The Freezing Tolerance of Alfalfa Nodules Depends on Sinorhizobium Meliloti Strains

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The increase in frequency of freeze-thaw episodes combined with the reduced snow cover protection due to climate change compromises winter survival of alfalfa (*Medicago sativa* L.). The establishment of an early symbiosis in cold soils in the spring that continues to be efficient in the fall would allow alfalfa to benefit from an extended growing season and potentially from an additional cut and higher yields. Furthermore, adapted rhizobia strains have been shown to directly affect alfalfa physiology and enhance its tolerance to freezing stress. In order to identify efficient symbiotic partners under cold, two alfalfa populations bred to differ in their levels of freezing tolerance (A-TF0 and A-TF7) were inoculated with five cold-tolerant strains of *Sinorhizobium meliloti* (A2, NRG34, I1, S27, Rm1521) and a commercial stain (B399) and plants of each different association were grown for eight weeks in a growth chamber before being exposed to temperatures promoting their acclimation to cold. Plants were then exposed to a freezing stress (-11ºC) and regrown for three weeks. Shoot, root and nodule biomasses were measured before cold acclimation and three weeks after the freezing stress. In addition, we proceeded with a detailed phenotyping of the above and belowground parts of the plants of the different populations/strains associations three weeks after the freezing stress. We observed that alfalfa shoot regrowth after exposure to a freezing stress was 35 % larger for the freezing tolerant alfalfa population A-TF7 inoculated with strain NRG34 isolated from Northwestern Canada than for the freezing sensitive alfalfa population inoculated with the commercial strain B399. Distinct profiles in the percentage of distribution of nodules shapes were observed among the strains. Plants inoculated with strains NRG34 and S27 had higher proportions of palmate-coralloid nodules which is the largest size of nodules observed. It could be supposed that palmate-coralloid nodules stored more resources to ensure the regrowth after freezing and represent a stronger carbon sink to sustain spring regrowth than smaller nodules. Freezing damages on nodules differed according to the strain in symbiosis with alfalfa, as shown by different proportions of undamaged nodules, nodules with regeneration zones, and necrotic nodules observed after the freezing stress. Plants inoculated with strain NRG34, had the largest proportion of active nodules showing no damage or with a large regeneration zone after the freezing stress (85%) along with the greatest shoot regrowth three weeks after freezing (+19%) than plants inoculated with any other strain (65% of active nodules in average). The strong positive relationship between shoot regrowth and nodule regrowth after a freezing stress highlights that nodule freezing tolerance differs according to the strain in symbiosis with alfalfa, and that partially necrotic nodules have a capacity of regeneration after freezing. These are the first *in vivo* observations of the variability of freezing tolerance among *S. meliloti* strains. Alfalfa regrowth after freezing relies in part on nodule tolerance to freezing stress which in turn depend on *S. meliloti* strains. The choice of freezing tolerant *S. meliloti* strain in association with a freezing tolerant alfalfa population could present synergistic positive effects on the winter survival and spring regrowth of alfalfa.