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PLANT GROWTH IN MICROGRAVITY FOR BLSS: GENERAL ISSUES AND THE ITALIAN CONTRIBUTION

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ABSTRACT

Plants are among the key organisms in Bioregenerative Life Support Systems (BLSS) in Space because they have a role in the regeneration of resources and in the psychological support of the crew. The design of an efficient BLSS cannot be irrespective of the profound knowledge of the functioning of the vegetal systems under the effect of Space factors. From an evolutionary perspective, reduced gravity can be considered one of the factors driving the evolution of plants in Space.

In this paper, we outline the need for plant-based BLSS to sustain exploratory-class manned missions in Space. After some evolutionary considerations about future plant development in Space, we also report a synthesis of the results of case studies performed by Italian research groups aiming to understand the effects of simulated or real microgravity on various aspects of plant growth and reproduction. We conclude by emphasizing how plant research in Space should address both the improvement of the knowledge of basic biological processes and the development of new agro-technologies. Efforts to engage in a multidisciplinary approach to understand the effects of Space factors on plant growth are needed considering that such factors affect the *biological systems contemporarily at molecular, biochemical, morphostructural and physiological levels.*

Keywords: *agrotechnology, altered gravity, plant anatomy, plant evolution, plant space biology, species selection*

RAST RASTLIN V MIKROGRAVITACIJSKEM OBMOČJU ZA BLSS: SPLOŠNA VPRAŠANJA IN PRISPEVEK ITALIJANSKIH RAZISKOVALCEV

IZVLEČEK

Rastline spadajo med ključne organizme v sistemih za bioregenerativno ohranjanje življenjskih funkcij (BLSS) v vesolju, saj skrbijo za regeneracijo virov in za psihološko podporo posadki. Oblikovanje učinkovitega sistema BLSS ne sme spregledati natančnega znanja o delovanju vegetativnih sistemov pod vplivom dejavnikov v vesolju. Iz evolucijske perspektive se zmanjšana gravitacija lahko upošteva kot eden izmed dejavnikov, ki vodijo evolucijo rastlin v vesolju.

V tem prispevku predstavljamo potrebo po sistemu BLSS, ki bi temeljil na rastlinah, da bi ohranili raziskovalne misije v vesolju. Po nekaterih evolucijskih obravnavah prihodnjega razvoja rastlin v vesolju prav tako pričamo o sintezi rezultatov študij primerov, ki so jih izvedle italijanske raziskovalne skupine, ki so želele razumeti učinke simulirane ali resnične mikrogravitacije na različne vidike rasti in razmnoževanja rastlin. Naš prispevek zaključimo s poudarkom na tem, kako bi raziskovanje rastlin v vesolju moralo upoštevati tako izboljšanje znanja o osnovnih bioloških procesih kot razvoj novih agrotehnologij. Potrebno bo vložiti veliko truda v multidisciplinarni pristop k razumevanju učinkov vesoljskih dejavnikov na rast rastlin, saj je potrebno upoštevati, da ti dejavniki vplivajo na biološke sisteme že na molekularni, biokemični, morfološko-strukturalni in fiziološki ravni.

Ključne besede: *agrotehnologija, spremenjena gravitacija, anatomija rastlin, evolucija rastlin, vesoljska biologija rastlin, vrste, selekcija*

PLANT-BASED BIOREGENERATIVE LIFE SUPPORT SYSTEMS (BLSS)

Plants are key organisms on Earth since they provide us with oxygen and occupy the first trophic level. It is therefore natural to ask why plants should be unnecessary in any environment other than Earth. The use of plants to support life in Space is not a novelty, and many studies have been carried out in the second half of the twentieth century with extensive ground-based demonstrations throughout the world (e.g. Myers, 1954; Gitelson et al., 1989; Gitelson, 1992; Tako et al., 2007; Wheeler, 2010). Such widespread interest arises from the objectives of the international Space exploration programs which include manned long duration missions. The permanence of humans in Space for long periods of space travel, onboard orbital platforms or on Lunar and Martian stations depends upon the possibility of overcoming challenges in engineering and medical research as well as in plant space biology and agro-technology (De Micco, Aronne, Colla, Fortezza, & De Pascale, 2009). Indeed, the possibility of creating an Earth-like environment where resources can be regenerated has an impact not only on the economy of space exploration, because it minimizes the need for external supply of resources, but also on the well-being of astronauts in Space. It is well accepted that plants may play a key role for the regeneration of resources in BLSS because they accomplish four main functions: a) regeneration of the atmosphere in the pressurized modules through the depletion of CO_2 and the release of O_2 by photosynthesis, b) recycling the liquid and solid wastes of the crew, c) recovering water through transpiration and d) production of edible biomass to reintegrate the astronauts' diet with fresh food (Wheeler et al., 1996). Although algae and bacteria could be used in bioregenerative systems, higher plants could certainly provide tastier and more attractive fresh food. Moreover, higher plants in Space have been demonstrated to also play a role in mitigating the stress of isolation suffered by astronauts (Williams, 2002). For these reasons there is an interest in having higher plants in closed or semi-closed support systems (also when they are designed as modular systems where chemical, physical and biological subsystems based on microorganisms are integrated). These compartmentalized systems are intended to reproduce the natural cycles occurring on Earth in a technological and reduced scale characterized by high robustness and safety (Lasseur et al., 2010).

Considering that there is common belief that plants can survive in Space, at the present the issue is to optimize plant growth to maximize the production of edible biomass, oxygen, clean water and the removal of carbon dioxide and wastes (Galston, 1992). However, ground-based experimentation cannot be irrespective of the possible growth reactions of plants due to the harsh conditions of Space characterized by different levels of environmental factors including pressure, radiation and gravity (De Micco, Arena, Pignalosa & Durante, 2011; Wheeler, Wehkamp, Stasiak, Dixon & Rygalov, 2011).

WHICH FACTORS PRIME EVOLUTION IN SPACE?

Every organism is ideally designed to fulfill precise metabolic and physiological processes in specific environmental conditions. Focusing on higher plants, there is evidence of how specific traits arose to fulfill specific needs dictated by changing environmental factors: for example, the development of complex vascular systems consisting of specialized cells with lignified walls is a means to guarantee water transport and mechanical support in organisms leaving the water environment to colonize lands (Raven, 1977). During the evolution of higher plants, the key moment when organisms moved away from the aquatic environment to colonize the land was obviously marked by the need to solve the conflict between water retention and the metabolic requirement to exchange gases with the atmosphere to accomplish photosynthesis (Niklas, 1986). Moreover, there is also evidence that many other factors, including solar radiation and gravity, may have played a fundamental role in shaping the structure and function of higher plants (Graham, 1993; Bateman et al., 1998). In a simplified model aimed at explaining the variation of gravity and radiation during higher plant evolution on Earth, we might consider that gravity has increased (because of the lack of buoyancy balancing the gravity force during submersion in water) while radiation has decreased (due to the augmentation of atmospheric oxygen and the thickness of the ozone layer) (Graham, 1993; McGinley & Weis, 2009). In the further evolution of higher plants in Space, we might expect that the plants would face again environmental conditions similar to those of remote past times at least with regards to reduced gravity and increased radiation (De Micco et al., 2009). These new levels of gravity and radiation are known to cause alterations in various aspects of plant growth. Consequently, growth reactions of plants to such factors must be taken into account in the design of space greenhouses and in the choice of species and cultivars for BLSS.

MAIN TOPICS IN PLANT SPACE BIOLOGY AND THE ITALIAN CONTRIBUTION

For organisms well-adapted to live in a given environment, any changes in biotic and abiotic factors can be perceived as stress which will likely alter growth and reproduction. Within this scenario, Space can be considered a novel environment where plants are subjected to multiple stressors which exert direct or indirect effects on developmental processes (De Micco et al., 2009). Among those factors already present on Earth, the different perception of gravity can be considered one of the main constraints to organism development in Space. Reduced levels of gravity are known to affect many processes including gene expression, cell division (affecting both vegetative growth and reproduction), gravisensing, vascular development and cell wall deposition (Brinckmann, 2007; De Micco, Aronne, Joseleau & Ruel, 2008; Paul et al., 2011).

Moreover, altered gravity can interfere with physical processes such as fluid-dynamics, thus affecting plant growth both directly (due to effects on gas diffusion) and indirectly (because of alteration of hardware functioning) (Musgrave & Kuang, 2003; Kitaya & Hirai, 2008; De Micco & Aronne, 2008a).

Over the last decades, several Italian research groups have been working on projects funded by the Italian Space Agency (ASI) and/or the European Space Agency (ESA) to address various issues of plant space biology. Three main ambitions have been pursued: a) the advancement of scientific knowledge about the effects of reduced gravity on specific biological processes, b) the choice of biological systems and species to integrate the astronauts' diet with fresh food produced onboard, and c) the development of new agro-technologies to support plant growth in Space. Experiments aiming to understand the effects of altered gravity on plant development were focused on specific processes during both vegetative growth and the reproductive cycle. Studies aimed at optimizing plant growth in Space were directed both at the identification of objective procedures for cultivar selection and to the preparation of cultivation protocols with a focus on soilless systems. (De Micco, Buonomo, Paradiso, De Pascale & Aronne, 2012; Paradiso, Buonomo, De Micco, Aronne, Palermo, Barbieri, De Pascale, 2012).

In the following paragraphs, the results from some experiments performed in simulated and real microgravity are summarized.

Studies on the effects of microgravity on seedling development.

Several experiments have been performed in Space or on Earth with the uni-axial clinostat to simulate weightlessness, albeit with the awareness that such a facility can simulate but not replicate real microgravity (Aronne et al., 2003). Most of these studies were aimed at investigating the possibility of producing sprouts in Space as highly nutritional "edible vegetal systems" that can be easily produced in few days, in a small physical volume and with low energy, to integrate fresh food into the diet of the crew (De Micco, Aronne & De Pascale, 2006a; De Micco, Aronne, Scala, Castagnolo & Fortezza, 2006b; De Micco & Aronne, 2008a, b). Although not being ideal candidates for BLSS, the production of sprouts would be desirable in Space because they are characterized by a low content of antinutritional compounds and a high content of proteins, vitamins, minerals, phenolics and other compounds having protective effects on the human body. Moreover, they can be considered "functional" foods because they seem to improve health and well-being while reducing the risk of human disease (Zieliński, Frias, Piskuła, Kozłowska & Vidal-Valverde, 2005). The choice of species might improve the beneficial effects of sprouts on the health of astronauts who are more exposed to microgravity-induced diseases such as osteoporosis and muscle atrophy. For instance, there is evidence that specific compounds absorbed through a soy-based diet can protect bones from osteoporosis (Taku, Melby, Nishi, Omori & Kurzer, 2011). Moreover, it has been proven that rats fed with soy protein isolate are less exposed to muscle atrophy when subjected to weightlessness (Tada & Yokogoshi, 2002).

Several experiments were carried out with the aim of studying the morpho-anatomical development of bean and soy seedlings under simulated microgravity (ASI project "Morphological and Physiological response of seedlings to a low-gravity environment") and in Space (ESA – SAYSOY project, Foton-M2 mission) (Aronne et al., 2003; De Micco & Aronne, 2008a, b). Specific attenion was paid to root gravitropism, vascular development, cell wall deposition and lignification, accumulation of phenolic compounds and starch metabolism (both in statoliths and in storage organelles). Observations by means of light, epi-fluorescence and transmission electron microscopy, combined with digital image analysis, allowed quantifying anatomical and cytological parameters. Experiments on bean germinating on the clinostat showed that prolonged clinorotation determines modifications during root development including a loss of orientation of the statocytes in the columella, a decrease in the starch content and changes in cell size in various regions of the root (Figure 1) (Aronne et al., 2003).

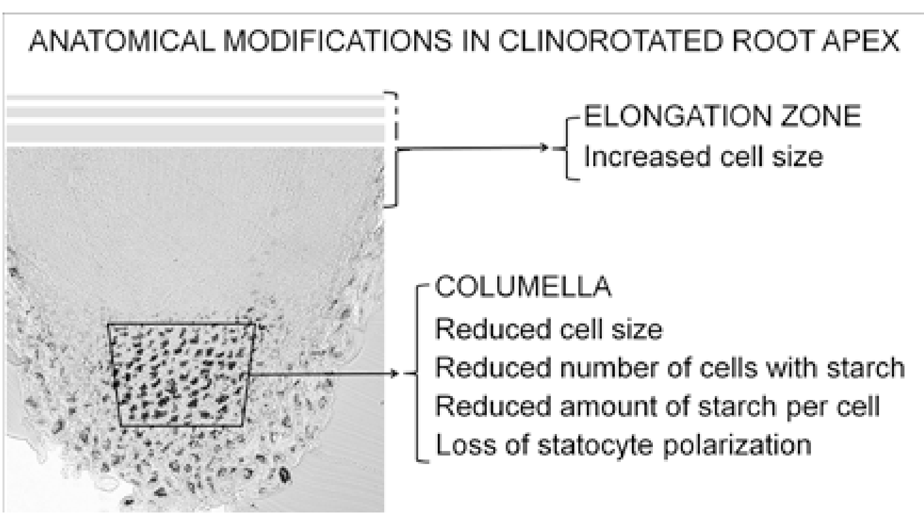

Figure 1: Graphic representation of the modifications happening in clinorotated roots of Phaseolus vulgaris L.

At the vascular level, perturbations have been found in soy seedlings developed both on Earth under clinorotation and in real microgravity in Space (De Micco & Aronne 2006a; De Micco et al., 2006a, 2008). At the ultrastructural level, alterations in the deposition of cellulose microfibrils were evidenced in the cell walls of soy seedlings growing in Space. These alterations are in agreement with the broadened view of the microtubule/microfibril paradigm which links the ordering principles of the deposition of cellulose microfibrils with the orientation of cytoskeleton microtubules. However, these perturbations were evident only in primary cell walls at early stages of development, while ordering principles seemed to be restored during the deposition of secondary wall layers (De Micco et al., 2008). The delay in the deposition of compact cell walls might be responsible for the delay in morphological development in Space and for the development of larger cells due to the lack of mechanical constraints ascribed to cell walls at the beginning of cell enlargement.

Morpho-anatomical modifications due to microgravity have been demonstrated to be dependent also on interactions with other environmental factors: for example, sub-optimal temperatures were responsible for more evident alterations of the morphological development in clinorotated soy seedlings (De Micco et al., 2006a). Such a sensitive interaction between gravity and other environmental factors, as well as other sources of variability (e.g. the use of different hardware, protocols, biological sources), might be responsible for the contrasting results often reported in the literature.

Studies on the effects of microgravity on pollen germination.

Several experiments have been performed to understand the effects of simulated microgravity on pollen germination and pollen tube development in many herbaceous and woody crops (ASI project "Morphological and Physiological response of seedlings to a low-gravity environment"). These experiments were characterized by a double approach: a) to evaluate the possible influence of microgravity-induced alterations on the completion of the reproductive cycle and b) to investigate the possibility of applying methods for gametophyte selection in Space (Figure 2). Results of these experiments showed that reduced gravity can alter nuclei formation and migration during pollen tube development; moreover, the lack of coordination between the formation of callose plugs and nuclei migration might impede nuclei to reach tube tip thus preventing fertilization. These modifications might be the consequence of alterations of cytoskeleton organization. However, it is clear that these perturbations were strictly dependent on the species (De Micco, Scala, & Aronne, 2006c, d).

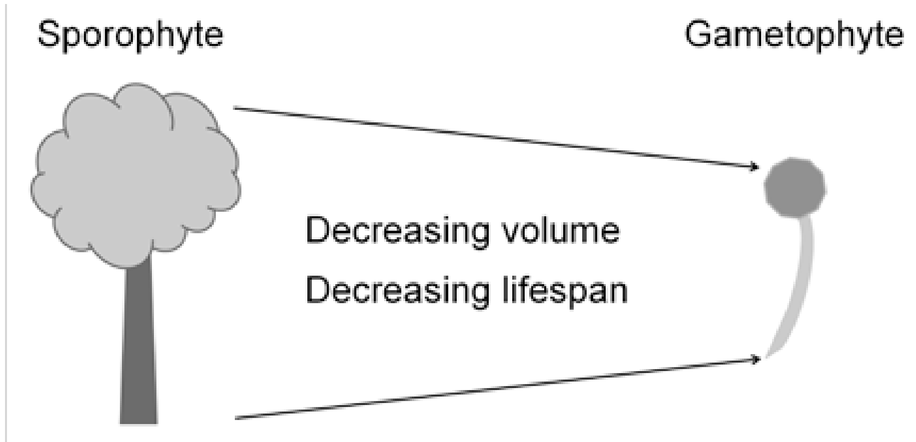

Figure 2: Sporophyte (diploid mother plant) versus male gametophyte (aploid germinated pollen): during the evolution of higher plants, gametophyte has diminished in terms of both size and longevity compared to the sporophytic generation. The selection among genotypes can be done by screening the pollen produced by mother plants (male gametophyte selection) with advantages in terms of time and volume which are two main constraints in Space.

FURTHER PERSPECTIVES

The leitmotiv of the studies on the response of biological systems to microgravity mentioned in this paper was the focus on those biological processes that not only affect the normal course of plant growth, but have also an importance on the properties of fresh food. For instance, vascular development and lignification processes affect the palatability of the fresh food due to their influence on tissue softness. On the other hand, changes in the content of phenolics, which is a common phenomenon in stressful conditions such as those experienced in Space or Space-like environments (Figure 3), affect the nutritional value of seedlings because such compounds have antioxidant properties and some of them confer a bitter taste to the food. Results from these studies open up interesting perspectives in the vision of space explorations in which the astronauts' diet can be supported with fresh food produced onboard in BLSS or in simple automatic hardware, such as the SAYSOY hardware designed and built within an ESA-Education Project (SAYSOY – Space Apparatus to Yield SOYsprouts) (De Micco & Aronne, 2008b).

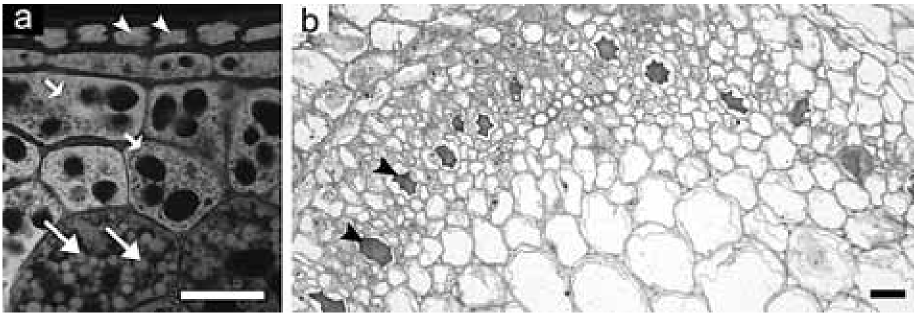

Figure 3: Epi-fluorescence (a) and light (b) microscopy views of soybean cotyledons (a) and hypocotyl (b) grown in Space. Phenolic compounds are present in epidermal cells (a, arrowheads), subepidermal cell layers (a, short arrows) and in parenchyma cells (a, long arrows) of cotyledons. Phenolic bodies are around the stele in hypocotyl (b, arrowheads). Microgravity determines increased content of phenolic compounds in both cotyledons and hypocotyls (De Micco & Aronne, 2008a). Bars = 50 microns.

Further studies aimed at fulfilling the requirements for the productivity and quality of biological systems subjected to Space factors are needed to produce the ideal BLSS which can be considered a stable Space platform where all vegetal organism needs are supplied with optimal water, light, temperature and microgravity ranges, according to the space environmental conditions. In the future perspectives, the study of the effect of microgravity on photosynthesis is needed because it is a key process for the building of an efficient BLSS. Although deriving from limited spaceflight experiments, information on the effects of microgravity on photosynthesis suggest that photosynthetic machinery can be altered at different steps (Stutte, Monje, Goins & Tripathy, 2005). Studies conducted onboard the International Space Station on wheat have demonstrated that the response of plants to microgravity in terms of net photosynthesis, water use efficiency and electron transport activity depend on light intensities experienced during the experiment (Monje, Stutte & Chapman, 2005; Stutte et al., 2005). Moreover, changes in stomatal resistance and gas exchanges due to altered fluid-dynamics in microgravity might be taken into account (Kirkham, 2008).

It is worth highlighting that based on available knowledge, we are beyond plant survival in Space: future goals will be to module plant systems and agrotechnologies to reach high efficiency in BLSS. The latter can be achieved only through tight cooperation between the various disciplines within plant biology, agronomy and technology. Plant biologist in Space have to consider that microgravity and other environmental factors deeply influence organism growth at molecular, biochemical, morphostructural and physiological levels with interactions that can be considered additive, antagonistic or synergic. Finally, considering that experiments in Space and in simulated space conditions are costly and constrained by opportunities, there is a need to dedicate experiments starting from a well-selected biological source (species/cultivar), focusing on specific developmental processes that affect the optimal growth of plants, but have also influence on the nutritional quality of the fresh food.

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