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The genetics of self-incompatibility in white clover



Key external stakeholders:
Plant Breeders, seed companies.

Practical implications for stakeholders:

Self incompatibility, the genetic mechanism preventing self fertilisation, is a major feature of the reproductive biology of plants. Understanding the genetic basis of this important process in white clover will provide a basis for the potential manipulation of the trait in the future, impacting the way in which white clover is bred. In the longer term, it could form part of the basis of the development of F1 hybrid varieties of white clover.

Main results:

Self-incompatibility (SI) is the genetic mechanism by which outbreeding plants prevent both self fertilisation and the fertilisation of related plants, thus preventing inbreeding. At the outset of the project, the knowledge of the genetic control of SI in white clover came largely from classical genetical studies. These suggested that white clover exhibited a mechanism called gametophytic self incompatibility, and that this was controlled by a single locus. Our goal was to confirm this hypothesis and to identify the specific chromosomal location of the genetic locus via a process called genetic linkage mapping. Using a white clover population developed by our collaborators in IBERS, we successfully identified a single locus controlling SI on white clover homeologue-group (chromosome) 1. This represents the first step in identifying the genes underlying SI in white clover.

Opportunity / Benefit:

Identification of the genes underlying self-incompatibility in white clover, the logical next step to the research described above, will be central to the routine development of self-fertile lines of white clover in which SI has either naturally broken down, or in which knowledge of the genes has allowed the SI mechanism to be overcome artificially. This in turn will lead to an ability to produce inbred lines of the species. This is not currently possible for clover because of SI, and clover varieties are produced by selecting and vegetatively propagating several superior parents, and performing a random "bulk-polycross" of these parents to produce seed. While many high-performing clover varieties have been produced using this method, it results in an inherent genetic and phenotypic variability in clover varieties that represents a distinct limitation in addressing certain performance barriers such as increased persistency and variable seasonal response. Inbred lines offer advantages such as completely uniform varieties of bi-parental origin, with key traits fixed in every plant from the variety, F₁ hybrid varieties exhibiting hybrid vigour, and the effective exploitation of rare traits, by releasing, and then capturing hidden recessive genetic variation.

Collaborating Institutions:

Institute of Biological, Environmental and Rural Studies (IBERS), UK

Teagasc project team: Dr. Dan Milbourne (Co-PI)
Dr Susanne Barth (Co-PI)
Ms Nora Casey

External collaborators: Dr Daniel Thorogood, IBERS, UK.

1. Project background:

Self-incompatibility (SI) is considered to be one of the most important strategies used by flowering plants to circumvent the tendency toward self-fertilization. Classic genetic studies in the early 20th century revealed two major classes of SI systems, gametophytic and sporophytic. In many cases, SI is controlled by a single genetic locus, the S-locus consisting of a large number of alleles. In most families, GSI has been found controlled by a single locus (the S-locus) which harbours separate pistil and pollen specific S-genes. Self incompatibility is determined by the haploid genotype of the pollen, relative to the diploid genotype of the pistil. Two mechanistically different systems have been investigated extensively at the molecular level. One is the S-RNase system, originally found in members of the Solanaceae and more recently reported in the Rosaceae, Scrophulariaceae and Campanulaceae (McCubbin & Kao, 1999). The other system is found in the Papaveraceae (Hiscock & McInnis, 2003).

In the S-RNase-based SI system, incompatible pollen germinates and grows normally on the stigma, and the pollen tube is arrested by the time it has reached about one-third of the way through the style. Studies in *Nicotiana glauca* led to the cloning and sequencing of the first pistil specific S-gene, and this allowed the identification and cloning of a large number of alleles of this gene from members of the Solanaceae and other species. The genes involved encode highly polymorphic RNases (called S-RNases) with two hypervariable regions (HVa and HVb) and five highly conserved regions (C1 to C5). These S-RNases are thought to cause premature termination of the pollen tube in incompatible reactions by degradation of ribosomal RNA in the developing tube. The most likely candidate for the pollen S-determinant in the S-RNase mechanism is an F-box gene SLF (S-locus F-box), first identified from *Antirrhinum hispanicum* (Scrophulariaceae) and recently obtained from several *Prunus* (Rosaceae) species. The identity of these F-box genes as the pollen specific S-factor is supported by the fact that they have been located at the S-locus, at physical distances between only 400bp and 50kb of the pistil specific S-RNase genes in a variety of plant species, including *Antirrhinum*, sweet cherry and *Prunus*, (eg. Lai *et al.* 2002).

The body of knowledge regarding self-incompatibility in white clover is not extensive. Studies on the inheritance of SI in white clover suggest that SI mechanism is gametophytic, and that the trait is indeed controlled by a single locus in this species. Interestingly, work by Lawrence *et al.* (1996) suggests that both white and red clover have amongst the highest number of S-alleles (in excess of 100) in any species studied to date. Although, anecdotally, white clover has a very strong self-incompatibility system, work in the breeding programme at Oak Park has shown variation in the apparent strength of the SI response in breeding material, and work at both Oak Park and IBERS in Wales has resulted in the identification of a limited number of consistently self-fertile lines (Connolly 2001). Despite the difficulties associated with inbreeding depression as a result of repeated rounds of self-pollination, at IBERS this work has progressed to the development of a number of stable, morphologically uniform inbred lines, which when crossed, exhibit strong hybrid-vigour in the F1 generation. Taken together, these observations suggest the existence of allelic variants at the S-locus in white clover that effectively represent self-fertility (SF) alleles rather than SI alleles.

References:

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Hiscock SJ, McInnis SM. (2003) Pollen recognition and rejection during the sporophytic self-incompatibility response: Brassica and beyond. Trends Plant Sci. (12):606-13. Review; Erratum in: Trends Plant Sci. 2004 Feb;9(2):64

Lai Z, Ma W, Han B, Liang L, Zhang Y, Hong G, Xue Y. An F-box gene linked to the self incompatibility (S) locus of *Antirrhinum* is expressed specifically in pollen and tapetum. 2002 Plant Mol. Biol. 50:29-42

Lawrence, M. J.; Afzal, M., and Kenrick, J. Genetic-Control of Self-Incompatibility in *Papaver-rhoeas*.

Heredity. 1978; 40:239-253.

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2. Questions addressed by the project:

- Is the single locus model of SI in white clover suggested by classical genetical studies correct?
- If the answer to the above is yes, where in the white clover genome is this locus?

3. The experimental studies:

An F1 mapping population consisting of 94 progeny individuals was created by crossing two highly divergent white clover genotypes referred to as S1S4 (female parent) and R3R4 (male parent). To identify the genetic location of the S locus, a genetic linkage map of each parent was constructed using 64 white clover SSRs and 339 AFLP markers. The presence of SSR markers from previously published maps of white clover on the maps of S1S4 and R3R4 allowed the identification of resulting linkage groups (LGs) belonging to the eight homoeologue groups (HG or chromosomes) of white clover. Plants in the mapping population, identified by numbers 1 through 94, were cross-pollinated by hand to determine incompatibility relationships within the population. Cross-pollinations were scored as compatible or incompatible after ca. 7 days by checking the seed pods of pollinated flowers visually and by tactile examination to determine whether or not seeds were developing. In some cases, this was verified by dissection using a scalpel and a stereo microscope. An absence of seeds was taken to indicate a self-incompatible reaction resulting from the parental plants sharing the same S genotype. Presence of seeds indicated a compatible reaction, indicating that the parents possessed at least one different S haplotype. A scoring matrix identifying the segregation of four different S alleles was developed from these data, and incorporated with the marker segregation data used to develop the genetic linkage map, allowing the location of the S-locus on the genetic map to be determined via linkage analysis.

4. Main results:

Using the genetic mapping approach described above, we have identified the chromosomal location of the locus determining self incompatibility in white clover. The S-locus is located on the distal end of one of the linkage groups comprising Homoeologue Group 1 in white clover.

White clover is allotetraploid ($2n=4x=32$), comprising two well preserved ancestral subgenomes each with a basic (haploid) complement of 8. The extant representatives of the ancestral genomes are thought to be *Trifolium occidentale* and either *T. pallescens*, *T. nigrescens* or an unidentified species. The ancestral genomes assort independently on meiosis, but are very similar in terms of gene content and order, and thus genetic maps reveal 8 homoeologue groups, each consisting of two linkage groups (chromosomes), one derived from each of the ancestral donors. The S-locus maps to "Chromosome 1" of one of the 2 ancestral subgenomes. At the time of writing, it is not possible to distinguish the ancestral origin of linkage groups comprising HGs. Interestingly however, modern *T. occidentale* is self compatible, while *T. pallescens* and *T. nigrescens* are self-incompatible, suggesting that the latter are the most likely donors of the trait to white clover.

5. Opportunity/Benefit:

Most seed-dispersed crops are inbreeders, allowing the production of uniform varieties by reducing or eliminating heterozygosity through inbreeding, and the fixing of many important characteristics in the population because individual plants are completely or nearly genetically identical (clonal). In addition, in inbreeding systems, all of the previous characteristics can be combined with the advantages offered by maximising heterosis by producing F₁ hybrid varieties from genetically divergent inbred parents. This is not possible for clover because of SI, and clover varieties are produced by selecting and vegetatively propagating several superior parents, and performing a random "bulk-polycross" of these parents to produce seed. While many high-performing clover varieties have been produced using this method, it results in an inherent genetic and phenotypic variability in clover varieties that represents a distinct limitation in addressing certain performance barriers such as increased persistency and variable seasonal response.

The ability to produce inbred-lines of white clover would offer the possibility of exploring a different strategy for the production of varieties that would have many of the advantages listed above. Inbred lines offer advantages such as completely uniform varieties of bi-parental origin, with key traits fixed in every plant from

the variety, F₁ hybrid varieties exhibiting hybrid vigour, and the effective exploitation of rare traits, by releasing, and then capturing hidden recessive genetic variation. Inbred lines also offer a tool to more easily dissect the genetics underlying complex polygenic traits in white clover, facilitating the use of marker-assisted selection (MAS) based approaches for breeding in the species. The discovery of the chromosomal location of the S-locus in white clover is the first step in the development of the tools required for biotechnology-based approaches for the routine production of inbred lines in white clover. The data developed in this project are an ideal starting point for the isolation of the genes underlying SI and their subsequent exploitation for breeding.

6. Dissemination:

The results were of a scientific/technical nature and were disseminated via the scientific literature.

Main publications:

Casey NM, Milbourne D, Barth S, Febrer M, Jenkins G, Abberton MT, Jones C, Thorogood D. (2010)
The genetic location of the self-incompatibility locus in white clover (*Trifolium repens* L.).
Theor Appl Genet. 121(3):567-76.

7. Compiled by: Dan Milbourne, Susanne Barth.
