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ScienceDirect
South African Journal of Botany 73 (2007) 600 – 605
SOUTH AFRICAN JOURNAL OF BOTANY
www.elsevier.com/locate/sajb

Growth media effects on shoot physiology, nodule numbers and symbiotic nitrogen fixation in soybean

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Received 20 December 2006; received in revised form 24 May 2007; accepted 29 May 2007

Abstract

Several research groups (both in South Africa and other countries) are currently involved in research aimed at improving symbiotic nitrogen fixation (SNF) and root nodule sustainability in soybean [*Glycine max* (L.) Merr.]. In many of these experiments potted plants are used, and in this paper the importance of careful selection of growth media is demonstrated. *Bradyrhizobium japonicum*-inoculated soybean seedlings were cultivated in pots containing N-free growth media (sand, fine vermiculite or coarse vermiculite) or a growth medium containing low concentrations of water-soluble nitrogen predominantly in the form of ammonium (mixture of potting soil, sand and vermiculite). The effects of growth media on shoot physiology were assessed by measurement of plastochron index, chlorophyll content and CO_2 assimilation rates. Nodule numbers, nitrogenase activity and nodule ureide content were also determined. Although similar source–sink relationships were maintained in plants cultured in the various growth media, large effects on nodule numbers and SNF were observed. Shoot phenotype and physiology did not provide any insight into these belowground effects. The presence of mineral N, or sand as culture medium, led to the formation of more abundant nodules but with low SNF activity. Vermiculite, irrespective of particle size, resulted in plants with root systems housing nodules with high SNF activity. It is concluded that choice of growth media for cultivating soybean plants under controlled growth conditions is an important consideration, especially in multi-institution collaborations where comparability between experiments is a pre-requisite.

Keywords: Nitrogenase; Nodulation; Root nodules; Soybean [*Glycine max* (L.) Merr.]; Symbiotic nitrogen fixation; Ureides

1. Introduction

Symbiotic nitrogen fixation (SNF) by annual legume crops such as soybean plays a key role in the maintenance of global food production (Bordeleau and Prévost, 1994; Herridge and Rose, 2000). It has been realised for some time that enhancement of SNF in agriculture would become increasingly important as N-fertiliser derived from fossil fuels becomes more expensive (LaRue, 1978).

The symbiotic association between the roots of soybean and *Bradyrhizobium japonicum* bacteria leads to formation of specific organs, called root nodules, where SNF takes place. The main products of SNF in soybean root nodules, namely ureides (allantoin and allantoic acid), are exported to the rest of the plant where it is incorporated into amino acids and proteins. The establishment and maintenance of an effective symbiosis depends on several factors of which a favorable environment, that will allow maximum N_2 fixation, is extremely important (Bordeleau and Prévost, 1994). Several environmental factors such as soil pH, soil fertility, drought stress and temperature extremes impose limitations on the symbiotic association between the host plant and microsymbiont (Vessey and Waterer, 1992; Bordeleau and Prévost, 1994; Serraj et al., 1999; Van Heerden et al., 2003).

Even under optimal growth conditions the functional lifespan of root nodules is relatively short where after the symbiosis is terminated through a process known as nodule senescence. In the case of soybean nodules, senescence begins at the center of the nodule and progressively spreads to the outside. A major problem in agriculture is that nodule senescence can be induced prematurely by various factors such as temperature extremes and drought stress (Gogorcena et al., 1995; Gonzalez et al., 1998).

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doi:10.1016/j.sajb.2007.05.009

Various observations suggest that shoot and root derived signals are major determinants of the nodule senescence process, but the factors that orchestrate this process, and the identity of the signals involved, have received relatively little attention (Puppo et al., 2005).

Because of the problems associated with premature nodule senescence and the agricultural importance of soybean, it is not surprising that several research groups (both in South Africa and other countries) are currently involved in research aimed at improving soybean production, SNF and nodule sustainability. In many of these experiments potted soybean plants are used, and in this paper it is demonstrated how different growth media markedly affects nodule numbers and SNF without any apparent effects on shoot development and physiology. A very sensitive indicator of shoot development, namely the plastochron index (Erickson and Michelini, 1957) was used to quantify any effects on shoot growth. The time interval between the initiation of consecutive leaves on a plant is termed the "plastochron". The plastochron index (PI) is considered a very sensitive indicator of plant development and has been used extensively for various physiological investigations, especially in legumes grown under controlled environmental conditions (Snyder and Bunce, 1983; Yourstone and Wallace, 1990; Jamadagni et al., 1995; Ade-Ademilua and Botha, 2004). Among its applications, the PI enables precise quantification of shoot growth rates (Snyder and Bunce, 1983). The results reported in this paper emphasise the need for very careful growth media selection and standardisation, especially in the case of multi-institution collaborations where uniform plant culture among the various participating laboratories is an absolute prerequisite for studies on nodulation and symbiotic nitrogen fixation.

2. Materials and methods

2.1. Plant growth

Seeds of the South African soybean genotype PAN809 were sown in 2 dm^3 plastic pots containing the following growth media: (a) sterile river sand; (b) fine vermiculite (FV;±2 mm diameter); (c) coarse vermiculite (CV;±5–7 mm diameter) and (d) potting soil without added fertiliser (Culterra, PO Box 982, Muldersdrif 1747, South Africa) mixed with river sand and vermiculite (ratio of 4:2:1 respectively). The total water soluble N-content of this potting soil mixture (PSM) was 4.9 mg/l whereas the other growth media can be regarded as N-free. In all cases the seeds were inoculated with *Bradyrhizobium japonicum* (bacterial strain WB 74) at the time of sowing to ensure root nodule development and SNF. Seedlings were grown in a Conviron PGV 36 growth chamber (Controlled Environments Ltd., Winnipeg, MB, Canada, R3H 0R9) under rigorously controlled growth conditions: 15 h/9 h and 26 °C/20 °C light/dark cycle with a light intensity of 1000 μmol m^{-2} s^{-1} at the level of the plant canopy. Illumination was provided by a combination of fluorescent (Sylvania Cool White VHO, 215 W) and incandescent (Sylvania, 100 W) lamps. The incandescent lamps were included as an enriching source of red light to minimise any growth abnormalities known to occur when plants are grown under artificial illumination. The fluorescence lamps were replaced on a regular basis to ensure maximal and comparable light intensities during all experiments. Potted plants were rotated daily to compensate for any variations in light intensity, especially along the sides of the growth chamber. Seedlings were watered daily and supplied with N-free (to prevent inhibition of SNF) full-strength Hoagland nutrient solution (Hoagland and Arnon, 1950) three times a week. Two seeds were sown per pot but seedlings were thinned out to one plant per pot after emergence to minimise effects that the pots could have on plant development.

2.2. Quantification of vegetative development

The vegetative development of the plants cultivated in the four different growth media (sand, FV, CV and PSM) was quantified by repeated measurements of the plastochron index (Erickson and Michelini, 1957). All trifoliate leaves with central leaflets exceeding a reference length of 25 mm (Snyder and Bunce, 1983) were counted. The length of the youngest central leaflet longer than or equal to 25 mm, as well as the length of the central leaflet (shorter than 25 mm) on the next trifoliate leaf was measured. The PI of each plant was calculated using the following formula:

$$\mathrm{PI} = n + (\log L_n - \log L_{\mathrm{ref}})/(\log L_n - \log L_{n+1})$$

where n = number of trifoliate leaves with central leaflets longer than the reference length of 25 mm (L_{ref}), L_n = length of the central leaflet on trifoliate leaf L_n (which by definition is longer than or equal to L_{ref}) and L_{n+1} = length of the central leaflet on trifoliate leaf L_{n+1} (which by definition is shorter than L_{ref}). Shoot growth rates (SGR = increase in plastochron index units per day) during the experimental period (between 12 and 32 days after sowing) were determined by calculating the slopes of linear regressions constructed by plotting plastochron index values for each growth media treatment against time. In all cases the increase in plastochron index values over time was highly linear ($r^2 > 0.96$) validating the use of this method for determination of SGRs.

2.3. Measurement of chlorophyll content

In a separate experiment, the chlorophyll content of leaf discs harvested from soybean leaves of different stages of development (young to senescent) were measured with a hand-held chlorophyll Content Meter (CCM-200, Opti-Sciences, Inc., 164 Westford Road #4, Tyngsboro, MA 01879, USA), which recorded a chlorophyll content index (CCI) for each leaf disc. The total extractable chlorophyll content of each of these leaf discs was determined according to the method of Wintermans and De Mots (1965). The actual chlorophyll content of each leaf disc was plotted against its corresponding CCI value. This calibration curve revealed a highly linear relationship ($r^2 > 0.97$) between actual chlorophyll content and a wide range of recorded CCI values, thus justifying the use of the measured index values. In the growth media experiments, the CCI values of the first trifoliate leaf of plants were measured at regular intervals during leaf development to assess any effects on chlorophyll content.