack of residual independence problems in the data. Quadratic regressions were estimated using the Generalized Linear Model procedure of SAS with plant age as the independent variable and nodule length as the dependent variable, for each plant species and root temperature combination. These regressions were conducted three ways, using different nodule groupings in each case. These groupings were (1) all nodules as a single population (full), (2) all nodules from a given plant as a population (by plant) or (3) each individual nodule (by nodule). The regressions obtained for by plant and by nodule populations were compared with those obtained for the full population. Differences among means are only discussed when statistically significant at $P < 0.05$.

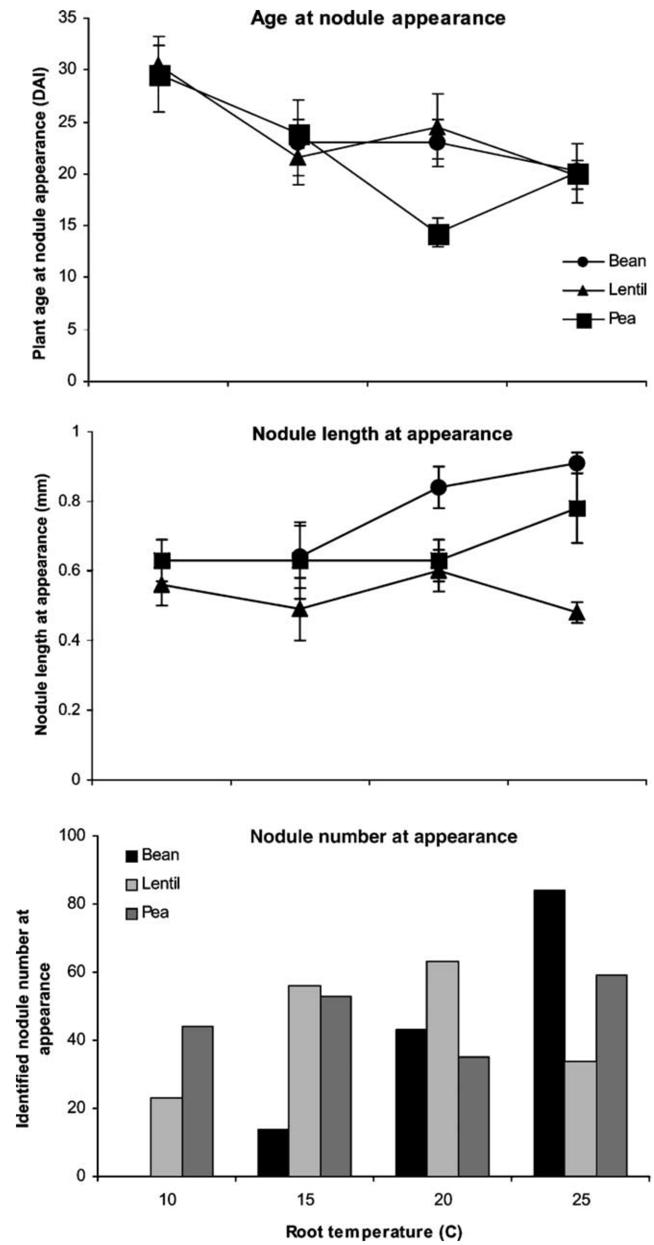

Fig. 1. Effects of root temperature on plant age at nodule appearance, nodule length and number of nodules for bean, lentil and pea at the time when they were first large enough to be identified. Vertical bars indicate the 95% confidence limits of plant age at nodule appearance and initial nodule size. Initial number of nodules is a count from all plants, and thus was not subjected to statistical analysis, and is used for comparative observation only.

3. Results

A temperature effect on nodulation was observed (Fig. 1). Except for pea, there were few differences between 20 and 25 °C, however, nodule appearance was generally slower for plants at root temperatures below 20 °C. For pea (Fig. 1) nodulation was most rapid at 20 °C, with delays at both higher and lower root temperatures. Bean did not form any nodules at 10 °C, and there was a slight reduction in time required for nodulation as root temperature increased above 20 °C.

There was also an effect of root temperature on length and number of nodules at the time when they were first visible (Fig. 1). This followed the same pattern as the temperature effects on nodulation, in that temperatures outside the optimum range retarded development.

There was a strong effect of nodule population on regressions for nodule development over time (Table 1); this effect was variable among plant species and individual nodules. When nodule population (by nodule) was