



Classification and Applied Use of Self-Incompatibility in Outcrossing Grass Species

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Abstract:

Self-incompatibility (SI) prevent self-fertilization in a few species of Poaceae, many of which are economically important foods, bioenergy energy and grasses. Your incompatibility ensures pollen diversity and genetic diversity but limits the ability to modify useful genetic diversity. In many domesticated plants it is possible to develop highly efficient homozygous parent lines, which facilitates the formation of more efficient F1 hybrids, a condition known as heterosis. The inability to fully exploit heterosis in transit grasses contributes to lower levels of development in breeding systems compared to breeding plants. However, SI can be overcome by eating grass to build harmonious communities. This generates interest in understanding the basics of self-adaptation (SC), its importance in reproductive techniques and its exploitation for plant development, especially in the context of F1 hybrid breeding.

Keywords:

Suitability, self- incompatibility, breeding, Poaceae, introgression



Introduction

Your own incompatibility (SI) is the most common way to allow you to skip hermaphrodite flowering plants. Described by Charles Darwin as ‘the ability of some plants to reproduce their pollen’, SI is considered to be a system for the synthesis of ancestral angiosperms (Allen and Hiscock, 2008). Early flowering plants retained SI in order to increase the variety of pollen and genetic diversity to adapt and expand in many habitats. The autoimmune systems in angiosperms, in most cases studied, are controlled by a single polymorphic S locus that contains at least two closely related genes, encoding male and female SI signals that link self-attention / non-pollen attention. When the S-alleles of pollen and pistil match, the pollen appears to be 'the best', thus preventing reproduction and reproductive effect.

The decline of SI has led to a shift from exit to self-fertilization in the evolutionary history of many flowering plants (Vekemans et al., 2014). During angiosperm emergence and expansion, SI has been irreversibly lost due to loss of function (Igic et al., 2008). Self-compatibility (SC) emerged as a plant reproductive strategy as, among other factors, adapting to the cultivation of domestic plants (Iriondo et al., 2018). As a result of the high rate of reproduction caused by self-fertilization, a tragic change in traits was revealed that early growers chose against propagating plants with flexible alleles that contribute to beneficial features (Abbo et al., 2012; Kantar et al., 2017).

Understanding how SC is developed is important for crop rotation, population density and crop farming studies. In fact, SC has resulted in the production of high-resolution integrated lines in removable alleles. Homozygous lines from different domains can be crossed to form F1 hybrids with increased activity, a phenomenon known as heterosis. Understanding this situation has been enhanced by the development of map statistics that include homozygous lines in which a combination of alleles from opposite parents is corrected. These lines can be permanently propagated as seeds.

Therefore, genetic mapping and agronomic markers have been instrumental in the development of techniques aimed at DNA selection in many domesticated plants by breeding long-lasting



immunodeficiency genes based on line. These include integrated rows (RILs) produced by reproductive cycle (Burr et al., 1988) and isogenic lines (NILs) produced by repeated RIL interactions in other populations and self-replicating to produce an equal population varying the presence of segments genomic imported from the first RIL near a specific target area (Kaeppler et al., 1993). In addition, homozygous lines can be produced quickly and in very small numbers through double haploid techniques (Guzy-Wrobelska and Szarejko, 2003). These figures cannot be reproduced by transient species, and in grasses, such as perennial ryegrass (*Lolium perenne*), genetic mapping studies use heterozygous pseudo-F1 or pseudo-F2 for people in need of joint care. This requires a lot of labor and may jeopardize human genetics over time.

Current SI and SC information on grass and we discuss how recent advances in genomics and DNA sequencing technologies will help expose the novel SC sources in the grass. In conclusion, we are exploring the possibilities, so far considered impossible, for the SC to direct them to the state of grassroots development programs. In this last section we will focus on how using SC in cabbage grass will help further understanding the basics of heterosis and the ability to control it with new ways to make full use of potential genetic diversity.

Self-incompatibility in the grasses

The SI genes in angiosperms are phylogenetically differentiated and are usually family-specific, reflecting a number of evolutionary mechanisms (Bateman, 1952). Physiological SI processes can be classified as gametophytic or sporophytic, but the specific mechanisms, as well as the number of genes involved, vary between families. The identification of genes that determine SI is achieved only in SI-controlled, SI systems, such as those of Solanaceae, Rosaceae, Plantaginaceae, Brassicaceae and Papaveraceae, discussed in detail in a recent review (Muñoz-Sanz). Despite the involvement of different molecular actors, the basic genetic makeup of all single-locus SI systems is the same. The S locus contains at least two closely related genes that form an unrelated S-haplotype: one gene encapsulates the female SI separator, which is expressed in the pistil, and the other gene encloses the male SI, expressed. in pollen (gametophytically controlled SI) or tapetal cells (spatially controlled SI). In the pollen cycle, male and female decision-making products interact and trigger the SI-blocking response (Brennan et al., 2011).



The gametophytic SI system of Poaceae is composed of two independent loci, designated S and Z, located on chromosomes 1 and 2, respectively (Thorogood et al., 2002). Self-fertilization is prevented when the same haplotypes of S and Z are shared by pollen and discrimination. This dual system was first reported 60 years ago in sunolgrass (*Phalaris coerulescens*) (Hayman, 1956) and rye (*Secale cereale*) (Lundqvist, 1956), later confirmed in *L. perenne* (Cornish et al., 1979). Despite several attempts to identify genes that determine SI, their identity and function remain to be fully elucidated. However, significant improvements have been made in recent years. Using a combination of fine mapping, genetic sequencing, transcriptome analysis and comparative sequence analysis, Manzanares et al. (2016a) identified and evaluated potential gene expression in the L-segment. *perenne* pollen S-locus. Three genes were isolated from the S locus and were further expressed in reproductive tissues, two were regulated in the genitals and one gene controlled by pollen. The gene encoding a protein code (LpSDUF247) containing the DUF247 domain of unknown function appears to be the candidate most likely to determine pollen SI as it has the high level of allelic variability expected in the SI determined genetic structure affected by selective choice, 1994) (Fearon. LPSDUF247 was predicted to have C-terminal trans membrane helix and extracellular extraction, suggesting that it may act as a ligand in the pollen region (Manzanares et al., 2016a). The differences between the allele were not evenly distributed throughout the protein sequence, and the region from the trans membrane domain to the C-terminus was largely preserved. In addition, all the appropriate grass species from which the LPSDUF247 sequence of orthologue was obtained had a codon suspension alteration or significant removal of the predicted protein sequence (Manzanares et al., 2016a). However, no genes were proposed as a S stigma separator candidate, presumably because a gap in the tracked sequence of the S-location identified may prevent its identification.

The use of SC has been used in dietary grasses

In this review section we focus on strategies for using SC in the grass to utilize genetic diversity that is not readily available in a variety of transit grasses. Although challenging to solve and adapt, genetically modified species provide broader sources of genetic diversity than breeding species (Hamrick and Godt, 1996).



Inherited lines and F1hybrids for plant breeding

As forage crops, species of the Poaceae family are economically important, providing feed for ~80 % of bovine milk production (Huyghe et al., 2014) and 70 % of meat production (Wilkins and Humphreys, 2003) worldwide. According to figures from the European Seed Certification Agencies Association (ESCAA) on the area utilized for the production of certified forage seeds in the European Union in 2018, species of the Poaceae are the most important (229 838 ha), together with the Fabaceae (229 878 ha). In addition, they are sown as turf grasses in stadiums and golf courses, parks and domestic lawns, where they are used for sport and recreation.

Currently, forage grasses are mainly improved as populations and released as synthetic cultivars. Cultivar development begins with the evaluation of widely spaced plants for traits such as flowering time, plant habit, disease resistance, abiotic stress tolerance and nutritive value. Selected plants are then polycrossed to create a base population, which is highly heterozygous and heterogeneous. As some traits, notably biomass, cannot reliably be evaluated in individual spaced plants, synthetic populations are produced by crossing selected individuals to produce seed for plot trials that are more representative of grass pastures. Individual full- or half-sib family progeny seeds are collected from polycrossed plants and evaluated in plot trials. Only families from the best progeny plots and those that indicate the best performance when put together (combining ability) proceed to the next generation. Rounds of recurrent phenotypic selection are made to gradually increase performance and at the same time populations are tested for uniformity. When the desired levels of performance and uniformity are reached the population is then intermated in isolation from possible contaminant pollen from outside sources for several generations to produce commercial quantities of seed. Levels of genetic improvement in forage grass yield have been noticeably modest in comparison with other crops (Pembleton et al., 2015). Recent estimates indicated an annual genetic gain for biomass yield of 0.45 % in *L. perenne* and from 0.27 to 0.37 % for *L. multiflorum*, amongst the lowest compared with other major crops (Laidig et al., 2014; McDonagh et al., 2014), including forage *Z. mays* (Taube et al., 2020), where hybrid production technologies have been applied. To redress this deficiency, genomic selection technologies, developed in animal breeding, are currently being applied to recurrent selection programmes of outbreeding temperate forage grasses, most recently by Danish (Esfandyari et al., 2020), French/Belgian (Keep et al., 2020), New Zealand (Arojju et al., 2020), Australian (Jighly et al., 2019) and UK (Grinberg et al., 2016) research groups and breeding companies. Four reviews of the technology applied to outcrossing grasses have been published (Hayes et al., 2013; Yabe et al., 2013; Lin et al., 2014; Talukder and Saha, 2017).



Although not yet used on a commercial scale, hybrid schemes based on the intercrossing of two synthetic populations have been proposed for *L. perenne* (Foster, 1971b), switchgrass (*Panicum virgatum*) (Martinez-Reyna and Vogel, 2008) and other forage crop species (Brummer, 1999). These are assumed to produce ‘chance’ hybrid populations, where up to half of the progeny derives from a direct hybridization of the two populations. More sophisticated strategies were also proposed based on selective restriction of SI diversity (England, 1974; Posselt, 1993; Pembleton *et al.*, 2015) and on within-population crossing using cytoplasmic male-sterile plants (Rouwendal *et al.*, 1992; McDermott *et al.*, 2008; Islam *et al.*, 2014; Vogt *et al.*, 2020). These are predicted to substantially increase the proportion of hybrid seed produced.

The systematic development and use of self-compatible germplasm expands the possible hybrid breeding strategies available for forage grasses. Specifically, it offers the opportunity to design inbred line-based F1 hybrid breeding strategies by producing elite homozygous parental lines capturing favourable genetic diversity. These lines can be crossed for testing heterotic combinations to maximize combining ability in F1 hybrids. Although the challenges are considerable, the transition from populations to inbred line-based F1 hybrid breeding is gaining traction (Begheyn *et al.*, 2016; Do Canto *et al.*, 2016; Herridge *et al.*, 2019) and provides a long-awaited route to increased rates of genetic gain.

A major concern concerning the formation of homozygous inbred lines in forage grasses is the reduced fitness due to repeated cycles of self-pollination, a phenomenon called ‘inbreeding depression’. Inbreeding depression is caused by the unmasking of recessive deleterious mutations through inbreeding, that have accumulated over generations in obligate outcrossers. In outcrossing individuals, their deleterious effects are buffered as they exist mostly in the heterozygous state (Charlesworth and Willis, 2009). One way to overcome inbreeding depression is by eliminating, or ‘purging’, recessive deleterious alleles. This can be achieved by recurrent self-pollination of plants showing high performance at each selfing generation, until fully homozygous plants are created with a similar fitness to the original, highly heterozygous parent. This is particularly important in perennial species, like many of the forage grasses, that regularly reproduce asexually, accumulating recessive deleterious somatic mutations (Zhang *et al.*, 2019).



In grasses, inbreeding depression is severe and dependent on the genotype and number of cycles of inbreeding (Do Canto et al., 2016). Moreover, because of SI, purging deleterious alleles is challenging. Only few and heavily depressed inbred progenies can be obtained by forced self-pollination in self-incompatible plants or by exploiting environmental conditions that favour pseudo-self-compatibility. This has impeded grass breeders in their efforts to produce highly homozygous lines, purged of their genetic load. In self-incompatible genotypes, the process of purging may be facilitated by the fact that, by self-pollination of a single plant, it is possible to produce a larger progeny pool to select from at each self-pollination cycle. Selfing is efficient in eliminating deleterious alleles whose effect on the phenotype are clearly visible and can be selected against, but less effective on mildly deleterious alleles (Boakes and Wang, 2005). As a result, while selfing helps to reduce a large part of the mutation load, it can also accelerate the fixation of deleterious alleles that are difficult to detect. Also, repeated selfing from a highly heterozygous genotype likely results in a reduction of genome size due to the purging of transposable elements and chromosomal knobs, as shown in *Z. mays* (Roessler et al., 2019). Given the absence of empirical data on the impact of systematic selfing in forage grasses, it is difficult to envisage the impact of purging efforts and their effect on traits of agronomic importance. However, as recently demonstrated in potato (*Solanum tuberosum*), overcoming inbreeding depression by incorporating SC in selected germplasm is feasible and provides the possibility of creating novel, valuable inbred germplasm for F1 hybrid breeding (Lindhout et al., 2011; Jansky et al., 2016).

Inherited genes and F1hybrids for plant breeding

As fodder plants, species of the Poaceae family are economically important, providing feed for 80 80% of cow's milk production (Huyghe et al., 2014) and 70% of meat production (Wilkins and Humphreys, 2003) worldwide. According to figures from the European Seed Certification Agencies Association (ESCAA) in the area used for certified fodder production in the European Union in 2018, the species of Poaceae are the most important (229 838 ha), and the Fabaceae (229 878 ha). In addition, they are planted as turf grass in stadiums and golf courses, parks and indoor grasses, where they are used for sports and recreation.

Currently, cabbage grass is highly developed as a population and is being released as a cultivar. Growth of a farmer begins with the evaluation of a separate crop to determine such factors as flowering time, crop habits, disease resistance, abiotic stress tolerance and nutritional value.



Selected plants are then covered with polycrossed to form a population, which is very heterozygous and varied. Since some factors, especially biomass, can not be reliably tested on individual plants, artificial concentrations are generated by cross-pollination in order to produce seed test specimens that strongly represent grassy pastures. Each of the whole family seeds or part of the egg is collected from polycrossed plants and tested on a structural test. Only families from the genealogy and those who show the best performance when combined (the ability to integrate) pass on to the next generation. Cycles of repeated phenotypic selection were made to gradually increase performance and at the same time communities were tested for similarities. When the desired levels of performance and similarity are reached the population is then separated by pollutant pollutants from external sources for several generations to produce commercial seed prices. Genetic improvement rates in cabbage grass harvest have been significantly modest compared to other crops (Pembleton *et al.*, 2015). Recent estimates have shown annual genetic profits with a biomass yield of 0.45% *L. perenne* and from 0.27 to 0.37% *L. multiflorum*, among the very low compared to other large plants (Laidig *et al.*, 2014; McDonagh *et al.*, 2014)), which includes fodder *Z. mays* (Taube *et al.*, 2020), in which hybrid production technology is used. To address this deficiency, genomic selection technology, developed in breeding animals, is currently being used in repetitive selection of low-quality cool grass, most recently by Danish (Esfandyari *et al.*, 2020), French / Belgian (Keep *et al.*, 2020), New Zealand (Arojju *et al.*, 2020), Australian (Jughly *et al.*, 2019) and the UK (Grinberg *et al.*, 2016) research teams and breeding companies. Four reviews of the technology used in the past grass have been published (Hayes *et al.*, 2013; Yabe *et al.*, 2013; Lin *et al.*, 2014; Talukder and Saha, 2017).

Although they have not yet been used on a commercial scale, a hybrid strategy based on a combination of two artificial intelligence suggested by *L. perenne* (Foster, 1971b), switchgrass (*Panicum virgatum*) (Martinez-Reyna and Vogel, 2008) and other fodder plants. species (Brunner, 1999). These are thought to produce the 'opportunity' of the mixed multitude, when it comes to half of the genealogy found in the direct union of the two tribes. Sophisticated strategies are also proposed based on selective prevention of SI diversity (England, 1974; Posselt, 1993; Pembleton *et al.*, 2015) and cross-breeding using sterile cytoplasmic plants (Rouwendal *et al.*, 1992; McDermott; McDermott; ; *et al.*, 2008; Islam *et al.*, 2014; Vogt *et al.*, 2020). This is predicted to significantly increase the proportion of hybrid seeds produced.



Systematic development and the use of a moving germplasm amplify the hybrid breeding techniques available for cabbage grass. Specifically, it offers the opportunity to design hybrid breeding strategies based on the F1 line by producing elite homozygous parent lines that capture good genetic diversity. These lines can be crossed to test for heterotic compounds to increase fusion potential in F1 hybrids. Although the challenges are great, the transition from human to mixed breeding based on the F1 line is steadily increasing (Begheyn *et al.*, 2016; Do Canto *et al.*, 2016; Herridge *et al.*, 2019) and provides long-awaited results. the path to increased levels of genetic profit.

The main thing about the formation of homozygous ornamented lines on cabbage grass is weight loss due to repeated cycles of dexterity, something called 'inbreeding depression'. Inbreeding depression is caused by the disclosure of excessive changes in breeding, which has accumulated over generations from outsiders. In passersby, their harmful effects are prevented as they are most common in the heterozygous state (Charlesworth and Willis, 2009). Another way to overcome the stress of inbreeding is to eliminate, or 'cleanse', the alleles that remove. This can be achieved by multiplying the plants themselves by showing high performance in each individual generation, until fully homozygous plants are created in the same proportions as the original, highly heterozygous parent. This is especially important for long-lasting species, such as large cabbage grasses, which are often sexually active, accumulating genetic mutations (Zhang *et al.*, 2019).

Conclusion

Identifying and exposing the SC base in grass helps to provide a better understanding of the genes involved in SC implantation, as well as information on how SC develops from often incompatible species can help biologists determine the pathways involved in the SI system in grass. This provides details about the natural evolution of the SI system and other SI systems and, more generally, other cell recognition processes in living organisms. Subsequent exploitation of SC has the potential to promote effective breeding methods based on population development or F1 hybrids that use genetic diversity due to mixed power. A groundbreaking study of the heterosis gene conducted by SC will be important for practical application. A systematic study of the agronomic performance of hybrids and the design of efficient seed



production systems will be critical to the successful production of first-generation hybrids or hybrid plants. Given the modification of SC, a study examining heterosis in species that often jumps, and high levels of accumulated heterozygosity and genetic diversity, offers hope of a major contribution to our understanding of heterosis currently found in species and fertile species.

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