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PRODUCTION OF FOOD FOR SPACE MISSIONS: SOYBEAN (*GLYCINE MAX* L.) ADAPTABILITY TO HYDROPONIC CULTIVATION

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FOREWORD

Food in space

Since the first moon-landing in 1969, one of the most important problems of space exploration by humans has been the generation and maintenance of optimal living conditions during the transit to and/or a stay on a planet (Gazenko and Shepelev, 1972; Grigoriev and Gazenko, 1998). One of the most difficult technical challenges for scientists is represented by the diet of the crew: nutrition, food storage and sanitation are important objectives to be reached.

ESA (European Space Agency) and NASA (National Aeronautics and Space Administration) have been tried to improve food quality for human in space, considering not only nutrition but also the psychological well-being of astronauts: good food could provide psychological support for the crew, away from Earth for years.

Weightlessness and long time living in a closed environment create additional requirements during the planning of astronaut nutrition. Food has to be nutritionally balanced, tasty and appealing, together with a degree of thickness suitable to be consumed in weightlessness, specially packed and easy to store for a long time. Today all the food for astronauts is brought from Earth, but this is not possible for longer missions (www.esa.int).

The launch needs (propellant) and consequently the costs of a space mission is determined by the amount of total mass that have to be put into Earth orbit, propelled towards the targeted planet, enter into orbit again and finally transported back to Earth. A single crew needs on average 3 Kg of combined food and water a day, so for a typical Mars mission of minimum 2 years (730–1000 days) about 12 tonnes of 'food' (consumables) would be needed. The frequent consumable re-supply is costly and it becomes logistically difficult as missions extend further. To keep the weight down, foods are eaten in their packaging (plastic pouches).

Another issue is the food storage: many processed foods and reconstituted beverages do not retain their nutrition or palatability for even a year, turning off-colour or becoming mushy or tasteless.

Moreover, studies conducted by NASA scientists proved that, after one year, space food shows notable losses of vitamin A, folic acid and thiamine, and the vitamin C totally disappears. Astronauts could use multi-vitamin pills, but the efficacy of vitamins diminishes faster in pills than in foods. Using current propulsion systems, crews will take between 6 and 8 months to travel each way to Mars. Since Mars and Earth come close to each other only once every year, crews will have to stay on the planet surface for 18 months before coming

back home. Scientists' goal is the development of foods that will remain both safe and appetizing for at least five years. Canned food has a good shelf life, but can not be heated in microwaves and is considerably heavier than the plastic pouches.

Crews need to eat every bite of what they open up. Crumbs from cookies or crackers can get into someone's eyes or create problems to the equipments. Wet foods that aren't completely gobbled up will eventually go bad and stink up a spacecraft: astronauts do not take out the trash every day but bring it back home with them or hold it for months until they can pack it into a craft directed towards Earth's surface (but actually incinerate in the atmosphere).

Since journey to Mars is still many years away, probably 2035 at the earliest, there should be ample time to find an optimal strategy for food supplying in space missions.

An alternative to bringing food from Earth could be the development of human Life Support Systems (LSS), that can allow a continual recycling of system resources. This could be achieved with physical-chemical approaches where, for example, CO_2 is chemically reduced with H_2 using the Sabatier process (Sabatier and Senderens, 1902) to produce methane and water, after which the water is electrolytically split to provide O_2 for the humans (Jones and Ingelfinger, 1973).

Tough the efforts spent to build a closed physical-chemical system in the last decades, there exist only different components (subsystems, units, aggregates etc.) of physical-chemical regenerative systems but not a complete system. The experiments carried out showed that it would be easier to design a biological regenerative system than a physical-chemical one (Gitelson *et al.*, 1975).

In example plant based life support processes could be the optimal tool for CO_2 reduction and O_2 production (Myers, 1954). In addition to the atmospheric regeneration, plants could be used to produce food and purify waste water (trough transpiration); this concept has been called "Bioregenerative Life Support System", or "Controlled Ecological Life Support System" (CELSS) or "Environmental Closed Life Support System" (ECLSS). These terms have generally been used in a more inclusive sense for both biological and physical-chemical approaches.

By growing enough plants to cover around 40% of what the crew eats, humans could get 'for free' the oxygen and water needed to live. Although still on the drawing board, ESA has already started research to see what could be grown on other planets - and what a self-supporting eco-system might look like on Mars (www.esa.int)

The use of plants for bioregenerative life support for space missions was first studied by the US Air Force in the 1950s and 1960s. Extensive testing was also conducted from the 1960s

through the 1980s by Russian researchers at the Institute of Biophysics in Krasnoyarsk, Siberia, and the Institute for Biomedical Problems in Moscow. NASA initiated bioregenerative research in the 1960s but it did not include testing with plants until about 1980, with the start of the CELSS Program (Wheeler *et al*., 2003). The overall objective of these studies was to define optimal environmental conditions for growth and high yields of crops that were identified as candidate species (Tibbitts and Alford, 1980) for a CELLS.

Many LSS, containing biological regeneration components (e.g. micro algae, higher plants, fish) have been proposed and discussed (Krall and Kok, 1960; Gitelson *et al.,* 1975; Gros *et al*., 2003; Sychev *et al.,* 2003). At the present time, the most promising and the best studied biological regeneration components of life support systems are microalgae (Gitelson *et al.*, 1975;) and higher plants (Gitelson *et al.,* 2003; Gros *et al.,* 2003; Salisbury *et al.,* 2002).

However, CELSS have not been used in space yet because of their high energy consumption; the big area and volume required for their placing together with the high weight are a big trouble too. Moreover, no technologies are available to handle and maintain organisms within such systems and it is still a question whether living organisms will be functional in the biological matter cycle during in microgravity (Sychev *et al*., 2003). To bring closed regenerative life support technology to a level of development suitable to space applications, further research is needed to fully understand and characterize the involved biological and physicochemical processes. In past and current missions physicochemical life support systems were sufficient to provide air and a limited amount of water to the crew, while food and drinkable water were re-supplied and waste discarded. As human missions extend further into space, physicochemical and biological processes are likely to be combined to achieve a highly self-sustaining and robust regenerative life support system.

Two French companies (ADF and GEM), working in a previous ESA project, tried to create some recipes for the astronauts. The challenge for the chefs was to offer astronauts well-flavoured food, made with only a few ingredients that could be grown on Mars. The result was 11 recipes that could be used on future ESA long-duration space missions. The menus were all based on nine main ingredients that likely could be grown in greenhouses of future colonies on Mars or other planets. The nine basic ingredients are rice, onions, tomatoes, soybean, potatoes, lettuce, spinach, wheat and spirulina – all common ingredients except the last. Spirulina is a blue-green algae, a very rich source of nutrition with lots of protein (65% by weight), calcium, carbohydrates, lipids and various vitamins that cover essential nutritional needs for energy in extreme environments. The nine must comprise at least 40% of the final

diet, while the remaining (up to) 60% could be additional vegetables, herbs, oil, butter, salt, pepper, sugar and other seasoning brought from Earth.

Plant production in a CELLS

If plants are to be used for human life support in space, growing conditions will differ from those typically encountered in the field. Plant growth and crop yield and quality are very dependent on the climatic conditions experienced from seeding to harvest. Protected agriculture in greenhouses optimises plant growth conditions and allows shortening growth cycles and guaranteeing a stable product quality. For the application of plant growth and associated food production as a subsystem in an CELSS setting, characterisation of crop food, potable water, oxygen and crop waste production rates and dynamics, as well as harvested food nutritional content and plant nutrient and CO_2 assimilation rates are a necessity. Datasets combining all these aspects are lacking.

The resources needed for plant cultivation also depend on the location. CO_2 as carbon source will always be available from crew respiration. Water could be recycled from the consumed food that was brought from Earth or used from in-situ planetary sources (when available). Nutrients for start-up can be transported from Earth, to guarantee the needed purity. In-situ extraction would require more equipment and analysis which is not realistic in the first steps of exploration. The utilisation of substrates available on planetary surfaces for plant growth would need a detailed characterisation of possible nutrient absorption or liberation, in order to keep the composition of the nutrient solution optimal for food production.

After considering a range of horticultural approaches, a recirculating nutrient film technique (NFT) was selected as the method for water and nutrient delivery, to minimize the substrate for plant growth (Steinberg *et al.*, 2000) and allow both air and liquid to contact the roots, to minimize the needed water volume for growth and avoid anoxic conditions at the root level (Monje *et al.*, 2000).

On the other hand, because of the limited water volume and buffering capacity, NFT systems are susceptible to crop stress or possible loss if some malfunctions happen (e.g. loss of circulating pumps). Moreover, using the NFT system requires monitoring and control of solution pH, EC and water volume.

Under optimal and constant environmental conditions, plant growth usually results in higher yield and shorter life cycles than the ones obtained in traditional agriculture. Depending on the ecological niche where a plant is growing on Earth (sun, shadow, latitude) plant photosynthetic carbon assimilation operates at its highest yield at 500-2000 μmol $m^{-2}s^{-1}$, but is

close to zero assimilation (i.e. compensation point) when the light level drops below 50-100 μmol $m^{-2}s^{-1}$ (Taiz and Zeiger, 2006). Hence, light availability is critical and will be limited by power availability during the space missions to a level of 500-1000 μmol $m^{-2}s^{-1}$, which is enough for medium/high yield, depending on the crop.

Furthermore, photosynthetic assimilation is directly related to the CO_2 concentration in the atmosphere. CO_2 concentrations below the atmospheric level will limit photosynthesis. For many plant species, a CO_2 concentration of 100 ppm will reduce photosynthetic assimilation by 60-70 %, and in combination with low light levels, this can even result in a more drastic reduction of net CO_2 fixation. When light is absent, respiration leads to a net carbon loss, irrespective of the CO_2 concentration. As a general rule, canopy photosynthesis is always less than the sum of photosynthetic assimilation by individual leaves and plants (Taiz and Zeiger, 2006). Partial shading and leaf movements influence light interception and canopy photosynthetic assimilation. Minimization of Leaf Area Index (LAI) could be a valuable strategy to maximize yield with a minimum non-edible part production.

A drop in photosynthesis also results in a decline of oxygen production, since for every mole of CO_2 and H_2O, about one mole of O_2 is released. Water oxidation by a light energy catalysed enzymatic reaction results in both oxygen production and in the reduction of CO_2 to sugars through the Calvin-Benson cycle. Thus, a reduction in CO_2 assimilation by a factor of 2 will result in only half the O_2 formed.

But a high light level is not always useful: in addition to the need for shielding the crew members from the radiation caused by solar flares and cosmic radiation, plants will also need to be protected against the mutagenic effects of the cosmic radiation, especially if long term seed-to seed production is envisaged whereby mutations can accumulate that likely would change the productivity or composition of the crop. It has to be mentioned however that crop sensitivity to radiation is far less than for humans, so a trade off with mass requirements and acceptable radiation level has to be investigated for FPPS concepts, based on a detailed literature study. Otherwise re-supply of standardised seed from Earth can be preferable if shielding needs outweigh the costs for consumable re-supply.

To guarantee acceptable air and food quality for the crew, it is necessary the creation of a plant chamber with light, CO_2, temperature and humidity level set points fit for efficient food production, and a control strategy for modifying these parameters within limits that do not affect plant product composition significantly but that will enable to stabilise O_2 and CO_2 levels.

To illustrate the potential impact of non-optimised environmental factors on crop yield, a number of examples are given below. Salinity, an excess of ions at the root level (species-dependent, for example 40mM salts for rice, 50mM for lettuce) results in a 50% reduction in crop yield. Lethal temperature conditions for most crops start from an exposure of 10 minutes at 45 °C (Levitt, 1980). Optimum temperatures for plant growth depend on the species considered; for agricultural crops the optimal range is from 15 to 35 °C. In addition, if plant growth hardware can provide an accurate and homogenous temperature and humidity control, the response of all plants grown within this controlled environment will be similar, and this will eventually be reflected in their homogeneous composition and nutritional content.

On Earth, the impact of both biotic and abiotic factors is known to severely affect the process of plant growth. The crop-specific bio safety issue should be considered for long-term missions. Infection of the crop by plant-pathogenic micro-organisms would induce a major perturbation in the system, potentially leading to the temporary loss of life support function.

Many of the risks related to plant pathogens are reduced in a relatively closed system. Despite this, sterilization protocols for much of the materials handling were considered at first, then they were abandoned in favour of sanitation and avoiding over risks (plants and seeds certification, seeds sterilization, cleaning of all the materials used in each cultivation cycle…). In addiction, extraneous plant materials (including lunch vegetables and cigarettes) and soil were not permitted in or near the chamber. Initial studies included disinfecting the hydroponic systems with either a hypochlorite solution or nitric acid between planting, but this was later abandoned in favour of physically cleaning and flushing the system. High-efficiency particulate air (HEPA) filters were used in the air ducts to remove airborne microbes and spores, but these were removed with little consequence on atmospheric and surface microbial counts.

The relative infrequency of obvious pathogens suggests that using cleaning procedures and maintaining a diverse and stable microflora in the root zones (Strayer, 1994) may avoid many pathogen problems. Yet the possible serious consequences of an aggressive pathogen must be taken seriously into account and counter measures or contingencies should be part of the mission planning (Nelson, 1987; Schuerger, 1998). This might include the use of interplantings with multiple species and/or isolating different growing environments to reduce overall system risks, or implementing disinfections procedures if recirculating nutrient solutions are used (e.g. filtering nutrient solutions or treating solutions with hydrogen peroxide or ozone). Another possibility would be to use fungicides, but few of them are cleared for use on hydroponic crops.

In addition, in Earth circumstances plants have an associated microflora, in the rhizosphere as well as on the leaf surface area, colonizing the surfaces and avoiding or limiting ingress of pathogens by competition. Research is ongoing on formulating microbial inoculants for hydroponic culture that would avoid infection, especially by water moulds (*Oomycetes* such as *Pythium*: Johnstone *et al.*, 2005). Combining the possible preventive measures with a disinfection method that leaves no toxic residue, such as aqueous ozone treatment, will guarantee long-term plant disease-free crop production. The root associated microflora is also strongly involved in nutrient exchanges.

Most of the activities made during the cultivation of a crop in a growth chamber are carried out manually and are labour intensive (particularly sowing and harvesting). Further mechanization or automation should be considered for more sophisticated life support systems, to reduce the crew time requirements. Moreover, activities like threshing and cleaning the seeds are dusty processes; they could be carried out in a ventilated settings but in a closed life support system it will be a serious issue.

In most of the studies, the entire chamber was either planted or harvested in a single day. This resulted in episodic, labour-intensive events. In addiction, this approach required dedicated storage capabilities for keeping the biomass. A more manageable approach would be to stagger the plantings and harvests over more frequent intervals, which would reduce the single-day labour requirements and allow a reduction in the processing and storage system components.

Soybean: hydroponic cultivation for space purpose

Soybean is a candidate crop species because of the relatively high lipid and protein content of its seeds and high nutritional quality (Hoff *et al.,* 1982). Proteins, lipids, and other seed components may change in concentration with growing environment.

Traditionally, soybean is an open field species. Recirculating hydroponic systems are necessary to allow the soybean cultivation during long duration space missions. Several studies on improving production efficiency of commercial systems are available for many crops. On the contrary, hydroponic soybean culture has been mostly used to answer physiological questions rather than for commercial production. Thus, there are few reports that directly apply Advanced Life Support (ALS) crop production goals (Wheeler *et al*., 1993).

Plant description and crop requirements

Soybean is an annual plant. The root system is mostly represented by secondary roots, which first grow horizontally for 40 cm, and then they go deep in the soil up to 150 cm. If soil depth is restricted, the tap root is less evident and lateral roots are more developed (www.fao.org).

The stem can reach up to 150 cm height and is widely branched. Soybean cultivars may have undetermined or determined development. Generally, leaves are shed before pod ripening, when seeds have more than 20% humidity. Flowers are joined in racemes containing 2 to 35 flowers. They firstly bloom on the stem base. Usually, from 20 to 80% of flowers abort, especially in cultivars characterised by many flowers per node. The flowering may last 3-5 weeks or more, depending on the cultivar and sowing age. Soybean is an autogamous species; heterogamy is less than 1%. The pods are little; each pod contains 1-5 seeds. Seed size is variable (the weight of 1000 seeds ranges between 50 and 450 g). The bigger seeds are usually used for human nutrition.

Germination occurs 5-10 days after sowing, when the soil moisture and the temperature are adequate. In hydroponic systems, soybean seeds can be sown in trays and germinated with nylon wicks in hydroponic tray inserts (Prince and Knoot, 1989). Seedling is covered with white translucent tray covers for the first 4 days after planting, to maintain high humidity and aid establishment. After 10 days from planting, seedlings are thinned; plant density usually varies from 12 to 20 plants m^{-2}. A 4 h pre-soak in deionised (DI) water can be performed, in order to improve the germination performances (Mackowiak *et al.,1999)*.

In commercial controlled environment agriculture, seedlings are typically started in a nursery under lower light intensity and grown in a dense spacing. The seedlings are then transplanted to a wider spacing in a production environment just prior to when the shoots begin to grow rapidly. Assuming a single transplant step could save 12 days in each production cycle, edible biomass productivities and radiation use efficiencies would have improved by 13% for soybean (Wheeler *et al*., 2008).

For most of commercial cultivars, lifecycle (from sowing to harvesting) lasts 80–120 days. Seeds are ready to be harvested when pods are completely ripened and seed humidity is about 12-14 %. Seeds can be stored for a season (humidity 14%), 1 year (humidity 13%), about 3 years (humidity 12%) or about 4 years (humidity 10%). Grain yield is about 35-45 q/ha. Regarding the climatic requirements, the minimal temperature for growing is about 4-6 °C; a mean temperature of 24-25 °C seems to be the optimum for most cultivars. Lower temperatures determine delays in the flowering.

Considering photoperiod, soybean is a short-day plant, but response to day-length varies according to the cultivar and temperature: many cultivars need 10 hours of dark to bloom. All cultivars bloom faster under 8 hour photoperiod. Soybean shows a high sensitivity to water deficit in particular developmental phases. If the water shortage is experienced during the flowering or at the beginning of the pod formation, flowers abortion and pods shedding can occur. A long flowering time allows the plant to tolerate low levels of water deficit. The maximum decrease of seeds production occurs when water deficit is experienced during the last week of pod development and during seed formation. Less damage occurs at the beginning of flowering and in the final stage of seed formation. Soybean can grow in a wide range of soil types: from clay to organic and poorly fertile. Optimum pH of soil is 6 to 6.5. (Baldoni and Giardini, 1989).

The species is sensitive to water logging, but moderately tolerant to soil salinity. Yield decrease due to soil salinity is the following: 0% at ECe 5 dS/m, 10% at 5.5; 25% at 6.2; 50% at 7.5 and 100% at ECe 10 dS/m (www.fao.org).

Lighting

In a experiment (Jurgonski *et al*., 1997), light supply was performed by cool-white fluorescent lamps, 250 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF); Wheeler *et al.* (2008) used 400 W High Pressure Sodium lamps (HPS) or Metal Halide lamps (MH), or a combination of them, and light was cycled to provide a 12 h light/12 h dark or 10 h light/14 h dark photoperiod. Canopy PAR ranged from 477 to 815 μmol m^{-2} s^{-1}, depending on the combination of the lamps. The highest photosynthetic rate occurred during the experiments with HPS lamps, which had the highest PPF, while the lowest rate occurred during the experiment with MH lamps, which had the lowest PPF. A drop in photosynthetic rate was observed corresponding to a lodging event (stand collapse). It suggests that light harvesting efficiency of the canopy was adversely affected by the lodging and that is important to maintain stable canopy structure to sustain maximum photosynthesis rates (Wheeler *et al.*, 2004).

Short-stature, high yielding cultivars are required for controlled environments because volume is often limited. However, soybean grown in controlled environments is taller than field-grown plants (Downs and Thomas, 1990). Red:far red ratios, specifically phytochrome 660:730 nm, have been implicated as the cause of internode elongation (Pausch *et al.*, 1991), although soybean may also respond to a balance of red and blue light (Britz and Sager, 1990). Wheeler *et al.* (1991) reported that a threshold intensity of blue light (30 μmol m^{-2} s^{-1}) was

necessary to reduce stem elongation. This minimum requirement could be met in three ways: using blue-rich lamps (e.g. fluorescent); supplementing low-level HPS lighting with sufficient blue fluorescent (BL); increasing the PPF of HPS to provide sufficient BL.

Assuming the HPS PPF contains about 7% BL, Wheeler *et al.* suggested that a minimum of 430 μmol m^{-2} s^{-1} (7% = 30 μmol m^{-2} s^{-1}) would be needed to provide sufficient BL to prevent excessive stem growth. However elongation is also dependent upon the total photosynthetic photon flux from lamps (Tibbitts *et al.*, 1983). Dougher and Bugbee (1997) studied the soybean canopy height, carbon partitioning and yield under HPS and MH lamps. A PPF of 450 μmol m^{-2} s^{-1} was maintained at the top of the canopy. This supplied approximately 40 and 140 μmol m^{-2} s^{-1} of blue light in HPS and MH lamps, respectively. The photoperiod was 12 h. MS lamps significantly reduced canopy height but slightly increased relative branch length. The main stem of HPS lamps was 87% the length of the longest branch while MH main stem was 75% of the longest branch. MH canopy height was taller than the longest branch length because canopy height included petiole length. Although not measured, petioles appeared to contribute more to height in MH canopies. Plants grown under MH lamps had 14% less biomass compared to plants under HPS lamps. Reduced stem length mass in MH plants was associated with an increase of harvest index (HI).

According to Wells *et al.* (1993), a positive correlation between plant height and seed yield was found. Net Photosynthesis (P_{net}) measurements were consistent with the yield differences between lamp types. Taller plants under HPS lamps may have had better light interception. Higher P_{net} , longer internodes, larger leaves and more rapid canopy closure suggest that there is better light distribution and capture in the HPS canopy. Increasing plant density under MH lamps might overcome canopy closure differences but this would probably have increased stem elongation after canopy closure, which would have reduced the height advantage.

The canopy gases exchange rates, although the irradiance was controlled to 450 μmol m^{-2} s^{-1} PPF for all treatments, increased slightly faster during early growth under warm temperatures (e.g.,. 29 °C) and HPS lighting (Dougher and Bugbee, 1997).

Temperature

Soybean perform well under warm temperatures (>24 °C). Mackowiak *et al.* (1999) reported values of 26/22 °C (light/dark), while Wheeler *et al.* reported 26/20 °C (light/dark). Also in Jurgonski *et al*. experiment (1997), temperature ranged from 26 °C (day) to 22 °C (night). Dougher and Bugbee (1997) studied the effects of 5 day/night temperature regimes,

maintaining the root temperatures constant at the average daily temperature of the shoot. They found that higher temperature increased seed yield via increased number of pods per square meter and seeds per pod, while cooler temperatures reduced the seed fill period. Total biomass and harvest index (Table 4) tended to decrease with lower temperatures. Percent leaf mass decreased with increasing temperature probably because of delayed leaf senescence. Warm temperatures also tended to decrease percent root mass. The day/night scheme did not affect canopy height.

The canopies at +4 DIF were not significantly taller than at 0 DIF. The lack of significant effect on plant height indicates temperature could be manipulated to some extent to maximize yield without increasing canopy height.

Relative humidity

In previous experiments, relative humidity (RH) levels ranged from 65 to 75 %. Jurgonski *et al*. (1997) maintained the RH at 70% while Mackowiak *et al.* (1997) reported values of 67±6% of RH.

Carbon dioxide

Wheeler *et al.* (2008) refer that carbon dioxide (CO_2) uptake by the plants was offset by controlled injections of pure CO_2 to hold a set point of 1000 or 1200 μmol mol^{-1} during the light cycles, while CO_2 was allowed to accumulate from plant respiration during the dark cycles. When the lamps came on in the morning, CO_2 concentrations quickly drew down to a set point, where controlled injections began. These drawdowns lasted approximately 60-90 min depending on how much CO_2 accumulated during the dark cycle, which in turn depended on the length of the dark period and the respiration rate of the crop.

Wheeler *et al* (1990, 1994) studied the proximate composition and caloric value of seeds, pods, leaves and stems of soybean grown at four CO_2 levels (500, 1000, 2000 and 5000 ppm). They found significant differences between soybean cultivars in proximate composition and some effects of CO_2 levels on protein, lipid, and crude fibre contents. Later, Wheeler *et al.* (1996b) reported higher crude fibre contents of soybean seeds from the controlled environmental conditions than from field literature values. Regarding the effects of the 4 CO_2 concentrations on soybean yield, Wheeler *et al.* (1993) reported that they depended on the cultivars used in the experiment: higher levels did not show significant differences on a dwarf, determinate cultivar, while promoted more vegetative growth and consequently greater yields on an indeterminate cultivar.

In Jurgonski *et al*. experiment (1997), CO_2 levels ranged from 350 to 1000 ppm. Seeds, stems, and leaves of plants grown at 350 ppm CO_2 had significantly higher protein N contents than did those grown at 1000 ppm. This reduced protein content of leaves at high CO_2 levels is consistent with lower levels of Rubisco (rubilose-1, 5-biphosphate carboxylase) at high CO_2.
In testing 'McCall' and 'Pixie' soybeans at 500, 1000, 2000, or 5000 ppm CO_2 (Wheeler *et al*., 1990, 1994), seed protein levels (by standard Kjeldahl procedure) were highest at 1000 ppm (39.3 and 41.9%, respectively) and lowest at 2000 ppm (34.7 and 38.9%, respectively). Protein content of leaves, stems, and pods also varied with CO_2, level (Wheeler *et al.*, 1990, 1994). Wheeler *et al.* (1990, 1994) reported increased rather than decreased levels of leaf protein with increased CO_2 levels, but only for 5000 ppm CO_2. Seeds accumulated less NO_3-N than did any other plant part. This is consistent with the findings of McKeehen *et al.* (1996a) who showed that the typical edible portions of rice, wheat, and potato do not accumulate NO_3-N when grown with excess N. Vegetative material is known to accumulate nitrate and other Non Protein Nitrogen (NPN) (Aldrich, 1980). However, soybean leaves, stems, and pods accumulated less NO_3 as a percentage of total N than did the vegetative portions of other CELSS candidate crops (McKeehen *et al.,* 1996 a, b).
The higher total N and protein N contents of seeds and leaves from plants grown at 350 ppm CO_2 compared to 1000 ppm CO_2, translated to increased amino acid levels in 350 ppm CO_2 leaves, but such increases were not the case for 350 ppm CO_2 seeds. Jurgonski *et al*. (1997) also reported that lipid contents of seeds and leaves were seemingly influenced by CO_2 level, but ash contents were not. Both trends are consistent with reports by Wheeler *et al.* (1990, 1994).
Increasing CO_2 concentration decreased the total dietary fibre (TDF) content of soybean leaves, as was the trend for lettuce leaves (McKeehen *et al.,* 1996b). Wheeler *et al.* (1990, 1994) reported increasing amounts of crude fibre in 'McCall' soybean leaves with increasing CO_2 levels. However, because crude fibre and TDF assays differ in what they measure, values cannot be compared directly.

Nutrient solution and plant nutrition

Wheeler *et al.* (2008) used the following starting nutrient concentration of the solution: 7.5 mM N, 3.0 mM K, 0.5 mM P, 2.5 mM Ca, 1.0 mM Mg, 1.0 mM S, 60 μM Fe, 7.4 μM Mn, 0.96 μM Zn, 1.04 μM Cu, 7.13 μM B and 0.01 μM Mo. The composition of the solution is

slightly different from the concentration of a trial in 1996, when Wheeler *et al.* used a concentration of 50 μM Fe and 9.50 μM B.

Nutrient solution volumes were maintained at a constant level either through daily addiction of deionized water or continuous recycling of condensate water (water transpired by plants was condensed on the cooling coils of the heating system). Following water adjustments, nutrient stock solution was added to maintain an electrical conductivity of 1.2 dS m^{-1}. Stock solution nutrient concentrations were: 70 mM N, 56 mM K, 10 mM P, 12mM Ca, 10 mM Mg, 10 mM S, 134 μM Fe, 96 μM Mn, 12,5 μM Zn, 13.5 μM Cu, 93 μM B and 0.13 μM Mo (Wheeler *et al.*, 1999). Nutrient solution pH was controlled to 5.8 using automatic addictions of 0.4 mM nitric acid. In 1996 Wheeler *et al*. used a stock solution having a different composition: 75 mM N, 68 mM K, 7.5 mM P, 7.5 mM Ca, 9.8 mM Mg, 9.8 mM S, 199 μM Fe, 68 μM Mn, 8.8 μM Zn, 9.5 μM Cu, 87 μM B and 0.1 μM Mo. The EC was maintained close to 1.2 dS m^{-1}.

Jurgonsky (1997) used the following concentration of elements for starter and refill solution: reported in Table 1:

Table 1. Starter and refill solutions used by Jurgonsky (1997) for hydroponic cultivation on soybean

	Starter solution	Refill solution
	mM	
$Ca_3(NO_3)_2$	1.0	1.0
KNO_3:	1.0	5.0
KH_2PO_4:	0.5	0.75
$MgSO_4$:	0.5	0.75
K_2SiO_3:	0.1	0.1
	μM	
$Fe(NO_3)_3$	5.0	1.5
Fe-HEDTA:	15.0	5.0
$MnCl_2$:	6.0	9.0
$ZnSO_4$:	6.0	2.0
H_3BO_3:	40.0	40
CuSO4:	0.6	0.6
$NaMoO_4$:	0.09	0.06

Soybean, Rhizobium and nitrogen fertilization interactions

The soybean's seeds generally contain 37% to 45% protein. Some of the nitrogen (N) needed by plants comes from the oxidation of soil organic matter with the balance produced by bacteria (*Rhizobium*) living in nodules on the plant's roots. Each legume species requires different bacteria to fix nitrogen from the air and produce ammonium (NH_4^+) to be used by plant (Beuerlein, 2004).

The bacterial strain of *Rhizobium* used by soybean is *Bradyrhizobium japonicum,* and the process by which it establishes a symbiosis with the plant is very complex, but known. During germination, soybean plants release chemical signals (flavonoids) into the soil, recognized by the bacteria, that produce a return signal "Nod factor signal" to the plant. This return signal results in the plants' roots preparing for infection. The Nod factor signal makes the root hairs curl, trapping rhizobia present on the root surface. An infection thread develops, and the rhizobia multiply until they reach the inside of the root. The cells of the root also begin to divide and form a specialized structure called a nodule. The rhizobia continue multiplying inside the nodules, increasing their size. The enzyme (nitrogenase) that fixes nitrogen from the air to produce NH_4^+ can not work in the presence of oxygen, so the plant must produce leghaemoglobin inside the nodule to absorb oxygen in the root to avoid its interference with the nitrogen fixation process. Since leghaemoglobin is red, the inside of an actively fixing nodule is pink. Sugars produced in the leaves move into the nodules travelling through the root system and provide energy to the rhizobium for extracting nitrogen from the air and make the nitrogen compounds which the plant then uses to produce protein. Both plant and bacteria genes govern the nitrogen fixation cycle. Stresses on the bacteria or the plant such as cold, flooding, drought, and low soil pH can all interfere with this process. Cold temperatures delay the recognition of both the plant and bacteria signals. The plants and bacteria have to produce more signals in order to begin nodulation, which delays the onset of nitrogen fixation. Once enough signal is received and nodules develop, NH_4^+ is produced and provided to the crop (Beuerlein, 2004).

Drought reduces the moisture in the soil that protects the bacteria while it lives on the seed surface after planting. A dry seedbed and dry seeds will quickly draw moisture from the inoculation material causing the bacteria on the surface of the seed to dry and die. If enough bacteria die, there will be little nodule formation and insufficient ammonium production for a good yield.

The symbiosis provides usable nitrogen for the host and a reproductive niche for the bacterium. Because the nodulation-nitrogen fixation process is energetically costly to the host

(Ryle *et al.*, 1984, Raven 1985), the plant seems to inhibit or repress nodulation when a sufficient quantity of usable nitrogen is present in the soil (Kohl *et al*., 1980; Rennie, 1982).

Nitrogen fixation is known to be adversely affected by nitrate (NO_3^-) in at least two major ways: the continued presence in the rooting medium of a modest concentration of nitrate represses nodule formation (Ralston and Imsande, 1982), and the presence of a high concentration of nitrate in addition slowly inhibits the functioning of mature nodules and may eventually promote nodule senescence (Streeter, 1985).

Nitrogen is the only essential element that can be absorbed as both as cation and anion. Plants absorb NH_4^+ much faster than NO_3^-, and the form of N have a significant effect on the uptake of other nutrients by competitive inhibition. The absorption or translocation of NO_3^- or NH_4^+ has been investigated extensively in hydroponic culture at either seedling stage or reproductive stage of vegetable crops (Shelp, 1987; Liu and Shelp, 1993; Kosola and Bloom, 1996).

The utilization of these ions was influenced by plant genotype (Gabelman *et al.*, 1986), solution pH (Yokota and Ojima, 1995), solution temperature (Ikeda and Osawa, 1984) and was also affected by the growth stage in lima beans (McElhannon and Mills, 1978) and sweet corn (Mills and McElhannon, 1982).

The form of N absorbed also affects the pH of the rhizosphere. Absorption of NH_4^+ results in a efflux of H^+ and the rhizosphere pH can shift downward as much as two units (from 7 to 5) in a short time. In contrast, absorption of NO_3^- results in an efflux of OH^-, increasing rhizhosphere pH (Marschner, 1995). Balancing the proportion of NO_3^-/NH_4^+ in the nutrient solution can be used to control pH (Lea-Cox *et al.*, 1999).

Stabilization of growth medium pH can also be achieved by using chemical compounds having buffering capacity. One of these is the 2-(N-morpholino)ethanesulphonic acid (MES), that appears to be biologically inert and does not interact significantly with other solution ions. (Bugbee and Salisbury, 1985; Ewing and Robson, 1991).

Although mineral N generally has a negative effect on legume-rhizobia symbioses, experiments in hydroponic culture in laboratory (Waterer *et al.*, 1992) demonstrated that low concentrations of NH_4^+ can stimulate nodulation in pea (*Pisum sativum* L.). This stimulation is not an artefact of hydroponic culture, but also occurs in a solid rooting medium such as sand (Gulden and Vessey, 1997).

There is evidence that NH_4^+ has less of an inhibitory effect on legume-rhizobia symbioses than NO_3^- (Streeter, 1988). In many legumes, the inhibitory effects of NO_3^- on nodulation occur at much lower concentrations than NH_4^+, or, at similar concentrations of NO_3^- and

NH_4^+, the inhibitory effects can be much more severe with NO_3^- compared to NH_4^+ (Chen and Phillips, 1977; Rys and Phung, 1984; Silsbury *et al.*, 1986; Svenning *et al.*, 1996; Waterer and Vessey, 1993a,b). However, although less common, some studies indicate a more inhibitory effect of NH_4^+ on nodulation as compared to NO_3^- (Guo *et al.*, 1992).

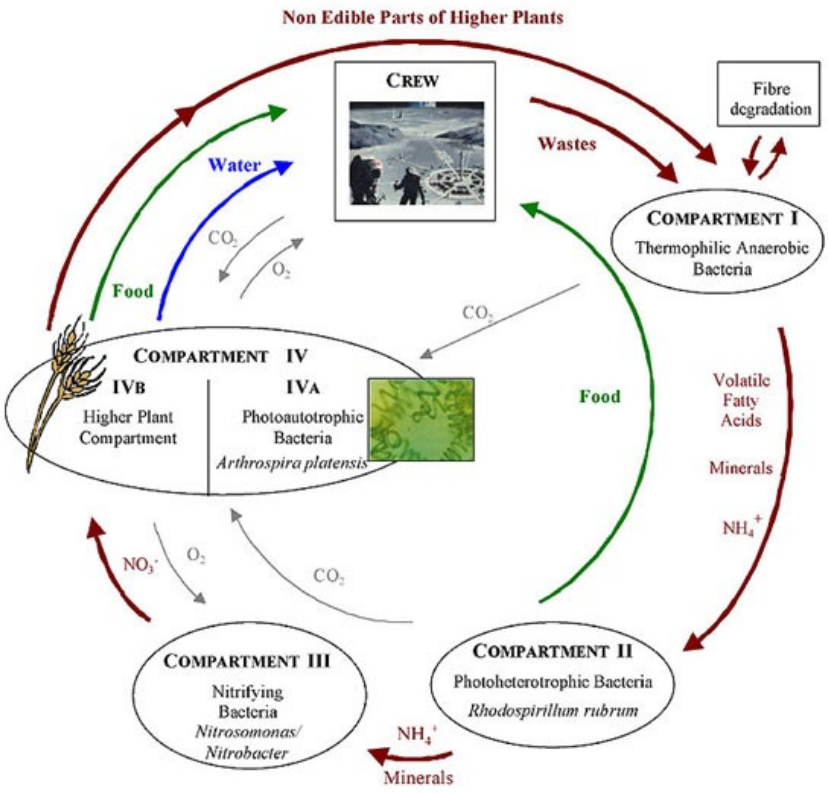
Vigue *et al.* (1977) and Imsande (1988) reported that urea in absence of nitrate in the nutrient solution do not inhibit nodulation.

The MELiSSA Project

MELiSSA (Micro Ecological Life Support System Alternative) has been conceived as a micro-organisms and higher plants based ecosystem intended as a tool to gain understanding of the behaviour of artificial ecosystems, and for the development of the technology for a future regenerative life support system for long term manned space missions, e.g. a lunar base or a mission to Mars.

The driving element of MELiSSA is the recovering of edible biomass from waste, carbon dioxide and minerals, using light as source of energy to promote biological photosynthesis. Based on the principle of an "aquatic" ecosystem, MELiSSA consists of 5 compartments colonised respectively by thermophilic anoxygenic bacteria, photoheterotrophic bacteria, nitrifying bacteria, photosynthetic bacteria, higher plants, and the crew (Fig. 1).

Figure 1. MELiSSA ecosystem loop.



Waste products and air pollutants are processed using the natural function of plants, which also provide food and contribute to water purification and oxygen for air revitalization.

The aim of the technology developed is the understanding, modelling and controlling of every single unit, to obtain the closure of the ECLSS loop; consequently, many processes have to be coupled together. Regarding food sources, mainly higher plants, this level of characterization

is far from being complete and from allowing a good understanding and a proper modelling approach.

The first task within MELiSSA project is the elaboration of a functional concept for a Food Production and Preparation System (FPPS) to be operational at the Moon (180 days) or Mars surface (360 days). During long term human space missions, such a FPPS should provide a significant share of the human diet as vegetable-derived products with high nutritional value and psychological acceptance for the crew. Four energy-and/or protein-rich crops with world-wide usage have been chosen to initiate and validate the proposed approach (wheat, durum wheat, potato and soybean).

The key issue is to be able to guarantee food production in a future FPPS, stable both in quantity and in quality.

The latter requirement implies: the choice of a cultivar adaptable to the required growth conditions and with a suitable nutrient composition in agreement with dietary requirements; the subsequent detailed characterization of the produced food for validation or update of formerly published results (even obtained under different growth conditions); the characterization of the plant growing environment, which directly influences the final crop yield and the waste composition.

Modelling of crop growth is useful for the characterisation and functional understanding of food production. The aim is to develop a model that will be based on plant metabolism and its regulation by environmental factors.

The choice of the crops and crop cultivars is based primarily on their capacity to provide high yield and also to provide oxygen, recycle fresh water and consume carbon dioxide.

Cultivar water and nutrients supply needs are to be considered a selection criterion of minor importance, since these are supposed to be optimal in alternative life support systems which are based on hydroponic cultures.

The mission requirements (especially limitation on energy use) and scenario (environmental conditions) should be taken into account for the cultivar choice: maximal yield under the mission imposed environment would thus provide a starting point. In this respect, the MELiSSA crops are all C3 plants.

C4 crops (and CAM plants) can attain a higher productivity through suppression of photorespiration by a sub-cellular concentration mechanism of the CO_2 to be fixed by photosynthesis. This improvement however needs additional energy to function; hence these crops only thrive under warmer, high light conditions. C4 plants are also adapted to cope with less water than C3 plants. However high light and temperature are - from a closed

environment energy viewpoint - not the most efficient option for plant growth. Moreover, the productivity advantage of C4 over C3 plants disappears under atmospheres enriched with CO_2 at levels of 700 ppm or higher (Zhu *et al.*, 2008).

The selection of the 4 crops in the project (potato, soybean and 2 wheat species) is based on worldwide acceptance and relative ease of food production, while providing the largest share of nutritional needs. For wheat additional variability was introduced by selecting 2 species (durum wheat and bread wheat). Moreover, the combination of the 4 crops delivers an appropriate protein composition (Schaafsma, 2000).

The crops considered for this study are not the most 'simple' living plants: potato has the particularity of tuber formation on subterranean stalks, while wheat and soybean are seed production crops where induction of flowering should be assured with the correct lighting conditions, to provide acceptable yields.

At the end of the first phase of MELiSSA, preliminary protocols for reproducible growth and analysis of cultivars from 4 crops should be available, along with design plans plus a simulation model for a dedicated plant growth chamber intended for the continuation of this activity. In addition, a preliminary approach for mechanistic, metabolism-based modelling of plant growth will have been validated with the first obtained growth data. These results will provide a basis for the characterisation of crop cultivar performance under completely controlled conditions.

Crop productivity data derived from growth trials under completely controlled conditions are lacking for most crop species, so an extensive characterisation under optimal production conditions is needed. Such datasets can be useful for predicting food, potable water and oxygen production rates and dynamics, which in a closed regenerative life support system will enable closure of the air, water and nutrient loops, based on the calculation of mass balances.

The MELiSSA project is organised in four phases: *Phase 1*: Ground demonstration; *Phase 2*: Preliminary flight experiments; *Phase 3*: Space adaptation; *Phase 4*: Technology transfer.

The experiments described in the following chapters are part of the first phase of the MELiSSA project. At first, a preliminary survey was carried out to select the 4 European soybean cultivars to be grown. Before the comparison among the selected cultivars on hydroponic cultivation, a germination test was performed on the seeds. Moreover, a comparison between hydroponic systems on a Canadian soybean cultivar was carried out.

Preliminary survey for the soybean cultivars selection

Introduction

A methodology for objective soybean cultivar selection was developed, based on the requirements advised by ESA (Tab. 2). It has to be said that many of these parameters are not available in the scientific literature and not provided by breeders, however they were considered in the view of future studies. Moreover, the selection method can be refined if needed, considering that it appears unlikely that the relevant criteria and their relative weight can be properly selected in early stage of the research. Among criteria initially proposed, the following ones were not considered: volatile organic compound (VOC) production and root exudates production (no instrumentation available); resistance to stress (plants during bench test should be cultivated under optimal conditions and should not be subjected to any stress); behaviour in extraterrestrial environments (no flight experiments were planned).

Table 2. Crop cultivar selection criteria to be considered during the cultivation test according to ESA requirements.

Criteria	Major parameter(s)	Associated parameter
Crop cultivar stature	Growth space	Handling (harvest)
	Growth period length	Crop senescence
Cultivar harvest index	Waste production	Waste degradability
	Influence of plant growth system	
Cultivar nutritional composition	Absence of anti-nutritional compounds	Pro-nutritional compounds
Cultivar edible part composition	Processability	Possible conflict with levels of pro-nutritionals
	Storage stability	
	Palatability	Storage time
Water use efficiency	Growth efficiency	High water turnover rate
		Regeneration rate
	Atmosphere regeneration capacity	O_2 production
Volatile organic compound (VOC) production	Ethylene	Growth inhibition
Root exudate production	Allelochemical inhibiting plant growth	Interspecies compatibility
Resistance to stress	Abiotic stress	System cleanness maintenance
	Biotic stress	
Pollination	Self-pollinating cultivar requirements	
Propagation	Seed to seed or vegetative	Seed handling/storage
Behaviour in extraterrestrial conditions	Reduced gravity / reduced pressure	Radiation influence

Materials and methods

According to the Official Journal of the European Union of 20.11.2008 (http://eur-lex.europa.eu), the total number of soybean cultivars admitted by the EU is 297.

Information about the main European companies and the most commercialized cultivars was found.

Beside, a literature study was carried out to look for data about environmental needs and yields of soybean cultivars already tested in cultivation trials. However, most cultivation trials were carried out on genetically modified cultivars not admitted in EU, while only scarce information was available for a limited number of cultivars admitted in EU.

Information about cultivars derived from different sources, often from different countries; cultivation trials were performed by different farmers and researchers in several places. As a consequence, available information about all the considered cultivars was very heterogeneous.

Table 3 reports the number of parameters for which there are available data per each considered cultivar; cultivars whose information is available also in catalogues are starred (*); cultivars admitted and cultivated in more than one European country are reported in blue; cultivars not admitted in EU are reported in red.

Conversely, table 4 summarizes, per each considered parameter, the number of cultivars for which there is available information. For some specific parameters, data are available only for a few cultivars. It is likely that these parameters will not be considered in the final choice of the candidate cultivars unless they are fundamental for the successful cultivation in ecologically closed systems. For some cultivars, data are available only for a few parameters. It is likely that these cultivars will not be considered in the final choice of the candidates unless they present specific parameters that make them particularly suitable for the successful cultivation in an environmental closed life support system.

To select the 4 candidate cultivars of soybean with an objective method, an algorithm was created to attribute a score to the different cultivars.

Starting from the availability of characteristics about the different cultivars, a priority factor (P) was assigned to each characteristic. This factor is based on the relevance of each feature for the choice. The highest value (P=3) was assigned to the characteristics considered very important (i.e. group, size, destination, antinutritional factors content, suitability to industrial uses, tolerance to abiotic and biotic stresses, yield, protein content). A factor P=2 was assigned to the feature having a medium relevance (e.g. branching, colour of *hilum*, sensitivity to lodging, stalk rating) and a factor P=1 was attributed to those characteristics less

important for the cultivar growth in a closed system (e.g. defoliation, insertion of first pods) (Tab. 4).

According to available information reported above, each feature was characterized by a different measure unit that is expressed as continuous values or in defined classes, depending on data sources. In order to standardize the values attributed to each parameter, per each feature, also those data expressed with continuous values were summarized into classes. Moreover, considering that the number of classes is not equal per each parameter, the score attributed to different classes was expressed as a percentage factor (X). The highest level of satisfaction corresponds to X=1, while the absence of information corresponds to X=0 (Tab. 5).

A final score (S) was calculated per each cultivar, based on the available characteristics, the priority factor and the percentage factor, to classify them in decreasing order and to choose the four showing the highest values, according to the following formula:

$$S = \Sigma (P_i * X_i)$$

Table 3. Number of parameters for which there is available information per each cultivar

Cultivar	# parameters	Cultivar	# parameters	Cultivar	# parameters
Aires *	14	Landor	7	Sumatra *	10
Albinos *	11	London *	5	Supra	9
Alisa	5	Lory	4	Taira	5
Alma ata	10	Lotus	12	Tea	11
Anita 66	5	Magnum	4	Toliman	4
Ascasubi *	12	Manuela	3	Tundra	9
Askja *	10	Meli	5	Amphor	8
Astafor	7	Merlin *	5	Dekabig *	11
Atlantic *	14	Milor	3	Essor	6
Avila	3	Neoplanta	9	Fukui *	8
Bahia *	7	Nikir *	13	Giulietta *	13
Batida	7	Nikita	3	Nikko	7
Borneo *	14	Norma	3	Pr92b63 *	15
Brillante *	12	Oac erin *	9	Safrana *	10
Celior	5	Oac vision	12	Samurai	3
Clara *	12	Orion	4	Sapporo	8
Colorado *	12	Orlanda *	8	Sarema	4
Condor *	14	Osaka	3	Sekoïa *	10
Cresir *	13	Pacific *	13	Shama *	15
Dekafast	5	Paoki	5	Sponsor	4
Demetra *	13	Pedro *	13	Toyama	7
Ecudor	7	Pr91b92 *	12	Zen	8
Fasto *	7	Pr91m10 *	15	Aveline *	-
Fiume	4	Primus	8	Balkan	-
Fly	3	Pronto	6	Blanca	-
Fortezza	11	Protina *	9	Blancas *	-
Gallec *	5	Queen gt 450	5	Capnor	-
Goriziana	4	Quito	6	Casa	-
Hilario *	13	Regir *	13	Cataline *	-
Igor	3	Sakai	4	Mariana	-
Indian *	12	Sake	4	Mitsuko	-
Isidor	8	Salta	3	Proteinka	-
Katana	6	Santana *	9	Sigalia	-
Klaxon *	13	Sepia *	8	Vanessa *	-
Korada	9	Soledor	5	Venera	-
Lanca *	9	Splendor	3		

Table 4. Priority factors (P) and number of cultivars for which information is available per each considered parameter.

Characteristic	# cv	P
Destination	30	3
Group	107	3
Level of antinutritional factors	6	3
Protein content	57	3
Sensitivity to *Sclerotinia*	40	3
Size	78	3
Suitability to industrial uses	6	3
Tolerance to *Diaporthe*	19	3
Tolerance to *Phytophthora*	33	3
Tolerance to *Rhizoctonia*	7	3
Tolerance to stresses	13	3
Yield	70	3
1000 seeds weight	73	3
Branching	11	2
Colour of *Hilum*	30	2
Sensitivity to lodging	84	2
Stalk rating	3	2
Defoliation	19	1
Dehiscence	16	1
Insertion 1st pods	41	1

Table 5. Classes and percentage factors (X) per each feature

Characteristics	Classes	X
Destination	first crop-first and second crop	1
	second crop	0.5
Group (earliness)	000 to 00	1
	0 to 0+	0.75
	1- to 1+	0.5
	1/2 to 2	0.25
Level of antinutritional factors	very low	1
	low	0.5
Protein content	very high (>44 %)	1
	high (42-44 %)	0.75
	medium (39.4-41.9 %)	0.5
	low (38.8-39.3 %)	0.25
Sensitivity to *Sclerotinia*	low	1
	medium	0.66
	high	0.33
Size	low (68-78 cm)	1
	low-medium (79-90 cm)	0.8
	medium (91-102 cm)	0.6
	medium-high (103-114 cm)	0.4
	high (>114 cm)	0.2
Suitability to industrial uses	suitable	1
Tolerance to *Diaporthe*	high-excellent (score 9-10/10)	1
	medium-good (score 8/10)	0.5
Tolerance to *Phytophthora*	high-excellent (score 8-9/10)	1
	medium-good	0.5
Tolerance to *Rhizoctonia*	high	1
	good	0.5
Tolerance to stresses	very high (score 10/10)	1
	high (score 8-9/10)	0.66
	medium (score 7/10)	0.33
Yield	very high (>45 q/ha)	1
	high (score 8-8.5/9; 39-45 q/ha)	0.66
	good (score 7/8; 33-38 q/ha)	0.33
1000 seeds weight	very high (>217 g)	1
	high (201-217 g)	0.8
	medium (183-200 g)	0.6
	low (165-182 g)	0.4
	very low (147-164 g)	0.2
Branching	high	1
	medium-good	0.66
	low-absent	0.33
Colour of *Hilum*	colourless	1
	yellow-imperfect yellow	0.75
	fair	0.75
	slightly brown	0.5
	brown	0.25
Sensitivity to lodging	very low (7- 18 %)	1
	low (19-30 %)	0.75
	medium (31-43 %)	0.5
	high(>43 %)	0.25
Stalk rating	excellent-strong	1

	good	0.5
Defoliation	optimal	1
	very rapid	1
	rapid	0.5
Dehiscence	resistant (score 10/10)	1
	tolerant	1
	medium high (score 9/10)	0.66
	medium (7)	0.33
Insertion 1st pods	high (15-19 cm)	1
	medium (score 6/9 or 13-14 cm)	0.66
	low (11 cm)	0.33

Results

The cultivars were classified in decreasing order, according to the calculated score, as reported in table 6.

107 cultivars were selected among the total amount of cultivars admitted in EU, according to the criteria suggested by ESA and the information available. Unfortunately, although they were inserted in the Official Journal of the European Union and they were used in field trials in 2008, the seeds of some cultivars were not available. Consequently, we focused our attention on the first 10 cultivars, to evaluate their actual availability. The first 10 cultivars are commercialized by the companies illustrated in table 7.

Table 6. Cultivar classification based on their final score.

Cultivar	Score	Cultivar	Score	Cultivar	Score
Pr91m10	30.75	Alma ata	15.57	Fiume	9.68
Clara	24.18	Korada	15.57	Goriziana	9.68
Regir	23.42	Supra	15.30	Astafor	9.65
Atlantic	23.05	Askja	14.93	Pronto	9.61
Cresir	22.37	Protina	14.91	Alisa	9.53
Colorado	22.16	Tea	14.12	Meli	9.35
Pr91b92	20.80	Nikko	13.83	Celior	9.21
Aires	20.42	Sapporo	13.51	Magnum	9.00
Condor	20.30	Fukui	13.48	Bahia	8.98
Albinos	20.20	Batida	13.47	Toliman	8.50
Klaxon	20.12	Gallec	13.29	Lory	8.48
Indian	20.09	Orlanda	13.22	Sponsor	7.98
Pr92b63	19.72	Santana	13.01	London	7.59
Fortezza	19.38	Zen	12.72	Merlin	7.59
Neoplanta	19.29	Amphor	12.62	Sakai	7.38
Hilario	19.06	Katana	12.58	Avila	7.23
Nikir	18.80	Dekabig	12.54	Dekafast	7.18
Borneo	18.51	Toyama	12.30	Sake	6.88
Pedro	18.46	Sekoïa	12.24	Orion	6.70
Lotus	17.71	Tundra	11.88	Samurai	6.70
Demetra	17.64	Quito	11.71	Fly	6.60
Pacific	17.36	Oac erin	11.63	Norma	6.20
Sumatra	17.34	Anita 66	11.48	Soledor	6.04
Giulietta	17.33	Sepia	11.35	Igor	6.00
Safrana	17.28	Essor	10.79	Milor	5.73
Primus	16.92	Fasto	10.76	Salta	5.50
Oac vision	16.66	Sarema	10.59	Splendor	5.49
Brillante	16.40	Paoki	10.23	Manuela	5.45
Isidor	16.38	Ecudor	9.91	Nikita	4.25
Ascasubi	15.96	Taira	9.75	Osaka	4.24
Lanca	15.59	Landor	9.73		

Table 7. Companies selling the first 10 selected cultivars.

Cultivar	Company
Pr91m10	Pioneer
Clara	Panam Seeds
Regir	Pioneer
Atlantic	Venturoli
Cresir	Pioneer
Colorado	SIS
Pr91b92	Pioneer
Aires	SIS
Condor	Agroservice
Albinos	RAGT Semences

Experiment I. Germination test

Introduction

Seed germination includes all the events beginning with water uptake by the dry quiescent seed and ending with the root apex protrusion out of the seed coat as the result of the elongation of the embryonic axis (Bewley, 1997).

The tight control of seed germination is fundamental for both plant establishment (and consequent crop development) and derived food characteristics.

The first point to be addressed is then the analysis of seed germination of the selected cultivars.

Materials and methods

Seeds of the first 4 selected cultivars ('Atlantic', 'Clara', 'Pr91m10' and 'Regir') were germinated according to the Fernandez-Orozco *et al.* (2008) germination method.

Ten grams of seeds were soaked for 30 min with 50 ml of 0.07% sodium hypochlorite. Seeds were drained and washed with distilled water until neutral pH. Afterwards, seeds were soaked with 50 ml of distilled water for 5 h and 30 min and shaken every 30 min, then they were put in imbibed tissue paper, into an environmental chamber (20 °C, 99% relative humidity, darkness).

According to *International Rules for Seed Testing* (1999) the seeds were tested into 4 replications of 100 seeds.

The germination percentage and the Mean Germination Time (MGT) were evaluated at the end of the test (8 days). The MGT was calculated by counting the number of germinated seeds every day and according to the following formula:

$$MGT= \Sigma (n*g)/N$$

Where:

n = number of germinated seeds per day

g = number of days from the begin of the test

N = total number of germinated seeds

Results and discussion

The MGT did not show any differences among the cultivars, with a value of 4.3 days on average (Tab. 8). The percentage of germination after 8 days in environmental chamber was high for all the cultivars but 'Clara' (Tab. 9), probably due to the scarce quality of seeds received (if seeds are too old, the fats can go rancid and prevent the germination process). In this respect, a further germination test was performed on 'Cresir' (the following cultivar in the selection list) (Table 8 and 9).

On the basis of the results obtained in the test, the cultivars chosen for the following hydroponic cultivation were 'Atlantic', 'Cresir', 'Pr91m10' and 'Regir'.

Table 8. Mean Germination Time (MGT) of the selected soybean cultivars (Mean±St.Err.).

Cultivar	MGT (days)
Atlantic	4,2 ± 0,1
Clara	4,4 ± 0,2
Pr91m10	4,2 ± 0,1
Regir	4,4 ± 0,3
Cresir	4.2 ± 0.3

Table 9. Percentage of the selected soybean cultivars seeds germinated at the end of germination test (8 days) (Mean±St.Err.).

Cultivar	%
Atlantic	93.5 ± 2.8
Clara	17.5 ± 2.3
Pr91m10	88,7 ± 2.9
Regir	94.3 ± 4.7
Cresir	86.8 ± 1.3

Experiment II. Comparison among European cultivars on hydroponic cultivation

Materials and methods

Growth chamber and hydroponic system

The 4 selected cultivars of soybean ('Atlantic', 'Cresir', 'Pr91m10', 'Regir') are indeterminate, early (groups 1, 0^+, 0^+ and 1, respectively) and photoperiod-independent. The experiment was carried out in a 16 m^2 walk in growth chamber, equipped with a recirculating NFT system. Plants were grown in plastic double gullies, the density was 43 plants m^{-2} (Fig. 2). Sowing was performed in place, at 20 °C, in the darkness. Seeds were placed in a small plastic basket in the gully holes, on perlite, keeping the moisture of the substrate constant. Seeds were covered with white acrylic covers, for 4 days after planting, to shade seedlings and maintain high humidity. Three seeds were used per each hole and were thinned to one after the plantlet emergence (approximately 8 days after sowing). Plants were sealed in the gully holes using a two sided polyethylene film, with the white one outside, to reflect light.

Fertigation was performed with one separate nutrient solution reservoir per each double gully, equipped with its own submerged pump, in order to work independently. Nutrient solution returned to the reservoir by gravity dependent flow. The nutrient solution was based on the standard Hoagland solution 1/2 strength (Hoagland and Arnon, 1950), modified by Wheeler *et al.* (2008), according to specific requirement of soybean. The recipe and the salt composition of nutrient solution are reported in tables 10 and 11, respectively.

EC and pH were kept at 2.0 dS m^{-1} and 5.8, respectively and they were controlled manually and adjusted every two days by adding deionized water and/or fresh nutrient solution and nitric acid (65% concentration) respectively in the storage tanks. Water depletion was measured every two days and the volume of the nutrient solution was kept at a constant level. The nutrient solution was completely replaced once during the experiment, on 50 days after sowing (DAS).

The experiment was carried out under controlled environmental conditions. Light was provided by High Intensity Discharge lamps (HID), working on hot vapour light emission. Particularly, High Pressure Sodium lamps (HPS, 400 W) were used. The emission spectrum of lamps is shown in Figure 3.

A minimum PAR of 350 μmol $m^{-2}s^{-1}$ was obtained at the canopy level, according to a day/night regime of 12/12 hours. Temperature regime was established at 26/20 °C (light/dark) and relative humidity (RH) was kept within the optimum range of 65-75% using a fog system (Fig. 2). Air

change and dehumidification was guaranteed by two air extractors. The experiment was carried out under ambient CO_2 and O_2 concentration and atmospheric pressure.

Table 10. Nutrient solution recipe.

	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo
	mM						μM					
Nutrient solution	7.5	0.5	3	2.5	1	1	60	7.4	0.96	1.04	7.13	0.01

Table 11. Salt composition of nutrient solution.

Salts
Calcium nitrate
Potassium nitrate
Monopotassium phosphate
Potassium sulphate
Magnesium sulphate
Iron chelate (Fe EDDHA)
Boric acid
Copper sulphate
Zinc sulphate
Manganese sulphate
Ammonium molybdate

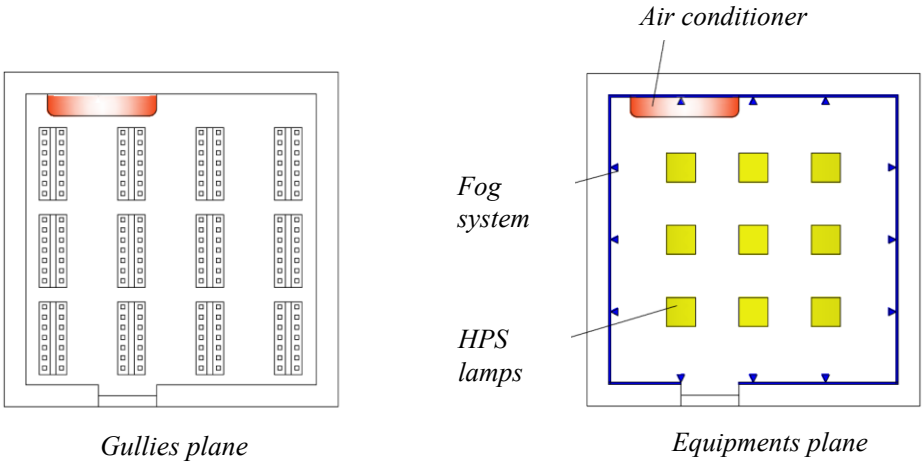


Figure 2. Layout of the gullies and the climate control devices.

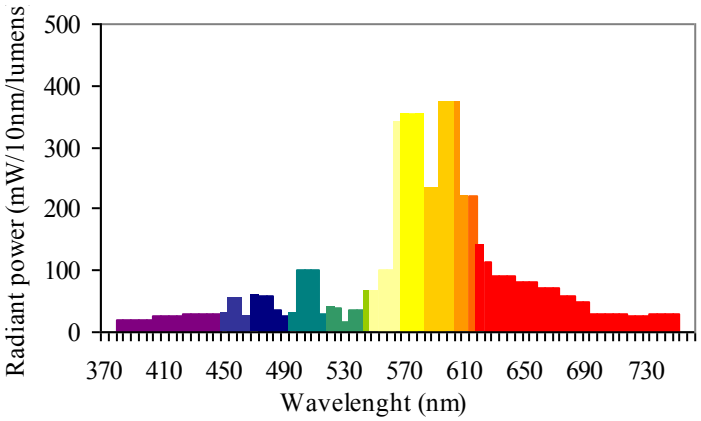


Figure 3. Typical emission spectrum of HPS lamps

Sampling and Measurements

Plant growth and yield

The plant growth and development were measured at 7-day intervals.

Growth analysis was based on non-destructive measurements of plant height, number of nodes and leaves. Plant leaf area (LA) was estimated by measuring leaf length and width, taking into account the specific leaf shape (Wiersma and Bailey, 1957). Measurements were carried out on 6 plants per each cultivar.

Plant tissue analysis (N, P, K, Ca, Mg, S, Cl) were performed on stems and leaves at the beginning of pods formation (48 DAS) and at the harvest stage. Analyses were carried out using a spectrophotometer Hach DR 2000, on the DM of 6 plants per cultivar. Organic nitrogen was determined by Kjeldahl Method. Spectrophotometer readings were performed on water extracts, according to the protocols provided by the factory for plant tissues (Hach, 1997).

Plant dry weight (DM) (after oven-drying at 60°C until constant weight) and carbon partitioning (organ DM/ total DM) were measured separately for the different organs, at the beginning of pods formation (48 DAS) and at harvest.

At harvest, the number of pods and seeds was measured on a unit area basis for the different cultivars compared. Proximate analyses on seeds (protein, fat, carbohydrates, fibre) were performed on 3 samples per cultivar.

Data were analyzed with ANOVA and means were compared by the LSD test.

Water consumption, biomass accumulation rates and efficiency indexes

Crop water consumption was calculated on the basis of the water balance of each double gully, by measuring the volume of the supplied and the drained nutrient solution, assuming the water uptake unaffected by evaporation (due to the plastic covering). At the end of the growing cycle, total and edible biomass accumulation rates, as g of biomass per m^{-2} d^{-1} were estimated, and the following efficiency indexes were calculated: Water Use Efficiency (WUE), expressed as g of edible DM per kg of nutrient solution; Radiation Use efficiency (RUE), as g of edible DM per mole of PAR; finally, as nitric acid was used in order to control the pH in the nutrient solution, we also estimated the Acid Use Efficiency (AUE), as g of edible DM per mmole of H^+.

Data were analyzed with ANOVA and means were compared by the LSD test.

Results and discussion

Plant growth and yield

The time from sowing to flowering varied among the cultivars: 'Cresir' was the earliest (33 days) compared to the others (40 days on average). In our experiment, harvest was staggered to simulate a situation in which there is no much space for storing the seeds and to create a more manageable approach (harvesting in a single day would result in a higher single-day labour for astronauts). Harvests began on 90 DAS in 'Cresir', confirming its earliness compared to the other cultivars (94 days on average), also in terms of duration (24 *vs* 39 days on average) (Tab. 12).

As expected, the plant growth rate did not diminish at flowering because of the indeterminate stem growth (Fig.4a); 'Cresir' was the highest, with more nodes and the highest maximum leaf area, compared to the other cultivars. 'Pr91m10' was the shortest (Tab. 13, Fig 4a). According to its earliness 'Cresir' showed the higher rate of leaf falling (Fig. 3b), indicating a trend of early leaf senescence; moreover, it shown an excessive internodes elongation, probably because this cultivar has a limited adaptability to the reduced light intensity adopted during the experiment.

Plant fresh and dry weight were not influenced by the cultivar, both at the beginning of pods formation and at harvest (fresh weight was 1012.6 and 1596.2 respectively, on average, data not shown) (Tab. 14 and 15); moreover, differences were not found at 48 DAS in the % DM content in the different plant organs (data not shown). Harvest data expressed on a unit area basis for each cultivar are shown in Table 15. The cultivars did not show any differences in terms of yield (532 g m^{-2} of seed at 14% of water content, on average), and neither the number of pods and seeds per square meter nor the number of seeds per pods were influenced by the cultivars.

Plant biomass at harvest was lower than that obtained by Wheeler *et al* in 1999 and 2007 on soybean grown in hydroponic cultivation but probably the lighting conditions in Wheeler experiments were more favourable to the plant growth (500 to 800 μmol m^{-2} s^{-1}). DM partitioning in the different tissues varied during the cycle: in all the cultivars, at 48 DAS the most of DM was stored into leaves compared to the other plant portion; particularly, 'Pr91m10' showed the highest DM percentage in leaves and the lowest into stem, compared to the other cultivars. At harvest, the higher values of DM were found in seeds in all cultivars; 'Atlantic' accumulated more DM in inedible plant organs (stem-pods) and showed the lowest percentage on DM in seeds (harvest index, HI) (Fig. 5a and 5b); even though these values matched with the ones listed for hydroponically-grown soybean (Dougher and Bugbee, 1997) while HI was slightly higher (56%) for the other cultivars.

Concentrations of elements in stems and leaves on 48 DAS and at harvest are reported in Tables 14 and 15. Plants did not have any nutrient deficiencies during the growth cycle, all the values of main nutrient being included into the range considered optimal for plant growth (www.omafra.gov.on.ca). Nitrogen content of steams and leaves decreased over time, indicating that these tissues were the main source of nitrogen for the pods. NO_3/total N rate was always higher in stems than in leaves, confirming that the former were the plant portion that generally move the nitrate through the plant while the latter are the plant tissue where the nitrate is transformed in organic nitrogen. A little amount of chloride was found, maybe due to its presence as an impurity in the fertilizers used in the experiments (e.g. potassium nitrate).

Proximate analysis on seeds is reported in Table 17. DM percentage varied among the cultivar, with 'Cresir' having the highest value. Protein content was higher in 'Pr91m10' and lower in 'Regir', that also showed the highest fibre content. Concentration of fat was not affected by the cultivars.

Table 12. Beginning of harvest (DAS) and growth cycle length of hydroponically-grown soybean cultivars .

	Beginning of harvest (DAS)	Growth cycle length (days)t
Altantic	93	133
Cresir	90	114
Pr91m10	97	133
Regir	93	114

Table 13. Main growth parameters of tested soybean cultivars (Mean values; ns = not significant; * = significant at P≤0.05). ([1] lsd).

	Plant height (cm)	Maximum n. of leaves	Number of nodes	Maximum LA (cm^2)
Atlantic	96.2 b	24.7 a	12.5 a	1005 b
Cresir	121.7 a	24.2 a	12.3 a	1237 a
Pr91m10	57.8 c	16.3 b	11.0 b	854 b
Regir	99.3 b	22.3 a	10.2 c	990 b
Significance	* (14.65[1])	* (5.89)	* (0.55)	* (189.7)

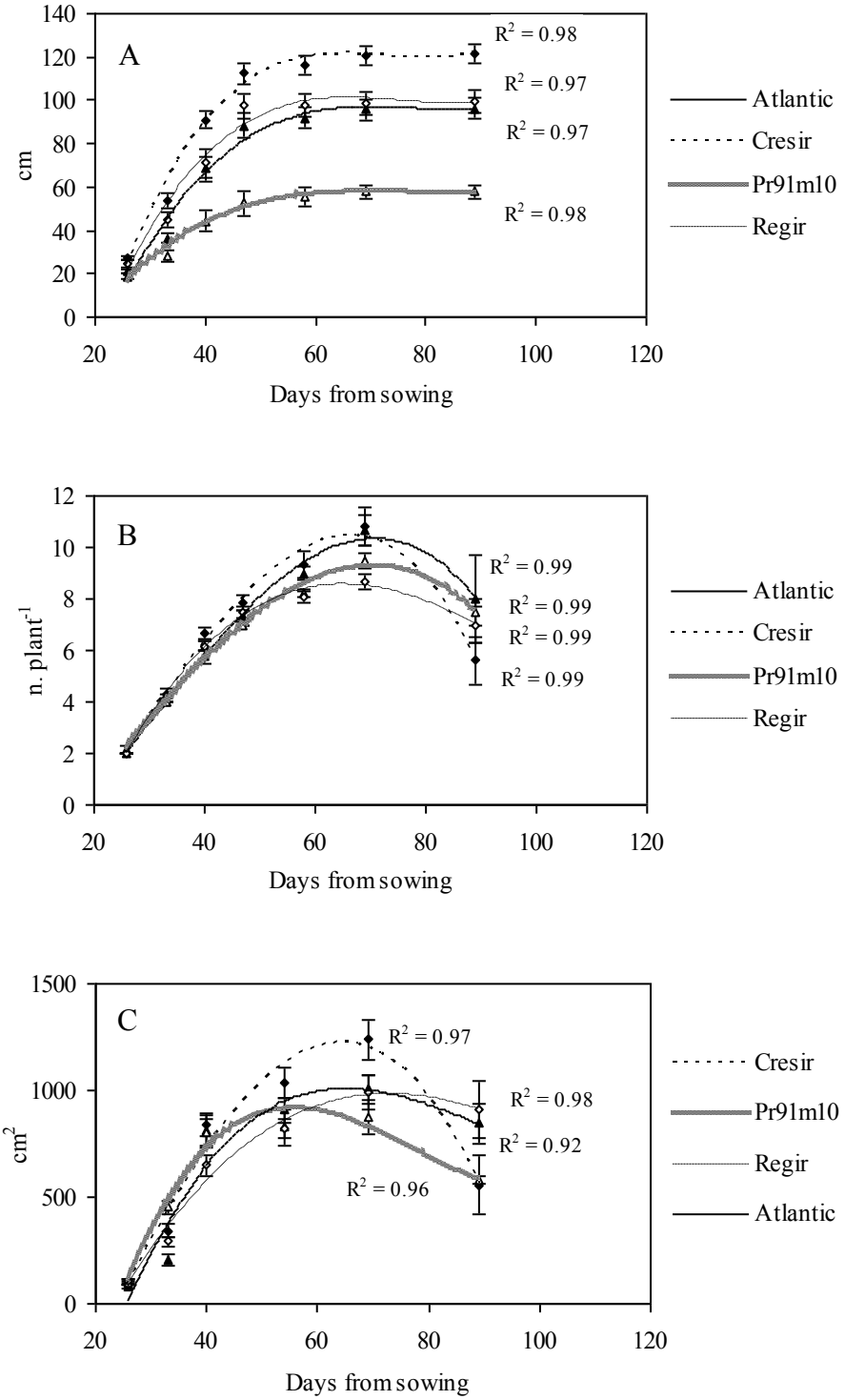


Figure 4. Trend of plant height (A), number of trifoliate leaves (B) and leaf area (C) of tested soybean cultivars.

Table 14. Total plant dry weight and dry weight of different soybean tissues calculated on a per area basis (48 DAS). (Mean values; ns = not significant; * = significant at P≤0.05).

	Total DM ($g\ m^{-2}$)	DM stem ($g\ m^{-2}$)	DM leaves ($g\ m^{-2}$)	DM pods ($g\ m^{-2}$)	DM flowers ($g\ m^{-2}$)
Atlantic	223.6	81.7	133.3	3.01	3.01
Cresir	219.3	73.1	133.3	9.03	2.58
Pr91m10	167.7	43.0	116.1	2.58	3.87
Regir	172.0	64.5	103.2	3.44	3.87
Significance	n.s.	n.s.	n.s.	n.s.	n.s.

Table 15. Total biomass and yield data calculated on a per area basis at harvest (Mean values; ns = not significant; * = significant at P≤0.05).

	Total DM ($g\ m^{-2}$)	Yield 14% water content ($g\ m^{-2}$)	n. pods m^{-2}	n. seeds m^{-2}	Seeds pod^{-1}
Atlantic	951.3	506	1323	2224	1.7
Cresir	906.5	542	1247	2225	1.8
Pr91m10	908.7	546	1253	2184	1.7
Regir	912.5	535	1580	2928	1.8
Significance	n.s.	n.s.	n.s.	n.s.	n.s.

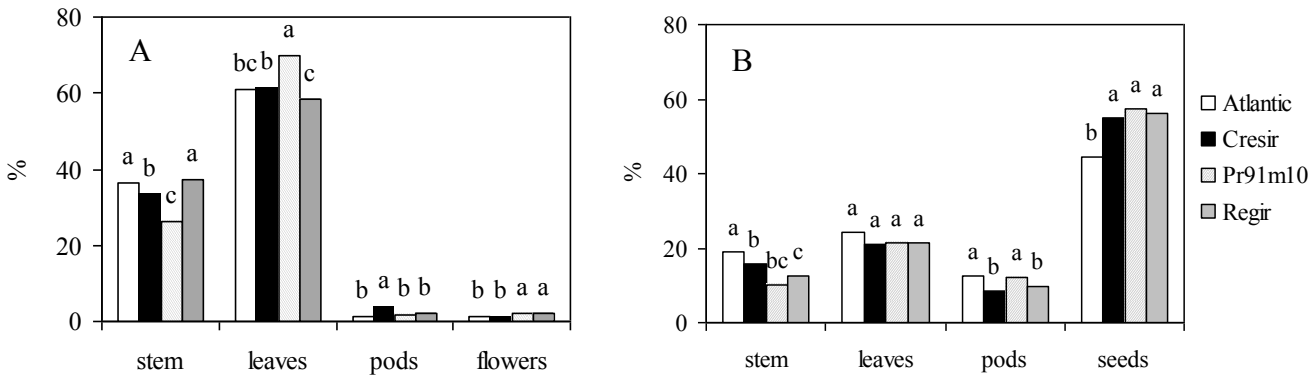


Figure 5. Dry matter partitioning in different plant organs at the beginning of pods formation (A) and at harvest (B). Values in column with differing letters are significantly different (p < 0.05).

Table 16a. Chemical composition of soybean stems and leaves at the beginning of pods formation (48 DAS) and at harvest.

	N (%)		P (%)		K (%)		NO3/Ntot	
	48 DAS	harvest	48 DAS	harvest	48 DAS	harvest	48 DAS	harvest
Atlantic	3.7	2.4	0.4	0.3	2.9	2.4	3.7	4.3
Cresir	3.4	1.9	0.4	0.2	2.6	2.4	4.6	6.2
Pr91m10	3.7	2.6	0.4	0.3	2.5	1.9	6.8	7.7
Regir	3.8	2.6	0.3	0.2	2.8	2.1	8.2	7.1
stem	2.9	2.2	0.5	0.3	2.3	2.3	8.9	8.4
leaves	4.3	2.5	0.3	0.2	3.0	2.1	2.7	4.2

Table 16b. Chemical composition of soybean stems and leaves at the beginning of pods formation (48 DAS) and at harvest.

	Ca (%)		Mg (%)		S (%)		Cl (%)	
	48 DAS	harvest	48 DAS	harvest	48 DAS	harvest	48 DAS	harvest
Atlantic	0.8	1.4	0.4	0.5	0.2	0.2	0.4	0.9
Cresir	0.9	1.3	0.5	0.5	0.2	0.2	0.4	1.0
Pr91m10	0.8	0.7	0.4	0.5	0.2	0.2	0.3	0.8
Regir	0.9	1.0	0.4	0.4	0.2	0.2	0.5	1.0
stem	0.5	0.6	0.4	0.4	0.2	0.3	0.2	0.5
leaves	1.2	1.6	0.5	0.5	0.1	0.2	0.5	1.3

Table 17 Proximate composition of soybean seeds.
(Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	DM (%)	Protein (%)	Fat (%)	Fibre (%)
Atlantic	88.1 b	33.8 b	22.1	27.5 b
Cresir	89.1 a	34.1 b	22.1	27.4 b
Pr91m10	88.1 b	35.6 a	21.2	27.6 b
Regir	88.8 ab	32.0 c	22.5	31.5 a
Significance	* (0.75[1])	* (0.31)	n.s.	* (1.48)

Water consumption, biomass accumulation rates and efficiency indexes

Nutrient solution uptake followed the trend of leaf area, increasing rapidly during early growth as the total evaporating surface of foliage increased, reaching the maximum after about 65 days after sowing (19 litres m^{-2} on average), then declining with age. The trend for'Pr91m10'was slightly different, showing the maximum of water uptake about 5 days later than the other cultivars with a slow decrease when the leaf fall began (Fig. 6a). Total water consumption did not vary among the cultivars (348 litres m^{-2} on average) and was less of that reported by Wheeler *et al.* (1999). This difference was probably due to the lower total biomass production in our experiment (Fig 6b). EC control strategy was very efficient in keeping the target values, with an average EC of 2 in the different storage tanks after adjustments (Tab. 18); on the other hand the EC values before the adjustments indicated a preferential uptake of water respect to mineral elements. Solution pH tended to rise requiring acid for pH control (Tab. 18). This result is consistent with previous experimental evidence, indicating a preferential uptake of anions over cations from a nitrate-based nutrient solution throughout the growing cycle (Willumsen, 1980; Marschner, 1992). The acid requirements ranged from 15.1 mmol m^{-2} d^{-1} in 'Atlantic' to 11.5 mmol m^{-2} d^{-1} in 'Pr91m10' (Tab. 18). The high water and acid use during early vegetative growth was consistent with results from other studies on hydroponically-grown soybean (Vessey *et al.*, 1991; Grusak and Pezeshgi, 1994).

Data on biomass accumulation rate and efficiency indexes are shown in Table 19. The dry mass accumulation rate in the seeds was 3.55 g m^{-2} d^{-1} on average, without any differences among the cultivars. Water use Efficiency (WUE) and Radiation Use efficiency (RUE) were not affected by the cultivars, with 1.35 g of seed per litre of nutrient solution and 0.23 g of seed per mole of PAR respectively, on average. These values are comparable to those reported by Wheeler *et al.* (2003 and 2008) and Dougher and Bugbee (1997) in similar experiments on hydroponically-grown soybean. Acid Use Efficiency (AUE) was 0.27 g of DM seeds per mol H^+ on average, according to the values obtained by Wheeler *et al.* in 2003.

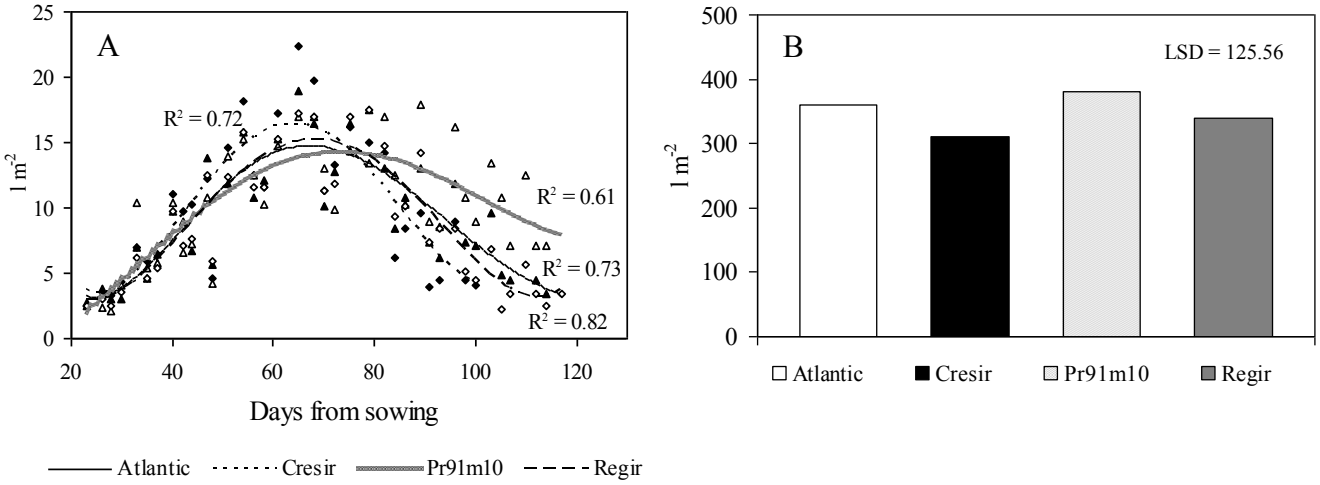


Figure 6. Trend of water use throughout the growing cycle (A) and cumulative water use at harvest (B).

Table 18a. Average fluctuation of nutrient solution EC and pH (with their coefficients of variation) during the growing cycle.

	EC before adjustment	CV%	EC after adjustment	pH before adjustment	CV%	pH after adjustment	CV%
Atlantic	2.36±0.02	10.2	2.00	7.37±0.06	6.4	5.37±0.04	5.6
Cresir	2.40±0.03	12.2	2.00	7.05±0.07	7.9	5.21±0.05	8.0
Pr91m10	2.41±0.02	8.1	2.00	7.15±0.06	7.2	5.25±0.05	7.2
Regir	2.38±0.03	10.8	2.00	7.25±0.05	6.2	5.24±0.05	7.7

Table 18b. Acid and water use during the growing cycle.

	Nitric acid (mmol m^{-2} d^{-1})	Water consumption (l m^{-2} d^{-1})
Atlantic	15.1	2.7
Cresir	13.2	2.7
Pr91m10	11.5	2.9
Regir	13.1	2.6

Table 19. Biomass accumulation rate and efficiency indexes of selected soybean cultivars (Mean values; ns = not significant; * = significant at P≤0.05).

	Growth cycle (days)	Total DM (g m^{-2} d^{-1})	DM seeds (g m^{-2} d^{-1})	WUE seeds (g l^{-1})	RUE seeds (g mol^{-1})	AUE seeds (g $mmol^{-1}$)
Atlantic	133	7.2	3.2	1.2	0.2	0.2
Cresir	114	8.0	4.0	1.5	0.3	0.3
Pr91m10	133	6.8	3.6	1.3	0.2	0.3
Regir	133	6.9	3.4	1.4	0.2	0.3
Significance		n.s.	n.s.	n.s.	n.s.	n.s

Conclusions

Biological life support systems where higher plants are used as component in order to provide useful resources for humans (by photosynthesis) have been demonstrated to have good prospects. The NFT system and the nutrient solution management proved to be efficient in growing healthy soybean plants, without any nutrient deficiency or other kind of stresses (e.g. anoxic condition for the roots).

The use of nitrate as the only N source in our experiments (like in all the previous studies on cultivation of vegetable species for space missions) is reliable, but a great input of acid is needed to control the nutrient solution pH. We used nitric acid to maintain pH levels suitable for plant growth, with a substantial increase of the acid requirements during periods of heavy nutrient uptake.

For example, if an entire hectare of wheat is grown hydroponically with nitrate as the sole N source, this would require about 25 L of concentrated nitric acid per day to control solution pH (assuming PAR levels ranging from 500-800 μmol m^{-2} s^{-1}) (Wheelet *et al.,* 1999). This provides a strong argument for reducing the acid requirement in a CELSS, which could be achieved by using a certain percentage of NH_4-N to maintain a better charge balance in the root zone (Marschner, 1995).

In a CELLS, plant size is one of the most important characteristics to be considered, because available volume is often limited. 'Cresir' was the earliest cultivar but it reached the greatest plant dimension; 'Pr91m10 showed the smaller size compared to the other cultivars, thus being the most fit for the growing in a closed environment. Total inedible biomass is representative of the production of waste in a CELLS, so it is a further parameter to be taken into account; the tested cultivars did not show any differences in terms neither of inedible nor in edible biomass, even though 'Atlantic' showed the higher rate between inedible/edible part. The proximate composition of seeds revealed a higher protein content in 'Pr91m10'.

Water use and efficiency indexes did not vary among the cultivars, with values comparable to those obtained in similar experiments on hydroponically-grown soybean.

In conclusion, among the 4 tested soybean cultivars, 'Pr91m10' could be the most suitable for the cultivation in a CELLS, coupling good yield and high resource use efficiency with short size and good quality of seeds (highest protein content).

Experiment III. Comparison between hydroponic systems on a Canadian cultivar.

Introduction

Urea is one of the most important nitrogen fertilizers used for vegetable production in the field (Vavrina and Obreza, 1993). It is seldom used in hydroponic cultivation for vegetable production, although a few successes have been reported in reducing nitrate accumulation in leafy vegetables by partial replacement of nitrate with urea in the nutrient solution (Gunes *et al.*, 1994). In recent years much attention has been focused on whether urea should be used as the sole hydroponic N source for vegetables, especially for the leafy vegetables (Luo *et al.,* 1993; Khan *et al.*, 1997; Zhu *et al.*, 1997). Studies of the utilization of hydroponically applied urea by fruit vegetables have been limited at seedling stage (Kirkby and Mengel, 1967; Gerendas and Sattelmacher, 1997). According to their findings, urea was not a suitable hydroponic N source compared to nitrate. Similar results were also obtained in experiment with tomatoes at seedling stage (Ikeda and Tan, 1998). The response of fruit vegetables at different growth stages to the utilization of urea in hydroponic culture has received much less attention.

In a life support system, urea is about 85% of the recycled nitrogen available for plant growth, (Wydeven and Golub, 1990). Water purification is particularly relevant in a regenerative system since liquid wastes (urine and wash water) will be the dominant waste streams. Estimates of urine production range from 1.3-2.1 L person^{-1} day^{-1}, while gray water production (e.g., liquid waste from the shower, clothes washer, and dishwasher) is estimated in the range of 25 L person^{-1} day^{-1} (Wydeven and Golub 1990). Combined, the mass of liquid wastes will be 50 times greater than inedible plant biomass and 1000 times greater than faecal dry matter. A biomass production system scaled to meet food requirements for one human would produce approximately 40 L of atmospheric condensate per day (Muhlestein *et al.,* 1999).

Results from plant growth studies indicated that the costs of storage or re-supply to provide plant nutrient requirements would be significant. Mackowiak *et al.* (1996) estimated that the mass of reagent-grade salts required to support plant growth would be equivalent to 30% of the mass of human food requirements. The high nutrient demand of the system appears to be partially a result of significantly higher accumulation of nutrients in hydroponically-grown plants relative to field-grown crops (McKeehen *et al*., 1996a). Decreasing nutrient levels in the hydroponic solutions may reduce excessive nutrient uptake, but may also increase the potential risk of nutrient deficiency. Even if plant uptake can be minimized, inorganic nutrient requirements will remain a significant mass flux in the system.

Urine recycling is desiderable because it represents a significant source of water and nitrogen within the system. Based on an average urea content of 7000 mg L^{-1} (Putnam, 1971), urine production would contain nearly 900 mmol of N per person per day. Average N use for crops such as wheat and potato used in previous experiments equals approximately 35 mmol per square meter per day (Mackowiak *et al.*, 1996a). Assuming a crop growth area requirement of 40 m^2 per person, the estimated N flux in urine is over half of the plant N requirement in the system. The simplest approach for recycling the nitrogen and water within urine would be direct incorporation into the plant growth system. The major problems with direct recycling are potential phytotoxic effects of ammonium as N source (urea can be readily converted to ammonium by micro-organisms), and NaCl accumulation. NH_4^+ is also toxic to plants, but this may be not true if pH is rigorously controlled (Lahav *et al*., 1976). Physical-chemical methods for removal of NaCl from the urine prior to recycling, and microbiological conversion of ammonium to nitrate (nitrification) could eliminate potential phytotoxicity. (Garland *et al.*, 1997)

Results from our previous experiment confirmed the highest acid requirement for the hydroponic cultivation of crops for a CELLS, due to the preferential uptake of anions over cations from a nitrate-based nutrient solution. On the other hand the need of minimize the weight of fertilizers brought from Earth pushes forward the potential employment of urea as N source. The following experiment, aimed to assess the effects of urea as the sole nitrogen source on productive behaviour and N nutrition of soybean in hydroponic cultivation. Moreover as urea, compared to nitrate, does not inhibit the nodulation (Vigue *et al.*, 1977; Imsande, 1988), the interaction between nitrogen source and inoculation with a *Rhizobium* strain was tested, both on a solid growth medium and NFT.

Materials and methods

Growth chamber and hydroponic system

The experiment was carried out at University of Guelph, Ontario, Canada...

Soybean plants (*Glycine max* L. Merr. cultivar 'OT9814') were grown in a walk in growth chamber using metallic gullies equipped with a recirculating hydroponic system. Growth conditions were kept constant during the cycle: the temperature regime was 26/18 °C (light/dark) and the relative humidity (RH) was kept within the range of 70-85%. Light was provided by fluorescent lamps setting photosynthetic active radiation (PAR) at 750 μmol cm^{-2} s^{-1} according to a day/night regime of 16/8 hours. Lamps could be moved towards above in order to keep the PAR constant at the canopy level during the cycle. Plant density was 50 plants m^{-2}.

The influences of the nutrient solution, the inoculation with *Bradyrhizobium japonicum* and the growing media on plant growth and yield were tested.

Two different nutrient solutions were compared, differing in the source of nitrogen. The first one (W) was based on the standard Hoagland solution 1/2 strength (Hoagland and Arnon, 1950), modified by Wheeler *et al.* (2008), according to specific requirement of soybean and containing nitrogen as nitrate. The second one (U) was obtained from the first, by replacing the nitrate with urea (Tab. 20); consequently its salt composition was slightly different, in order to obtain the same concentration of the nutrients; moreover, in the solution containing urea, the concentration of sulphur and molybdenum were slightly increased, in order to support the bacteria infection (Tab. 20). In both the nutrient solutions, the 2(N-Morpholino)ethanesulfonic acid (MES) was added as a buffering agent, in order to stabilize the solutions pH (Tab. 20). Since urea hardly dissociate in a water solution (in absence of specific enzymes), causing a delay in plant growth at least in the early developmental stage, the effect of inoculation with a *Bradirhizobium japonicum* strain BUS-2 was also tested

Concerning the substrates, previous studies about hydroponic cultivation of crops for space explorations have been usually performed using the NFT system. In this experiment, we compared the NFT system with the cultivation in rockwool (Grodan©) on inoculated plants, to evaluate their effects on plant development and plant-bacterium interactions (as *Rhizobium* usually lives in the soil).

Resuming, the following 6 treatments were tested, in a two factor incomplete randomized block design with 3 replicates:

- inoculated plant grown in NFT with Wheeler solution (INW);
- inoculated plant grown in NFT with Urea solution (INU);
- not inoculated plant grown in NFT with Wheeler solution (NNW);

- not inoculated plant grown in NFT with Urea solution (NNU);
- inoculated plant grown in rockwool with Wheeler solution (IRW);
- inoculated plant grown in rockwool with Urea solution (IRU).

Sowing was preceded by a sterilization (Somasegaran and Hoben, 1994) and inoculation procedure (Vincent, 1970): seeds were rinsed in 95% alcohol for 20 seconds to remove waxy materials, and then they were completely immersed in a sodium hypochlorite solution (2.5%) and gently swirled to bring the seeds and the disinfectant in contact. After 5 minutes, sodium hypochlorite was drained off and the seeds were rinsed 6 times in sterile H_2O. All the seeds were incubated overnight in sterile H_2O at room temperature in darkness. Inoculation was performed on a part of seeds using 200 ml of deionised water containing 0.5 g of peat carrier, and then all seeds were placed into the Petri dishes, with agar as growing media, at 27 °C in the darkness.

After two days, the seedlings to be grown in NFT were moved to autoclaved glass tubes, wrapped in aluminium foil to protect roots from light, with seedling agar as growing media (Tab. 22). The plants to be grown in rockwool were directly transplanted in rockwool cubes and irrigated constantly with nutrient solution containing the same salts of the seedling agar. A 12 h photoperiod was provided until 1 week after germination, when the plantlets were moved to the growth chamber. Before the transplant, the roots of inoculated seedlings were infected again dipping them in 200 ml of deionised water containing 10% sucrose and 0.5 of peat carrier. On not-inoculated plants only a 10% sugar solution was used on roots.

Gullies were sealed using a two sided polyethylene film, with the black inside, to reduce the lighting to the roots, and the white one outside, to reflect light.

Fertigation was performed with one separate nutrient solution reservoir per each treatment. Each reservoir was equipped with its own submerged pump, in order to work independently. Nutrient solution returned to the reservoir by gravity dependent flow.

EC and pH target were 1.2±0.1 dS m^{-1} and 5.8±0.2, respectively and they were controlled manually and adjusted every day by adding HNO_3 0.5M (Wheeler solution) or H_3PO_4 0.5M (Urea solution) or KOH 0.5M respectively in the storage tanks. At the beginning of the experiment, reservoirs were filled with *nutrient solution* (Tab.20), while the volume of the nutrient solution was kept at a constant level by adding deionised water and *stock solution* (Table 20). Water depletion in each reservoir was measured twice a week.

Table 20. Wheeler and Urea nutrient solutions recipes.

	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo	MES
Wheeler solution			mM						μM				mM
Nutrient solution	7.5	0.5	3	2.5	1	1	60	7.4	0.96	1.04	7.13	0.01	2
Stock solution	70	10	56	12	10	10	134	96	12.5	13.5	93	0.13	-
Urea solution			mM						μM				mM
Nutrient solution	12	0.25	4.3	3	1	3	54	7.4	0.96	1.04	7.13	0.05	2
Stock solution	70	10	56	12	10	31	134	96	12.5	13.5	93	0.65	-

Table 21. Salt composition of Wheeler and Urea nutrient solutions

Urea solution	Wheeler solution
Urea	
Calcium chloride	Calcium nitrate
Monopotassium phosphate	Monopotassium phosphate
Dipotassium phosphate	
Magnesium sulphate	Magnesium sulphate
Potassium sulphate	Potassium nitrate
Iron chelate (Fe-EDTA-7% Fe)	Iron chelate (Fe-EDTA-7% Fe)
Boric acid	Boric acid
Manganese chloride	Manganese chloride
Zinc sulphate	Zinc sulphate
Copper sulphate	Copper sulphate
Ammonium molybdate	Ammonium molybdate

Table 22. Seedling agar recipe

	$g\ l^{-1}$
Agar	10.0
$CaHPO_4$	1.0
$K2HPO_4$	0.2
$MgSO_4\ 7H_2O$	0.2
NaCl	0.2
FeCl	0.1

Sampling and Measurements

Plant growth and yield

The plant growth and development were measured at 7-day intervals until the beginning of pods filling, and then were measured at 21-day intervals.

Growth analysis was based on non-destructive measurements of plant height, number of nodes and leaves carried out on 6 plants per treatment.

Additionally, the following growth indexes were calculated on 30, 52 and 80 days after sowing (DAS) on the basis of the collected data: Specific Leaf Area (SLA), as the Leaf Area/ DM of leaves ratio; Leaf Area Ratio (LAR), as the Leaf Area/total DM ratio, to assess how the plant's total stock of organic material is divided between photosynthetic organs (leaves) and the rest of plant parts.

The chlorophyll content was estimated using a colorimetric method (CCM-200 chlorophyll meter, Opti-Sciences, Inc.), on the middle leaflet of the second and third fully expanded trifoliate leaves from the top of 2 plants per each treatment replicate (2 measurements per leaf).

Destructive measurements (height and diameter of stem, number of leaves, fresh and dry weight) were performed on 30, 52 and 80 DAS (at the full vegetative growth, at the beginning of pods formation and at the beginning of pods filling). Plant LA was measured by using a leaf area meter (LI-3100, Li-Cor, Lincoln, USA). Fresh weight, dry weight (after oven-drying at 50°C), % of DM and DM partitioning were measured; plant tissue analysis was performed on 30, 52 and 80 DAS leaves, to determine the main nutrient concentration (N, P, K, Ca, Mg), according to the AOAC 990.03 and AOAC 985.01 protocols (AOAC, 1990). Analyses were carried out on the DM of 3 plants per each combination nutrient solution x growth medium x inoculation.

At harvest, yield was measured on a unit area basis for the different treatments compared and the harvest index (HI) was calculated by dividing the edible DM by the total DM in order to evaluate the plant yield performances. Moreover, 100 seeds weight (14% water content) was calculated. Chemical analysis (N, P, K, Ca, Mg) on seeds was performed according to the AOAC protocols (AOAC, 1990): nitrogen was detected by Kjeldahl method; the other elements were determined using inductively coupled argon plasma (ICAP) analysis. Proximate analysis on seeds (protein, fat, carbohydrates) was made following standard AOAC (1990) procedures and included ash by muffle furnace, protein by total Kjeldahl N (6.25 as conversion factor for protein), fibre by digestion and gravimetric technique, fat by acid hydrolysis and ether extraction, and carbohydrate by difference. All the analyses were carried out on 3 seed samples per each treatment.

Data were analyzed with ANOVA and means were compared by the LSD test.

Nodules

Trend of number and dry weight of nodules per plant was determined on 30 and 52 DAS and at harvest (122 DAS). ANOVA was performed on number and dry weight of nodules per plant and on the mean nodule dry weight on 52 DAS; means were compared by the LSD test.

Gas exchanges

Gas exchange measurements were performed using an open flow gas exchange system (*Li-6400*; *Licor*, Lincoln, NB, USA) on the middle leaflet of the second and third fully expanded trifoliate leaves from the top of 3 plants per each treatment, on 33, 47 and 66 DAS. Net photosynthesis rate (*NP*), transpiration rate (Tr), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and leaf temperature (T_{leaf}) were determined at CO_2 concentration of 400 μmol mol^{-1} and 700 μmol m^{-2} s^{-1} of PAR.

Data were analyzed with ANOVA and means were compared by the LSD test.

Water consumption and biomass efficiency indexes

Crop water consumption was calculated throughout the growth cycle by measuring the volume of the supplied and the drained nutrient solution, assuming the water uptake unaffected by evaporation (due to the plastic covering). At the end of the growing cycle, the cumulative water consumption was calculated and the following efficiency indexes were estimated: Water Use Efficiency (WUE), expressed as g of edible DM per kg of nutrient solution; Radiation Use Efficiency (RUE), by dividing edible productivities by daily PAR; moreover, considering that the control of pH was performed by adding acid or base during the whole experiment, and as the acid (and base) budget for a hydroponic cultivation has to be considered for space missions, Acid Use Efficiency (AUE), as g of edible DM per mmole of H^+, and Base Use Efficiency (BUE), as g of edible DM per mmole of OH^-, were estimated.

Data were analyzed with ANOVA and means were compared by the LSD test.

Results

Plant growth and yield

A comparison of main growth parameters is shown in Table 23 and Figure 7. The highest values of stem length and diameter, maximum number of leaves (before leaf fall) and maximum leaf area (before leaf fall) occurred with plants grown in the nutrient solution containing nitrate (W). Nutrient solution containing urea (U) decreased all the biometric parameters; these plants also were the smallest in terms of total dry weight (Tab. 26-28). Thus, stem lengths in U plants were probably shorter because of slightly delayed development.

Wheeler solution increased the number of leaves, even though the rockwool enhanced the number of leaves in both the nutrient solutions; values obtained in inoculated plants grown in rockwool fed urea (IRU) were comparable to those obtained in NFT fed nitrate (both inoculated (INW) and not inoculated (NNW) (Tab. 23).

Growth indexes (SLA, LAR), calculated on the basis of values on 30, 52 and 80 DAS, varied during the growth cycle and were influenced by the nutrient solution, while were unaffected by the other treatments (Tab. 24).

Considering the SLA, during the growing cycle, as the plants become bigger and heavier their leaves become larger, and a necessary part of the architecture of these larger leaves is represented by the midrib and the main veins, whose presence naturally lowers the leaf area per unit leaf dry weight. Also, individual leaves, once they have ceased to expand, tend to become gradually heavier, and this reinforces the fall in SLA (Evans, 1972).

On 30 and 52 days from sowing, SLA was higher in W compared to U, but there were no differences on 80 DAS.

LAR decreased during the plant growth, depending on SLA and on the proportion of dry matter going to leaves during the lifecycle of the plant (Evans, 1972). The index was significantly affected by nutrient solution on 30 DAS showing the highest values with W; an interaction was found on 52 DAS, when the lowest LAR values were recorded in inoculated plants grown in urea and plants grown in NFT supply with nitrate, with no differences among the treatments; the highest values were recorded in NNU and IRW (Fig. 8). All the differences disappeared during the following stages of growth.

The estimated chlorophyll content (measured at 22, 40 and 54 DAS) showed an interaction only in the first measurement, when NNU had the lowest value (18.4 CCI units), while there were no differences among the other treatments (20.2 CCI units on average) (Fig.9). In the following measurements, the difference disappeared (Tab. 25). This result seems to indicate a difficulty of not inoculated plants in uptaking nitrogen from urea during the early growth.

Data about the dry mass are shown in tables 26 to 28. Considering the leaves, the dry weight decreased during the pod-filling (80 DAS, Tab. 28). On 30 days from sowing, plants supply with nitrate accumulated more biomass than the ones fed with urea, while the percentage of dry mass was always higher in plant U. Moreover, rockwool increased the plant biomass compared to the NFT (Tab. 26). On 52 days after sowing, nutrient solution and other treatments still had an influence on the measured parameters: considering DM content in pods, Wheeler nutrient solution had a negative effect in the inoculated plants compared to not inoculated ones, while in Urea plants there were no differences between inoculated and not inoculated. (Fig 10a). Moreover, nutrient solution did not have any effect on inoculated plants , while the growth in a substrate significantly increased the DM content in pods when plants are fed urea and showed a trend of higher values in W plants (Fig 10a). The total DM percentage also showed an interaction among the treatments: U plants tended to reach the highest values compared to W ones, especially when inoculated and grown in NFT (Fig. 10b). These differences were particularly evident observing the DM percentage in leaves and pods (Fig.10c, d). Data reported seemed to indicate that Wheeler solution is able to feed plants in a better way than Urea solution that produces plants with reduced water content. NFT in association with urea and inoculation does not allow the bacteria using efficiently the urea, while the rockwool enhances the dry matter accumulation in plants fed urea and seemed to reduce the inhibitory effect of nitrate on nodulation in plants supplied with Wheeler solution. Besides, rockwool in general seemed to anticipate plant development, allocating more biomass in pods compared to the other treatments.

On 80 DAS Wheeler solution still recorded higher values of biomass; there were no differences between the nutrient solutions in terms of total DM percentage even though U showed higher values of DM percentage in pods and seeds (Tab. 28); dry matter content in stem and leaves were similar between the nutrient solutions, probably because the senescence of these plant organs began, as the photosynthetates were moving towards pods and seeds. The other treatments did not have any effects in this time of growing cycle (tab 28), indicating that the rockwool earliness in biomass accumulation in pods was made up from the other treatments.

Considering the DM partitioning, as expected (Evans, 1972) the fraction of DM in leaves decreased with plant age, because as the dry weight increased, the proportion of dry matter going to new leaves steadily declines throughout the life of the plant. Moreover, on 30 DAS, U had higher DM percentage in leaves and W in the stem (Tab. 29), while the other treatments did not show any differences; in the following stages of growth, this trend was kept, even though an interaction was found in pods on 52 DAS (Fig 11), when the values were similar to the DM content in pods (see Fig. 10a). These results seemed to indicate again an early in biomass

allocation in pods when plants are grown in rockwool. In addition, on 80 DAS, IR treatment showed higher DM percentage in seeds compared to the others, and IN showed the lowest (Tab. 29), even though the differences between NN and IN disappeared at harvest (Tab 30).

Flowering started on 35 DAS in all treatments; the entire growth chamber was harvested in a single day, on 122 DAS. Yield was affected by all the treatments, particularly W produced more than U and plants in rockwool had the highest value; the weight of 100 seeds did not vary among the treatments (25.4 g on average) and was higher than that obtained from cultivation trial in open field (Tab 30). The number of seeds per pod did not vary among the treatments (2.4 seeds $pods^{-1}$), so it's thanks to the number of pods that yield was different (data not shown). The Harvest Index (HI) was lower in U plants compared to W; the growth in rockwool increased the HI compared to the growth in NFT. Nevertheless, the results compared favourably to those obtained in previous experiments on hydroponically-grown soybean (Wheeler *et al.* 2003 and 2008).

Results from the growth indexes (see Tab. 24) indicate a delay in leaf biomass production during the early phases of growth; this gap seemed to be only partially closed in the following developmental stages, because both total biomass production and yield were affected.

A possible explanation of increased seed yield for nitrate-fertilized soybeans may be that such plants received a better start, since nodules are not visible on soybean seedlings until about 9 days after planting and N_2 fixation under favourable moisture and temperature begins at about 14 days (Ham *et al.*, 1975).

Compositional data about leaves showed that all the plants did not have any nutrient deficiencies during the growth cycle, all the values of main nutrient being higher than the critical concentration estimated for soybean (www.omafra.gov.on.ca) (Tab. 31a,b). The nitrogen accumulation in leaves over time showed a pattern similar to that observed for accumulation of dry weight, declining during pod-filling, indicating that leaves were sources of N for the pods (Tab. 31a).

During vegetative growth plants supplied with urea had less nitrogen in leaves compared to W but the difference diminished with plant age: on 52 DAS, only plants fed urea grown in rockwool had less nitrogen in leaves compared to plants grown in NFT (Fig.12a) and on 80 DAS U plants showed higher nitrogen content in leaves when grown in NFT (Fig.12b). On this last sampling, IR plants fed urea continued to have less nitrogen in leaves, comparable to the values obtained from W plants, indicating a higher leaf senescence, according to the data on DM partitioning (see Tab. 29). (Tab. 31a and Fig. 12b). Plants fed nitrate did not show any statistical differences when inoculated. These results confirmed that urea is not able to provide a high amount of nitrogen at

least in the early stages of plant growth, according to the estimated chlorophyll content in leaves (see Tab. 25 and Fig. 9). The higher reduction of nitrogen content in plants supplied with Wheeler solution showed a greater mobilization (probably also due to a greater availability) towards the reproductive organs.

Considering the other mineral nutrients in leaves, phosphorous content on 30 DAS was higher in U not inoculated plants and the lowest values were recorded in W plants (Fig. 13a). The reduced P content in IN plants was also observed on 52 DAS, even though there were no differences among all the treatments on 80 DAS (Tab. 31a). Calcium content on 30 DAS was the lowest in IRU plants, but on the other sampling dates the differences were found only between the nutrient solutions, with highest values in W. Magnesium content on 30 DAS was lower in U plants, particularly in inoculated ones; in the following samplings only the nutrient solution had an effect, with lower values in Urea. The higher rates of Mg and Ca intake in the solution containing NO_3 as the sole N source can be attributed to reduced competition in the absorption process.

Analyses on seeds showed higher nutrient content compared to reference values (www.omafra.gov.on.ca) but compared favorably with those obtained by Wheeler *et al*. in several experiments.

Nitrogen content in seeds (and consequently the protein content) was higher in plants U (that also showed the lowest phosphorous and potassium content) and in plants grown in NFT(Tab. 32). The highest nitrogen content could be explained considering the low yield obtained from these treatments, so the total nitrogen had to move towards a reduced number of seeds. Moreover, plants fed nitrate had a higher total carbohydrates content compared to U plants (Tab. 32), that showed a lower fat content when plants were grown in NFT, while plants in rockwool showed values of fat percentage comparable with W plants (Fig. 15).

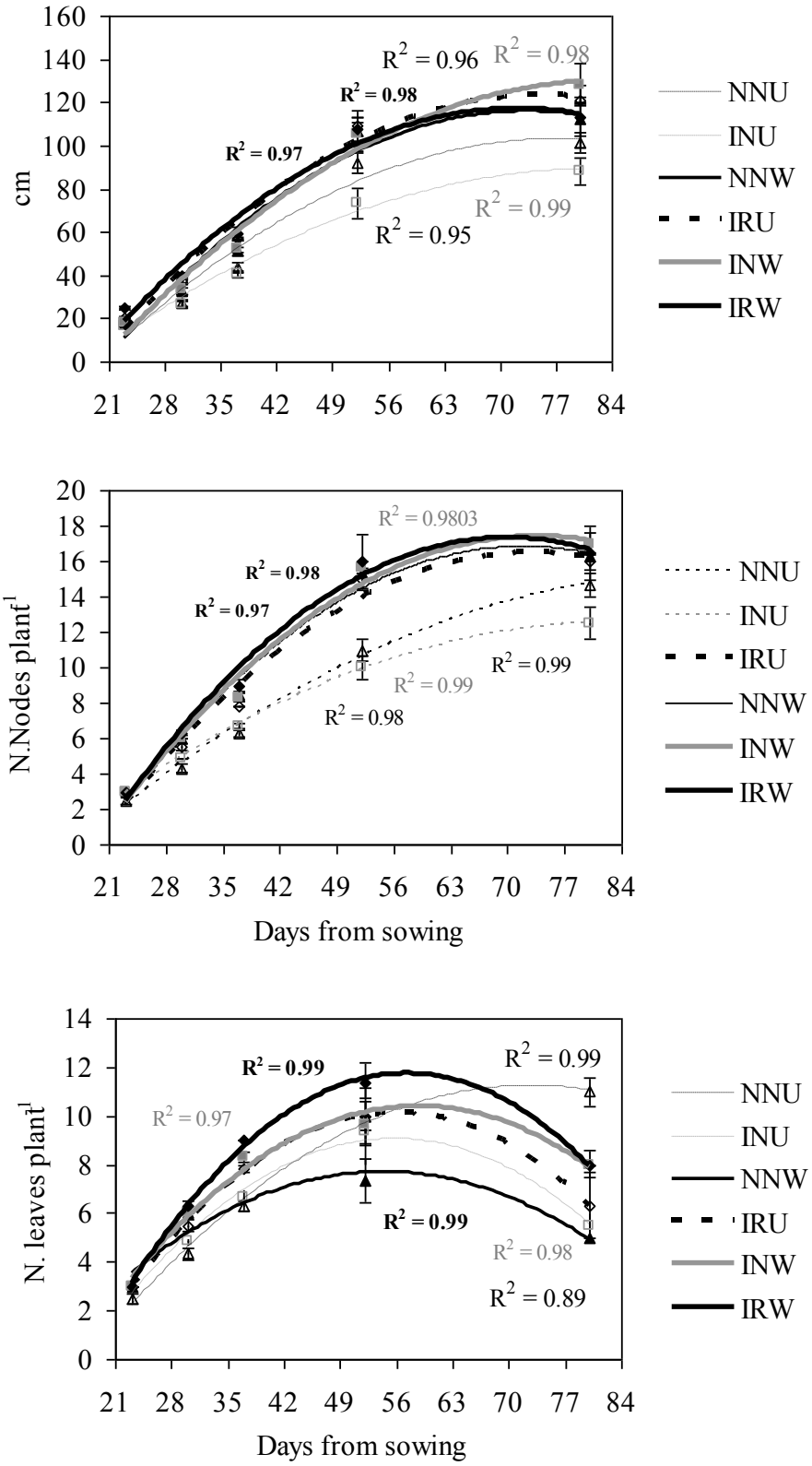


Figure 7. Trend of soybean plant height, number of trifoliate leaves and leaf area throughout the growing cycle for the different treatments.

Table 23. Main biometric characteristics of soybean plants in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Plant height (cm)	Number of nodes	Stem diameter (mm)	Maximum number of leaves	Maximum leaf area (cm^2)	LA/leaf (cm^2)
Nutrient solution						
U	98.3 b	13.4 b	3.2 b	22.0 b	1242 b	56.5 b
W	118.0 a	16.2 a	4.1 a	43.6 a	3837 a	88.0 a
Treatments						
NN	106.8	15.5	3.4	28.2 b	1629 b	57.8 b
IN	108.4	14.8	3.8	27.8 b	1726 b	62.1 b
IR	109.2	14.2	3.8	42.3 a	3715 a	87.8 a
Significance						
Nutrient solution	*	*	*	*	*	*
Treatments	n.s.	n.s.	n.s.	* (9.47[1])	* (552.1)	* (16.8)
Interaction	n.s.	n.s.	n.s.	* (13.4)	n.s.	n.s.

Table 24. Growth indexes of soybean plants calculated on 30, 52 and 80 DAS in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	30 DAS		52 DAS		80 DAS	
	LAR ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)
Nutrient solution						
U	180.6 b	229.6 b	163.8	247.5 b	69.2	213.0
W	223.0 a	292.7 a	176.8	320.6 a	61.0	235.0
Treatments						
NN	206.3	269.0	178.8	303.0	67.3	215.7
IN	206.5	267.7	154.2	255.5	63.5	206.7
IR	192.5	246.8	177.9	293.7	64.7	249.7
Significance						
Nutrient solution	*	*	n.s.	*	n.s.	n.s.
Treatments	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	* (30.87[1])	n.s.	n.s.	n.s.

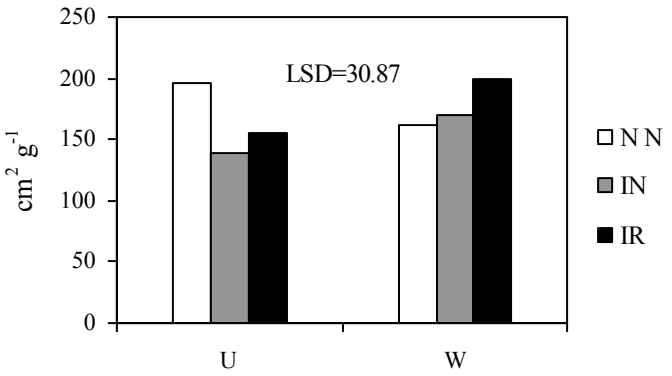


Figure 8. Nutrient solution x growth medium x inoculation interaction on LAR on 52 DAS.

Table 25. Estimated chlorophyll content in soybean leaves on 22, 40 and 54 DAS in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	22 DAS	40 DAS	54 DAS
Nutrient solution			
U	18.8	24.7	36.2
W	20.8	25.2	41.0
Treatments			
NN	18.4	25.2	38.0
IN	20.4	25.1	37.8
IR	20.7	24.5	40.1
Significance			
Nutrient solution	*	n.s.	n.s.
Treatments	* (1.8[1])	n.s.	n.s.
Interaction	* (2.76)	n.s.	n.s.

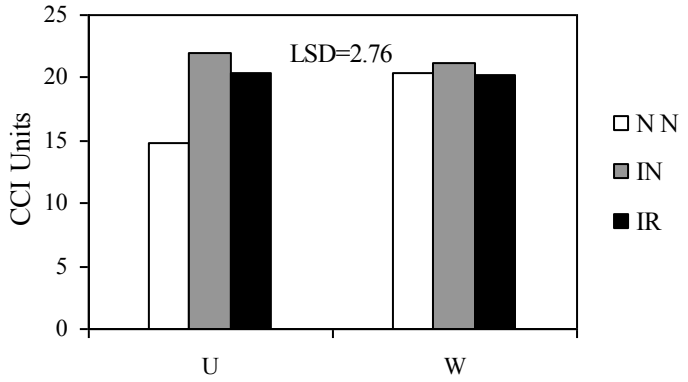


Figure 9. Nutrient solution x growth medium x inoculation interaction on chlorophyll content on 22 DAS.

Table 26. Total DM and DM of different organs on 30 DAS of soybean plants in response to nutrient solution, growth medium and inoculation (values per plant) (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Total DM (g)	DM stem (g)	DM leaves (g)	Total DM (g 100 g^{-1} FW)	DM stem (g 100 g^{-1} FW)	DM leaves (g 100 g^{-1} FW)
Nutrient solution						
U	2.2 b	0.5 b	1.7 b	16.9 a	14.5 a	17.9 a
W	4.0 a	1.0 a	3.1 a	14.0 b	11.6 b	15.0 b
Treatments						
NN	2.7 b	0.6 b	2.0 b	15.6	13.1	16.5
IN	2.8 b	0.7 b	2.1 b	15.4	12.9	16.4
IR	3.9 a	0.9 a	3.1 a	15.4	13.1	16.4
Significance						
Nutrient solution	*	*	*	*	*	*
Treatments	* (0.76[1])	* (0.20)	* (0.58)	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

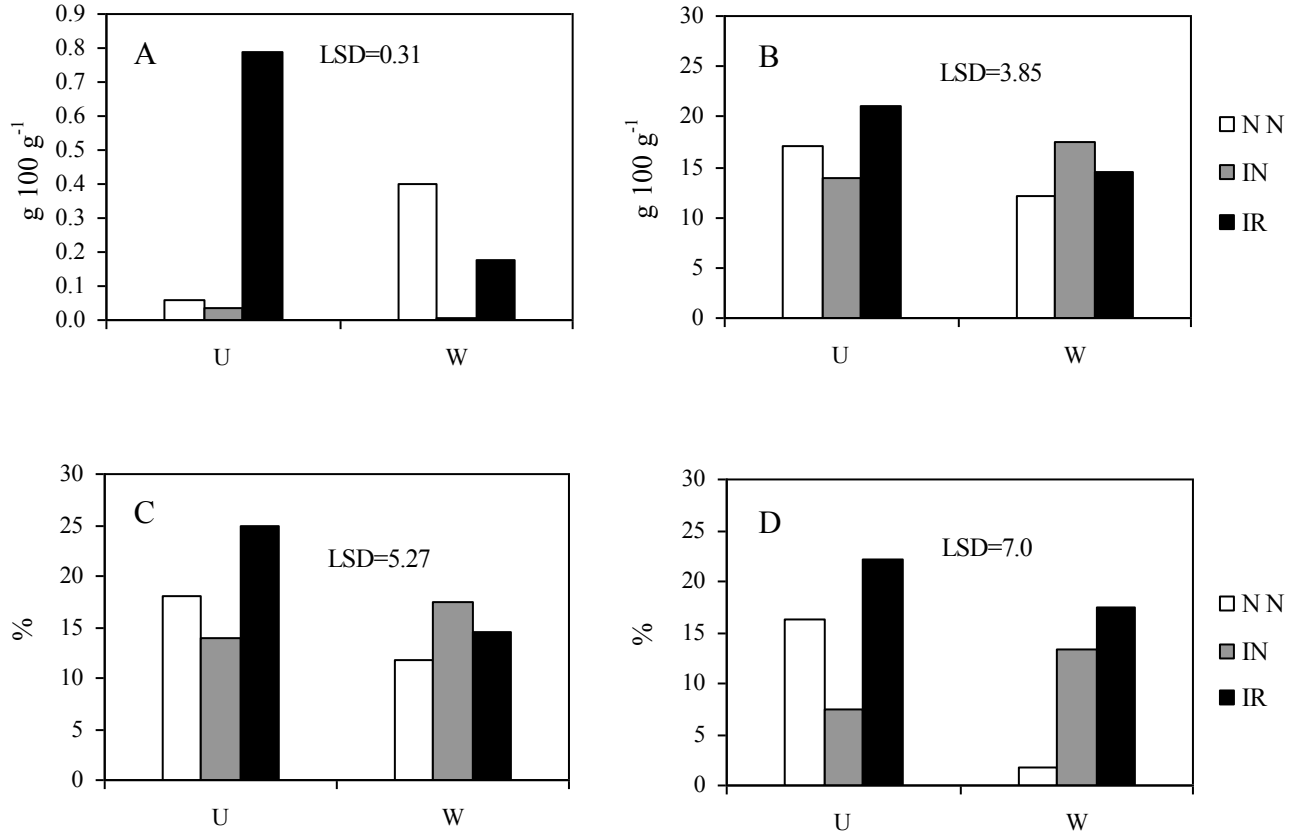


Figure 10. Nutrient solution x growth medium x inoculation interaction on pods DM (A) total DM (B), DM percentage in leaves (C) and pods (D) on 52 DAS.

Table 27. Total DM and DM of different organs on 52 DAS of soybean plants in response to nutrient solution, growth medium and inoculation (values per plant) (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	DM total (g)	DM stem (g)	DM leaves (g)	DM pods (g)	Total DM (g 100 g^{-1} FW)	DM stem (g 100 g^{-1} FW)	DM leaves (g 100 g^{-1} FW)	DM pods (g 100 g^{-1} FW)
Nutrient solution								
U	10.3 b	3.3 b	6.7 b	0.29	18.5 a	16.4	20.2 a	17.3 a
W	25.5 a	10.4 a	15.0 a	0.20	13.5 b	14.8	13.4 b	8.9 b
Treatments								
NN	17.6	6.9	10.6	0.23 b	15.5	15.7	16.0	11.9
IN	13.4	5.3	8.1	0.02 b	16.5	14.5	18.3	12.0
IR	22.7	8.3	13.9	0.49 a	15.9	16.5	16.0	15.3
Significance								
Nutrient solution	*	*	*	n.s.	*	n.s.	*	*
Treatments	n.s.	n.s.	n.s.	* (0.21[1])	n.s.	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	n.s.	* (0.31)	* (3.85)	n.s.	* (5.27)	* (7.0)

Table 28. Total DM and DM of different organs on 80 DAS of soybean plants in response to nutrient solution, growth media and inoculation (values per plant). (Mean values; ns = not significant; * = significant at P≤0.05).

	DM Total (g)	DM stem (g)	DM leaves (g)	DM pods (g)	DM seeds (g)	DM total (g 100 g^{-1} FW)	DM stem (g 100 g^{-1} FW)	DM leaves (g 100 g^{-1} FW)	DM pods (g 100 g^{-1} FW)	DM seeds (g 100 g^{-1} FW)
Nutrient solution										
U	14.4 b	3.8 b	4.8 b	2.7 b	3.2	15.8	19.6	18.7	17.7 a	27.2 a
W	47.1 a	18.4 a	12.9 a	10.4 a	5.4	14.7	17.9	17.2	14.3 b	17.7 b
Treatments										
NN	28.7	10.4	8.6	5.9	3.8	15.8	19.5	19.5	16.0	20.8
IN	29.9	11.5	9.3	6.1	3.0	14.5	17.9	14.9	15.7	21.7
IR	33.7	11.5	8.6	7.6	6.1	15.5	18.9	19.6	16.2	24.9
Significance										
Nutrient solution	*	*	*	*	n.s.	n.s.	n.s.	n.s.	*	*
Treatments	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 29. DM partitioning in the different organs on 30, 52 and 80 DAS in response to nutrient solution, growth media and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	30 DAS		52 DAS			80 DAS			
	stem	leaves	stem	leaves	pods	stem	leaves	pods	seeds
Nutrient solution									
U	21.3 b	78.7 a	31.7 b	66.4 a	1.9	34.8 b	44.2 a	21.0	20.7 a
W	23.8 a	76.2 b	43.6 a	56.0 b	0.4	47.0 a	29.6 b	23.4	11.1 b
Treatments									
NN	23.1	76.9	39.1	60.4	0.7	37.9	38.3	23.9	15.9 b
IN	22.6	77.4	37.9	61.9	0.3	45.7	36.5	17.8	11.2 c
IR	21.9	78.1	36.1	61.5	2.5	39.1	36.0	24.9	20.7 a
Significance									
Nutrient solution	*	*	*	*	*	*	*		*
Treatments	n.s.	n.s.	n.s.	n.s.	* (0.74[1])	n.s.	n.s.	n.s.	* (4.04)
Interaction	n.s.	n.s.	n.s.	n.s.	* (1.1)	n.s.	n.s.	n.s.	n.s.

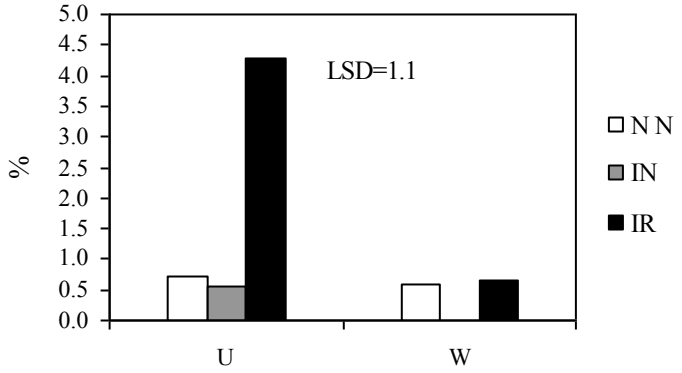


Figure 12. Nutrient solution x growth medium x inoculation interaction in DM partitioning in pods on 52 DAS

Table 30. Yield, 100 seeds weight and Harvest Index of soybean plants in response to nutrient solution, growth media and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Yield ($g\ m^{-2}$)	100 seeds weight (g)	HI
Nutrient solution			
U	183.8 b	24	0.30 b
W	1000.7 a	27	0.41 a
Treatments			
NN	315.6 b	32	0.29 b
IN	291.8 b	21	0.33 b
IR	1169.3 a	23	0.44 a
Significance			
Nutrient solution	*	n.s.	*
Treatments	* (250.6[1])	n.s.	* (0.09)
Interaction	n.s.	n.s.	n.s.

Table 31a. Chemical analysis on leaves of soybean plants in response to nutrient solution, growth media and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	N			P			K		
	30 DAS	52 DAS	80 DAS	30 DAS	52 DAS	80 DAS	30 DAS	52 DAS	80 DAS
Nutrient solution									
U	4.5 b	6.8	6.1	0.91	0.64 b	0.8	3.5 b	2.7 b	2.8
W	6.2 a	6.7	3.8	0.65	0.78 a	0.6	4.7 a	4.0 a	2.7
Treatments									
NN	5.1	6.9	5.3	0.83	0.83 a	0.9	3.9	3.1	2.6
IN	5.3	7.2	5.2	0.75	0.61 b	0.5	4.3	3.6	2.8
IR	5.6	6.3	4.3	0.77	0.69 a	0.8	4.0	3.5	2.8
Significance									
Nutrient solution	*	n.s.	*	*	*	n.s.	*	*	n.s.
Treatments	n.s.	n.s.	n.s.	* (0.03)	* (0.45)	n.s.	n.s.	n.s.	n.s.
Interaction	n.s.	* (1.04[1])	* (1.04)	* (0.05)	n.s.	n.s.	n.s.	n.s.	n.s.

Table 31b. Chemical analysis on leaves of soybean plants in response to nutrient solution, growth media and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Ca			Mg		
	30 DAS	52 DAS	80 DAS	30 DAS	52 DAS	80 DAS
Nutrient solution						
U	1.4	1.2 b	1.2 b	0.3	0.3 b	0.3 b
W	1.8	1.8 a	2.9 a	0.4	0.4 a	0.7 a
Treatments						
NN	1.8	1.5	1.9	0.4	0.3 b	0.4
IN	1.6	1.5	1.8	0.3	0.3 b	0.4
IR	1.4	1.4	2.5	0.4	0.4 a	0.6
Significance						
Nutrient solution	*	*	*	*	*	*
Treatments	*	n.s.	n.s.	*	*	n.s.
Interaction	* (0.28[1])	n.s.	n.s.	* (0.03)	n.s.	n.s.

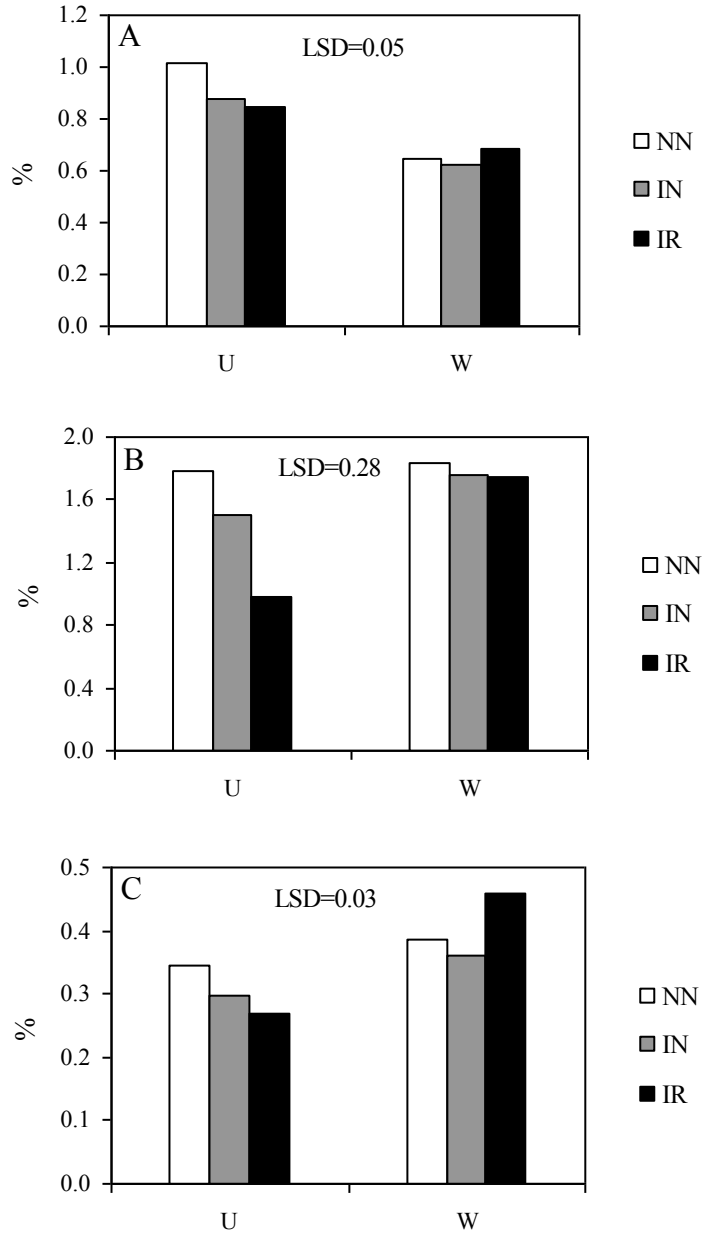


Figure 12. Nutrient solution x growth medium x inoculation interaction on Phosphorus (A), Calcium (B) and Magnesium (C) content in leaves on 30 DAS

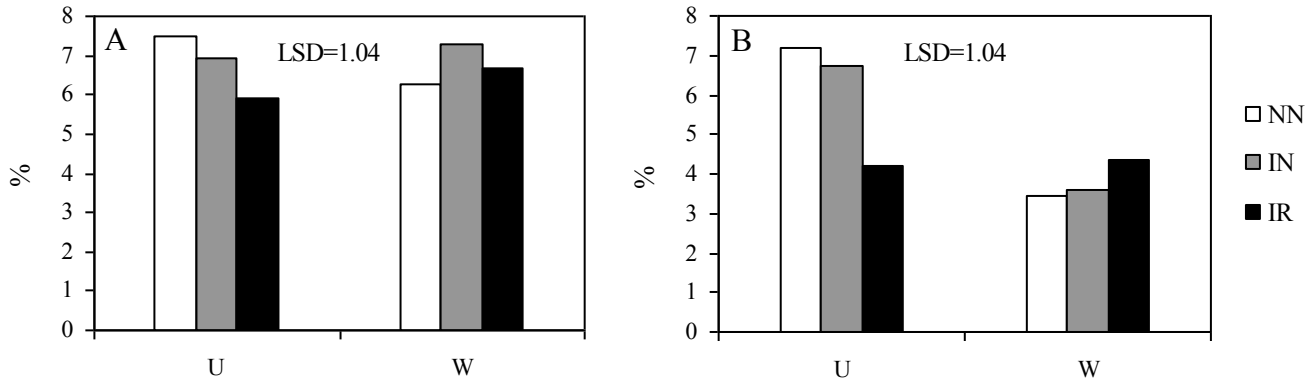


Figure 13. Nutrient solution x growth medium x inoculation interaction on nitrogen content in leaves on 52 (A) and 80 (B) DAS

Table 32. Chemical and proximate composition of seeds of soybean plants in response to nutrient solution, growth media and inoculation (Mean values; ns = not significant; * = significant at P≤0.05;). ([1] lsd).

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Protein (%)	Fat (%)	Carbohydrates (%)
Nutrient solution								
U	7.6 a	0.7 b	1.9 b	0.12	0.3	46.2 a	12.2	31.0 b
W	6.5 b	0.8 a	2.3 a	0.14	0.3	39.9 b	12.9	35.9 a
Treatments								
NN	7.1 a	0.8	2.1	0.14	0.3	43.3 a	12.2	33.5
IN	7.1 a	0.8	2.1	0.12	0.3	43.9 a	12.3	32.8
IR	6.8 b	0.7	2.1	0.13	0.3	41.9 b	13.2	34.1
Significance								
Nutrient solution	*	*	*	n.s	n.s	*	n.s	*
Treatments	* ($0.18^{[1]}$)	n.s	n.s	n.s	n.s	* (1.34)	n.s	n.s
Interaction	n.s.	n.s	n.s	n.s	n.s	n.s	* (1.31)	n.s

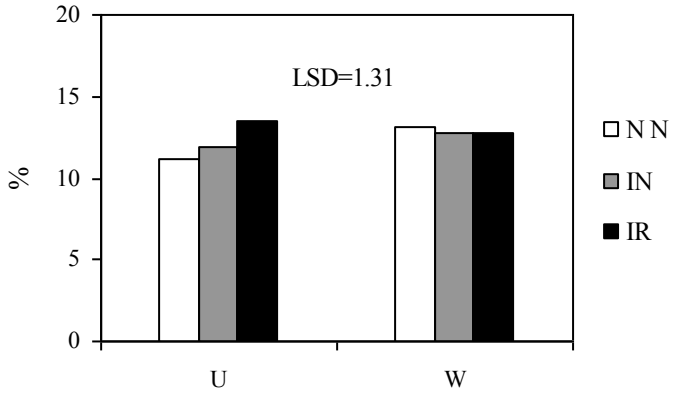


Figure 14. Nutrient solution x growth medium x inoculation interaction on fat content in soybean seeds.

Nodules

Nodule count reflected the efficiency of rhizobia. As expected, NN plants were poorly nodulated; the other treatments showed an increasing in number of nodules, followed by a reduction during the pod-filling (Fig. 16). A decline in the rate of nitrogen fixation per plant with the onset of pod filling has been reported for peas grown under field conditions (Dean and Clark, 1980) and under controlled-environment conditions (Bethlenfalvay and Phillips, 1977; LaRue and Kurz, 1973). There are similar reports for soybeans and common beans (Bethlenfalvay and Phillips, 1977; Quebedeaux *et al.,* 1975).

On 52 days after sowing, inoculated plants in urea reached the highest nodule number (Fig.15a); in plants fed nitrate there were no differences between inoculated and not inoculated plants. Colonization was greater for IR plants respect to IN when fed nitrate and comparable to inoculated plants fed urea (Fig.15a). Urea increased the total dry weight of nodules per plant; rockwool also had a positive effect on this parameter, while the lowest values were obtained in not inoculated plants (Tab. 33).

Dry weight per nodule showed a statistical interaction among the treatments: urea increased the weight in association to all the other treatments, with highest values in NN plants; IR only increased the weight in plants fed urea compared to IN, while there were no differences among the treatments in plants fed nitrate, showing the lowest values (fig.15b). Considering the data obtained, urea seemed to promote or at least to not inhibit the nodulation compared to nitrate, that decreased the rate of nodule formation and also reduced the dry mass of nodules. These results are in agreement to that reported in previous studies (Vigue *et al.*, 1977; Imsande, 1988). The growth in rockwool seemed to reduce the inhibitory effect of nitrate on nodulation even though it did not allow the nodule biomass accumulation in presence of nitrate.

Well nodulated plants did not have more dry matter than the poorly nodulated (see Tab. 26-28), probably because of the lower energy requirement for deriving organic N from NH_4^+ than from N_2,balanced advantage of inoculation in terms of nitrogen uptake. These results are in agreement with those reported by Bethlenfalvay *et al.* (1978) on pea.

Table 33. Number and DM of nodules per plant and nodule average DM on 52 DAS in response to nutrient solution, growth media and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Number nodules plant $^{-1}$	Total nodule DM (mg plant $^{-1}$)	Dry weight (mg nodule^{-1})
Nutrient solution			
U	144.8	256.8 a	2.53
W	107.9	111.4 b	0.97
Treatments			
NN	19.0	48.3 c	2.70
IN	139.0	189.0 b	1.13
IR	221.0	315.0 a	1.42
Significance			
Nutrient solution	n.s.	*	*
Treatments	* (67.07[1])	* (110)	* (0.56)
Interaction	* (94.8)	n.s.	* (0.79)

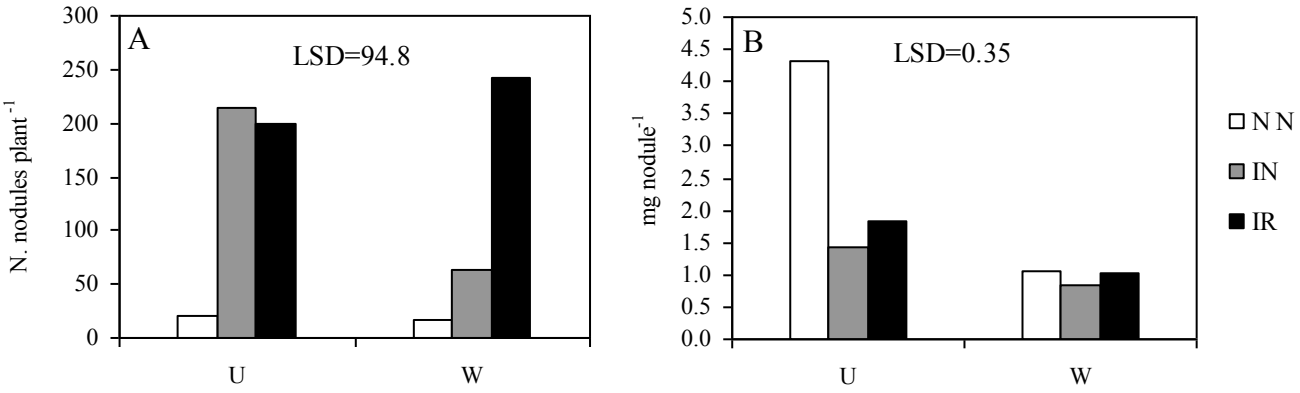


Figure 15. Nutrient solution x growth medium x inoculation interaction on number of nodules per plant (A) and nodule average DM (B)

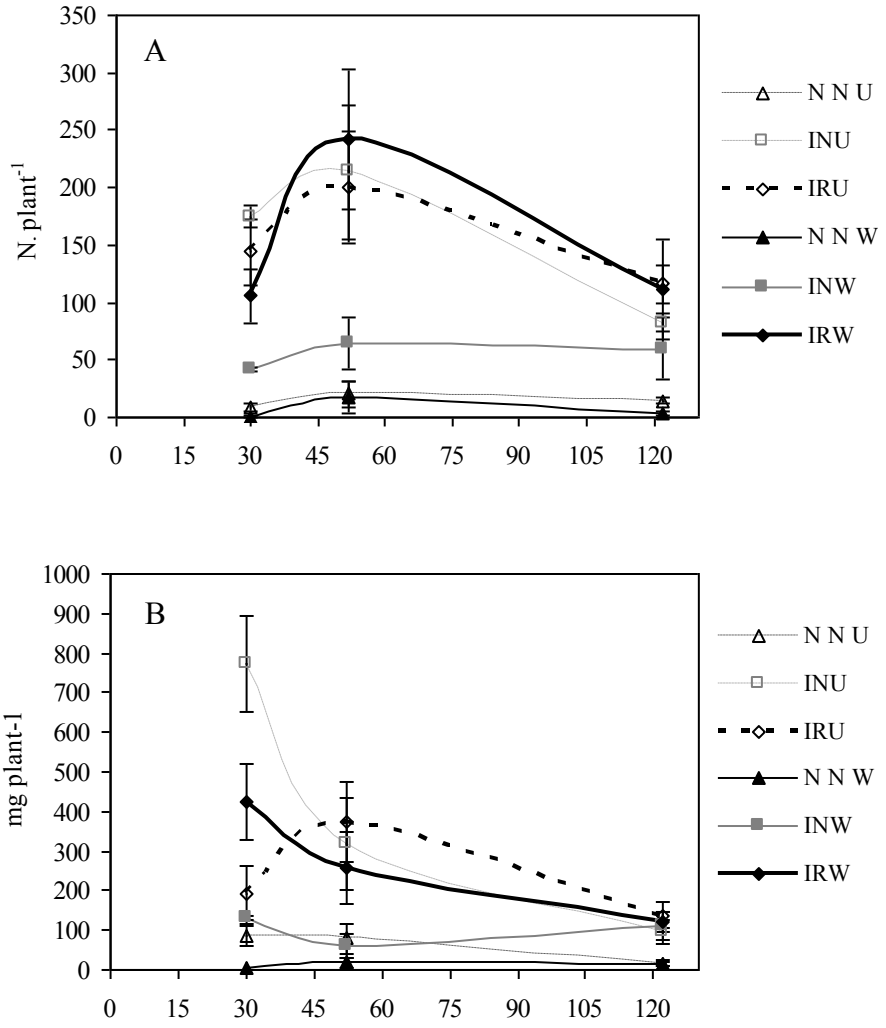


Figure 16. Trend of number (A) and DM (B)of nodules per plant throughout the growing cycle in response to nutrient solution, growth media and inoculation (Mean±St.Err).

Gas exchange

Data on net photosynthesis (NP), stomatal conductance (g_s), transpiration rate (Tr), CO_2 concentration (C_i) and leaf temperature (T_{leaf}) are shown in Tab. 34a,b. Net photosynthesis was always included in the values considered optimal for soybean (Salisbury and Ross, 1992) and ranged from 16.8 μmol CO_2 m^{-2} s^{-1} (33 DAS) to 13.8 μmol CO_2 m^{-2} s^{-1} (66 DAS) on the average among the treatments; showing the higher values in plants fed nitrate compared to urea.

Stomatal conductance and transpiration rate followed a similar trend during plant growth; IN showed lower values in the average of all measurement dates, compared to the other treatments. CO_2 concentration also was lower for IN on the average, indicating a probable difficulty for inoculated plants grown in NFT, where roots are submerged during the whole cultivation cycle.

Table 34a. Net photosynthetic rate (*NP*), stomatal conductance (g_s) and transpiration rate (Tr), in response to nutrient solution, growth media and inoculation (Mean values; ns = not significant; * = significant at P≤0.05). ([1] lsd).

	NP (μmol CO_2 m^{-2} s^{-1})			g_s (mol m^{-2} s^{-1})			Tr (mmol m^{-2} s^{-1})		
	33 DAS	47 DAS	66 DAS	33 DAS	47 DAS	66 DAS	33	47	66
Nutrient solution									
U	16.2 b	14.0 b	12.3	0.4	0.5	0.2	4.8	5.2	3.5
W	17.4 a	15.3 a	15.4	0.4	0.5	0.4	5.3	6.4	5.6
Treatments									
NN	17.0	13.4 b	13.7	0.5	0.5	0.4	6.5	6.0	5.5
IN	17.1	16.1 a	12.6	0.3	0.5	0.2	4.1	5.4	2.7
IR	16.2	14.6 ab	15.2	0.4	0.5	0.4	4.7	6.0	5.5
Significance									
Nutrient solution	*	*	*	n.s.	n.s.	*	*	*	*
Treatments	n.s.	* (1.67[1])	n.s.	* (0.09)	n.s.	*	* (0.60)	n.s.	* (0.64)
Interaction	n.s.	n.s.	* (3.47)	* (0.12)	* (0.12)	* (0.11)	* (0.73)	* (1.02)	* (0.91)

Table 34b. intercellular CO_2 concentration (C_i) and leaf temperature (T_{leaf}) in response to nutrient solution, growth media and inoculation (Mean values; ns = not significant; * = significant at P≤0.05;). ([1] lsd).

	C_i (μmol m^{-2} s^{-1})			T_{leaf}(°C)		
	33 DAS	47 DAS	66 DAS	33 DAS	47 DAS	66 DAS
Nutrient solution						
U	292.0	310.0	259.4	24.5	23.5	24.7
W	293.4	312.5	283.7	24.5	23.4	24.2
Treatments						
NN	307.0	321.1	312.3	23.9	23.4	23.9
IN	280.8	309.4	231.5	25.2	23.2	25.1
IR	290.2	303.3	270.9	24.6	23.8	24.3
Significance						
Nutrient solution	n.s.	n.s.	*	n.s.	n.s.	*
Treatments	* (12.17[1])	* (16.47)	* (19.41)	* (0.51)	* (0.34)	* (0.41)
Interaction	* (17.98)	* (23.29)	* (27.46)	n.s.	* (0.52)	* (0.58)

Water consumption and efficiency indexes

Rate and trend of nutrient solution uptake during the growing cycle varied among the treatments: in plants fed nitrate and in plants grown in rockwool with urea (IRU) it increased rapidly during early growth as the total evaporating surface of foliage increased, reached the maximum on about 60 DAS, and then declined with age. In NNU and INU, however, the water consumption was almost constant during the entire growing cycle (3 litres m^{-2} on average) (fig. 17a).

Total water use throughout the growth ranged from 89 (INU) to 599 litres m^{-2} (IRW) (Fig. 17b). The lower values of nutrient solution uptake in NNU and INU were related to the reduced number and surface of foliage. These two treatments also showed a reduced difference between the EC before and after the correction (by adding deionized water and fresh nutrient solution), indicating a difficulty in uptaking nutrients during the growth (Tab.35), probably due to the reduced urea hydrolysis in the nutrient solution. However, IRU plants showed a slightly greater water use compared to the other urea treatments, cushioning the negative effects of nutrient unavailability. The water use calculated on a per area and per day basis was consistent with date reported above (Tab. 35) and were comparable to those obtained by Wheeler *et al.* in 2008.

Solution pH tended to rise in Wheeler treatments requiring acid for pH control (44.5 mmol H^+ m^{-2} d^{-1}, on average), while a reduced amount of acid was used in Urea treatments (7.6 mmol H^+ m^{-2} d^{-1}, on average) (Tab 36). The maximum acid use was recorded in plants IRW. pH tended to decrease in Urea treatments, so a greater amount of KOH was used to control it, compared to Wheeler solution (12.9 *vs* 3.1 mmol OH^- m^{-2} d^{-1}, on average) (Tab 36). Moreover, plants grown in rockwool had a trend of greater acid requirement, in both Wheeler and Urea solution, and a higher base requirement in plants fed urea, compared to plant grown in NFT.

These results are consistent with previous experimental evidence, indicating a preferential uptake of anions over cations from a nitrate-based nutrient solution throughout the growing cycle (Willumsen, 1980), while for plants assimilating NH_4^+ and urea, in the absence of the readily permeable NO_3 anion, inorganic cation uptake exceeds inorganic anion uptake substantially, with a resulting acidification of the rooting medium (Lahav *et al.*, 1976; Breteler, 1973; Houba *et al.*, 1971; Kirkby and Mengel, 1967).

Data on biomass efficiency indexes are shown in Table 37. Plants grown in NFT fed urea had lowest values of Water use Efficiency (WUE) (0.33 g of seed per litre of nutrient solution, on average); the index was a little higher for U plants grown in rockwool and was the highest in W plants (Tab. 37). Thus, apart from NNU and INU plants, the results compared favourably to those obtained in previous experiments on hydroponically-grown soybean (Wheeler *et al.* 2003 and 2008).

In experiments on hydroponically-grown soybean, Wheeler *et al.* (2008) reported a value of Radiation Use efficiency (RUE) of 0.19 g seeds per mole of PAR and Dougher and Bugbee (1997) a RUE of 0.25-0.28 g seeds per mole of PAR. U plants showed lower RUE, particularly in NNU and INU (0.02 g seeds mol^{-1} PAR, on average). Plants fed nitrate recorded a higher RUE, particularly in IR (Tab. 37). Acid Use Efficiency (AUE) was very low in NNU and INU plants (0.12 g seeds $mmol^{-1}$ H^+ on average) but was the highest in IRU plants; nutrient solution containing nitrate showed an average value of 0.35 g seeds $mmol^{-1}$ H^+, comparable to that obtained by Wheeler *et al.* in 2003. NNU and INU also had low Base Use Efficiency (BUE), indicating a reduced uptake of NH^4-N (Tab. 37).

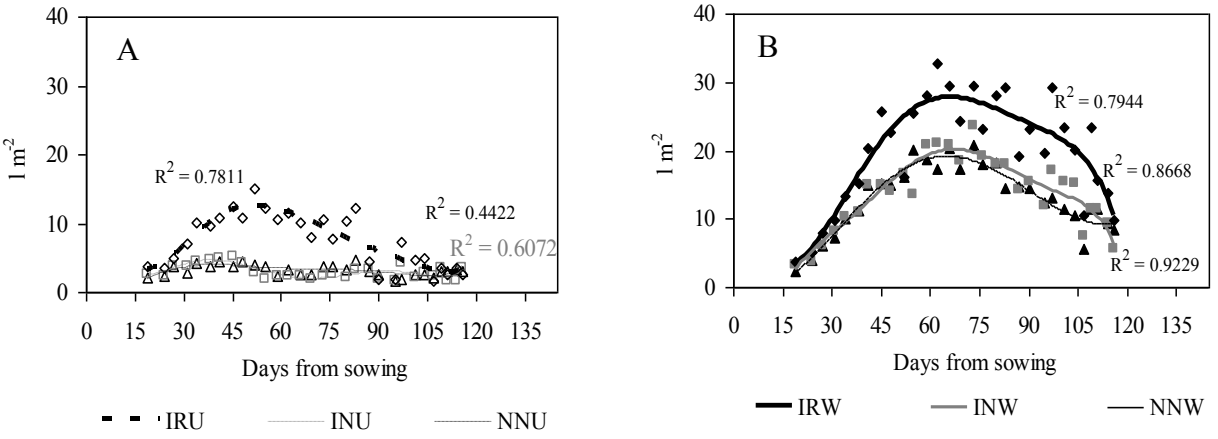
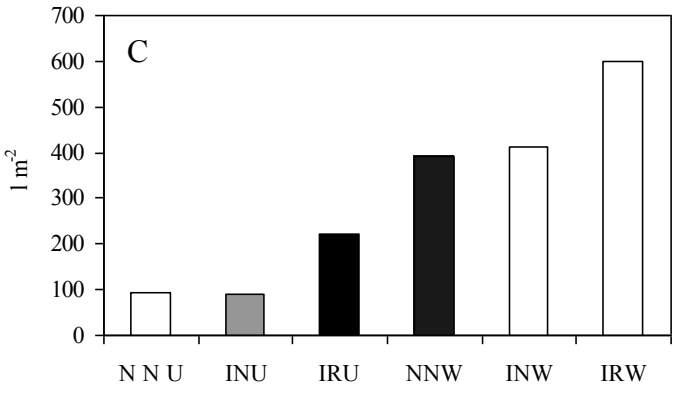


Figure 17. Trend of water use throughout plant growth in Urea (A) and Wheeler (B) solutions and cumulative water use at the end of the experiment (C) for the different treatments.

Table 35. Average fluctuations of nutrient solution EC (with their coefficients of variation) and plant water use on a per area and per day basis for the different treatments.

	EC before adjustment (dS m^{-1})	CV (%)	EC after adjustment (dS m^{-1})	CV (%)	Water consumption (l m^{-2} d^{-1})
NNU	1.73 ± 0.04	18.2	1.72 ± 0.06	17.4	2.6
INU	1.67 ± 0.04	23.0	1.56 ± 0.07	24.7	2.5
IRU	1.73 ± 0.04	19.5	1.45 ± 0.04	16.0	6.3
NNW	1.28 ± 0.02	12.0	1.15 ± 0.03	14.4	10.9



INW	1.34 ± 0.02	12.8	1.16 ± 0.03	14.3	11.4
IRW	1.42 ± 0.04	22.3	1.20 ± 0.04	18.0	16.5

Table 36. Average fluctuations of nutrient solution EC (with their coefficients of variation) and plant acid and base use on a per area and per day basis for the different treatments.

	pH before correction	CV (%)	pH after correction	CV (%)	Acid (mmol H^+ m^{-2} d^{-1})	Base (mmol OH^- m^{-2} d^{-1})
NNU	5.76±0.05	7.3	6.06±0.02	2.2	6.8	12.7
INU	5.90±0.05	7.1	6.04±0.03	3.9	7.4	7.4
IRU	5.75±0.05	8.4	6.07±0.02	3.0	8.5	18.6
NNW	6.42±0.06	8.9	5.92±0.02	3.4	38.7	4.3
INW	6.53±0.05	7.4	5.95±0.03	4.2	40.3	2.7
IRW	6.87±0.05	7.1	5.93±0.03	4.1	54.4	2.3

Table 37. Biomass efficiency indexes for the different treatments

	Growth cycle (days)	WUE seeds (g l^{-1})	RUE seeds (g mol^{-1})	AUE seeds (g $mmol^{-1}$)	BUE seeds (g $mmol^{-1}$)
NNU	122	0.33	0.02	0.13	0.07
INU	122	0.33	0.02	0.10	0.10
IRU	122	0.88	0.12	0.63	0.29
NNW	122	1.18	0.29	0.33	2.91
INW	122	1.12	0.29	0.31	4.64
IRW	122	1.29	0.51	0.40	9.59

Conclusions

The realization of CELSS based on higher plants is a complex objective as it involves the development of new technologies and the understanding of the effects of space factors both on the behaviour of biological systems and on the functioning of physical principles. (De Micco *et al*., 2009).

Apart from challenges in engineering research and medical aspects to face the constraints to the long permanence of the human body under space conditions, there are also many technical problems regarding the supplying of resources.

To bring down the materials to be brought in space for plant cultivation, a reduction in the amount of fertilizer or growth media can be supposed.

In our experiment, results from chemical and proximate analyses on seeds showed a higher mineral content compared to reference values, which could have been a result of the luxuriant nutrient uptake by the hydroponically grown plants. Methods for controlling excessive nutrient uptake by the plant might be explored. This could include selection of low nutrient accumulating cultivars and/or the use of less nutrient-rich solutions for growing plants, that would allow the reduction of fertilizers to be brought in space for plant cultivation.

The use of urea as nitrogen source for plant growth could be a good way to recycle human waste (urine) in a CELLS, but our experiment confirmed that is not able to provide a sufficient amount of nitrogen at least in the early stages of plant growth. This early disadvantage was not recovered by plant in the following phases of lifecycle, consequently, water use was very low and both growth and yield were reduced in plants fed urea as sole source of nitrogen; HI also was lower, indicating a higher production of inedible tissues (waste) in relation to the edible ones; however, the quality of seeds was enhanced thanks to the concentration of nitrogen in a reduced amount of seeds.

The inoculation with *Rhizobium* did not improve plant performances when fed urea, because symbiotic N fixation starts after nodule formation and is much slower in plant early stage (Brun, 1978), so plants were not provided with nitrogen at seedling stages (due to the reduced urea hydrolysis in nutrient solution); the positive effect of urea on nodulation was not sufficient to obtain good results in terms of plant yield. Failed bacteria positive effects on plants seemed to be related to the growth in NFT, (most of roots are submerged during the whole cultivation cycle), that in plants fed Wheeler solution are gathered to the nitrate inhibitory effect on bacteria.

Growth in substrate enhances the dry matter accumulation in plants fed urea and seemed to reduce the inhibitory effect of nitrate on nodulation in plants supplied with Wheeler solution.

Moreover, the growth in rockwool increased the HI compared to the growth in NFT and improved the plant efficiency in both Urea and Wheeler solution.

Considering the results obtained in this experiment, further research could be performed using a nutrient solution containing both urea and nitrate as sources of nitrogen, at least in early plant growth; moreover, the inoculation with *Rhizobium* could be involved, as during early developmental stages plants could use nitrate from the nutrient solution while bacteria are becoming active on root surface; urea could limit the inhibiting effect of nitrate on nodules. In following plant growth stages, when plants are well nodulated and nitrogen fixation has begun, nitrate supplying could be interrupted and urea could be used as sole nitrogen source.

Furthermore, as solid substrate seemed to have some positive effects on plant performances, even when nitrogen availability is limited (urea), further testing could be performer in order to evaluate the effect of other solid substrates, even having a reduced weight or being recyclables, and the use of enzymes (ureases) in the nutrient solution could be tested, in order to improve the efficiency of urea in plant nutrition.

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REFERENCES

Aldrich S.R., 1980. Protein, Nitrates, and Nitrites in Foods and Feeds. Nitrogen in Relation to Food, Environment, and Energy. Agricultural Experiment Station: 97-l10. University of Illinois, Urbana, IL.

AOAC, 1990. Official methods of analysis (14th edition). Association of Official Analytical Chemists, Washington DC, USA.

Baldoni R. and Giardini L., 1989. Coltivazioni erbacee. Patron Editore, Bologna.

Bethlenfalvay G.J. and Phillips D.A., 1977. Ontogenetic interactions between photosynthesis and symbiotic nitrogen fixation in legumes. Plant Physiol. 60: 419-421.

Bethlenfalvay G.J., Abu-shakra S.S., Phillips D.A., 1978. Interdependence of nitrogen nutrition and photosynthesis in *Pisum sativum* L. Plant Physiol. 62: 127-130..

Beuerlein, J. (2004) Soybean inoculation; its science, use, and performance. http:// agcrops.osu.edu/soybean/documents/ SoybeanInoculation.pdf.

Bewley, J.D. 1997. Seed germination and dormancy. The Plant Cell. 9: 1055-1066.

Beyer E.M., Sundin O.P., 1978. IJC,H, metabolism in morning glory flowers, Plant Physiol. 61: 896- 899.

Breteler H., 1973. A comparison between ammonium and nitrate nutrition of young sugar beet plants grown in nutrient solutions of constant acidity. Production of dry matter, ionic balance and chemical composition. Neth J Agric Sci 21: 227-244

Britz S.J., Sager J.C., 1990. Photomorphogenesis and photoassimilation in soybean and sorghum grown under broad spectrum or blue-deficient light sources. Plant Physiol., 94: 448.

Brun W.A., 1978. Assimilation. In: Norman A.G. (ed.): Soybean Physiology, Agronomy, and Utilization. Pp 45-76. Academic Press, New York, San Francisco, London.

Bugbee B.G., Salisbury F.B., 1985. An evaluation of MES (2(N-Morpholino)ethanesulfonic acid) and amberlite IRC-50 as pH buffers for nutrient solution studies. J.of Plant Nutr. 8 (79): 567-583.

Chen P.C. and Phillips D.A., 1977. Induction of root nodule senescence by combined nitrogen in *Pisum sativum* L. Plant Physiol. 59: 440-442.

De Micco V., Aronne G.; Colla G.; Fortezza R.; De Pascale S. , 2009. Agro-biology for bioregenerative Life Support Systems in long-term Space missions: General constraints and the Italian efforts. Journal of Plant Interactions, 4 (4): 241-252.

Dean J.R. and Clark K.W., 1980. Effect of low level nitrogen fertilization on nodulation, acetylene reduction and dry matter in fababeans and three other legumes. Can J. Plant Sci. 60: 121-130.

Dougher T.A.O. and Bugbee B., 1997. Effect of lamp type and temperature on development, carbon partitioning and yield of soybean. Adv. Space Res. 20 (10): 1895-1899.

Downs R.J., Thomas J.F., 1990. Morphology and reproductive development of soybean under artificial conditions. Biotronics, 19: 19.

Evans G.C., 1972. The quantitative analysis of plant growth. University of California Press, 734 pp.

Ewing M.A and Robson A.D., 1991. The use of MES buffer in early nodulation studies with annual Medicago Species. Plant and Soil 131: 199-206.

Fernandez-Orozco R., Frias J, Zielinski H., Piskula M.K., Kozlowska H., Vidal-Valverde C. 2008. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. emmerald, *Glycine max* cv. jutro and *Glycine max* cv. merit. Food Chem. 111: 622–630.

Gabelman W.H., Gerloft G.C., Schettini T., Coltman R., 1986. Genetic variability in root systems associated with nutrient acquisition and use. Hort. Sci. 21 (4): 971-973.

Garland J.L., Mackowiak C.L., Strayer R.F., Finger B.W., 1997. Integration of waste processing and biomass production systems as part of the ksc breadboard project. .Adv. Space *Res.* 20 (10): 1821-1826.

Gazenko O.G. and Shepelev Ye.Ya., 1972- Expansion of the ideas of K.E. Tsiolkovsky about the biological method of spacecraft habitability sustenance, in Proc., 6th readings devoted to advancement of the scientific heritage and expansion of ideas of K.E. Tsiolkovsky, pp. 3-6, Kaluga. (Russian)

Gerendas J., Sattelmacher B., 1997. Significance of N source (urea *vs* NH_4NO_3) and N supply for growth, urease activity and nitrogen metabolism of zucchini (*Cucurbita pepo* convar. giromontiina). Plant and Soil 196 (1): 217-222.

Gitelson I.I., Kovrov B.G., Lisovsky G.M., 1975. Problemy kosmicheskoi biologii (Problems of space biology) 28 Nauka, Moscow, Russia, p. 312.

Gitelson I.I., Lisovsky G.M., MacElroy R.D., 2003. Manmade Closed Ecological Systems. Taylor & Francis, London – NY, 402 pp.

Grigoriev A.I. and Gazenko O.G., 1998. Main areas and results of research at the Institute of Biomedical problems in 1961-1998, Aviakosmicheskaya i Ekologicheskaya Meditsina, 37 (5):4- 17. (Russian)

Gros J.B., Poughon L., Lasseur Ch., 2003. Recycling efficiencies of C, H, O, N, S, and P elements in a biological life support system based on micro-organisms and higher plants. Adv. Space Res. 31, 195–199.

Grusak M.A. and Pezeshgi S., 1994. Uniformly ^{15}N-labeled soybean seeds produced for use in human and animal nutrition studies: description of a recirculating hydroponic system and whole plant nutrient and environmental requirements. J. Sci. Food Agric. 64: 223-230.

Gulden R.H. and Vessey J. K., 1997. The stimulating effect of ammonium on nodulation in *Pisum sativum* L. is not long lived once ammonium supply is discontinued. Plant and Soil 195: 195–205.

Gunes A., Post W.N.K., Kirkby E.A., Aktas M., 1994. Influence of partial replacement of nitrate by amino acid nitrogen or urea in the nutrient medium on nitrate accumulation in NFT grown winter lettuce. Plant. Nutr. 17 (11): 1929-1938.

Guo R, Silsbury J.H., Graham R.D., 1992. Effect of four nitrogen compounds on nodulation and nitrogen fixation in faba bean, while lupin and medic plants. Aust. J. Plant Physiol. 19, 501-508.

Hach, 1997. HACH DR 2000 Spectrophotometer Handbook. Hach Company, Loveland, Colorado (USA).

Ham G. E., Liener I. E., Evans S. D., Frazier R. D., Nelson W. W., 1975. Yield and Composition of Soybean Seed as Affected by N and S Fertilization. Agron. J. 67: 293-297.

Haytowitz D.B. and Matthews R.H., 1986. Composition of foods. USDA agriculture Handbook No.8-16, United States Department of Agricolture, Washington DC, USA.

Hoagland D. R. and Arnon D. I., 1950. The water-culture method for growing plants without soil. College of Agriculture, Univ. of California, Berkeley, California, USA, Circular 347, 32 pp.

Hoff J.E., Howe J.M., Mitchell C.A., 1982. Nutritional and cultural aspects of plant species selection for a regenerative life support system. NASA Contract Report 166324, Moffet Field, California.

Houba V.F.G., Van Egmond F., Witrich E.M., 1971. Changes in production or organic nitrogen and carboxylates (C-A) in young sugar-beet plants grown in nutrient solutions of different nitrogen composition. Neth. J. Agric. Sci. 19: 39-47.

Ikeda H., Osawa T., 1984. Lettuce growth as influenced by N source and temperature of the nutrient solution. In: Proceedings of the Sixth International Congress On Soilless Culture, pp. 273-284.

Ikeda H., Tan X.W., 1998. Urea as an organic nitrose source for hysroponically grown tomatoes in comparison to inorganic nitrogen sources. Jpn. Soil Sci Plant Nutr. 44: 609-615.

Imsande J., 1986. Ineffective utilization of nitrate by soybean during pod fill Physiol. Plantarum 68: 689-694

Imsande J., 1988. Enhanced nitrogen fixation increases net photosynthetic output and seed yield of hydroponically grown soybean. J. Exp. Bot. 39: 1313-1321.

International Rules for Seed Testing, 1999 - ISTA International Seed Testing Association.

Jones W.L. and Ingelfinger A.L., 1973. Atmosphere control. In: J.F. Parker and V.R. West (eds.) Bioastronautics Data Book. 2nd edition. NASA SP-3006, Washington, DC.

Johnstone M., Chatterton S., Sutton J.C., Grodzinski B., (2005) Net carbon gain and growth of bell peppers, *Capsicum annuum* 'Cubico', following root infection by *Pythium aphanidermatum*. Phytopath. 95: 354-361

Jurgonski L.J., Smart D.J., Bugbee B., Nielsen S.S., 1997. controlled environments alter nutrient content of soybeans. Adv. Space Res. 20 (10): 1979-1988.

Khan N.K., Watanabe M., Watanabe Y., 1997. Effect of different concentrations of urea with or without nickel on spinach (*Spinacia oleracea* L.) under hydroponic culture. In: Ando T., Fujita K., Matsumoto H., Mori S., Sekiya J. (Eds.), Plant Nutrition for Sustainable Food Production and Environment. Kluwer Academic Publishers, Tokyo, pp. 85-86.

Kirkby E.A and Mengel K., 1967. Ionic balance in different tissues of the tomato plant in relation to nitrate, urea, or ammonium nutrition. Plant Physiol. 42 (1): 6-14.

Kohl D. H., Shearer G., Harper J. E., 1980. Estimates of N_2 fixation based on differences in the natural abundance of ^{15}N in nodulating and nonnodulating isolines of soybeans. Plant Physiol. 66: 61-65

Kosola K.R., Bloom A.J., 1996. Chlorate as a transport analog for nitrate absorption by roots of tomato. Plant Physiol. 110 (4): 1293-1299.

Krall A.R. and Kok B., 1960. Studies on algal gas exchangers with reference to space flight. Dev. Industrial Microbiol. 1: 33-44.

Lahav E., Harper J.E., Hagerman R.H., 1976. Improved soybean growth in urea with pH buffered by a carboxy resin Crop Sc. 16: 325-328

LaRue T.A.G. and Kurz W.G.W., 1973. Estimation of nitrogenase in intact legumes. Can. J. Microbiol. 19: 304- 305.

Lea-Cox J:D:, Stutte G:W:, Berry W.L., Wheeler R.M., 1999. Nutrient dynamics and pH/charge balance relationships in hydroponic solutions. Acta Hort. 481: 241-249

Levitt J., 1980. Responses of plants to environmental stresses. Academic press, New York, 497pp

Liu L., Shelp B.J., 1993. Nitrogen partitioning in greenhouse-grown broccoli in response to varying NH_4^+:NO_3^- ratios. Commun. Soil Sci. Plant Anal. 24 (1): 45-60.

Luo L., Lian Z.H., Yan X.L., 1993. Urea transformation and the adaptability of three leafy vegetables to urea as a source of nitrogen in hydroponic culture. J. Plant Nutr. 16 (3): 797-812.

Mackowiak C.L., Garland J.L., Sager J.C., 1996. Recycling crop residues for use in recirculating hydroponic crop production. Acta Hort. 440: 19-24.

Mackowiak C.L., Stutte G.W., Wheeler R.M., Ruffe L.M., Yorio N.C., 1999. Tomato and soybean production on a shared recirculating hydroponic system. Proc. Int. Sym. Growing Media and Hydroponics. ED. A.P. Papadopoulos. Acta Hort. 481, ISHS: 259-266.

Marschner H., 1995. Mineral nutrition of higher plants, Academic Press, New York, 889 pp.

McElhannon W.S., Mills H.A., 1978. Influence of percent NO_3/NH_4^+ on growth, N absorption, and assimilation by lima beans in solution culture. Agron. J. 70 (4): 1027-1032.

McKeehen J.D., Mitchell C.A., Wheeler R.M., Bugbee B., Nielsen S.S., 1996. Excess nutrients in hydroponic solutions alter nutrient content of rice, wheat, and potato, Adv. Space Res. 18, (4/5): 73-83.

McKeehen J.D., Smart D.J., Mackowiak C.L., Wheeler R.M., Nielsen S.S., 1996. Effect of CO_2 levels on nutrient content of lettuce and radish, Adv. Space Res. 18 (4/5): 85-92.

Mills H.A., McElhannon W.S., 1982. Nitrogen uptake by sweet corn. Hort. Sci. 17 (5): 743-744.

Monje O., Garland J., Stutte G.W., 2000. Factors controlling oxygen delivery in ALS hydroponic systems. SAE Technical Paper 2001-01-2425.

Muhlestein D.J., Hooten T.M., Koenig R., Grossl P, Bugbee B, 1999. Is nitrate necessary to biological life support? Society of Automotive Engineers, Inc.

Myers J., 1954. Basic remarks on the use of plants as biological gas exchangers in a closed system. J. Aviation Med. 25: 407-411.

Nelson B., 1987. The role of plant pathology in development of Controlled Ecological Life Support Systems. Plant Disease 71: 580-584.

Pausch R.C., Britz S.J., Mulchi C.L., 1991. Growth and photosyntesis of soybean in simulated vegetation shade: influence of the ratio of red to far red radiation. Plant, Cell and Env. 14: 647.

Prince R.P. and W.M. Knott, 1989. CELSS Breadboard Project at the Kennedy Space Center, In: D.W. Ming and D.L. Henninger (eds.), Lunar Base Agriculture, Amer. Sot. of Agronomy, Inc. Madison, WI, USA.

Putnam D.F., 1971. Composition and concentrative properties of human urine. NASA CR- 1802.

Quebedeaux B, Havelka U D, Livak K L, Hardy R.W.F., 1975. Effect of altered pO_2 in the aerial part of soybean on symbiotic N_2 fixation. Plant Physiol. 56: 761-764.

Baldoni R. and Giardini L., 1989. Coltivazioni erbacee. Patron Editore, Bologna.

Ralston E.J. and Imsande J., 1982. Entry of oxygen and nitrogen into intact soybean nodules. *J. Exp. Bot.* 33: 208-14.

Raven J.A,. 1985. Regulation of pH and generation of osmolarity in vascular plants: a cost-benefit analysis in relation to efficiency of use of energy, nitrogen, and water. New Phytol. 101: 25-77.

Rennie R. J., 1982. Quantifying dinitrogen (N_2) fixation in soybeans by ^{15}N isotope dilution: the question of the nonfixing control plant. Can. J. Bot. 60: 856-861.

Ryle G.J.A., Amott R.A., Powell C. E., Gordon, A. J., 1984. N_2 fixation and the respiratory costs of nodules, nitrogenase activity, and nodule growth and maintenance in Fiskeby soyabean. J. Exp. Bot. 35: 1156-1165.

Rys G.J. and Phung T., 1984 Effect of nitrogen form and counterion on establishment of the *Rhizobium trifolii–Trifolium repens* symbiosis. J. Exp. Bot. 35: 1811-1819.

Sabatier P. and Senderens J.B., 1902. Comptes Rendus Acad. Sci. 134: 689.

Salisbury F.B., Dempster W.F., Allen J.P., 2002. Light, plants, and power for life support on Mars. Life Support Biosph. Sci. 8: 161–172.

Salisbury F.B. and and Ross C.W., 1992. Fisiologia vegetale. Zanichelli Editore, Bologna. 790 pp.

Schaafsma G., 2000. The protein digestibility-corrected amino acid score. J. Nutr. 130 (suppl): 1865S-7S.

Schuerger A.C., 1998. Microbial contamination of advanced life support (ALS) systems poses a moderate threat to the long-term stability of space-based bioregenerative systems. Life Support and Biosphere Sci. 5: 325-337.

Shelp B.J., 1987. Plant characteristics and nutrient composition and mobility of broccoli (*Brassica oleracea* var. italica) supplied with NH_4^+, NO_3^- or NH_4NO_3. J. Exp. Bot. 38 (195): 1603-1618.

Silsbury J.H., Catchpoole D.W., WallaceW., 1986 Effects of nitrate and ammonium on nitrogenase (C_2H_2 reduction) activity of swards of subterranean clover, *Trifolium subterraneum* L. Aust. J. Plant Physiol. 13: 257–273.

Somasegaran P., Hoben H.J., 1994. Handbook for Rhizobia. Springer – Verlag, New York, 366-369.

Strayer R.F., 1994. Dynamics of microorganism populations in recirculating nutrient solutions. Adv. Space Res. 14: 357-366.

Strayer R.F., Finger B.W., Alzaraki M.K., 1996. Evaluation of an anaerobic digestion system for processing CELLS crop residues for resource recovery. Adv. Space Res. 19.

Steinberg S.L., Ming D.W., Hederson K.E., Carrier C., Gruener J.E., Barta D.J., Henninger D.L., 2000. Wheat response to differences in water and nutritional status between zeoponic and hydroponic growth systems. Agron. J., 92: 353-360

Streeter J.G., 1985. Nitrate inhibition of legume nodule growth and activity. II. Short term studies with high nitrate supply. Ibid. 77: 325-8.Streeter J., 1988. Inhibition of legume nodule formation and N2 fixation by nitrate. Crit. Rev. Plant Sci. 7: 1-23.

Svenning M.M., Junttila O., Macduff J.H., 1996. Differential rates of inhibition of N_2 fixation by sustained low concentrations of NH_4^+ and NO_3 in northern ecotypes of white clover (*Trifolium repens* L). J. Exp. Bot. 47. 729-738.

Sychev V.N.,. Levinskikh M.A, Shepelev Ye.Ya., 2003. The biological component of the life support system for a martian expedition. Adv. Space Res. 31 (7): 1693-1698.

Taiz L., Zeiger E., 2006. Plant physiology, fourth edition. Sinauer associates, Sunderland (Mass.). 690pp.

Tibbitts T. W. and Alford D. K., 1980. Controlled ecological life support system use of higher plants. NASA Conference Pub.2231, Moffett Field, CA.

Tibbitts T.W., Morgan D.C., Warrington I.J., 1983. Growth of lettuce, spinach, mustard and wheat plants under four combination of high pressure sodium, metal halide and tungsten halogen lamps at equal PPFD. J. Amer. Soc. Hort. Sci. 108: 622.

Vavrina C.S. and Obreza T.A., 1993. Response of Chinese cabbage to nitrose rate and source in sequential plantings. Hort. Sci. 28 (12): 1164-1165.

Vessey J.K., Raper C.D.Jr., Tolley-Henry L., 1991. Cyclic variations in nitrogen uptake rate in soybean plants: uptake during reproductive growth. J. Exp. Bot. 41: 1579-1584.

Vigue J.T., Harper J.E., Hageman R.H., Peters D.B., 1977. Nodulation of soybeans grown hydroponically on urea. Crop Sci. 17: 169-172.

Vincent J. M., 1970. A manual for the practical study of the root-nodule bacteria. International biological programme 7 marylebone road, London NW1. Blackwell scientific publications oxford and Edinburgh. Printed in Britain by Bugess and son (Abingdon) ltd, Berkshire, and bound by the kemp hall binder.

Waterer J.G and Vessey J.K 1993b. Nodulation response of autoregulated or NH_4^+-inhibited pea (*Pisum sativum*) after transfer to stimulatory (low) concentrations of NH_4^+. Physiol. Plant. 88: 460–466.

Waterer J.G. and Vessey J.K., 1993a. Effect of low static nitrate concentrations on mineral nitrogen uptake, nodulation, and nitrogen fixation in field pea. J. Plant Nutr. 16: 1775–1789.

Waterer J.G., Vessey J.K., Raper C.D. Jr., 1992. Stimulation of nodulation in field peas (*Pisum sativum*) by low concentrations of ammonium in hydroponic culture. Physiol. Plant. 86: 215–220.

Wells R., Burton J.W., Kilen T.C., 1993. Soybean growth and light interception: response to differing leaf and stem morphology. Crop Sci. 33: 520.

Wells R., Burton J.W., Kilen T.C., 1993. Soybean growth and light interception: response to differing leaf and stem morphology. Crop Sci. 33: 520.

Wheeler R.M., Mackowiak C.L., Sager J.C., 1990. Proximate composition of seed and biomass from soybean plants grown at different carbon dioxide (CO_2) concentrations. NASA Tech. Memo. 103496Wheeler R.M., 1996. Gas balance in a plant-based CELSS. In: Suge, H. (Ed.), Plants in Space Biology: 207–216.

Wheeler R.M., Mackowiak C.L., Siegriest L.M., Sager J.C., 1993. Supraoptimal carbon dioxide effects on growth of soybean. J. Plant Physiol. 142: 173-178.

Wheeler R.M., Mackowiak C.L., Sager J.C., Knott W.M. and Berry W.L., 1994. Proximate nutritional composition of CELSS crops grown at different CO_2 partial pressures. Adv. Space Res. 14 (1l): 171-176.

Wheeler R.M., Mackowiak C.L., Sager J.C., 1991. Soybean stem growth under high pressure sodium with supplemental blue lighting. Agron. J. 83: 903-906.

Wheeler R.M., Mackowiak C.L., Stutte G.S., Sager J.C., Yorio N.C., Ruffe L.M., Fortson R.E., Dreschel W.M., Knott W.M., Corey K.A., 1996. NASA's biomass production chamber: a testbed for bioregenerative life support studies. Adv. Space Res. 18 (4/5): 215:224.

Wheeler R.M., Mackowiak C.L., Sager J.C., Knott W.M., Berry W.L., 1996b. Proximate composition of CELLS crops grown in NASA'a biomass production chamber. Adv. Space Res. 18 (4/5), 43-47.

Wheeler R.M., Sager J.C., Berry W.L., Mackowiak C.L., Stutte G.W., Yorio N.C., Ruffe L.M., 1999. Nutrient, acid and water budgets of hydroponically grown crops. Acta Hort. (ISHS) 481: 655-662.

Wheeler R.M., Mackowiak C.L., Stutte G.S., Yorio N.C., Ruffe L.M., Sager J.C., Prince R.P., Peterson B.V., Goins G.D., Berry W.L., Hinkle C.R., Knott W.M., 2003. Crop production for advanced life support systems. Observations from the Kennedy Space Center Breadboard Project. NASA Tech. Mem. 211184, 58.

Wheeler R.M., Corey K.A., Volk G.M., Mackowiak C.L., Yorio N.C., Sager J.C., 2004. Soybean canopy gas exchange rates: effects of lighting. Eco-Engineering 16 (3): 209-214.

Wheeler R.M., Mackowiak C.L., Stutte G.S., Yorio N.C., Ruffe L.M., Sager J.C., Prince R.P., Knott W.M., 2008. Crop productivities and radiation use efficiencies for bioregenerative life support. Adv. Space Res. 41: 706-713.

Wiersma J. V. and Bailey T. B., 1957. Estimation of leaflet, trifoliate and total leaf areas of soybean. Agron. J. 67: 26-30.

Willumsen J., 1980. pH of the flowing nutrient solution. Acta Hort. 98: 191-199.

Wydeven T. and Golub M.A., 1990. Generation rates and chemical composition of waste streams in a typical crewed space habitat. NASA Tech. Mem. 102799.

Wydeven T.W. and Golub M.A., 1990. Generation rates and chemical compositions of waste streams in a typical crewed space habitat. NASA TM-102799.

Yokota S., Ojima K., 1995. Physiological response of root tip of alfalfa to low pH and aluminium stress in water culture. Plant and Soil 171 (1): 163-165.

Zhu Z.J., Gerendas J., Sattelmacher B., 1997. Effects of replacing of nitrogen with urea or chloride on the growth and nitrate accumulation in pak-choi in the hydroponics. In: Ando T., Fujita K., Matsumoto H., Mori S., Sekiya J. (Eds.), Plant Nutrition for Sustainable Food Production and Environment. Kluwer Academic Publishers, Tokyo, pp 963-964.

Zhu X.G., Long S.P., Ort D.R., 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Current Opinion in Biotechnology 19: 153-159.

http://eur-lex.europa.eu

www.esa.int

www.fao.org

www.omafra.gov.on.ca