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Rhizobium leguminosarum: Selecting Strain and Exploring Delivery Systems for White Clover

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Abstract : Leguminous crops can be self-sufficient for their nitrogen requirements when their roots are nodulated with an effective Rhizobium strain and for this reason seed or soil inoculation is practiced worldwide to ensure nodulation and nitrogen fixation in grain and forage legumes. The most widely used method of applying commercially available inoculants is using peat cultures which are coated onto seeds prior to sowing. In general, rhizobia survive well in peat, but some species die rapidly after inoculation onto seeds. The development of improved formulation methodology is essential to achieve extended persistence of rhizobia on seeds, and improved efficacy. Formulations could be solid or liquid. Most popular solid formulations or delivery systems are: wettable powders (WP), water dispersible granules (WG), and granules (DG). Liquid formulation generally are: suspension concentrates (SC) or emulsifiable concentrates (EC). In New Zealand, R. leguminosarum bv. trifolii strain TA1 has been used as a commercial inoculant for white clover over wide areas for many years. Seeds inoculation is carried out by mixing the seeds with inoculated peat, some adherents and lime, but rhizobial populations on stored seeds decline over several weeks due to a number of factors including desiccation and antibacterial compounds produced by the seeds. In order to develop a more stable and suitable delivery system to incorporate rhizobia in pastures, two strains of R. leguminosarum (TA1 and CC275e) and several formulations and processes were explored (peat granules, self-sticky peat for seed coating, emulsions and a powder containing spray dried microcapsules). Emulsions prepared with fresh broth of strain TA1 were very unstable under storage and after seed inoculation. Formulations where inoculated peat was used as the active ingredient were significantly more stable than those prepared with fresh broth. The strain CC275e was more tolerant to stress conditions generated during formulation and seed storage. Peat granules and peat inoculated seeds using strain CC275e maintained an acceptable loading of 108 CFU/g of granules or 105 CFU/g of seeds respectively, during six months of storage at room temperature. Strain CC275e inoculated on peat was also microencapsulated with a natural biopolymer by spray drying and after optimizing operational conditions, microparticles containing 107 CFU/g and a mean particle size between 10 and 30 micrometers were obtained. Survival of rhizobia during storage of the microcapsules is being assessed. The development of a stable product depends on selecting an active ingredient (microorganism), robust enough to tolerate some adverse conditions generated during formulation, storage, and commercialization and after its use in the field. However, the design and development of an adequate formulation, using compatible ingredients, optimization of the formulation process and selecting the appropriate delivery system, is possibly the best tool to overcome the poor survival of rhizobia and provide farmers with better quality inoculants to use.

Keywords : formulation, Rhizobium leguminosarum, storage stability, white clover
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