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Soybean cultivation for Bioregenerative Life Support Systems (BLSSs): The effect of hydroponic system and nitrogen source

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Abstract

Soybean [*Glycine max* (L.) Merr.] is one of the plant species selected within the European Space Agency (ESA) Micro-Ecological Life Support System Alternative (MELiSSA) project for hydroponic cultivation in Biological Life Support Systems (BLSSs), because of the high nutritional value of seeds. Root symbiosis of soybean with *Bradirhizobium japonicum* contributes to plant nutrition in soil, providing ammonium through the bacterial fixation of atmospheric nitrogen. The aim of this study was to evaluate the effects of two hydroponic systems, Nutrient Film Technique (NFT) and cultivation on rockwool, and two nitrogen sources in the nutrient solution, nitrate (as $Ca(NO_3)_2$ and KNO_3) and urea ($CO(NH_2)_2$), on root symbiosis, plant growth and seeds production of soybean. Plants of cultivar 'OT8914', inoculated with *B. japonicum* strain BUS-2, were grown in a growth chamber, under controlled environmental conditions.

Cultivation on rockwool positively influenced root nodulation and plant growth and yield, without affecting the proximate composition of seeds, compared to NFT. Urea as the sole source of N drastically reduced the seed production and the harvest index of soybean plants, presumably because of ammonium toxicity, even though it enhanced root nodulation and increased the N content of seeds. In the view of large-scale cultivation for space colony on planetary surfaces, the possibility to use porous media, prepared using *in situ* resources, should be investigated. Urea can be included in the nutrient formulation for soybean in order to promote bacterial activity, however a proper ammonium/nitrate ratio should be maintained.

Keywords: *Glycine max* (L.); Soilless culture; Nutrient Film Technique (NFT); Rockwool; Urea; Nitrate

1. Introduction

Higher plants play a key role in Bioregenerative Life Support Systems (BLSSs) for long term manned missions in Space and in the view of large-scale cultivation for space colony or planetary surfaces, by regenerating air through the photosynthetic CO_2 absorption and O_2 emission, purifying waste water through the transpiration, and recycling waste products through the mineral nutrition. In addition, plants could provide fresh food to integrate the crew diet and help to preserve the astronaut's wellbeing (De Micco et al., 2009). However, cultivation for food production in

Abbreviations: AUE, acid use efficiency; BUE, base use efficiency; BLSSs, bioregenerative life support systems; Cv, cultivar (cultivated variety); DAS, days after sowing; DM, dry matter; EC, electrical conductivity; HI, harvest index; LA, leaf area; NFT, nutrient film technique; PAR, photosynthetically active radiation; RUE, radiation use efficiency; RW, rockwool; WUE, water use efficiency

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BLSSs requires the proper choice of the technique, which must be feasible in the several constraints of Space and Space vehicles (Monje et al., 2003), and of the species and cultivar (cultivated variety, cv) which must be adaptable to the specific growth environment while providing a suitable nutritional quality to fulfil the astronauts needs (De Micco et al., 2012; Stasiak et al., 2012).

The most part of the studies aiming to characterize crop production under controlled conditions, in the context of BLSS, have been conducted using hydroponic (or soilless) culture, with recirculating nutrient solution (closed system) (Wheeler et al., 1996a, 2003). Nutrient Film Technique (NFT) is one of the most common hydroponic systems in ground-based experiments for Space research (Stutte, 2006). In NFT, plants are suspended in a slightly sloping channel, in which a shallow stream of circulating nutrient solution flows by gravity through the roots and is recovered and reused, after the needed adjustments (Jones, 2005). This system allows to eliminate the influence of substrate on plant growth and to guarantee root hydration and oxygenation, in order to prevent anoxic conditions (Monje et al., 2001). On the other hand, NFT is risky in case of malfunctions (e.g. loss of circulating pumps), because of the limited water volume and the absence of substrate buffering capacity. Furthermore, several crops have been proven to perform better in solid substrate (Xu et al., 1995). Rockwool is a chemically inert, rock-based fibrous material, considered a good substrate for greenhouse horticulture, because of the good physical properties and the absence of pathogens (Fonteno, 1996; Bougoul et al., 2005). However, inert substrates have been less investigated than NFT in Space oriented experiments (Stasiak et al., 2012; Page and Feller, 2013), since they have several constraints like the weight and the volume required for transport in Space.

Among the mineral elements, nitrogen (N) is crucial for plant growth and production, being a primary constituent of nucleotides, proteins, vitamins and hormones (Xu et al., 2012). N is the only essential element that can be uptaken as both the nitrate anion (NO_3^-) and the ammonium cation (NH_4^+). The ionic form influences the pH of circulating solution and rhizosphere, resulting in pH increase in case of NO_3^- depletion and in pH decrease for NH_4^+ depletion (Marschner, 1995). Nitrate based recipes are the most common in hydroponics. There is considerable research that indicates that the form of N supplied can have a significant effect on vegetative growth and fruit yield of hydroponically grown vegetables (Gorbe and Calatayud, 2010). A mixture of NO_3^- and NH_4^+ frequently results in better plant growth compared to NO_3^- as the only N source, if NH_4^+ does not exceed 25% (Tabatabaei et al., 2006). However, for some crops, such as tomato, NH_4^+ in the nutrient solution can increase the incidence of blossom-end rot (BER), a commonly occurring fruit disorder, therefore NH_4^+ should be supplied during the vegetative growth but not in the fruit setting period (Sandoval-Villa et al., 2001).

Soybean [*Glycine max* (L.) Merr.] is one of the species selected in the European Space Agency (ESA) Project MELiSSA – Micro-Ecological Life Support System Alternative – as a candidate for hydroponic cultivation in BLSS, because of the high nutritional value of seeds, rich in proteins and lipids, and the ability to produce further food products (fresh sprouts, soymilk, okara, whey) (Paradiso et al., 2013a). Similarly to other *Leguminosae*, soybean plants can obtain a certain amount of N from symbiotic fixation of atmospheric N_2 by *rhizobium* bacteria, living in nodules on plant roots (Jordan, 1984). *Rhizobium* species with specific mutualistic relationship with soybean is *Bradyrhizobium japonicum*. Nodulation is inhibited or repressed when the amount of N available in the growing medium is sufficient to fulfil the growth requirements (Kohl et al., 1980), nevertheless studies demonstrated that NH_4^+ has less negative effect on symbiosis than NO_3^- in soybean (Vigue et al., 1977; Imsande, 1988). In this respect, considering that urea from liquid wastes (urine- and wash-water) is about 85% of the recyclable N potentially available for plant growth in BLSSs (Wydeven and Golub, 1990), using urea would also represent a useful opportunity of resource recycling, which is a relevant topic in long-duration missions (Mackowiak et al., 1996). However, while the response of numerous crops to fertilization with urea has been extensively investigated in soil, where urea is hydrolysed to NH_4^+ from many soil bacteria producing the enzyme urease (Cheema and Ahmad, 2000), urea is seldom used in hydroponics (Gorbe and Calatayud, 2010) and only a few data are available on fertigation soybean plants grown hydroponically for seed production (Imsande and Schmidt, 1998; Paradiso et al., 2013b).

It is known from evidences in laboratory that solid media enhance the proliferation of some *rhizobia* compared to water solution (Goormachtig et al., 2004). Furthermore, numerous strains of *B. japonicum* are able to produce urease, thus to metabolize urea as a source of N (Sadowsky et al., 1983). We hypothesized that cultivation on solid medium could help root colonization by *B. japonicum*, while enhancing the bacterial contribution to plant nutrition, and improve soybean performance in hydroponics compared to solely the nutrient solution. In addition, we assumed that replacing nitrate with urea in the nutrient solution could enhance the plant-bacteria symbiosis and facilitate the pH control of recirculating solution reducing the acid requirement for pH adjustment, while offering a useful tool for liquid wastes recycling in BLSS. In this respect, the aim of this study was to evaluate the effects of two hydroponic systems, NFT and cultivation on rockwool, and of urea as an alternative N source to nitrate fertilizers, on root nodulation, growth and seed production of soybean plants, inoculated with *B. japonicum*.