Microgravity Effects on the Early Events of Biological Nitrogen Fixation in *Medicago truncatula*: Results from the SyNRGE experiment

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SyNRGE (Symbiotic Nodulation in a Reduced Gravity Environment) was a sortie mission on STS-135 in the Biological Research in Canisters (BRIC) hardware to study the effect of microgravity on a plant-microbe symbiosis resulting in biological nitrogen fixation. *Medicago truncatula*, a model species for the legume family, was inoculated with its bacterial symbiont, *Sinorhizobium meliloti*, to observe early biomolecular events associated with infection and nodulation in Petri Dish Fixation Units (PDFU's). Two sets of experiments were conducted in orbit and in 24-hour delayed ground controls. Experiment one was designed to determine if *S. meliloti* infect *M. truncatula* and initiates biomolecular changes associated with nodule formation. Roots of five-day-old *M. truncatula* cultivar Jemalong A17 (*Enod11::gus*) were inoculated 24 hr before launch with either *S. meliloti* strain 1021 or strain ABS7 and integrated in the BRIC-PDFU hardware, place in a 4°C Cold Bag for launch on Atlantis. Inoculated plants and controls were maintained in the dark at ambient temperature in the middeck of STS-135 for 11 days before fixation in RNALater™ by crew. Experiment two was designed to determine if microgravity altered the host plant and/or bacteria to induce nodule formation upon return to 1*g*. Seeds of two *M. truncatula* Jemalong A17 lines, the (*Enod11::gus*) used in experiment 1, and SUNN, a super-nodulating mutant of A17, were germinated on orbit for 11 days in the middeck cabin and returned to Earth alive inside of BRIC-PDFU's at 4°C . *S. meliloti* strains 1021 and ABS7 were cultivated separately in broth culture on orbit and also returned to Earth alive. After landing flight and ground-grown plants and bacteria were transferred from BRIC-PDFU's into Nunc™ 4-well plants for reciprocity crosses. Rates of plant growth and nodule development on Buffered Nodulation Media (lacking nitrogen) were measured for 21 days. Initial analysis of Experiment 1 confirms that the legumes and bacteria cultivated in space have potential to develop the symbiotic interaction leading to nitrogen fixation and the bacteria retain the ability to form nodules on *M. truncatula* roots upon return to 1*g*. Initial assessment of Experiment 2 suggests a microgravity effect on both the *M. truncatula* and *S. meliloti* that alters their ability to form nodules upon return to 1*g*. (Research supported by NASA ESMD/ Advance Capabilities Division grant NNX10AR09A)