The Legume Generation Clover Innovation Community

Boosting innovation in breeding for the next generation of legume crops for Europe

Charlotte Jones, Germinal Holdings (GER), UK; Stig Anderson, Aarhus University (AU), Denmark; Jos de Vega, Earlham Institute (EI), UK; Andrew Griffiths, AgResearch (AGR), New Zealand; Stephan Hartmann, Bavarian State Research Center for Agriculture (LfL), Germany; Catherine Howarth, Aberystwyth University (ABER) UK; Anelia Iantcheva, Agrobioinstitute (ABI, Bulgaria);David Lloyd, Germinal Holdings Ltd. (GER), UK; Heathcliffe Riday, United States Department of Agriculture, (USDA) US contact: charlotte.jones@germinal.com

Figure 1. Honeybee (*Apis mellifera*) on a white clover flower

Introduction: Red and white clover are out-crossing perennial forage species. They are used in agriculture as they are highly digestible and palatable; they contain high levels of protein and minerals reducing the need for expensive feed substitutes. Their root structures are able to ameliorate drainage problems and improve fertilizer uptake from the soil. As legumes, they reduce the need for synthetic fertiliser nitrogen thanks to their ability to bind nitrogen. They are also high in nectar and pollen and are therefore good for pollinator diversity on the farm (Figure 1).

Challenges in clover breeding: in Legume Generation we will address the following challenges faced by clover breeders

1. Low seed yield due to poor pollen viability.
2. Hybrid seed generation with low levels of intrapopulation seed.
3. Little pre-breeding investment due to cost and space requirements.

We as the **Clover Innovation Community** are a group of breeders and scientists from both commercial and academic organisations from around the globe working together to generate new breeding material that has both economic and environmental benefits for farmers, growers and the general public. Figure 2 and 3 illustrate how we will work together to bring about change in the breeding of both red and white clover.

The work is divided between the partners according to the specialities we bring to the project. Plot field work will be carried out in Wales (Germinal (GER), IBERS), Bulgaria (ABI) and in Germany (LfL). Molecular analysis will be carried out at Earlham Institute (EI), USDA (USA), Aarhus University (AU; Denmark) and AgResearch (New Zealand). The EUCARPIA Fodder Crops meeting will be in IBERS in 2025, this will enable the partners o meet and have practical and analysis advice from the USDA partner.

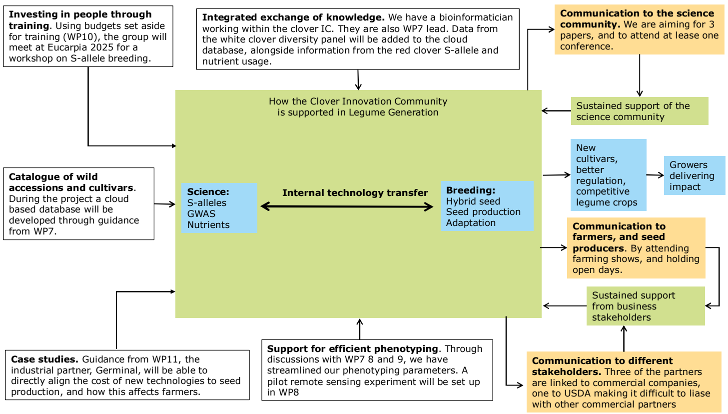

Figure 2. How the Clover Innovation Community is supported in Legume Generation

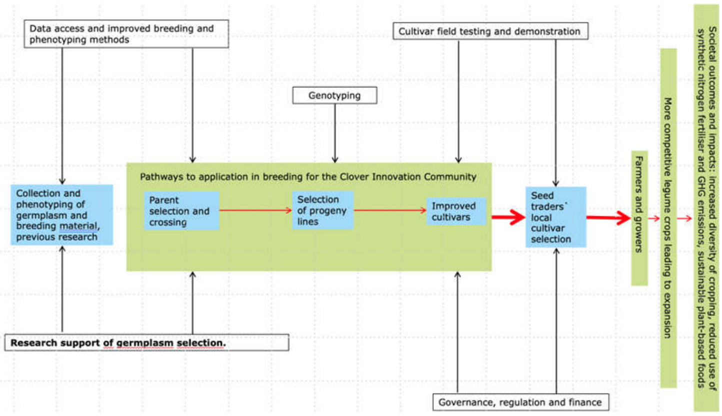

Figure 3. Pathways to application in breeding

Approaches

Low seed yield in red clover: carried out at ABI and GER/IBERS using varieties of low seed producing varieties. Boron application and analysis of pollen tube growth and seed development will be carried out in glasshouses and in the field (years 1 - 4).

Pre-breeding investment in white clover: carried out at LfL, GER/IBERS, EI. 200 accessions of wild collected and commercial varieties have been sown in augmented plot designs at LfL and GER in 4 contrasting environments. Yield data will be collected from plots, and further phenotyping on spaced plants will be carried out at GER (years 1 – 4). Whole genome sequencing and SNP discovery will be carried out at EI, the data will be used for GWAS (years 1 – 4).

Hybrid seed generation in red and white clover: markers developed at USDA will be used to genotype S-alleles in red clover, so that reduced numbers of S-alleles can be used to create populations with high levels of intrapopulation seed, and to improve seed yield (years 1 – 4). In white clover, work is being carried out at AgResearch and Aarhus University to develop markers around the S-alleles, these markers will be trialled in white clover towards the end of the program (years 1 – 4).

A quick guide to S-alleles: plants contain multiple self-incompatibility alleles (S-alleles), red clover has 143 – 193 unique S-alleles. These alleles can prevent both self-fertilization and cross fertilization by controlling the growth of the pollen tube. In monoecious plants these alleles cause male sterility and prevent inbreeding depression. Gametophytic tissue containing the same S-alleles as the sporophyte are discriminated against, and the pollen tube does not grow. Fig. 4 is a simplified representation of this mechanism.

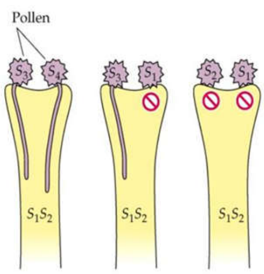

Figure 4. Self-incompatibility as shown by arrest of pollen tube growth (diagram from plantlet.org)

Legume Generation (Boosting innovation in breeding for the next generation of legume crops for Europe) has received funding from the European Union through Horizon Europe under grant agreement No 101081329 and co-funding from UK Research and Innovation (UKRI) from the UK government's Horizon Europe funding guarantee. It also receives support from the governments of Switzerland and New Zealand.

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