

## Molecular control of sexual reproduction in wild radish (*Raphanus raphanistrum*)

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**Summary** Here we describe our research aimed at developing technologies to control sexual reproduction in the cruciferous species wild radish (*Raphanus raphanistrum* Linnaeus), a major weed of grain crops that is subject to recurrent evolution of herbicide resistance. By acting selectively on either wild radish's self-incompatibility mechanism or on its ability to produce and disseminate viable male gametes and set seeds, these technologies should provide alternative control methods to herbicides that will be highly selective and not affect the surrounding crop.

**Keywords** Wild radish self-incompatibility, gibberellin, seed set, sexual reproduction.

### INTRODUCTION

Wild radish costs the Australian grains industry an estimated \$40 million each year due to yield loss and grain contamination. Recurrent and rapid evolution of multiple herbicide resistance has made control of this aggressive weed difficult and in this project we are seeking to develop alternative control methods based on reproductive interference. Our first approach to controlling sexual reproduction in wild radish (*Raphanus raphanistrum* Linnaeus) is through the self-incompatibility (SI) mechanism. SI is a genetically controlled means of preventing self-fertilisation found in many plant families. SI in *R. raphanistrum* is of the sporophytic type (Sporophytic SI or SSI) and is controlled by a single highly variable locus called the S locus, with fertilisation generally only possible between plants with entirely different sets of alleles at the S locus (e.g., pollen from an S<sub>1</sub>S<sub>2</sub> plant can fertilise an S<sub>3</sub>S<sub>4</sub> plant but not an S<sub>1</sub>S<sub>3</sub> plant). A complicating factor is the existence of S alleles that are dominant over other S alleles. That is, if S<sub>2</sub> is dominant over S<sub>1</sub>, then pollen from an S<sub>1</sub>S<sub>2</sub> plant can still fertilise an S<sub>1</sub>S<sub>3</sub> flower, even though both plants share the recessive S<sub>1</sub> allele.

The cell and molecular biology of SSI is well known in *Brassica oleracea* and its close relatives (Nasrallah 2002). In *Brassica* the products of the S locus are a large membrane-bound receptor kinase called SRK and a small, soluble protein known as SCR or SP11. Although SRK and SCR/SP11 from different

S alleles share certain features, each S allele encodes a uniquely different and co-evolved version of these proteins. SRK is found in the plasma membrane of the papilla cells that form the receptive surface of the stigma (Takayama *et al.* 2001) and SCR/SP11 is deposited on the surface of the pollen grain during pollen maturation (Schopfer *et al.* 1999). When a pollen grain alights on a stigmatic papilla cell it releases SCR/SP11, which binds to the external receptor domain of SRK when both proteins are products of the same S allele. SCR/SP11 binding activates the SRK kinase domain and initiates a signalling pathway that ultimately leads to highly localised changes to the papilla cell wall that prevent further growth of the pollen tube (Dickinson 1995).

To develop a control technology that targets SSI, we have determined at the phenotypic and molecular levels the number and distribution of S alleles in Australian populations of wild radish. We have also developed bacteria as a means of producing the SCR/SP11 protein from different *R. raphanistrum* S alleles and are testing whether this protein can activate the pollen rejection mechanism when applied to the stigma of a compatible plant. By preventing compatible pollinations, applying mixtures of SCR/SP11 from several wild radish S alleles can potentially reduce seed set.

The second approach to controlling seed set is through manipulation of gibberellins (GAs), which are a major class of plant hormone well known for their roles in regulating plant height and seed germination. GAs also have important roles in plant reproductive development and are essential for pollen grain development and for functioning of the anther (Swain and Singh 2005). In all plants, GAs are produced by a well-studied biosynthetic pathway and the genes for essential enzymes in this pathway are known (Olszewski *et al.* 2002).

The immediate goal is to develop *in vitro* assays to test various GA-related molecules (GA mimics) for their differential effects on a particular group of GA biosynthetic enzymes (the GA 3-oxidases) and receptor proteins for GA (GID1) from wild radish and selected grasses. Based on this knowledge, we can develop novel chemical inhibitors that selectively

inhibit only the weed GA 3-oxidase and/or *GID1*, and consequently only reduce seed set in wild radish.

#### MATERIALS AND METHODS

**Plant material** Wild radish seeds from WA, SA and NSW populations were obtained from Dr Lisa Crowsfoot (Ecology Australia) and Prof Roger Cousens (University of Melbourne) and new collections were made from populations in Victoria. Reciprocal crosses were performed using a  $15 \times 15$  plant diallel design, in which every individual was selfed and crossed with every other by rubbing pollen directly from the anthers of the pollen donor flower onto the stigma of the pollen recipient.

**Molecular methods** Primers for polymerase chain reaction (PCR) amplification were designed based on the sequences of target genes from other crucifers. All cloning and sequencing was done using standard recombinant DNA methods (Sambrook *et al.* 1989)

#### RESULTS

**SSI in wild radish** Across the eight  $15 \times 15$  diallels (~120 plants) the percent incompatible cross-pollinations ranged from 22% to 60%. Average incompatibility over all populations was 36%. The large numbers of incompatible pollinations suggests that Australian *R. raphanistrum* populations have fewer S alleles than is expected based on population size. *SRK* kinase domain sequences have been obtained from these plants. These sequences are related to authentic *SRK* kinase domains and cluster analysis shows that they resolve into several clades. Figure 1 shows that the rate at which new kinase domain sequences are being discovered in Australia reaches a plateau of 23, the current estimate for the number of *R. raphanistrum* S alleles in Australia. This number is similar to that obtained by different methods for Canadian populations of *R. raphanistrum* (Sampson 1967). Some S alleles are more common than others and several have been recovered from both WA and NSW. This is a good outcome since this approach to controlling *R. raphanistrum* fecundity is plausible only if there are dozens rather than hundreds of S alleles in Australia.

**GAs in wild radish** Following from the strategy of inhibiting GA production in wild radish by targeting the last stage of GA production, cDNAs for three *GA 3-oxidase* genes expressed in wild radish stamens have been recovered. These genes are closely related to known *GA3ox* genes

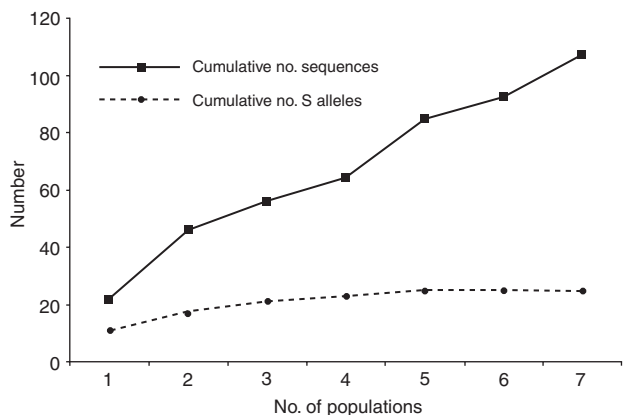
from *Arabidopsis* and other plants. To target the ability of wild radish to perceive active GAs, we have also cloned five cDNAs for the GA receptor (commonly known as *GID1*) from *R. raphanistrum* stamens. Since either decreased or increased GA activity in anthers will result in pollen sterility, both reducing and hyper-stimulating *RrGID1* can potentially lead to defects in anther function and result in fewer seeds.

To identify GA mimics that can specifically target wild radish, we have also developed a yeast two-hybrid method that allows the relative activity of *GA3ox* enzymes or *GID1* receptors from different sources to be compared. We are using this assay to determine whether any GA mimic specifically acts on the wild radish protein but not those from wheat and barley.

Finally, in addition to targeting anther and pollen function, we are also investigating other GA-regulated processes that can be potentially inhibited by GA mimics. We have shown that blocking GA production in wild radish greatly reduces plant growth and delays flowering, suggesting that targeting growth may reduce the ability of wild radish to compete with crop plants. Detailed analysis of *Arabidopsis* mutants lacking one GA 3-oxidase also suggests that reduced GA production in flowers can interfere with the functioning of the ovules (the site of the female gametophyte) and prevent seed set even in the presence of functional pollen.

#### DISCUSSION

Through the experiments outlined here we are testing the practicability of our concept that SSI and GA production and perception represent targets for developing novel and practicable ways of controlling



**Figure 1.** Cumulative number of sequences obtained from each population, and the number of newly discovered S allele sequences in each population. The curve for new S alleles plateaus at 23.

wild radish populations. The control methods we are proposing do not act by killing the plants to which they are applied, but by preventing seed production and should reduce the number of individuals over the medium to long term. Because they should act by triggering a reduction in population density below a certain threshold, these methods share much in common with biological control as a way of managing weeds. Host specificity is a key requirement of biological control, and we believe specificity can be achieved through our approach of targeting SSI and by identifying GA mimics that affect *Raphanus* proteins but not proteins from graminaceous crops. After this proof-of-concept stage has been completed, however, it is almost certain that further improvements will be needed before these technologies are suitable for use in an agricultural setting.

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