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Stability and field evaluation of rhizobia in alfalfa seed treatment

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Statement of the problem: Alfalfa cultivation in the world is necessary for forage uses in animal feeding. Nodulation by nitrogen-fixing bacteria is highly used in agriculture and it is well-known that the inoculation of leguminous plants increments nitrogen input in the plant increasing ultimately yield. However, the application of this kind of bacteria in the seed is relatively new. Seed Technology comprises a series of methods, techniques and protocols that allow the improvement of the seed after it is produced. Seed treatment, is a part of Seed Technology that it is defined by the use of different compounds adhered to the seed in order to improve its performance once cultivated. The aim of this study is to determine the dosage, stability through time and the effects on plant performance in the field of a seed treatment consisting of two rhizobia strains in alfalfa seeds.

Methodology & Theoretical Orientation: Formulation and coating of the seeds, plate-counting dilution protocol in rhizobia-specific medium, stability of the rhizobia inoculation in the seed through time, evaluation of germination and vigor protocols and field testing and evaluation of the seed treatment. Findings: The formulation protocol is effective and allows retention and survival of the bacterial strains through time, providing higher weight to the seeds and protecting them from external damage. Miniaturization of plate-counting traditional method allows quality control of the treated seeds in an easy and cost-effective manner. Rhizobia seed treatment showed increased germination (5-9%) and field testing showed increased plant stand (8.9%), percentage of nodulation (15.5%) and yield in dry weight per hectare (2%).

Conclusion and significance: In SEMILLAS FITO company we developed a seed coating for alfalfa inoculated with alfalfa-specialized rhizobium and free-living nitrogen-fixing bacteria that improved germination under chilling stress, prevents the seed from external damage, increases plant stand, percentage of nodulation and fresh weight of aerial parts.

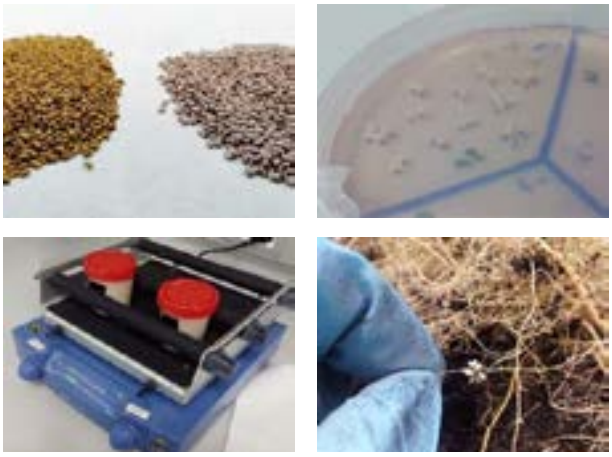

Fig 1. Upper left: seeds with or without the treatment. Upper right: colonies of rhizobia. Lower left: agitating rhizobia for plate-dilution method. Lower right: nodule of rhizobia in alfalfa roots

Recent Publications

1. Somasegaran P, & Halliday J (1982) Dilution of Liquid Rhizobium cultures to increase production capacity of inoculant plants. Appl. Environ. Microbiol. 44 (2) 330-333.
2. Corral Lugo A, Morales García YE, Pazos-Rojas LA, Ramírez Valverde A, Martínez Contreras D, Muñoz Rojas J (2012) Quantification of cultivable bacteria by the "massive stamping drop plate" method. Rev. Colom. Biotecnol. 14(2):147-156
3. Castillo M, Flores M, Mavingui P, Martínez-Romero E, Palacios R, Hernández G (1999) Increase in alfalfa nodulation, nitrogen fixation, and plant growth by specific DNA amplification in *Sinorhizobium meliloti*. Appl. Environ. Microbiol. 65(6):2716-2722.
4. Zeng ZH, Chen WX, HU YG. SUI XH, CHEN DM (2007) Screening of highly effective Sinorhizobium meliloti strains for "Vector" alfalfa and testing of its competitive nodulation ability in the field 17(2):219-228.
5. Lang-Unnasch N & Ausubel FM (1985) Nodule-Specific polypeptides from effective alfalfa root nodules and from ineffective nodules lacking nitrogenase. 77:833-839.

Biography

Virginia Estévez Geffriaud, BSc. In Microbiology (2014, Autonomous University of Barcelona, UAB); MSc. In Environmental Agrobiology (2015, University of Barcelona); Master Thesis in FUTURECO BIOSCIENCE (2015). Industrial PhD student at University of Barcelona (2016-); Project assistant and Trial Coordination at Seed Technology Dpt. (R&D) at FITO SEEDS (SEMILLAS FITÓ) (2016-); Last oral presentation: MICROPe (Microbe-assisted crop production-opportunities and challenges, Vienna 2018).

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