# GENETIC AND MOLECULAR GENETIC STUDIES OF SPOROPHYTIC SELF- INCOMPATIBILITY IN *SENECIO SQUALIDUS*




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A thesis submitted for the degree of MSc by Research

Under the supervision of Professor Simon J. Hiscock

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Michaelmas 2018

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## Abstract

Angiosperms are the most successful group of land plants in terms of their diversity, abundance and distribution. In order to ensure obligate outcrossing, hermaphrodite flowers have evolved elaborate systems of pollen-pistil recognition, including self-incompatibility (SI). Research on SI systems in many families such as Brassicaceae, Papaveraceae, and Solanaceae has shown that a range of divergent molecular mechanisms is involved in different families even when they share the same genetic basis. Therefore, to better understand the evolution of these processes in angiosperms and the molecular diversity of SI, one UK species, *Senecio squalidus* (Oxford Ragwort) is taken as an Asteraceae model species to investigate pollen-pistil recognition and SI. This project characterized further the genetics of sporophytic SI (SSI) in *S. squalidus*, in particular the dominance interactions

between *S* haplotypes. Among its low number of *S* haplotypes, novel dominance interactions were observed in pollen (male gametophyte). Then to identify the *S* locus, genomic maps of model plants in the Asteraceae family (sunflower, lettuce, chicory and *Tolpis coronopifolia*) that are reported to contain molecular markers or quantitative trait loci (QTL) associated with SI or self-fertility, were analyczed to identify any shared region. Pairwise comparisons between these genome sequences were conducted and aslo their synteny pattern with the draft genome sequence for *S. squalidus* were searched. Some synteny was revealed between lettuce and *Tolpis*. At the same time, a few linkage groups of *S. squalidus* are highlighted to contain the *S* locus potentially. Finally, the expression pattern and polymorphism between *S* genotypes were investigated for a candidate *S* gene belonging to the *Leucine-Rich Repeat Receptor-Like Kinase (LRR-RLK)* family. All the available *LRR-RLK* homolog sequences of *S. squalidus* were analyzed. Through molecular experiments, they shown to lack the tissue-specific expression pattern and polymorphism characteristics predicted for an *S* gene. So they are considered not to be the *S* determinant of *S. squalidus*. Together, these three lines of researches provide new insights into the genetic and molecular genetic basis of SSI in *S. squalidus*, and establish a better foundation for further research on SI in *S. squalidus* and Asteraceae generally.

## Acknowledgement

I would like to express my foremost heartfelt thank to my supervisor Prof. Simon Hiscock, for his patient encouragement and guidance all time throughout the study. I would also like to thank Prof. Dmitry Filatov and group member Dr. Bruno Nevado for their advice during developing analysis. Particular thanks also go to lovely friends: Edgar Wong, Heather Dun, Wenxi Ding and Gla Inwongwan for their suggestions and pleasant hanging out moments! And thank you to Gem Toes-Crichton for her help and warm chats!

I would also like to express sincere gratitude to Prof. Hugh Dickson and Dr. Adrian Brennan, for their valuable, practical and helpful advice on the big-picture of the study and experimental strategies. And a very special personal thanks to my parents and friends for their endless support and love, that make my journey and improvement possible.

Without any of these, or anyone from either college or university I met here, I would not be this far. I sincerely appreciate it.

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# Introduction

## A. Background

### a.1 Plant mating systems

Nowadays, there are over 300,000 species recognized in 416 families of flowering plants (APG IV). Regarding their diversity and distribution, flowering plants are the most successful group of plants dominating the earth's vegetation today. This success is, in part, the consequence of the evolution of a series of unique reproductive features (such as closed carpels that protect ovule from insects, double fertilization which gives birth to fertilized embryo), and an enormous diversity of sexual systems (Amasino et al. 2017). All these serve one primary function----reproductive success under distinct situations. Therefore, the origin, evolution and maintenance of flowering plants' reproductive systems have aroused tremendous attention.

To achieve the main goal----reproduction, and to meet a range of purposes and requirements, various mating systems have evolved in flowering plants. There are three main mating systems in flowering plants: outbreeding, inbreeding and apomixis (asexual reproduction)(Richards, 1997). They are very different from each other in terms of genetic and evolutionary consequences. So they all have costs or benefits (Barrett, 2010, 2013).

Outbreeding ensures plants mate with distantly related or unrelated individuals, leading to genetic diversity. Subsequently, increased dissimilarity of genes or alleles in the gene pool protects populations against extinction under complex

environments or unexpected pressures. While for inbreeding species, reproductive assurance is of priority. Despite the potential hazard of inbreeding depression, pollen discounting or genetic uniformity that might increase vulnerability when colonizing, inbreeding acts as an efficient and necessary method to produce offspring in severe environments where relative individuals are limited such as for short-lived plants, small populations, colonizing species, and polyploids (Shimizu & Tsuchimatsu, 2015). So approximately 20% of flowering plants still favor predominant selfing as the principal mechanism that drives their evolution (Spencer, 2002). For apomixis, namely agamospermy, populations are genetically invariable due to the mechanism that plants reproduce asexually. However, they have the advantages such as a very high proportion of loci fixed in the heterozygous phase (Richards, 1997).

Thereby, outcrossing accounts for the normal mode of reproduction in around 80% of all plants in the wild and is the most widespread mating system in flowering plants. In practice, about 33% of all flowering plants have a mixed reproductive strategy, allowing for some selfing in mainly outcrossing plants or some transition between selfing and outcrossing according to the environmental pressures (Barrett, 2002; Charlesworth, 2006; Fujii, Kubo, & Takayama, 2016; Richards, 1997). Nevertheless, since selfing populations are phenotypically less variable, a high level of potential for phenotypic plasticity would evolve with generations in response to selection. Hence, in agriculture, successful plant breeding programs often depend on the development of ‘pure breeding line’ by selfing, which is then crossed to produce F1 hybrid seed (Levin, 2001).

The majority (95%) of flowering plants are hermaphrodite (co-sexual), containing both male and female reproductive structure in the same flower. This is most likely the ancestral condition of angiosperms. Consequently, to promote outcrossing, flowering plants have evolved a range of strategies to avoid selfing from external morphological adaptations to internal genetic regulations. Heterostyly and dioecy are observable traits that plants evolve to avoid self-pollination by the spatial arrangement of female and male organs (Barrett, 2002; Charlesworth, 2006). Other flowering plants which show no such morphological adaptations have evolved a stricter and more effective genetically controlled system, specifically responsible for self/non-self-pollen recognition which prevents self-fertilization and inbreeding, and ensures obligate outbreeding among individuals (Hiscock & McInnis, 2003; Richards, 1997). Therefore, determining the ecological and genetic factors that govern the diversification of reproductive systems in flowering plants is a current central topic in evolutionary biology and ecology (Allen, Thorogood, Hegarty, Lexer, & Hiscock, 2011).

## a.2 Self-incompatibility (SI) systems

In flowering plants today, co-sexuality increases the efficiency of insect pollination but at the same time increases the risk of self-pollination. For this reason, self-incompatibility (SI) is favored and selectively maintained in co-sexual flowers leading to the evolution of diverse systems for pollen recognition. The majority of outcrossing is imposed by SI, while the majority of selfing species have evolved by the loss of the SI systems which are rarely regained (Barrett, 2010; Barrett, 2013; Charlesworth, 2006; Igic, Lande, & Kohn, 2008).

Self-incompatibility (SI) is defined as the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination (Richards, 1997). It was first observed by plant cultivators in the 18th century. Now it has been recorded in around 40% of flowering plants species, in at least 100 families (Fujii et al., 2016) and it is predicted most likely to be present in >60% angiosperms (Hiscock & McInnis, 2003). As a genetically determined pollen recognition system, SI prevents self-fertilization and fertilization by other individuals with the same *S*-phenotype (Hiscock & McInnis, 2003). SI is usually controlled by a single *S*-locus (or multiple *S*-loci) with multiple *S*-haplotypes/alleles. Each *S*-haplotype (or *S*-allele) carries both male and female specificity determining genes (*S*-determinants). Because of their physical proximity, *S*-determinants are genetically-tightly linked, inherited as a unit but expressed in the pistil and pollen respectively. Within a population, a large number of alleles at one locus can coexist because of frequency-dependent selection on *S* alleles. Alleles rare in pollen will be favored because they are unlikely to encounter the same allele in stigmas. Conversely, common alleles will be rejected more frequently as they will encounter the same cross-incompatible allele more often. With a few exceptions, all SI systems operate before fertilization, halting pollen development at one or more stages of pollen-pistil interactions such as pollen adhesion, pollen germination, pollen-tube growth or ovule fertilization (Allen & Hiscock, 2008). Discrimination of self or non-self is carried out by specific molecular interactions between the *S*-determinants pair.

SI systems exhibit much diversity regarding genetic and molecular mechanisms (Figure 1). Based on phylogenetic analysis multi-locus SI systems appear to be more ancient (‘primitive’) than single-locus systems because they are confined to the monocots and basal orders of eudicots. Following that, single locus

gametophytic SI (GSI) and sporophytic SI (SSI) systems are found throughout the higher Rosid and Asterid clades of eudicots (Allen & Hiscock, 2008; Hiscock & McInnis, 2003; Igic et al., 2008) (definitions of GSI and SSI are introducted in Chapter I). These distinct molecular mechanisms for avoiding selfing suggest that SI evolved independently and probably multiple times in different lineages of the angiosperms, going through convergent selective pressures (Fujii et al., 2016; Igic et al., 2008; Takayama & Isogai, 2005). Therefore, as SI systems appear to be complex with a collection of divergent mechanisms operating in quite different ways which at the same time same system sit in separate branches of families on the phylogenetic tree, it is hard to determine the ancestral system of SI (Barrett, 2010, 2013; Igic et al., 2008). In the meantime, frequent and irreversible transitions to self-compatibility are found several times independently through phylogenetic analysis (Barrett, 2013; Igic et al., 2008; Nasrallah, 2017). As the evolution of selfing is not merely a degradation of an outcrossing trait but a recurrent configuration that went through adaptation, questions on transitions from ancestral SI, like the evolutionary potentials of the lineages or under what natural selection, also attract much attention and questions (Charlesworth, 2002, 2006).

In brief, SI systems offer a macro-evolutionary advantage to outcrossing populations in terms of enhancing the barriers for pollination within or among species at a long-term time scale. They are also closely correlate with selfing when considering selfing as a breakdown of the SI systems. Based to these phenomena, much work has been done on resolving the underlying genetic and molecular mechanisms. But we seem to only uncover the corner of the whole picture of SI systems. There are still far more mysteries than answers to relevant evolutionary

process or genetic foundation. So it is crucial to characterize SI further at a molecular genetic level in more genera and families.

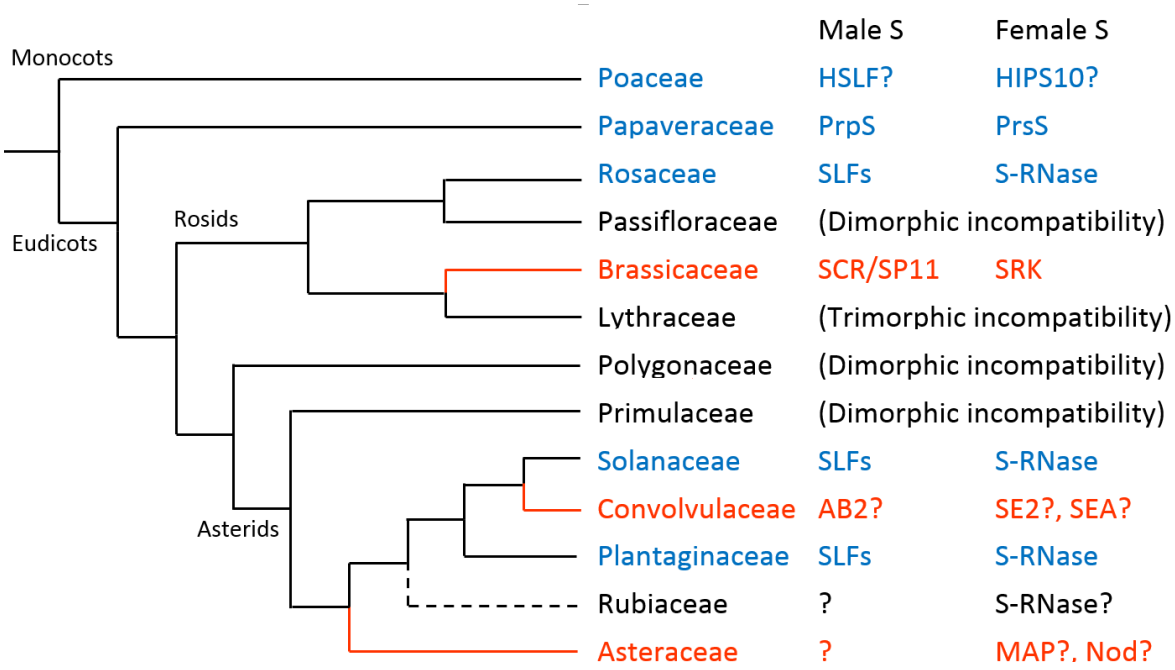



**Figure 1: Phylogenic distribution of different SI systems in flowering plants**. Families of plants and candidates for the male and female determinants of SI are listed. GSI species are marked in blue while homomorphic SSI plants are marked in orange branches, indicating probably derivation in Rosid and Asterid. Question marks indicate functionally untested candidates (for this reason, a dashed line indicates the branch leading to Rubiaceae). Heteromorphic SI systems in Passifloraceae, Lythraceae, Polygonaceae and Primulaceae are considered to have evolved independently. Candidates of the molecular determinants of SI in Poaceae, Convolvulaceae, Rubiaceae and Asteraceae are also listed here. The tree is based on APG IV (originally figure from Fujii et al. 2016).

## a.3 *Senecio squalidus*

*Senecio squalidus* belongs to the large, economically significant daisy family (Asteraceae). Observed as a novel sporophytic SI (SSI) system, understanding the SSI system of *Senecio* is of great potential magnitude for plant breeding research in the Asteraceae.

*Senecio* is one of the largest genera within the daisy family and one of the largest among all angiosperms (containing around 1250 species). It has a worldwide distribution and is one of the few genera occurring in all five regions with a Mediterranean climate (APG IV).

*Senecio squalidus L.* (Oxford Ragwort) is a common sight in Britain now. Originating on Mountain Etna in Sicily, it is a diploid hybrid species between Sicilian *S. aethnensis* (at high altitudes >2,000 m) and *S. chrysanthemifolius* (at low altitudes ≤1,000 m) and was collected for Oxford Botanic Garden in the late 17th century. It then escaped and spread across the UK with the help of the railway network, where the clinker beds provided a similar environment to the lava soils of Mt. Etna. During its establishment and invasion, *S. squalidus* has further hybridized with native self-compatible (SC) tetraploid species *S. vulgaris* to give rise to three new hybrid taxa: a radiate form of *S. vulgaris*, a tetraploid hybrid species (*S. eboracensis*) and an allohexaploid hybrid species (*S. cambrensis*) (Abbott et al., 2009). Furthermore, there has been frequent gene transfer (introgression) between *S. squalidus* and *S. vulgaris*, which has influenced key morphological and ecological traits during its recent evolution in the UK (Abbott, James, Milne, & Gillies, 2003; Kim et al., 2008).

It is believed that the entire UK population is derived from only a small number of individuals that established in allopatric isolation around 300 years ago despite a major population bottleneck (Abbott et al., 2009; Harris, 2002). Self-incompatible species are generally considered not to be good. According to Baker's Law, uniparental reproduction will be advantageous in colonizing populations where pollinators or partners for mating are scarce (Barrett, Colautti, & Eckert, 2008; Cheptou, 2012; Voillemot & Pannell, 2017). However, *S. squalidus* has undergone

a unique invasive history to become a successful colonizer of the British Isles. As a result, British *Senecio* has aroused intense research questions, such as why has *S. squalidus* been so successful as an invasive colonist while it is self-incompatible? What features of its mating system have facilitated it overcoming the severe population bottleneck? How has the self-incompatibility been maintained during its adaptive responses during colonization (Brennan et al. 2006, 2002; Hiscock et al. 2003; Hiscock 2000a; Hiscock 2000b).

Accordingly, *S. squalidus* has become a unique model for studying the interplay between mating system and dispersal, as well as being a model for studying the genetic and molecular basis of SSI. *S. squalidus* is thus an ideal system to explore insights into the short-term evolutionary dynamics of an SI mating system by investigating its successful colonization and the factors that were important in this process.

## B. Significance of researches on SI systems

Since the mating system of the Asteraceae implicates novel genetics and molecular mechanisms, its characterization will help expand the knowledge of the diversity and evolution of plant self-incompatibility systems and plant reproductive systems generally.

Most of the staple crops of the world (e.g., wheat, rice, maize, and beans) are seeds, the yield of which is a product of the breeding systems of the plants and their mating efficiencies. For SI crop plants, the production of pure breeding lines has proved to be a major obstacle to breeders. With a better understanding of the mechanisms of SI, it may be possible to develop a chemical treatment that leads to instant,

reversible self-compatibility to facilitate breeding. Therefore, the study of mating systems is of considerable applied interest.

In addition, as it is rare for a SI species to colonize allopatrically due to their mating restrictions, *Senecio squalidus* becomes intriguing for research as a successful colonizing and locally adapting species in the UK over a relatively short period. Consequently, this study will help to deepen our understanding of population genetics and evolution.

## C. Outline of the thesis

The overall aim of this research was to characterize sporophytic self-incompatibility (SSI) in *Senecio squalidus* further. Specifically, the project studied the genetic basis, genomic basis and molecular genetics underlying the SSI pollen-stigma recognition system of *S. squalidus.*

Results chapter I reports genetic analyses of dominance relationships among several *S* alleles in *S. squalidus.* Controlled crosses were designed, and the number of achenes (fruits) were recorded to determine the level of incompatibility or compatibility. On the basis of the results, genotypes, as well as phenotypes of the gametes, were deduced. Accordingly, novel levels of dominance for haplotypes acting in pollen were revealed.

Results chapter II sought to identify the *S* locus in *S. squalidus*. With the accumulation of genomic resources in Asteraceae and *Senecio*, analysis among species was carried out to assist in looking for the *S*-locus. Using a draft genome sequence for *S. squalidus*, a putative genetic map was generated, following a

comparative analysis with candidate markers or region for the *S* loci of other model species in the Asteraceae. The results do not indicate a united synteny region containing the *S*-locus but do highlight several promising linkage groups.

Results chapter III reports the identification and characterization in *S. squalidus* of the possible homologs of a candidate *S*-determinant gene from chicory (*Cichorium intybus*), a related species of Asteraceae. Following bioinformatics analysis on potential structure and function of the sequences, molecular experiments were conducted to investigate tissue-specific expression in reproductive tissues and polymorphism between individuals carrying different *S* haplotypes/alleles. None of the eight sequences satisfied these criteria needed for the *S* gene, which indicates that all the investigated sequences are unlikely to be involved directly as the *S* determinant in SSI in *Senecio*.

At the end of the thesis, an overall conclusion and general discussion are provided as well as some suggestions for future works on continually exploring the *S* gene in *S. squalidus.* With these efforts, this project contributes more information for understanding the mechanism of sporophytic SI in *S. squalidus* and in the Asteraceae generally.

# Chapter I

## Dominance relationships between *S* alleles in *Senecio squalidus*

### 1.1 Introduction

Conventionally, based on modes of genetic control of pollen self-incompatibility phenotype, SI is classified into two types, gametophytic SI (GSI) and sporophytic SI (SSI) (Figure 2)(Richards, 1997).

The most frequent form of SI in the angiosperms is GSI where the SI phenotype of the pollen is determined by its own gametophytic haploid genotype. The behavior of the individual pollen grain can be controlled by one-locus multi-allelic systems (such as in Rosaceae and Solanaceae) or multi-locus multi-allelic systems (such as two-locus system in the grasses, and four SI loci in Ranunculaceae, Chenopodiaceae and Liliaceae) (Richards, 1997).

In SSI, pollen *S*-specificity is determined by the genotype of the diploid parent anther (the sporophyte) that gave rise to it. SSI has been identified in the Brassicaceae, Asteraceae, Convolvulaceae, Betulaceae, Caryophyllaceae, Sterculiaceae and Polemoniaceae. Intriguingly, dominance relationships often exist between pairs of alleles, namely allowing successful mating to occur between individuals that share one recessive allele. Because dominance interactions between *S* alleles can act independently in pollen and pistil, it often results in complicated patterns of compatibility/self-incompatibility, such as reciprocal differences in compatibility between the same two parents and generation of individuals homozygous for a recessive *S* allele. Alleles that show dominance will be expressed most frequently

at the transcript level, leading to the uneven distribution of *S* allele frequency (Schierup, Vekemans, & Christiansen, 1997). Consequently, in SSI, allelic interactions are critically important for *S*-specificity determination (Mable, Schierup, & Charlesworth, 2003).

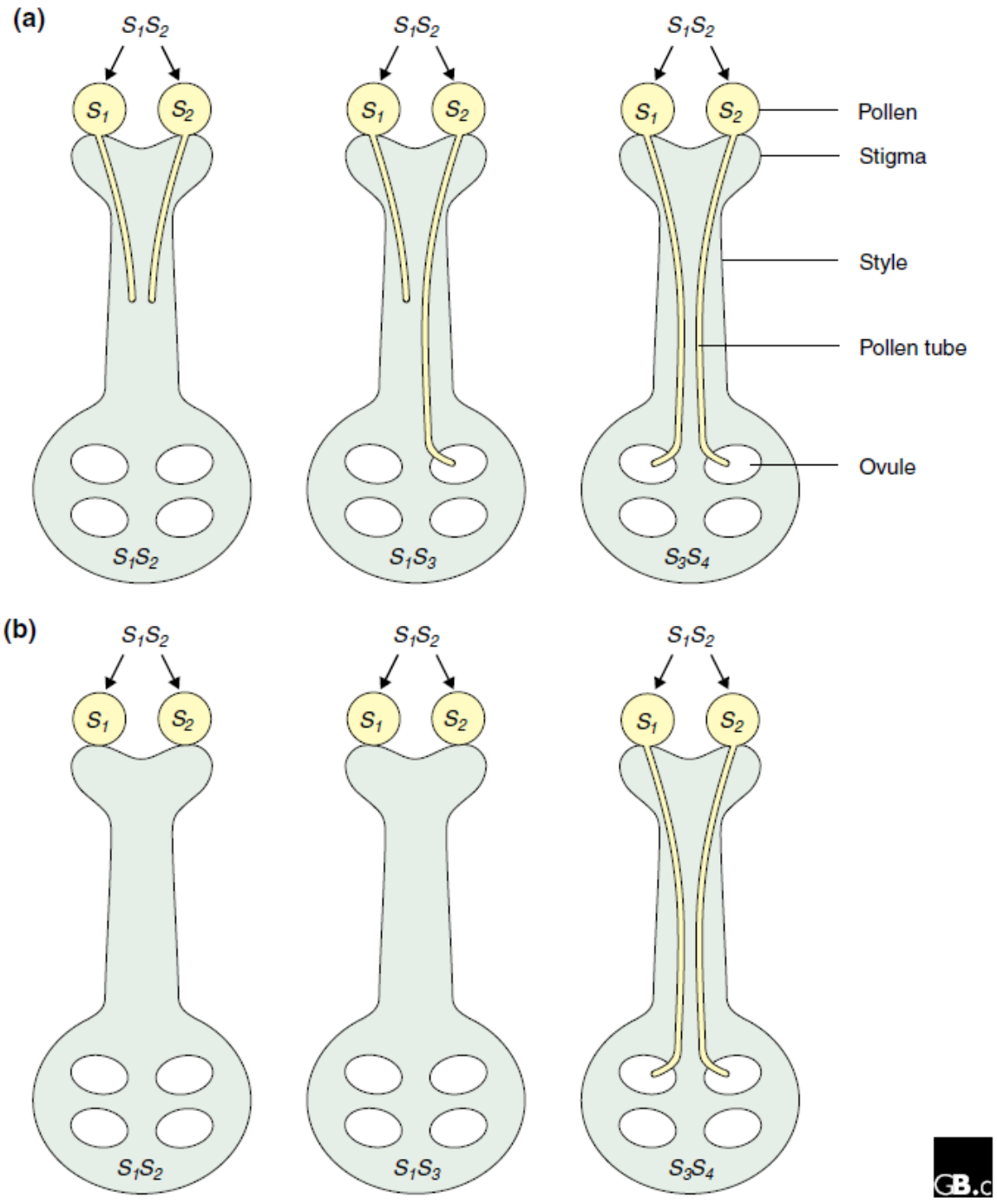


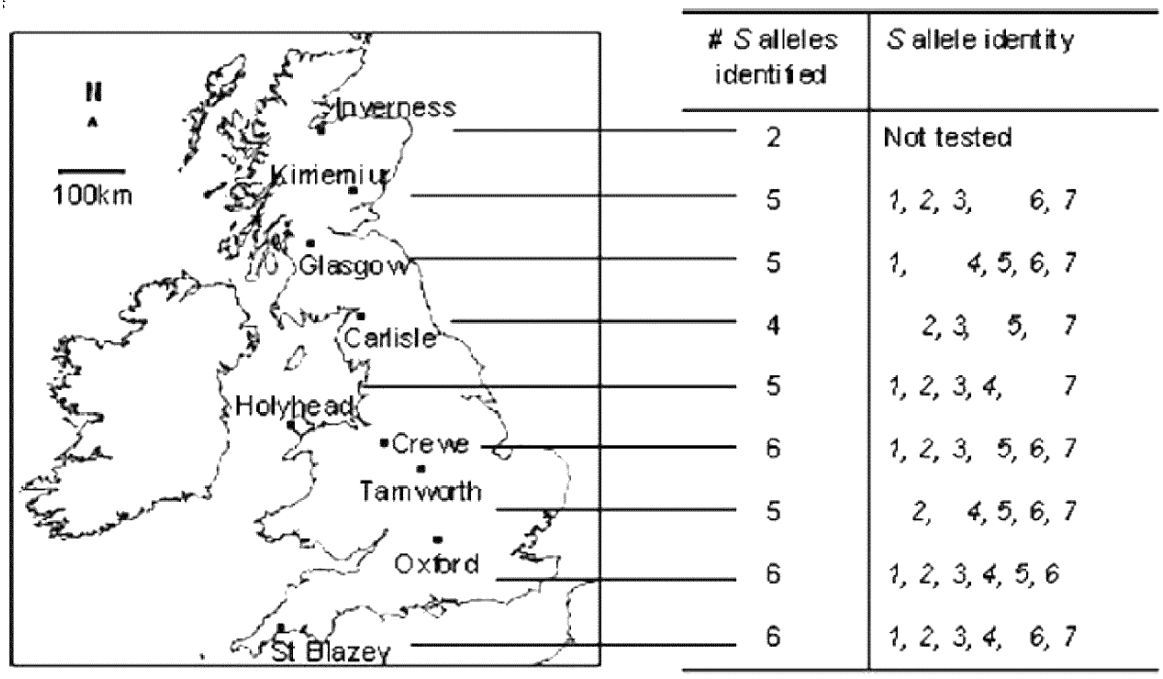

**Figure 2: Genetic control of self-incompatibility (SI).**

**Figure 2: Genetic control of self-incompatibility (SI).** When allelic forms (haplotypes) of the *S* locus are matched in pollen (male tissue) and pistil (female tissue) the pollen is rendered incompatible. The incompatibility response is mediated by a variety of molecular mechanisms in different species. (a) In GSI the SI phenotype of the pollen is determined by its own haploid genome, and the growing pollen tubes are usually inhibited in the style (the *S* alleles of the male and female parent plants and the haplotype of the pollen are indicated). GSI dictates that *S* alleles are expressed co-dominantly in the pistil. Note that 50% of pollen from an $S_1S_2$ individual is compatible with the $S_1S_3$ pistil because half the pollen grains express the $S_1$ allele and half the $S_2$ allele. (b) In SSI the SI phenotype of the pollen is determined by the diploid genome of its parent plant, so if *S* alleles are expressed co-dominantly 'in the pollen', 100% of the pollen from an $S_1S_2$ individual will be inhibited by an $S_1S_3$ pistil (in SSI, pollen is usually inhibited on the stigma). SSI is possible because pollen *S* alleles are expressed sporophytically in diploid cells of the anther tapetum that supplies the *S* proteins to the pollen coating (Hiscock, S. J., 2002).

From preliminary research, it was confirmed that regardless of the current location and time since the colonization of each population, *Senecio squalidus* throughout Britain possess a strong sporophytic SI system with a single multi-allelic *S* locus. This strong SI appears to have been maintained by strong inbreeding depression (Brennan et al. 2006; Hiscock 2000a; Hiscock 2000b). However, it shows typically low numbers of *S* alleles at 7-11 *S* alleles with an average of 5.3 *S* alleles for each population (Figure 3)(Brennan et al., 2002, 2006; Hiscock et al., 2003). This sets a new lower limit on the range of population S allele number estimates for species with SSI which previously ranged from 22 to 43 S alleles.

Besides, all seven *S* alleles in the British population were detected being involved in co-dominance or tissue-specific dominance interactions (Brennan, Tabah, Harris, & Hiscock, 2011; Brennan et al., 2006). For example, for six *S* alleles identified in the Oxford population, there are at least two dominance levels for alleles interacting in pistils ($S_2$, $S_3$, $S_4$, $S_5$, $S_6$ dominant to $S_1$) and at least three dominance levels for

alleles interacting in pollen (Brennan et al., 2011). High level of dominance may increase mate availability and reproductive assurance and then be selected for during colonization (Brennan et al. 2006). So there appears to be a positive relationship between the prevalence of S allele dominance interactions and population S allele number, with higher levels of dominance being associated with smaller numbers of S alleles when different species are compared. In addition, S allele dominance interactions were detected not to be fixed but vary between different populations and continually evolve under certain selection (Brennan et al. 2006, 2011), which can be explained by the independent evolution of S allele dominance within the different populations (Brennan et al. 2003).




| # *S* alleles identified | *S* allele identity |
|---|---|
| 2 | Not tested |
| 5 | 1, 2, 3, 6, 7 |
| 5 | 1, 4, 5, 6, 7 |
| 4 | 2, 3, 5, 7 |
| 5 | 1, 2, 3, 4, 7 |
| 6 | 1, 2, 3, 5, 6, 7 |
| 5 | 2, 4, 5, 6, 7 |
| 6 | 1, 2, 3, 4, 5, 6 |
| 6 | 1, 2, 3, 4, 6, 7 |

**Figure 3: The number, identity and distribution of self-incompatibility (*S*) alleles in *S. squalidus* across the United Kingdom.** Cities where *S. squalidus* samples were collected are indicated on the map. The accordingly number of alleles identified and their given identity are listed (Abbott et al., 2009; Brennan et al., 2006).

Thus, the increased dominance interactions between S alleles, may be an important short-term means of increasing mate availability when S allele numbers are low However, the dominance interactions among these alleles has yet to be fully determined. Additionally, it is suggested that the low *S* allele number might be a consequence of extreme population bottleneck conditions imposed upon individuals in founder populations. Correspondingly, it leads to some other specific features of SSI systems such as pseudo-self-compatibility (low but significant self-seed set in a predominantly SI individual or populations) as a short-term means by which *S. squalidus* escaped the constraints to increasing mate availability for a given number of *S* alleles (Brennan et al. 2006).

Another special event in genetic analyses of SSI is that during diallele crossing, a number of 'anomalous' crossing results were detected regularly. The scattered and seemingly unpredictable nature of these crossing anomalies could not be accommodated within models of SSI based on either one or two loci (Brennan et al. 2002; Hiscock 2000a; Hiscock 2000b). Therefore, anomalies strongly suggest there may be a 'gametophytic element', *G* gene, which may influence the outcome of crosses in certain genetic backgrounds. This possibility was first discussed by Lewis and co-workers in studies on Brassica Raphanus and Asteraceae (Lewis, Verma, & Zuberi, 1988; Zuberi & Lewis, 1988). They suggested a locus *G* that acts gametophytically in complementation to the *S*-locus. Crosses predicted to be cross incompatible were proved to be cross compatible because parents differ for *G* alleles (Richards, 1997). It is hypothesized that *G* is a relic from the original GSI system, which has persisted through into secondary SSI system. Concomitant G and S allelic matching is essential to give the incompatibility reaction in some matched combinations of S alleles. And *G* was shown to be linked loosely to the *S*

locus and to have very few alleles (possibly only two) that were usually present in a homozygous state so that it is hard to be detected in most crosses (Lewis, Verma, & Zuberi, 1988).

This chapter further explored the genetic basis of SSI in *S. squalidus* by attempting to characterize more dominance interactions (different reciprocal compatible/incompatible result pattern) between *S* haplotypes using controlled reciprocal crosses. With this, a more detailed illustration of *S* allele interactions in the Oxford population is achieved.

## 1.2 Materials and Methods

### 1.2.1 Plant materials

Achenes from previous controlled-crosses were planted. Their parental genotypes are listed in Table 1. The former genotype acted as females while the genotype after the crossing mark ‘x’ act as males. The soil media was prepared in a combination of one part of grit sand: one part of perlite: three parts of growing soil, mixed with Imidasect to control Sciarid Fly (0.28g/liter). *S* allele ‘tester plants’ (Brennan et al., 2011) with previously defined genotypes, were also used to assist verification of *S* phenotypes/genotypes deduced by crossing. Preventative biological control for thrips, Amblyline from Syngenta Bioline was used all times. Chemical pesticides (Gazelle SG by Certis, SAVONA by Koppert and Conserve by Fargro) were applied against aphids and thrips by a professional greenhouse manager when the situation became severe. Afterward, plants were given at least two weeks for recovery. All plants were exposed to 12 hrs. light per day. Cuttings and repotting were arranged periodically for maintenance.

**Table 1: Plant materials utilized in experiments.** The upper part of the table recorded the number and parental genotypes of seeds that were used for germination. The genotype in front of the crossing mark ‘x’ act as females while the latter genotype act as males. The lower part of the table displays genotype of the tester plants used and their naming that could be traced back to previous researches

| Seed resource | Female parent | Male parent | Number of Seeds |
|---|---|---|---|
| **$S_1S_1$x$S_1S_2$** | $S_1S_1$ | $S_1S_2$ | 20 |
| **$S_1S_2$x$S_1S_1$** | $S_1S_2$ | $S_1S_1$ | 20 |
| **$S_1S_1$x$S_1S_3$** | $S_1S_1$ | $S_1S_3$ | 20 |
| **$S_1S_3$x$S_1S_1$** | $S_1S_3$ | $S_1S_1$ | 20 |
| **$S_1S_1$x$S_1S_4$** | $S_1S_1$ | $S_1S_4$ | 10 |
| **$S_1S_1$x$S_1S_4$(B)** | $S_1S_1$ | $S_1S_4$ | 8 |
| **$S_1S_4$x$S_1S_1$** | $S_1S_4$ | $S_1S_1$ | 20 |
| **$S_1S_5$x$S_1S_1$(A)** | $S_1S_5$ | $S_1S_1$ | 20 |
| **$S_1S_5$x$S_1S_1$(B)** | $S_1S_5$ | $S_1S_1$ | 20 |
| **$S_1S_1$x$S_1S_6$** | $S_1S_1$ | $S_1S_6$ | 9 |
| **$S_1S_6$x$S_1S_1$** | $S_1S_6$ | $S_1S_1$ | 3 |
| **Genotype of tester plants** | | | **Source** |
| **$S_1S_1$** | | | Bill4 Self3 |
| **$S_1S_2$** | | | Bill4 x Ox2 |
| **$S_1S_3$** | | | Bill4 x Ox4 |
| **$S_1S_4$** | | | Bill4 x Ox22 |
| **$S_1S_5$** | | | Bill4 x Ox27 |
| **$S_1S_6$** | | | Bill4 x Ox19 |

### 1.2.2 Crossing design

When the plants started to grow buds, perforated bags were utilized with twist ties to cover the plants for avoiding contamination ---- unwanted pollination from insects or anemophily. When flower heads opened, pollinations were manually carried out by gently touching one flower head against another. Pollinated flower heads were then covered by emptied tea bags, and sealed with sewing pins to await maturity.

After around four weeks, tea bags with matured fruits were collected and the number of achenes counted. Fertilized achenes tend to appear dark color (brown, black and sometimes green) and plumper than unfertilized ones. When there are fewer than three achenes, it can be referred to strong incompatible result, 3-10 achenes indicate an intermediate situation, and more than ten achenes indicate a fully compatible crossing.

In order to define the genotypes and the phenotype for dominance relationships, inter-individual reciprocal crosses were conducted. First, self-pollinations were manually executed to discern any anomalous leaky SI results. At the same time, the tester plants were checked for their genotype by inter-individual crosses and crossing with tester plants. Then, crosses between tester plants and progenies were carried out to identify the phenotype of individuals. Finally, progeny reciprocal crosses were implemented to observe the tissue-specific dominance. Each cross has at least three replicates within the same parent individuals.

### 1.2.3 Determination on compatible/incompatible patterns

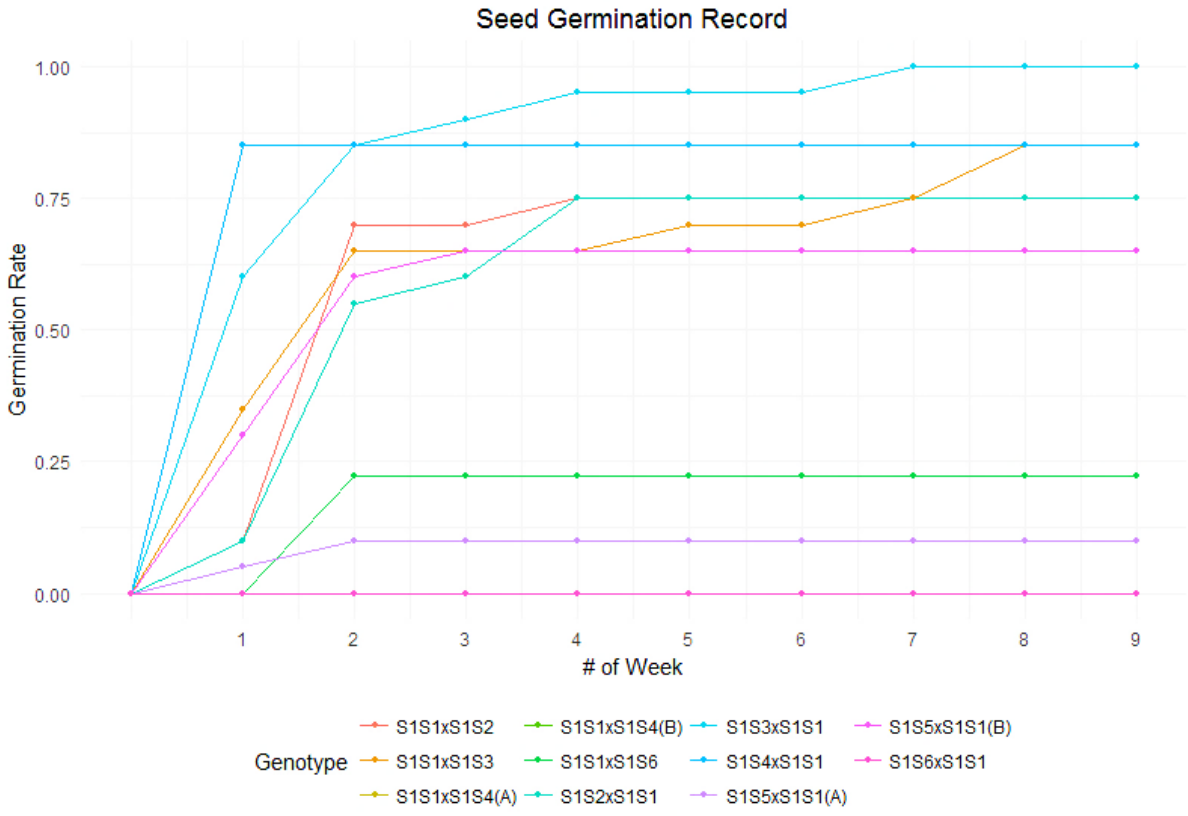
To classify the compatible/incompatible groups, the number of achenes were quantified: an incompatible result (less than three achenes) is marked as -0.5, intermediate situation (3-10 achenes) is marked as +0.5, and a fully compatible crossing (more than ten achenes) is marked as +1.0 score. More weight is given to compatible cross outcomes than intermediate and negative cross outcomes as failed crosses can occur for many reasons (attack by and treatment for pests, or other stress factors) while high seed set is a more reliable indicator of successful compatible pollination. After repetitions, marks were summed up to indicate the

conclusion for the certain pairs of crosses. When the absolute value of the final score is greater than 2.0, the extent of compatibility of this certain pair of the cross is confirmed as either strong compatible (>+2.0) or incompatible (<-2.0).

According to the result of compatible or incompatible pattern between pollen-pistil crossing pair, the phenotype of relevant male or female reproductive tissues are referred with the help of identified phenotype/genotype of tester plants. Then based on phenotypes of reproductive tissues, combining both male and female's *S* phenotypes, the genotype of the individual are indicated. By comparing the phenotype and the genotype, their dominance relationship between the two alleles in different tissue are then indicated. Namely the phenotypic *S* allele is dominant to another *S* allele in the specific reproductive organ. If the phenotypic *S* allele differs in male and female reproductive tissues, it means that they display adverse dominance relationship in reproductive tissues, which makes the dominance hierarchy different in male or female.

## 1.3 Results

From 170 seeds, 103 successfully germinated as displayed in Figure 4. Seeds started to sprout very soon after planting, with most of the tender shoots observed within two weeks. After nine weeks, all vigorous seeds succeeded in germination and no more germination was observed even after more time. Looking into each group for details, $S_1S_1$x$S_1S_4$ progenies failed to germinate. $S_1S_6$x$S_1S_1$ progenies also showed 0% germination. Two out of 20 $S_1S_5$x$S_1S_1$(A) offspring germinated, while 13 out of 20 $S_1S_5$x$S_1S_1$(B) germinated. Moreover, just two individuals germinated from $S_1S_1$x$S_1S_6$ cross. Afterwards, crossings were performed as described in the methods to determine their genotype and phenotype under certain dominance relationships.



| Parental genotypes | Number of Seeds | Number of Seeds Germinated | Naming |
|---|---|---|---|
| $S_1S_1xS_1S_2$ | 20 | 17 | pS2 |
| $S_1S_2xS_1S_1$ | 20 | 15 | sS2 |
| $S_1S_1xS_1S_3$ | 20 | 17 | pS3 |
| $S_1S_3xS_1S_1$ | 20 | 20 | sS3 |
| $S_1S_1xS_1S_4$ | 10 | 0 | pS4 |
| $S_1S_1xS_1S_4$**(B)** | 8 | 0 | pS4B |
| $S_1S_4xS_1S_1$ | 20 | 17 | sS4 |
| $S_1S_5xS_1S_1$**(A)** | 20 | 2 | sS5A |
| $S_1S_5xS_1S_1$**(B)** | 20 | 13 | sS5B |
| $S_1S_1xS_1S_6$ | 9 | 2 | pS6 |
| $S_1S_6xS_1S_1$ | 3 | 0 | sS6 |

**Figure 4: Records of seed germination.** The graph shows the germination rate during weeks after seeds were planted. The table displays the number of seeds sowed and the number of seeds germinated after nine weeks. Afterward, matured individuals were taken for crosses and labeled following the naming rules. For progenies coming from *S* alleles (except $S_1$) acting as a female, they were labeled as sSx, indicating progenies from stigma *Sx* haplotype. Conversely, individuals were named as pSx, indicating origination from pollen *Sx*.

Following the conversion process as mentioned in the method 1.2.3, data with the process of deduction of the *S* allele phenotypes are displayed in Supplementary 1. Taking the $S_6$ allele group as an example (Table 2), pollen donors are shown in the second row and pollen acceptors are listed in the second column. The dominant allele previously identified with confidence in female reproductive tissue is bold in the first row. Final scores are shaded in green if they can be confirmed as fully compatible and in grey if incompatible, whereas a blank cell indicates missing data. Accordingly, the phenotypes of gametes of every individual are inferred and filled in

the first row and the first column as in pollen and pistil separately. By this means, the pS6-1 individual was inferred to be an $S_6S_6$ homozygote and the pS6-2 individual an $S_1S_6$ heterozygote. More importantly, based on present data for pS6-2 and $S_1S_6$ test plants, it can be best interpreted that in the pollen, $S_1$ is dominant over $S_6$ since $S_6$ act as phenotypic allele, whereas in the stigma $S_6$ is dominant over $S_1$.

**Table 2: Crossing results in the pS6 group.** The second row represents males during reciprocal crosses while the second column represents females. Diagonal down border indicates self-incompatible result for self-fertilization. Marks of each pair of crosses are filled in the cell. When shaded in green, a complete compatible result is inferred. When shaded in grey, an incompatible result is settled. Blank cell is due to lack of crosses. Since *Senecio squalidus* exhibit dominance relationship of SSI system, the phenotypes of different reproductive tissue of each individual are showed in the first row and the first column. Alleles previously identified with confident are in bold. Some scores are larger than described in methods (+2.0) are due to more crosses for verification. For pS6-2 individual and $S_1S_6$ tester plant, according to the compatible/incompatible crosses result, their phenotypes of reproductive tissues are identified as S1 in pollen and S6 in stigmas, indicating that $S_1$ is dominant to $S_6$ in pollen while $S_6$ is dominant to $S_1$ in stigmas.

| **Male** / **Female** | **Phenotype** | S6 | S1 | S1 | **S1** |
|---|---|---|---|---|---|
| **Phenotype** | **Naming** | **pS6-1** | **pS6-2** | **Bill4xOX19 $S_1S_6$ tester plant** | **$S_1S_1$ tester plant** |
| S6 | **pS6-1** | | 3 | 2 | 1 |
| S6 | **pS6-2** | -3.5 | | 3 | 4 |
| **S6** | **Bill4xOX19 $S_1S_6$ tester plant** | -2 | -2 | | -1 |
| **S1** | **$S_1S_1$ tester plant** | -1 | -1 | -1.5 | |

With the same deductive process, $S_4$ and $S_5$ were also discovered to be recessive to $S_1$ in male gametes (as displayed in Supplementary 1). Thus far, dominance relationships among *S* alleles can be summarized as showing three apparent levels in male reproductive tissue (Figure 5). Accordingly, the theoretical compatible pattern of $S_4$, $S_5$ and $S_6$ groups are given in Table 3.

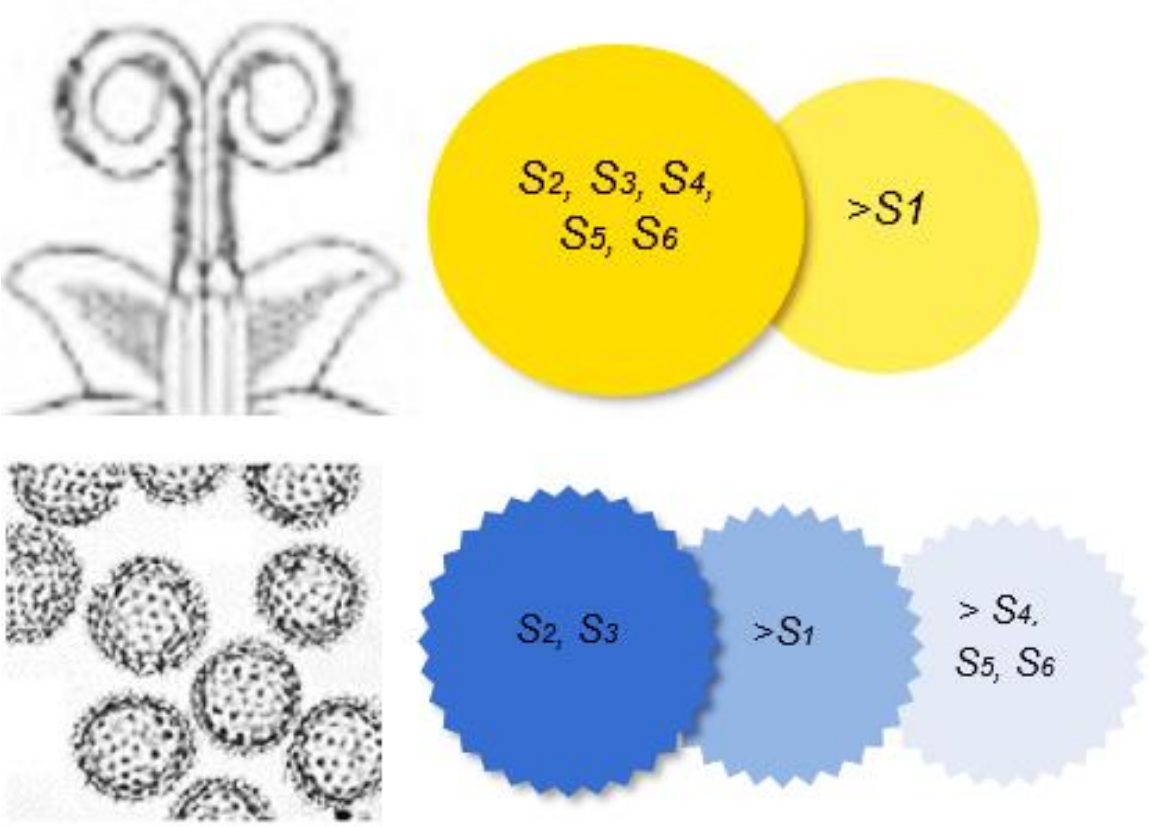



**Figure 5: Dominance relationships among *S* alleles in female and male reproductive tissues respectively.** In stigmas there are two levels of dominance, of which $S_2$, $S_3$, $S_4$, $S_5$ and $S_6$ are all dominant over $S_1$. In pollen, three levels are found, with $S_4$, $S_5$ and $S_6$ are the most recessive alleles to $S_1$, and $S_2$ and $S_3$ are still dominant to $S_1$. '>' indicates dominance relationship among different levels.

## 1.4 Discussion

From earlier work on *S. squalidus*, it is predicted that for the six *S* alleles identified in the Oxford population, there are at least two dominance levels for alleles interacting in stigmas and at least three dominance levels for alleles interacting in pollen (Brennan et al., 2006; Brennan & Hiscock, 2010). In the pistil, $S_2$, $S_3$, $S_4$, $S_5$ and $S_6$ were deduced to be dominant to $S_1$, especially $S_4$ and $S_5$ interact codominantly. Nevertheless, in pollen, $S_5$ is dominant to $S_4$ and was attributed to the most dominant group with the rest alleles.

Here, the dominance hierarchy of *S* alleles in the pistil is confirmed to be as the same pattern as the previous study with two clear levels (Brennan et al., 2011). Interestingly, distinct compatible results from diallel crosses were discovered in pollen as $S_4$, $S_5$ and $S_6$ are recessive to $S_1$. This difference is mainly because this study determined the genotype interaction by direct cross data from progeny with $S_1$ and another *S* allele. However, except for the relationship with $S_1$, other dominance levels were still conservatively evaluated by grouping *S* alleles together into the most dominant class. Hereafter, the results are not conflicting but compromise a more thorough and accurate picture of the dominance relationship from various methods. If combined with the conclusion of Brennan et al. (2011), there are now four dominance levels in pollen, which is that $S_2$, $S_3$ are dominant to $S_1$ respectively, $S_1$ is dominant to both $S_4$, $S_5$ and $S_6$, and $S_4$ is further recessive to $S_5$ (Figure 6).

Additionally, the newly discovered dominance levels are also evidenced by the data of seed germination in Figure 4. When $S_1S_4$ acted as a male parent in crosses with

$S_1S_1$, no seeds germinated but 85% germination rate was observed when $S_1S_4$ acted as a female parent. This is in line with the schematic pattern that when pollen *S*-phenotype is determined by a dominant $S_1$ allele, it leads to an incompatible result from crosses with an $S_1S_1$ female (as described in Table 3). The same pattern is also seen in the $S_6$ offspring groups, revealing the dominance of $S_1$ over $S_6$ in pollen. But it could also be possible that late-acting self-incompatiblity is involved to have a role on halting the maturity of the ovule (Allen & Hiscock, 2008).

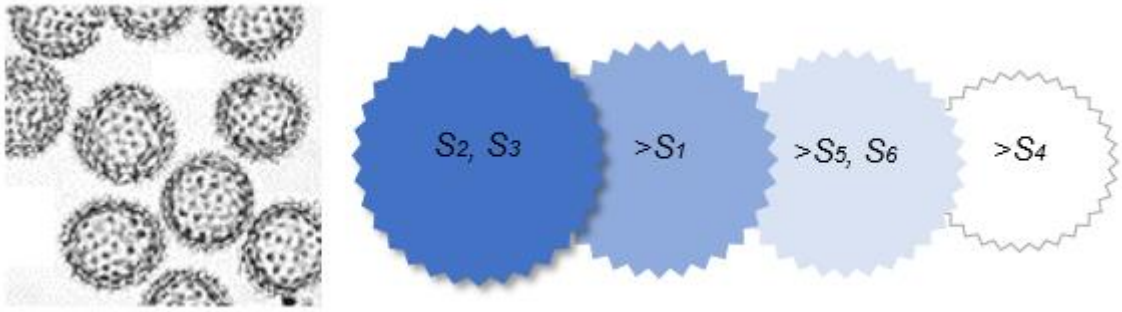



**Figure 6: Dominance relationship among alleles in male reproductive tissues when combining previous studies.** In pollen, four levels are found if merging results from Brennan et al. 2010, with $S_4$ being most recessive allele, $S_5$ and $S_6$ are recessive to $S_1$, and $S_2$ and $S_3$ are still dominant to $S_1$. '>' indicates dominance relationship among different levels.

**Table 3. Schematic diagram of theoretical compatible pattern of $S_4$ and $S_2$ allele.** '-' means incompatible result with no fertile achenes, '+' means compatible crossing with achenes. '\' showed the strong self-incompatible result when conducting self-pollination check. When shaded in green, a complete compatible result is inferred. When shaded in grey, an incompatible result is settled.

For $S_1S_4$ individuals, according to the compatible/incompatible crosses result, their phenotypes of reproductive tissues are identified as S1 in pollen and S4 in stigmas, indicating that $S_1$ is dominant to $S_4$ in pollen while $S_4$ is dominant to $S_1$ in stigmas. The patterns of $S_5$ and $S_6$ alleles are identified as the same as $S_4$ allele.

For $S_1S_2$ individuals, according to the compatible/incompatible crosses result, their phenotypes of reproductive tissues are identified as S2 in both pollen and stigmas,

indicating that $S_2$ is dominant to $S_1$ in both reproductive tissues. The pattern of $S_3$ is identified as the same as $S_2$ allele.

| **Male / Female** | **Phenotype** | **S1** | **S1** | **S1** | **S4** |
|---|---|---|---|---|---|
| **Phenotype** | Genotype | $S_1S_4$- individual 1 | $S_1S_4$- individual 2 | $S_1S_1$ **tester plant** | $S_4S_4$ **tester plant** |
| **S4** | $S_1S_4$- individual 1 | - | + | + | - |
| **S4** | $S_1S_4$- individual 2 | + | - | + | - |
| **S1** | $S_1S_1$ **tester plant** | - | - | - | + |
| **S4** | $S_4S_4$ **tester plant** | + | + | + | - |

| **Male / Female** | Phenotype | S2 | S2 | S1 | S2 |
|---|---|---|---|---|---|
| **Phenotype** | Genotype | $S_1S_2$- individual 1 | $S_1S_2$- individual 2 | $S_1S_1$ | $S_2S_2$ |
| **S2** | $S_1S_2$- individual 1 | - | - | + | - |
| **S2** | $S_1S_2$- individual 2 | - | - | + | - |
| **S1** | $S_1S_1$ | - | - | - | + |
| **S2** | $S_2S_2$ | - | - | + | - |

Furthermore, this kind of different dominance interactions of *S* alleles in reproductive tissues better assists *S. squalidus* in its invasive nature by increasing the proportion of compatible pollination outcomes. Individuals with the same heterozygous *S* genotypes can be fertile if their *S* alleles show different dominance in female and male gametophytes. Then given the small number of *S* alleles within the UK *S. squalidus* population, dominance interactions could be an adaptive feature to increase cross-compatibility to overcome potential mating constraints imposed by a low number of *S* alleles. However, they still follow strong incompatibility when self-pollinated. This counteracting results from parents with exactly the same *S* genotype

may indicate the existence of a *G* gene since *G* gene was responsible for generating the exceptional crossing behavior. Individuals with the same genotype containing $S_4$, $S_5$ or $S_6$ alleles can succeed in cross fertilizing but remain strongly SI when selfing, This could be explained by that where *S* alleles are shared but *G* alleles probably are different. Therefore, together with the *S* gene, the possible activity of a *G* gene provides high flexibility in the SSI system. Taken overall, the high frequency of dominance interactions is considered to be crucial to the success as a colonizer (Brennan et al. 2006; Hiscock et al. 2003;Hiscock 2000).

To sum up, with direct reciprocal crosses among $S_1$ and other *S* alleles, their dominance levels are clear in both female and male reproductive tissues. As reverse dominance relationship was found when doing reciprocol crosses, it is now possible to generate homozygotes ($S_4S_4$, $S_5S_5$ *and* $S_6S_6$). With precious homozygotes, their dominance relationship to each other can be easily studied. And with more individuals with various identified genotypes or allelic combinations, there are more dominance levels that can be identified further in the future.

Chapter II

# Genetic mapping and synteny analysis of the *S*-locus in *Senecio squalidus*

## 2.1 Introduction

The Asteraceae (commonly referred to as the daisy family) is a monophyletic group with worldwide distribution. It is one of the largest families of flowering plants in terms of numbers of currently accepted species (around 1620 genera/23,600 species)(Judd, Campbell, Kellogg, Stevens, & Donoghue, 2008). The family contains numerous economic plants and products for food resources, horticultural ornamentals, and herbal medicine, making it of great potential value in application while it also contains some invasive and weedy species. The Asteraceae, therefore, attracts enormous research interest into its economic species and their wild ancestors in terms of their genetics, physiology, mating system, ecology and evolution.

Research is ongoing on many members of the Asteraceae to investigate reproductive traits and mating systems (including SI), e.g., sunflower (Badouin et al., 2017; Gandhi et al., 2005), chicory (Cadalen et al., 2010; Gonthier et al., 2013), lettuce (Reyes-Chin-Wo et al., 2017), *Artemisia* (Graham et al., 2010), artichoke (Acquadro et al., 2017) and *Tolpis coronopifolia* (Koseva, Crawford, Brown, Mort, & Kelly, 2017). A review on the phylogenetic distribution of SSI within the Asteraceae estimated 63% of species in this family to be SI (presumably all SSI)(Hiscock & McInnis, 2003). Based on the monophyletic evolution (Figure 7), researches can be referred among species in the daisy family to look for the *S* locus.

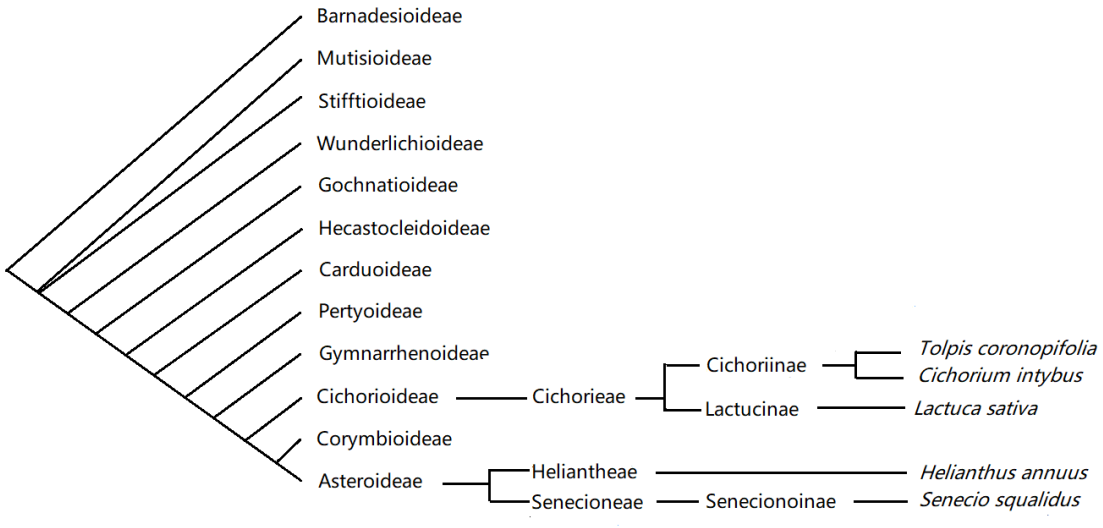



**Figure 7：Phylogenetic tree showing the relationship among Asteraceae species (APG IV).**

In sunflower, a quantitative trait locus (QTL) for self-pollination was mapped to linkage group (LG) 17 (64.6-107.5cM) based on a well-designed backcross population produced from a cross between an elite, self-pollinated line and a wild, self-incompatible population. Meanwhile, LG6 and LG15 are also found to contain the QTL for self-pollination (Gandhi et al., 2005). A decade later, a single QTL for self-compatibility in *Tolpis coronopifolia* was identified on linkage group 2 (Koseva et al., 2017). Two genes (*pectin methylesterase inhibitor* and *inositol polyphosphate kinase*) within this QTL exhibit clear evidence of having a dysfunctional mutation in SC species. Also, several other genes were annotated in pollen development, but details have not been confirmed in their function. In chicory (*Cichorium intybus*), through developed simple sequence repeat markers and sequence-tagged sites markers that are genetically linked with different *S* alleles, the *S*-locus has been mapped to a distal 0.8cM region of linkage group 2 (Gonthier et al., 2013). Comparative synteny mapping of homolog markers to the chicory *S*-locus were conducted in lettuce and has identified LG1 of lettuce to potentially contain the

ancestral *S* locus in this self-compatible species (personal communication with T. Hendriks and M. Quillet, the University of Lille).

Though no *S* determinant gene has been identified in the Asteraceae so far, the high-quality genome and genetic maps generated with developed markers can be of great help to achieve an integrative analysis in a search for the *S*-locus (Table 4). This chapter generated synteny analysis data among these SC/SI QTL and genomes, aiming to identify a putative location for the *S*-locus in *S. squalidus*.

**Table 4: Genome resources (QTL for self-compatible / LG for *S*-locus) used for comparative analysis.** The size of the whole genome, and the number of choromosomes/linkage groups of each species are also listed.

| Species | Genome size sequenced | Chromosome number | QTL for self-compatible / LG for *S*-locus | Reference |
|---|---|---|---|---|
| ***Senecio squalidus*** | 526Mb | 10 | / | / |
| ***Helianthus annuus*** | 3.6Gb | 17 | SC: LG17 (64.6-107.5cM) Selfing: LG6, 15 | Gandhi et al., 2005 |
| ***Tolpis coronopifolia*** | 1.18Gb | 9 | SC: LG2 | Koseva et al., 2017 |
| ***Lactuca sativa*** | 2.38Gb | 9 | SI: LG1 | Per. comm. |
| ***Cichorium intybus*** | 1.3Gb | 9 | SI: LG2 | Gonthier et al., 2013 |

## 2.2 Materials and Methods

### 2.2.1 Construction of linkage group of *Senecio squalidus*

Genomic data of *Senecio squalidus* were assembled by DovetailGenomics, on the individual 'Ox6' genotyped $S_1S_4$ (B. Nevado and S. Hiscock, unpublished). The total size of the genome data reached 526.12 Mb with 68966 scaffolds. Additionally, 699 transcripts single nucleotide polymorphism (SNP) markers, distributed all over the ten linkage groups were developed on hybrids of parental *S. aethnensis* and *S. chrysanthemifolius* (Chapman, Hiscock, & Filatov, 2016). Since the hybrids are ancestral plants of *S. squalidus*, these 699 transcript markers were utilized to construct an analog genetic map of *Senecio squalidus.*

The markers were first aligned to the genome data by BLASTN, setting a minimum threshold at 50 bp length and 95% identity. Markers that are not specific to a single genomic scaffold were discarded. Then the aligned scaffolds were sorted, removing those potential duplicates with a shorter length and lower score if multiple scaffolds hit the same marker. When scaffolds aligned to markers belonging to different linkage groups, the effective sum of the length of hits in each linkage group was calculated. If the sum of length of alignment hits is more than 10,000 bp, at the same time the sum belonging to one of the linkage group is more than doubled the sum length of the rest, the scaffold was included as an efficient alignment to one linkage group. After this series of tidying steps (Figure 8), the scaffolds were collated and visualized using ArkMAP (Paterson & Law, 2013).

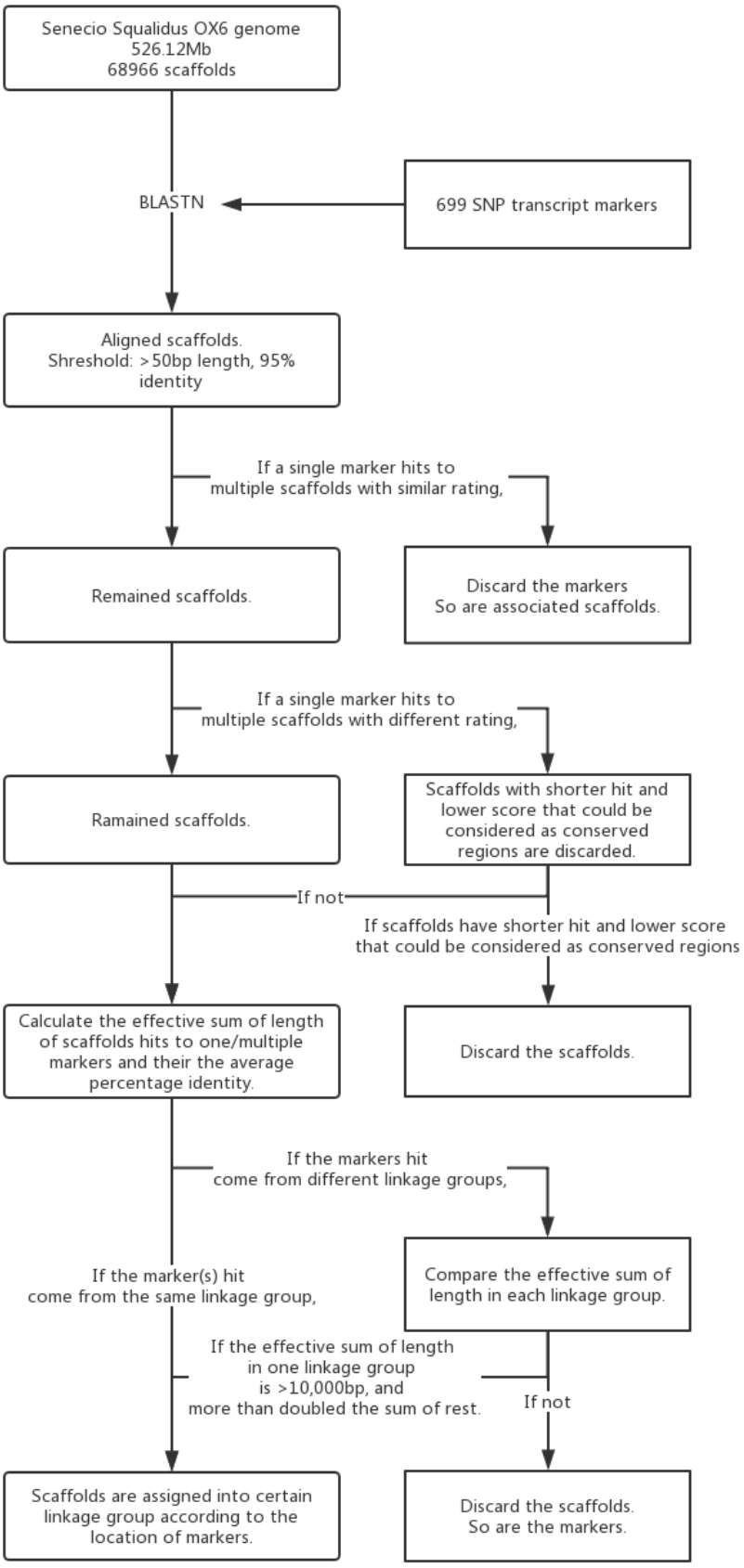



**Figure 8: The flow chart of tidying steps for generating the genetic map of *Senecio squalidus* by SNP transcript markers of hybrids.**

### 2.2.2 Comparative genome analysis

Whole genome sequence of sunflower *Helianthus annuus* was downloaded from NCBI (Badouin et al., 2017). Scaffold sequences and information of markers of *Tolpis coronopifolia* were kindly provided by Koseva and Kelly (Koseva et al., 2017). Genome sequences of chromosomes of lettuce *Lactuca sativa* were downloaded via CoGe website (https://genomevolution.org/coge/, *Lactuca sativa cv Salinas* V8 Plus unmapped sequences). All the information on the genome sequences such as the number of linkage groups and the position of QTL are listed as in Table 4. Together with the genome of *Senecio squalidus*, synteny maps were generated among these species by using default setting of SyMAP (Synteny Mapping and Analysis Program)(Soderlund, Bomhoff, & Nelson, 2011). The genome data of *Senecio* used is the same as described in 2.2.1.

Comparisons were first made between self-compatible QTLs to see if they shared synteny region. Then LG1 of lettuce that is proposed to contain self-incompatible locus were taken into comparison with genome sequences of every other species to study any synteny pattern on the *S* region.

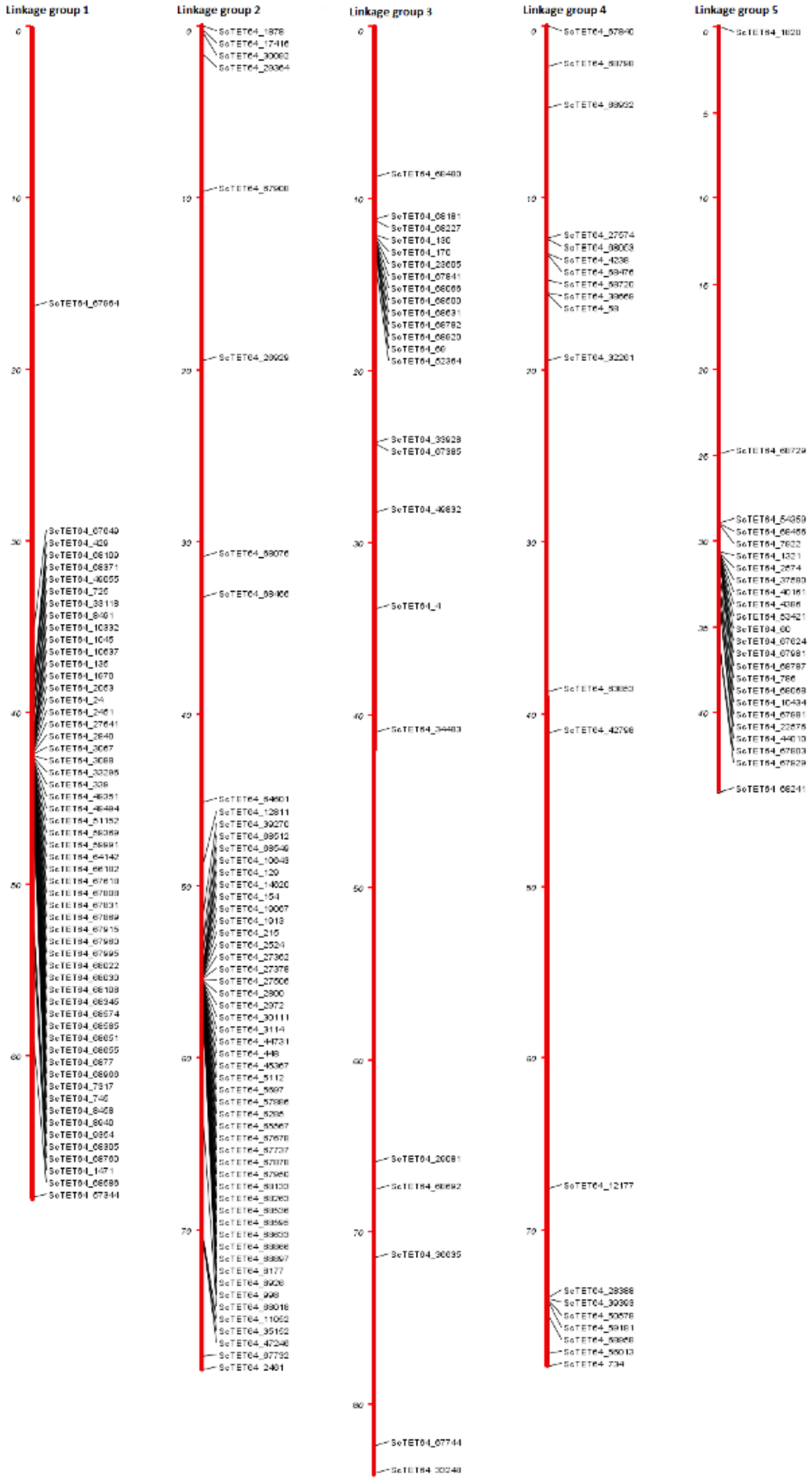
## 2.3 Results

### 2.3.1 Genetic map of *Senecio squalidus*

Among 699 original marker candidates, 567 markers were specifically aligned to the genome scaffolds. 380 scaffolds were found to have matched to the total ten linkage groups (Table 5, Figure 9). The total size of these scaffolds is around 180 Mb, accounting for around 34% coverage of the total of assembled genome. However, they were not equally distributed across linkage groups, with the least number (less than 10 Mb) in LG10 and LG8 (see Table 5). Detailed positions of each scaffold on the genetic map are shown in Supplementary 2.

**Table 5: Summary of linkage group of *Senecio squalidus.*** Number of markers available for *Senecio squalidus* after tiding, number of scaffolds that hit these specific markers, and the total size of sequences sitting in each linkage group are given.

| Linkage group | Number of markers | Number of scaffolds | Total size(bp) |
|---|---|---|---|
| **LG1** | 61 | 57 | 12,048,606 |
| **LG2** | 75 | 56 | 22,364,655 |
| **LG3** | 68 | 24 | 19,858,392 |
| **LG4** | 60 | 21 | 20,693,015 |
| **LG5** | 27 | 23 | 19,441,476 |
| **LG6** | 45 | 32 | 24,327,933 |
| **LG7** | 123 | 67 | 29,933,999 |
| **LG8** | 12 | 10 | 9,842,818 |
| **LG9** | 68 | 65 | 12,289,765 |
| **LG10** | 28 | 25 | 9,247,639 |


Linkage group 1
Linkage group 2
Linkage group 3
Linkage group 4
Linkage group 5

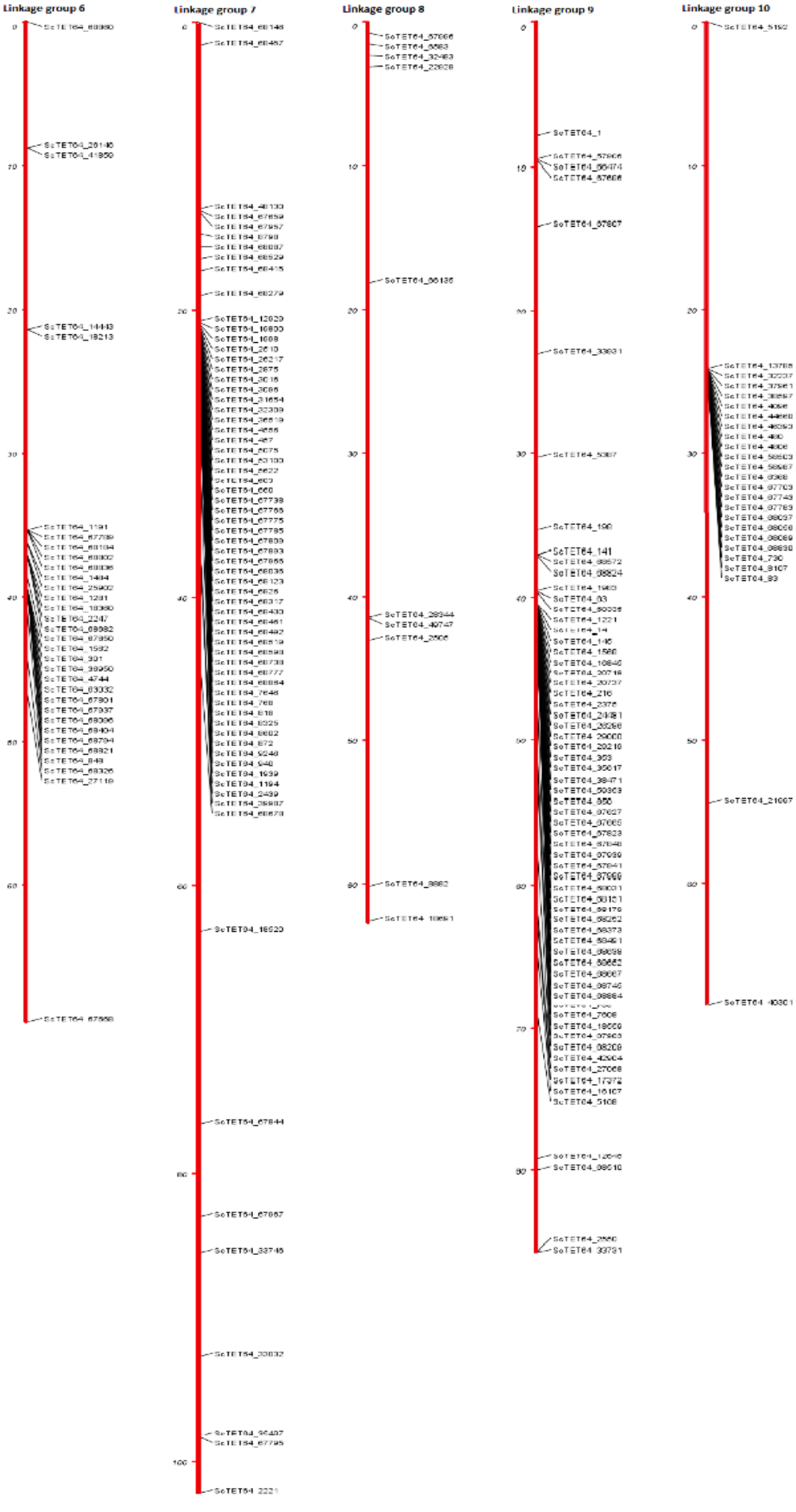

Linkage group 6
Linkage group 7
Linkage group 8
Linkage group 9
Linkage group 10

**Figure 9: Genetic map of *Senecio squalidus*.** Name and position of scaffolds aligned to the genetic map are diagramed. Detailed positions of each scaffold on the genetic map is shown in Supplementary 2.

### 2.3.2 Synteny map among Asteraceae species

To locate the *S*-locus of *S. squalidus*, a comparative genomic analysis among model species in the daisy family was carried out. As whole genome synteny analysis was conducted, the focus was placed on determining the comparative location of QTL associated with SI, 'self-pollination' and self-incompatibility.

First, taking the whole genetic maps of sunflower and *Tolpis*, their synteny map is shown in Figure 10c. When focusing on the linkage groups related to self-compatibility, namely *Tolpis* LG2 and sunflower LG17, they are not finely aligned with each other. For sunflower, LG17 is mainly matched to *Tolpis* LG3 and LG8. As it is also known in sunflower that LG6 and LG15 contain a QTL for self-pollination, their syntenic LG(s) in *Tolpis* were also investigated and found to align to LG3, LG4 and LG7 of *Tolpis* (Figure 10a). Conversely, *Tolpis* LG2 aligns with LG1, LG2, LG4 and LG11 of sunflower (Figure 10b). However, none of these regions have been found to be associated with QTLs involved in selfing or self-incompatibility in sunflower.

Taking the *Senecio squalidus* genome into the analysis, six scaffolds were found to align to *Tolpis* LG2 and 35 scaffolds aligned to sunflower LG17 (Sup 3). Among these scaffolds, 19 scaffolds are located to a certain linkage group of *Senecio*. Synteny regions to sunflower LG17 spread across the genetic map, sitting in six out of 10 *Senecio* linkage groups. One synteny scaffold to *Tolpis* LG2 locates in *Senecio*

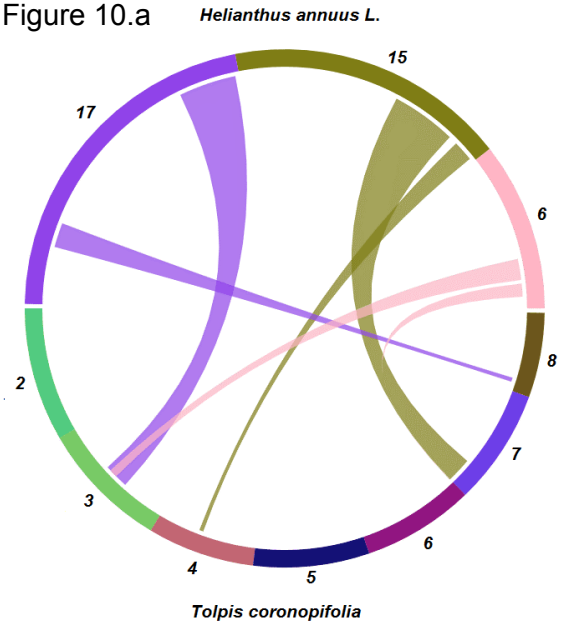
LG9 and one in *Senecio* LG10. Therefore, according to the random distribution of the synteny regions, it is hard to determine any unified loci for SC QTLs in these species.

Finally, all the genome data were taken to be aligned to LG1 of lettuce (Figure 11), which is identified as a homologous linkage group to LG2 in chicory to which the chicory *S*-locus maps. For sunflower, aligned regions fall into multiple chromosomes again (Figure 11a). However, for *Tolpis*, LG2 shows clear synteny with LG1 of lettuce (Figure 11b). As for the *Senecio* genome, 44 scaffolds were identified as syntenic regions to lettuce LG1 (Figure 11c, Figure 12). However, these scaffolds fall into nine out of 10 linkage groups. Several linkage groups, LG4, LG6, LG8, LG9 and LG10 contain similar numbers and sizes (8-10Mb) of synteny regions (Sup 3). Thus based on the data it is also hard to narrow down the site of the *S*-locus further in *S. squalidus*.

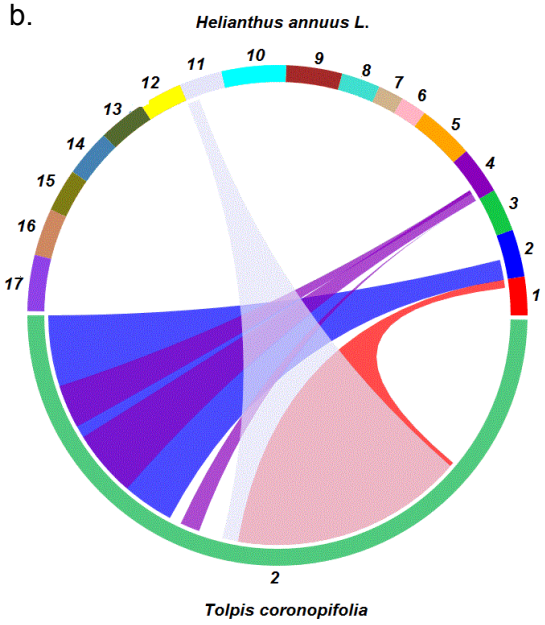
Intriguingly, the whole genome of lettuce and *Tolpis* display a certain synteny pattern (Figure 11d). Except for LG1, all of the *Tolpis* genome regions have a synteny region in lettuce. For instance, *Tolpis* LG2 is exclusively homologous to lettuce LG1, *Tolpis* LG5 is also a strict homolog of lettuce LG2 while *Tolpis* LG8 is aligned to lettuce LG4 with some duplicate regions. The rest of the linkage groups fall into no more than three lettuce linkage groups separately.

**Figure 10: Synteny analysis between *Helianthus annuus L.* and *Tolpis coronopifolia.*** For each circle, regions of upper semi-circle belong to one specie while the lower half indicate linkage groups of another specie. (a) Aligned regions in *Tolpis* (lower half) to sunflower LG6, 15 and 17 (upper half). (b) Aligned regions in sunflower (upper half) to *Tolpis* LG2. (c) Whole genome synteny pattern between two species. These figures show the lack of synteny between the SC QTLs of *Tolpis* and sunflower.

Figure 10.a




b.




c.

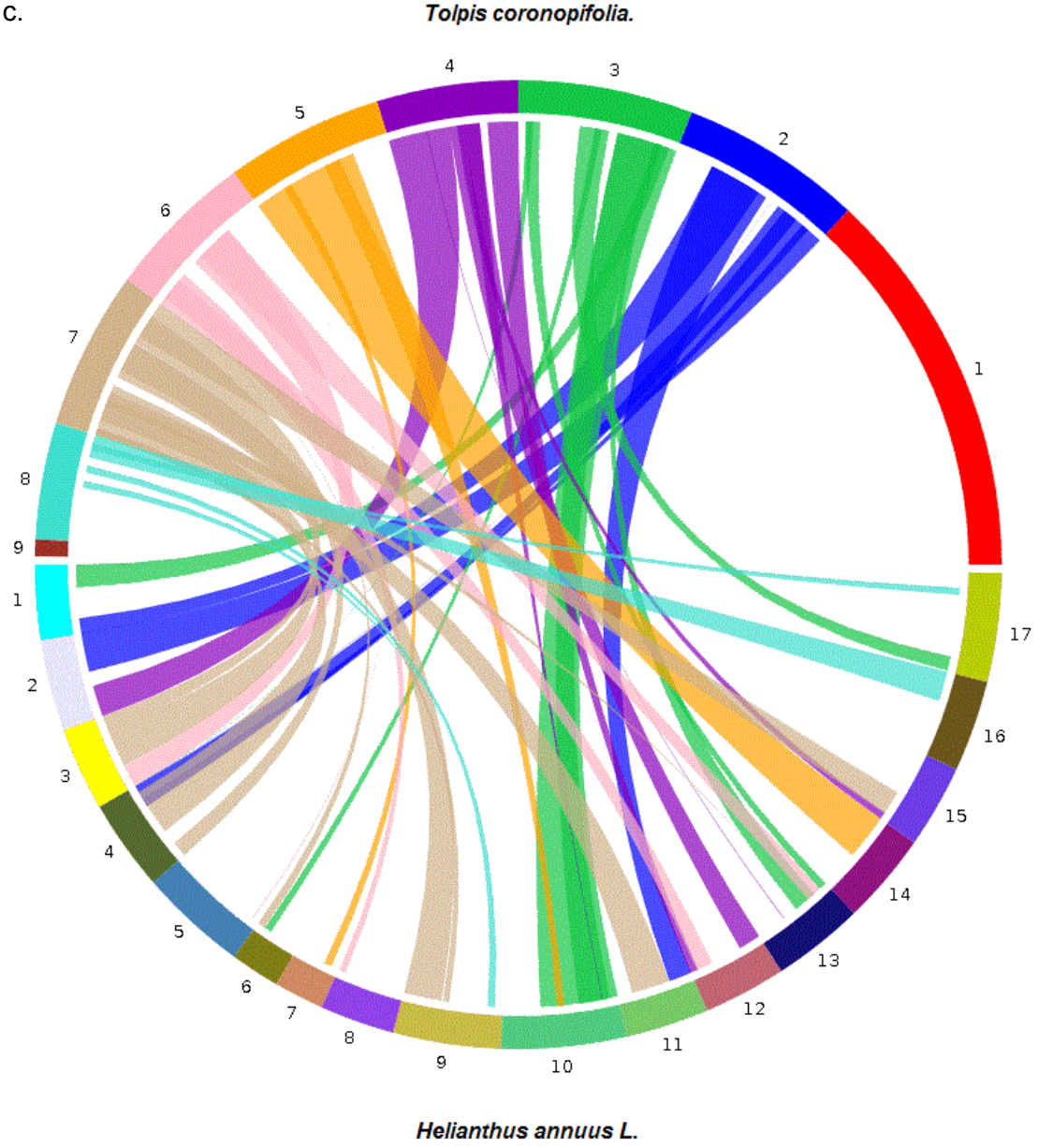
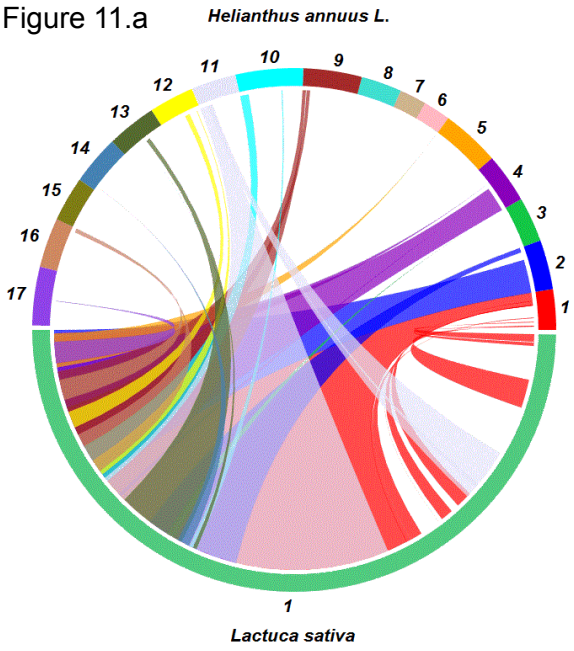
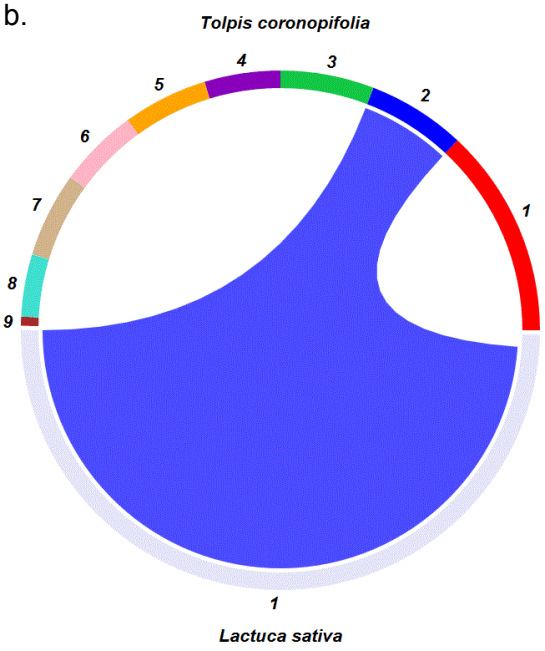


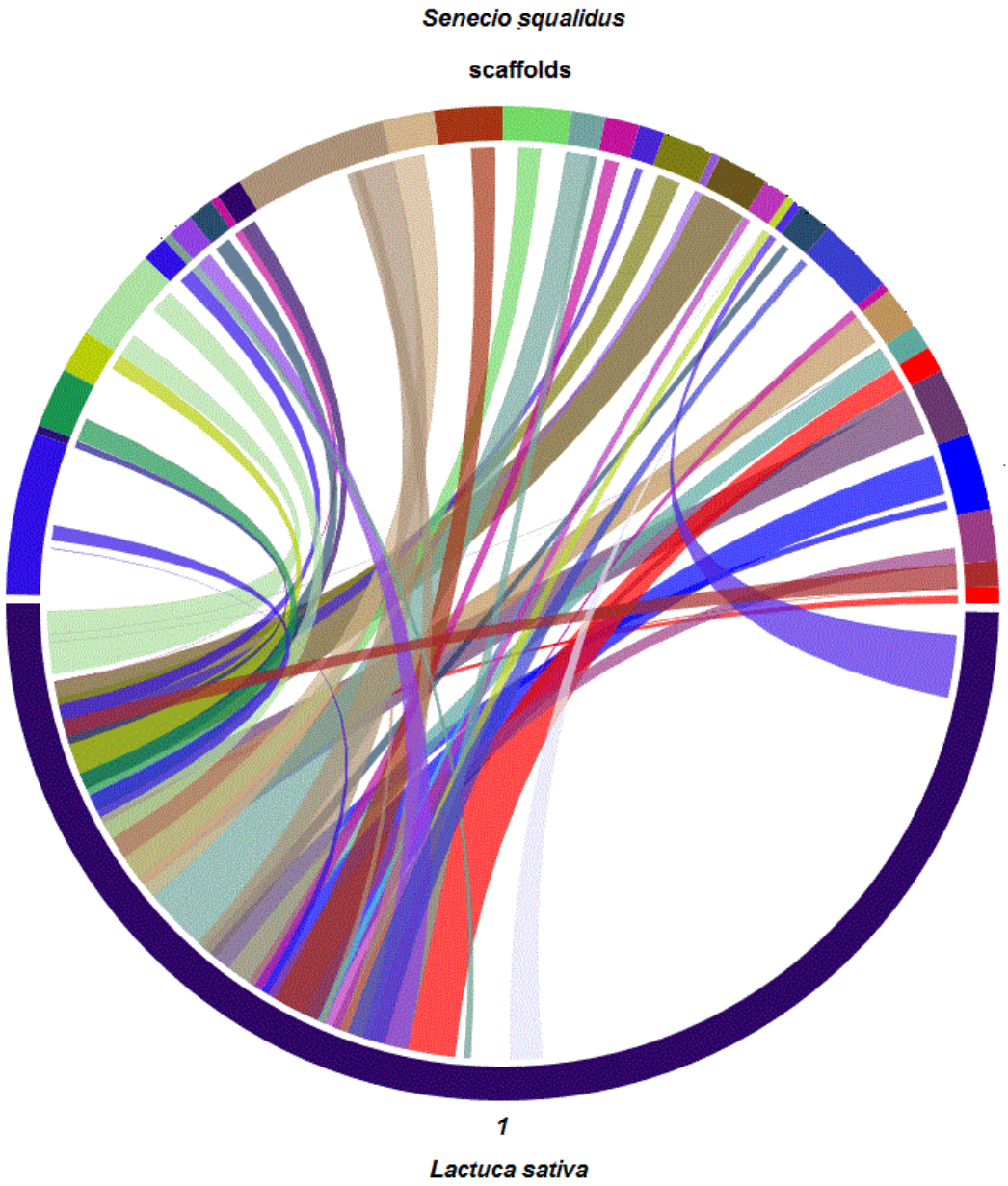
Figure 11.a




b.




c.



d.

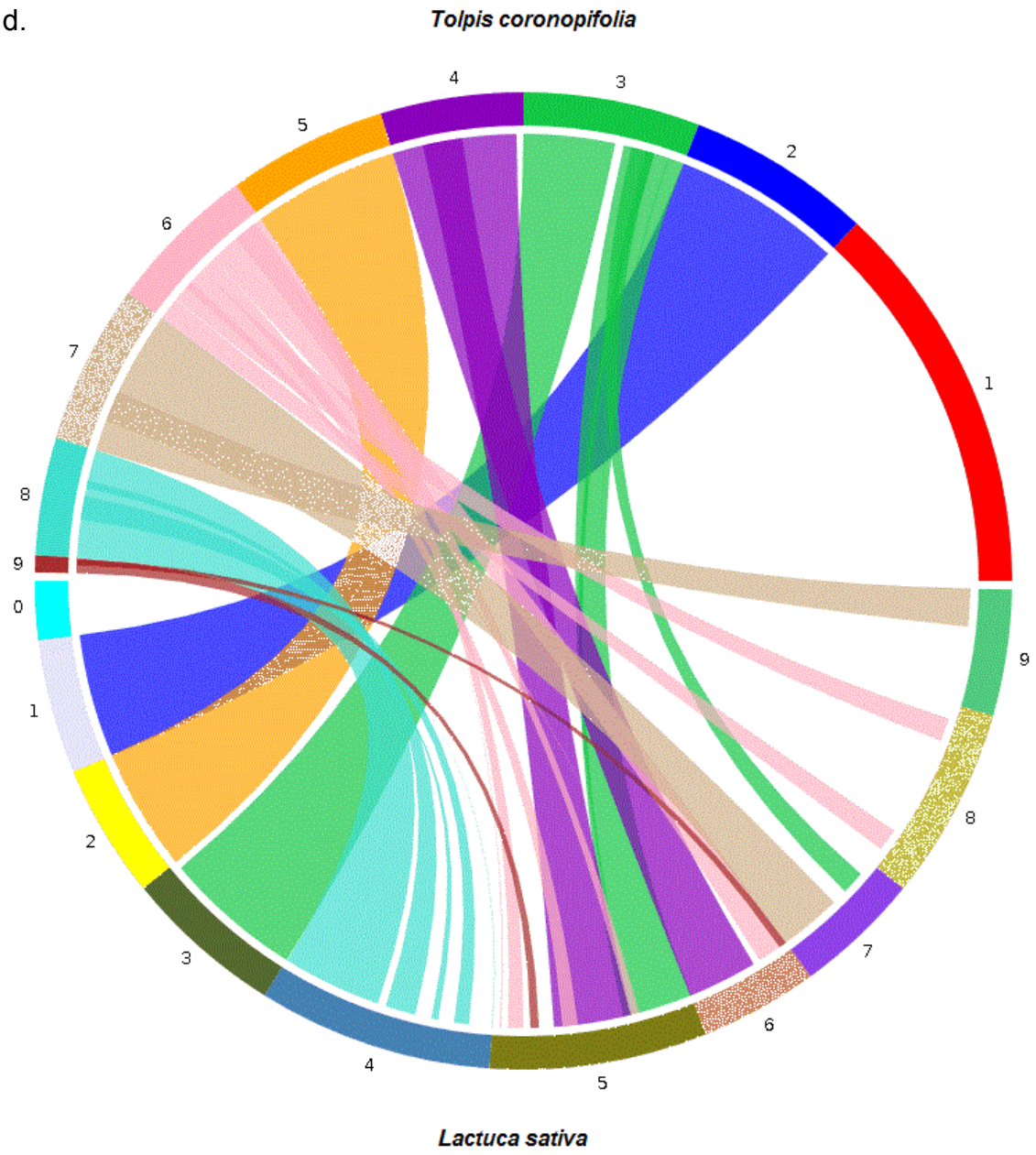
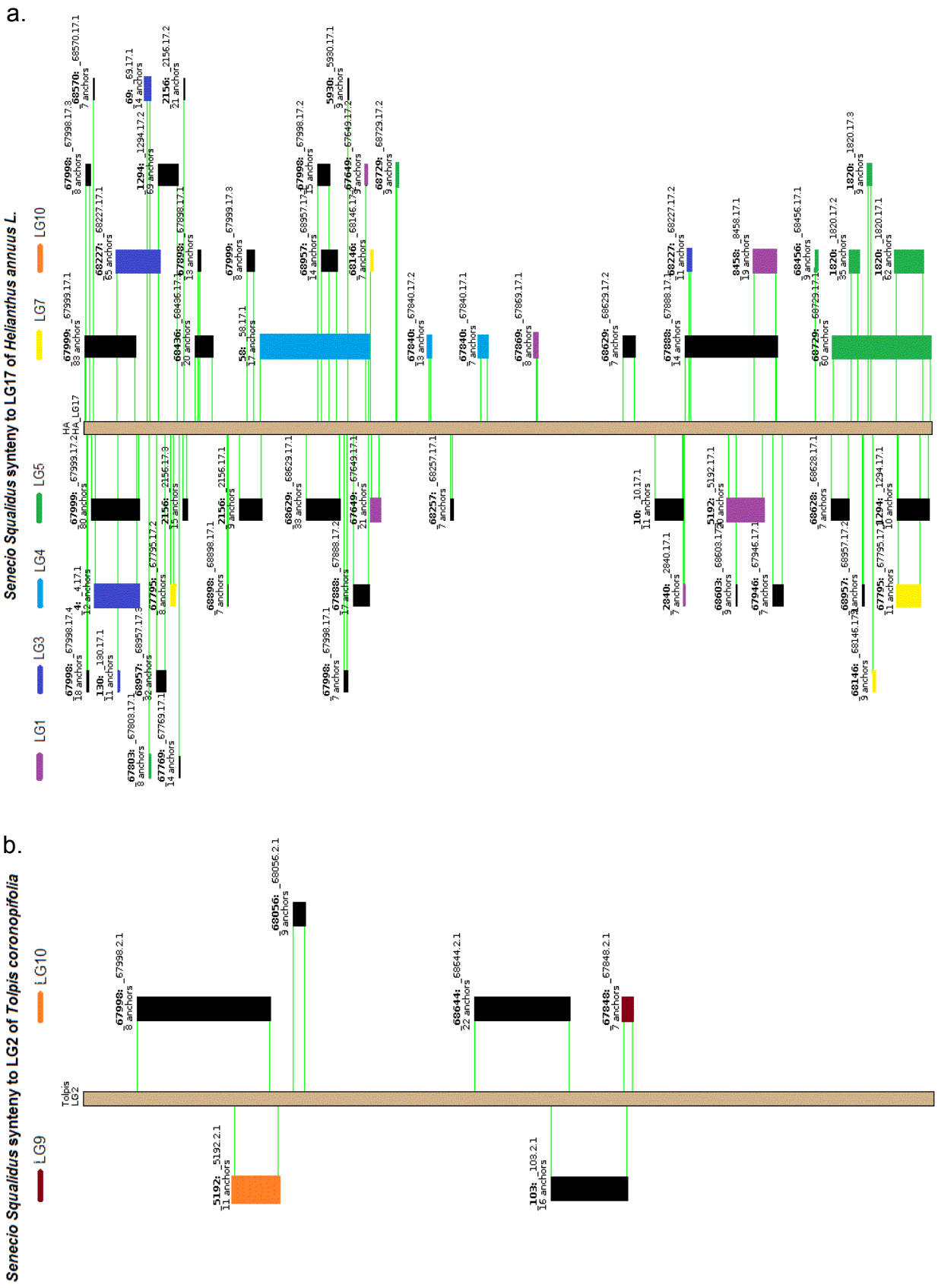



**Figure 11: Synteny regions of *Helianthus annuus L.*, *Tolpis coronopifolia* and *Senecio squalidus* to *Lactuca sativa* LG1. (**a) Sunflower's alignments among 17 chromosomes (upper half) to lettuce LG1. (b) Synteny regions of *Tolpis's* 9 linkgae groups (upper half) to lettuce LG1. (c) A range of aligned regions in *Senecio* to lettuce LG1. (d) Synteny map between *Tolpis coronopifolia* and *Lactuca sativa.*


a.
Senecio Squalidus synteny to LG17 of Helianthus annuus L.
LG1
LG3
LG4
LG5
LG7
LG10
HA_L617
b.
Senecio Squalidus synteny to LG2 of Tolpis coronopifolia
LG9
LG10
Tolpis LG2

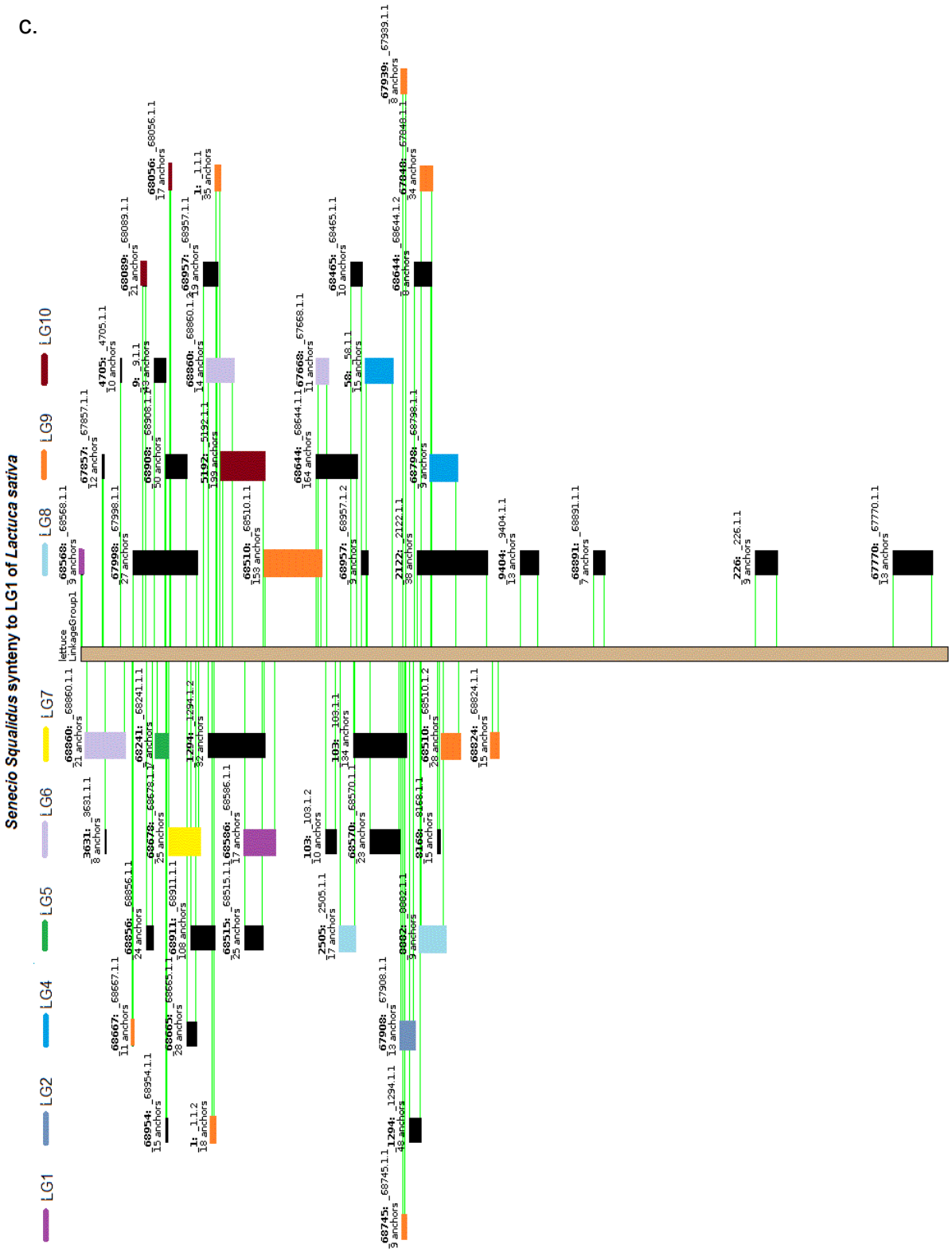



**Figure 12: Synteny map** of *Senecio squalidus* and (a) *Helianthus annuus L.* LG17, (b) *Tolpis coronopifolia* LG2 and (c) *Lactuca sativa* LG1 seperately. Color of blocks indicates the different linkage groups they belong to. Sscaffolds fall into multiple linkage groups, failing to give the location of the *S* locus confidently.

## 2.4 Discussion

The genomes of *Tolpis coronopifolia* and *Lactuca sativa* showed considerable synteny, most notably between LG2 of *T. coronopifolia* and LG1 of *L. sativa.* Therefore, the relationship between SC QTL of *Tolpis* and the ancestral SI locus of lettuce as well as SI locus of chicory could be explored. Accordingly, it is possible that self-compatibility of *Tolpis* is induced by a mutation at the *S*-locus (Koseva et al., 2017), as appears to be the case in lettuce.

Since little similarity was found between sunflower and *Tolpis* or lettuce, the linkage groups associated with selfing QTL in sunflower are probably not related to those associated with SC in *Tolpis* or to the lettuce *S* locus region. In this case, the main reason could be that the breakdown of self-incompatibility is not the result of a mutation or mutations in an *S* gene or at the *S*-locus. SC could result from mutation at a gene or genes (QTL) downstream of the SI recognition interaction (*S*-locus) pathway. On the other hand, since sunflower is more distantly related to *Tolpis* and lettuce than the latter are to each other (see Figure 7), there is still the possibility that QTL in sunflower contained the *S* locus but is undetected due to the diversity, especially when lettuce is self-compatible.

Comparative analysis with the *Senecio squalidus* genome sequence did not give a definitive result to clarify the linkage group that might contain the *S*-locus. It is partly because the number of chromosomes/linkage groups vary from species to species, with unknown duplication, rearrangements or downsizing during their evolution (Fujii et al., 2016). It is also owing to the fragmented nature of the scaffolds, which only allow for low coverage.

Based on the present analysis, LG4, LG6, LG8, LG9 and LG10 of *S. squalidus* are all of high probability in carrying the *S*-locus due to their alignment to lettuce LG1. More information from related species or a genetic map with better quality and coverage of the genome assembly of *S. squalidus* is needed to get a more precise location of the *S*-locus.

# Chapter III

# Characteristics of a candidate *S* gene in *Senecio squalidus*

## 3.1 Introduction

Discrimination of self or non-self is carried out by specific molecular interactions between the *S*-determining pairs of proteins during pollen-pistil interactions. Even though the same genetic basis is shared, different genes and molecules are involved in taking control of SI systems in different species (Figure 13). Among all these distinct SI systems, there are three molecular mechanisms of SI systems discovered now: *SLFs--S-RNase* based GSI in the Solanaceae, *PrpS—PrsS* based GSI in the Papaveraceae, and *SCR/SP11—SRK* based SSI in the Brassicaceae (Fujii et al., 2016; Hiscock & McInnis, 2003; Takayama & Isogai, 2005). They can be largely classified into self-recognition systems and non-self-recognition systems concerning the modes of matching between ligand and receptors (Fujii et al., 2016).

In Solanaceae, molecular recognition occurs when a male gametophyte enters non-self female tissues. Once the non-self element is recognized, the pathway is then triggered to execute detoxification which allows growth of a pollen tube through the style and a compatible interaction (Fujii et al., 2016). In Papaveraceae, the incompatible reaction also occurs in the pollen tube but it is through pollen inhibition and programmed cell death triggered by interactions between the same *S*-haplotype which results in self-rejection (Franklin-tong, 2006; Fujii et al., 2016; Stone & Goring, 2001). Interestingly, Rosaceae includes both strategies: a self-recognition system

in *Prunus* and non-self-recognition system in tribe *Pyreae*. Their *S-RNase*-based GSI is thought to be the common ancestor of Asterid and Rosid, but function in a different way. With SFB (*S*-locus F-box) recognizing self-S-RNase to block self-pollen tube, a group of SFBB proteins recognizes a subset of non-self-S-RNases (Sassa, 2016). The system in the Brassicaceae was the first and the only SSI system in which both pollen and stigma *S*-specificity determinants have been identified. The pollen tubes do not develop properly on the dry type stigma surface when there is a direct molecular interaction between SRK and SP11/SCR of the same haplotype (Hiscock & McInnis, 2003; Hiscock, 2002).

As SI encompasses a collection of divergent mechanisms operating in quite different ways despite the same classical genetic basis, the number of different mechanisms of SI is difficult to predict. Indeed, there are far more genera with distinct molecular mechanisms of SI that are underdeveloped. Therefore, it is still unclear how species transition from one type of SI system to another, or how SI systems can be lost and re-acquired although losses of SI appear far more common than gains (Igic et al., 2008). Therefore, more research on other taxa is of great necessity to gain insight into the macro-evolutionary biology of SI.

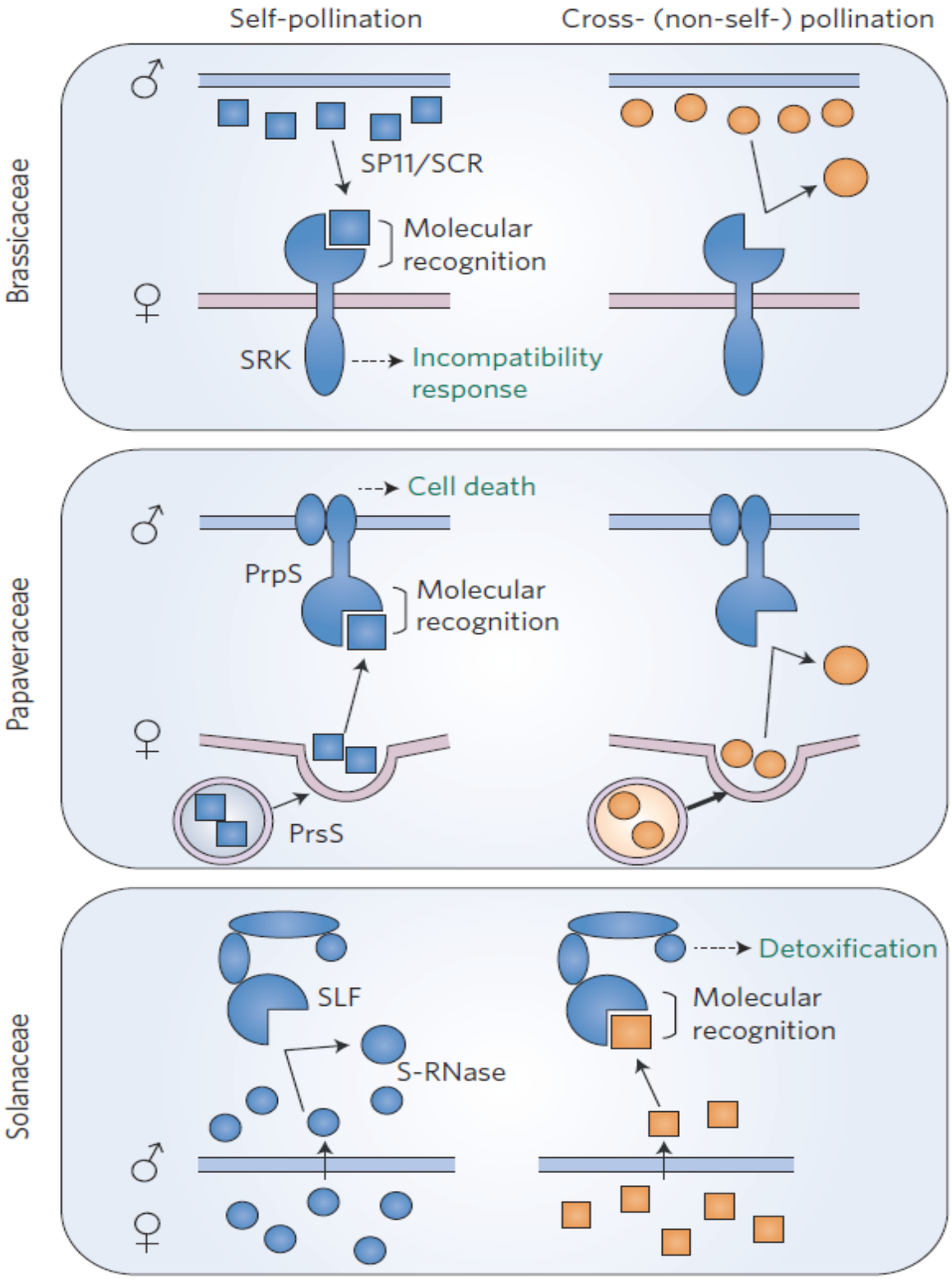



**Figure 13: Self/non-self discrimination in SI of three plant families.** In Brassicaceae and Papaveraceae, molecular recognition occurs when a male gametophyte encounters self female tissues. In Solanaceae, molecular recognition occurs when a male gametophyte enters into non-self female tissues. Blue, self; orange, non-self. (Fujii et al. 2016)

Many efforts have been made to identify the *S* genes controlling the pollen-stigma recognition system of *Senecio squalidus*. As a first step, because of the similarity in the cell biology of SI in *Senecio* and *Brassica*, stigma expressed homologs/orthologues of Brassica *SRK* genes were obtained: *Senecio S-Receptor-Like Kinases, SSRLK-1, SSRLK-2 and SSRLK-3* (Hiscock et al., 2003; Tabah, McInnis, & Hiscock, 2004). However, *SSRLKs* showed no allelic polymorphisms or exclusive expression to the stigma, which are expected characteristics of an *S* gene. Together with the sequence characteristics, *SSRLK* was considered to have a different function to *SRKs* involved in SI in *Brassica*.

As an alternative route, the protein profiles of stigmas from plants carrying defined *S* alleles $S_1$ and $S_2$ were analyzed using isoelectric focusing (IEF). Two potential stigma-specific *S*-linked proteins SSP1 and SSP2 (stigma *S* associated proteins) were identified by their association with *S* haplotypes $S_1$ and $S_2$, respectively. However, with further exploration on the level of polymorphism at the *SSP* locus, they were not associated exclusively with specific *S* phenotypes. Therefore, although *SSP* was expressed exclusively in stigmas and flower buds and located exclusively in the surface secretory papillae of the stigma (upon which pollen is captured and identified as compatible or incompatible), it was concluded not to be the female determinant of SSI. Instead, it turned out to be a new *Stigma-Specific Peroxidase*, the first tissue- and cell-specific class III plant peroxidase gene described. Nevertheless, its specific function is still to be determined (McInnis et al., 2006; McInnis, Costa, Gutiérrez-Marcos, Henderson, & Hiscock, 2005).

To identify more candidate genes involved in pollen-pistil interactions, comparative transcriptomic studies were carried out. A total of 174 stigma-specific genes were

isolated through the generation of pistil-enriched cDNA libraries and further subtraction analyses with its self-compatible relatives (Allen et al. 2011; Allen et al. 2010a; Allen et al. 2010b; Hiscock & Allen 2008). Among them, two related gene copies from *SF21 (sunflower-21)* gene family were identified for further investigation. They were found to be differentially expressed in pollen and pistil tissues: *ORSF21B (Oxford Ragwort SF21B)* is expressed exclusively in mature pollen, while *ORSF21A* is developmentally regulated in the transmitting tissue of the style. However, no polymorphism was present between different *S*-genotypes of *Senecio*. So they are unlikely to play a direct role in *S*-specificity. Nevertheless, many *S* candidates still exist including *membrane-associated protein (MAP)* and *nodulin/mtn3 (Nod)* gene (Allen et al., 2011).

A *leucine-rich repeat receptor-like kinase (LRR-RLK)* gene was first identified in chicory (*Cichorium intybus*). As another member of the Asteraceae family, wild chicory displays sporophytic SI controlled by a single multi-allelic *S* locus as in *Senecio* species and most members of the family. Thus, it is in all likelihood that they share the same genetic and molecular basis of the SSI system. Therefore, findings in chicory are of high relevance of *Senecio* and other Asteraceae. As a high-density genetic map has been generated for chicory (Gonthier et al., 2013) one of the amplified fragment length polymorphism (AFLP) markers and sequence-characterized amplified region (SCAR) markers show polymorphism associated with each of the four defined *S* haplotypes *($S_1/S_2/S_3/S_4$)* in chicory. It was then further characterized as a *leucine-rich repeat receptor-like kinase (LRR-RLK)* gene.

Leucine-rich repeat receptor-like kinases (LRR-RLKs) form one of the most abundant subfamilies of transmembrane receptor-like kinases in plants (Gou et al.,

2010). They are mainly structured with extracellular LRR motifs that may interact with ligands, a transmembrane helix that fixes the protein and a kinase domain. LRR-RLKs play a variety of roles as common components of signal transduction pathways as well as having many other functions in plant systems. Crucially, LRR-RLKs are involved in defense-related activities including host-specific and non-host specific defense responses (Dangl & Jones, 2001). As a 'non-self' surveillance mechanism in innate immunity, the evolutionary solution in plants to the problems of perceiving and responding to pathogens is conceptually similar to SI. This leads to speculation about a common ancestor for genes involved in SI and defense system and the hypothesis that SI evolved from the immunity system. So genes with pathogen defense activity are of particular interest as candidate *S* genes due to the similarity in molecule function between pathogen recognition and self/non-self-recognition functions in reproduction (Antolín-Llovera et al., 2014; Sanabria et al., 2017). Therefore, *LRR-RLK* is considered as a promising candidate for *S*-determinant.

To test the hypothesis that the *LRR-RLK* is the *S* gene, two criteria——tissue specificity and allelic polymorphism could be referred according to the characteristics of the *S* gene. This chapter details the bioinformatics search and moledular experiments on charaterising the *LRR-RLKs.*

## 3.2 Materials and Methods

### 3.2.1 Bioinformatic search

Sequences (in protein sequence format and in nucleotide format including un-translated regions (UTR) and introns) of the four allelic forms of chicory *LRR-RLKs* were kindly provided by T. Hendriks and M. Quillet, the University of Lille. Due to the low-complexity nature and the interspersed repetitive pattern of the leucine-rich repeat section of chicory *LRR-RLKs*, masked nucleotide BLAST databases were built according to the default program of NCBI on the genome database of *Senecio squalidus* (same as previously mentioned). To help improve the quality of hits, assure precision and avoid any omissions, tBLASTn was used against both dust-masked (mask low-complexity sequences only) and window-masked (mask the over-represented sequence data and low-complexity sequences to avoid spurious and confounded results) database as BLAST program offered. Sequences were then filtered by alignment length (>100 amino acid) and percentage identity (>50% identity).

However, as the assembly and annotation of the genome sequences are not of sufficient quality and integrity, these coding sequences were further taken as queries for blast search against the 'Ox6' transcriptome database (information see Table 6, personal communication with B. Nevado). Using de novo prediction via TransDecoder, long ORF (open reading frame) of sequences were extracted and the coding sequences were predicted. These coding sequences were further taken as queries for BLAST search against the 'Ox6' transcriptome database. The corresponding transcriptome contigs were then tested for their expression levels in

different tissues by calculated TPM (transcriptome per million) value from an alignment-based transcriptome abundance estimation method, Trinity (v2.4.0)(Haas et al., 2013). This acted as the preliminary step to assess the expected characteristics of an *S* gene (most notably either pollen or stigma-specific expression) by using distinct transcriptome libraries from different tissues(bud, floret, flower, leaf, root and young leaf).

**Table 6: Information about the transcriptome data used.** 'Trinity transcripts' and 'Trinity genes' mean transcripts/genes assebled and annotated by Trinity program defult algorithm.

| | Total assembled bases | Total number of 'Trinity transcripts' | Total number of 'Trinity genes' |
|---|---|---|---|
| **All tissue** | 95,312,727 | 111,297 | 80,498 |
| **Bud** | 69,578,746 | 74,934 | 53,769 |
| **Floret** | 63,581,780 | 69,505 | 50,535 |
| **Flower** | 62,835,774 | 65,703 | 46,784 |
| **Young leaf** | 60,378,478 | 64,764 | 46,966 |
| **Leaf** | 55,135,017 | 57,925 | 41,185 |
| **Root** | 62,290,320 | 69,660 | 49,746 |

### 3.2.2 Domain and structure prediction

To infer the potential function and structure of the sequences of *S. squalidus*, candidate *LRR-RLK* homologs were put in UniProtKB/Swiss-Prot data bank through BLASTP and the top hit was taken to infer the potential function as well as the kinase domain of *LRR-RLK* homologs. With the online program LRRfinder (http://www.lrrfinder.com/) and TMHMM2.0 (http://www.lrrfinder.com/index.php, Möller et al. 2001), leucine-rich repeats and transmembrane domain were detected.

Differences and divergence between homologs and the top-hit reference gene were analyzed using BLAST and phylogenetic anaylsis (Maximum Likelihood)(SeaView).

### 3.2.3 Tissue Collection

Tissue samples from buds, pollen, stigmas, ray flowers (petals), leaf and stems were collected from individuals of each genotype *($S_1S_1$, $S_1S_2$, $S_1S_3$, $S_1S_4$, $S_1S_5$)* for RNA extraction (QIAGEN, RNeasy Plant Mini Kit & RNase-Free DNase Set). Pure pollen was directly collected in 2ml microcentrifuge tubes through gently shaking the heads of fully opened flowers. The tops of stigmas was cut off quickly using a clean pair of scissors and collected into the tubes. The sample of stigmas contain pollens and anthers, which will affect the result of tissue-expression experiments by rt-PCR described below since results of stigmas come from both female and male reproductive tissues. However, it will not affect further analysis when outcomes were compared with those from pure pollen samples. Petal (ray flowers) were also collected by removing stigmas to limit the possibility of contamination with stigmas or pollen. All the samples were collected at the amount of 100 mg per 2ml microcentrifuge tubes.

### 3.2.4 Semi-quantitative reverse-transcription polymerase chain reaction (rt-PCR)

After getting good quality and quantity of RNA (measured with Nanodrop and Qubit), the first-strand cDNA was generated using SuperScript IV Reverse Transcriptase (Invitrogen). Around 15 ng cDNA was used as a template for semi-quantitative reverse-transcriptive PCR (rt-PCR). Primers were designed based on the

transcriptome sequences from transcriptome database via Primer Premier 6, followed by synthesis by Sigma-Aldrich. The regular reaction reagent was used at 3mM $MgCl_2$, 0.5 nM dNTP, 1 uM each primer and 1.25 Unit Biotaq DNA polymerase and assigned to 35 elongation cycles under appropriate annealing temperatures according to synthesis result. 18s-rRNA (forward: GCAAGGCTCAAACTTAAAGGAA, reverse: CCACCACCCATAGAATCAAGA, a standard version the lab used, personal communication with H. Dun) was used as the internal positive control. At least three replicates were carried out for confirming the PCR results. Products were confirmed by 1% agarose gel electrophoresis with ethidium bromide at 100 volts. Afterward, clear bands were extracted from the gel using the extraction kit (Thermo Scientific) following the manufacturer's protocol.

### 3.2.5 Degenerate polymerase chain reaction (PCR) amplification

To best extract all possible homologs/orthologues of chicory *LRR-RLKs*, degenerate primers were designed based on the conserved regions of chicory amino acid sequences (details given in the result session). Then, normal PCR was conducted as described above, followed by molecular cloning to separate possible multiple products for Sanger sequencing.

### 3.2.6 Molecular cloning

Taking around 50 ng gel clean-up products from PCR through degenerate primers, ligation was performed using pGEM-T Easy Vector System (Promega). After staying overnight at room temperature, 2.5 µl ligation products were mixed with 25 µl competent cells (α-Select Gold Efficiency Chemically Competent Cells, BIOLINE).

Then the cells were plated on LB culture dish containing 50 μl/ml ampicillin, 20 mg/ml X-gal and 1M IPTG. After overnight incubation at 37°C, single round white colonies were picked for further culture growth. Plasmids were extracted from bacterial culture eventually. And single (ApaI) and double digestions (EcoRI) using restriction enzymes from NEB were conducted to confirm the presence of the correct insert following 1% agarose gel electrophoresis check.

### 3.2.7 Sequencing and polymorphism analysis

Cleaned products (either gel extraction from PCR or plasmid extraction from bacterial culture) were sent for Sanger sequencing at the Oxford Zoology Sequencing Centre. PCR primers or normal plasmid primers for T7 and SP6 promoter were used for sequencing from both forward and reverse direction. Then, reads were capped and merged into the whole length, followed by manually double-checking the nuclear base calls with the chromatograms (BioEdit Sequence Alignment Editor). Afterwards, sequences were aligned and imported for generating the Maximum Likelihood divergence tree via SeaView default program.

## 3.3 Results

### 3.3.1 Homolog to chicory *LRR-RLK* in *Senecio squalidus*

Through the bioinformatics search, three scaffolds of *Senecio squalidus* (ScTET64_8882, ScTET64_21167 and ScTET64_2381) rank high in BLAST against the four chicory *LRR-RLK* alleles. They all hit the same contig (c36588_g2). Meanwhile, the aligned region of ScTET64_2381 scaffold hit an additional five contigs. From calculated TPM (transcriptome per million) value, expression patterns of annotated isoforms of these contigs are showed (Figure 14, Sup 4). Among them, four isoforms displayed a similar pattern as for a predicted *S* gene. The expression level of c36588_g2_i1 (an isoformatic sequence from transcriptomic annotation) appeared to show developmental regulation in reproductive organs, which starts to increase in the bud, reach the peak in florets, followed a drop when the flower matured but is not expressed in leaf or root. The isoform c36588_g2_i4 showed the same trend as i1 but is expressed at a much higher level, with the highest expression in floret. Expression of isoform c36588_g2_i10 is most abundant in the bud, slightly decreased in floret but still more than the amount in vegetative tissues. Finally, although little expression was found in leaf, c36143_g2_i1 is comparatively highly expressed in bud and flower, which still shows potential to being involved in flower development.

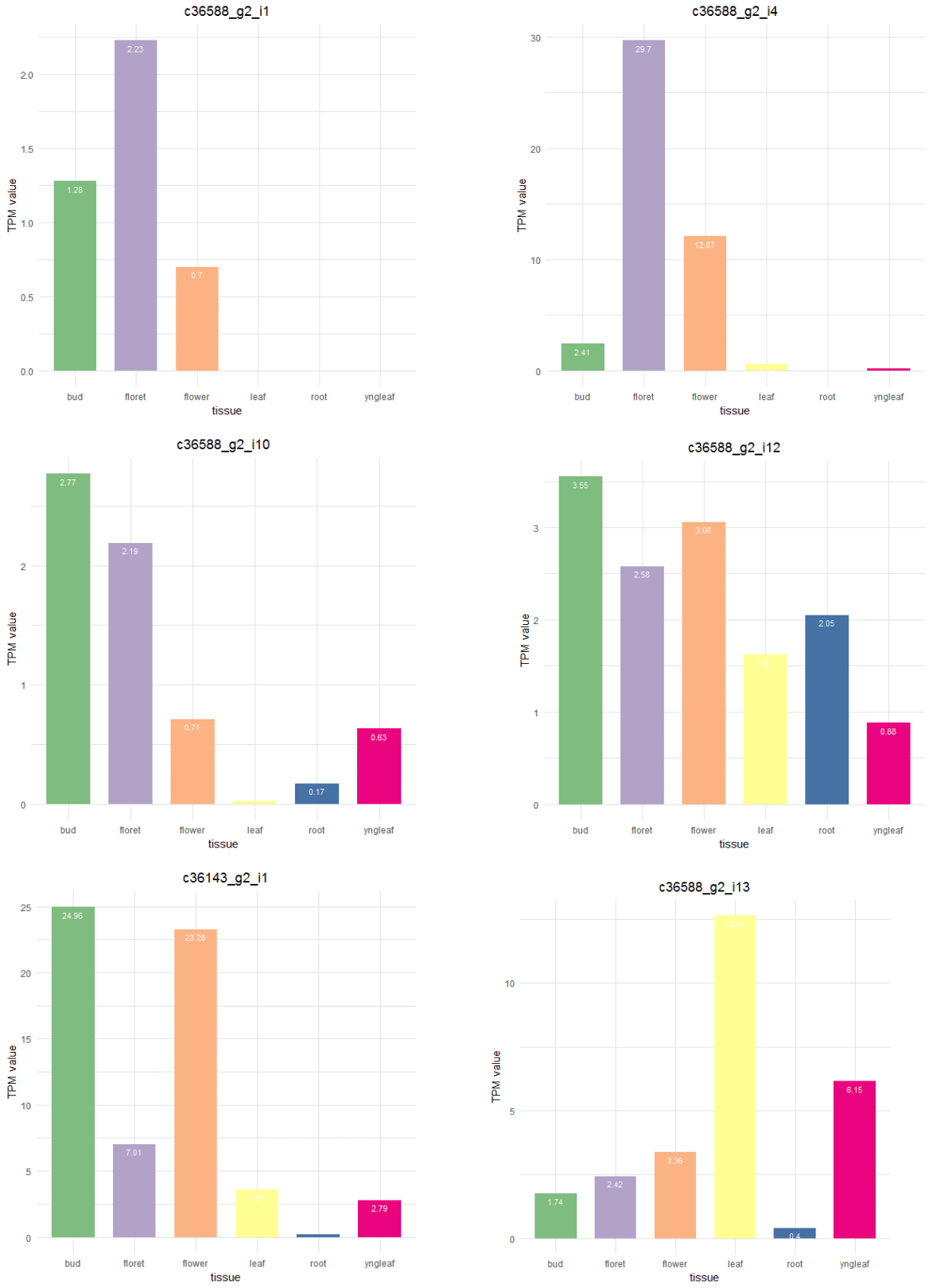



**Figure 14: Calculated TPM (transcriptome per million) values of key isoforms for different tissues (bud, floret, flower, leaf, root and young leaf).** Expression pattern of each isoform is shown. Extra results of the rest isoforms that display an unsimilar patten of the *S* allele are showed in the appendix.

Promising sequences were then examined in detail for potential domains and prediction in secondary structure as described in method 3.2.2. No leucine-rich repeats were found in contig c36143_g2_i1, so this contig was discarded. The rest of the contigs all contained a string of leucine-rich repeats and a transmembrane region. The divergence among these contigs is displayed in Table 7. *LRR-RLK* alleles in chicory share at least 70% identity. They can be further divided into two groups, with chicory $S_1$ and $S_2$ (91% identical) and $S_3$ and $S_4$ (87% identical), similarity between the two groups is around 75% similarity. In the meantime, the sequences of *S. squalidus* showed around 60% similarity with chicory *LRR-RLKs*, at the length of around 1,000 amino acids. Among all the isoforms of *Senecio*, they share at least 60% similarity within the species. i1 is 100% identical to i11 but with a shorter length. With a high identity at 98%, i1 can be grouped with i12. Similarly, i4 and i10 are grouped. Noticeably, although i12 displays high similarity with i1, their predicted differential expressions in tissues present a completely distinct pattern (Figure 15).

To further characterize the *LRR-RLKs*, homologs/orthologues of c36588_g2 in protein data bank were searched to infer their potential function. The top hit turned out to be *MDIS1-interacting receptor-like kinase 2-like* in *Helianthus annuus* (XP_022034232) from the refseq_protein database with at least 55% identity. Moreover, including the annotated XP_022034232, the most relevant protein from SwissProt in *Arabidopsis* direct to MIK2 (Q8VZG8, AT4G08850), with percentage identity around 45%, bitscore around 800, and e-value equaling 0. Taking LRR-RLKs of chicory as a query sequence for BLASTP, it also gave *MIK2* as the top hit. Therefore, MIK2 can be considered as the closest homolog so far for both chicory and *Senecio LRR-RLKs.*

**Table 7: Divergence among alleles of LRR-RLKs in chicory, isoforms of c36588_g2 and scaffolds in *Senecio*.** Percentage identity and the length of protein are shown. The shade of brown shows the level of percentage identity for each pair.

| | | chicory_S1 | | chicory_S2 | | chicory_S3 | | chicory_S4 | |
|---|---|---|---|---|---|---|---|---|---|
| name of seq. | length(aa) | pident (%) | length (aa) | pident (%) | length (aa) | pident (%) | length (aa) | pident (%) | length (aa) |
| chicory_S1 | 1038 | | | | | | | | |
| chicory_S2 | 1038 | 91.233 | 1038 | | | | | | |
| chicory_S3 | 1034 | 76.186 | 1033 | 76.767 | 1033 | | | | |
| chicory_S4 | 1033 | 74.225 | 1032 | 74.612 | 1032 | 87.911 | 1034 | | |
| c36588_g2_i1=i11 | 966 | 61.786 | 963 | 61.731 | 959 | 62.917 | 960 | 61.314 | 959 |
| c36588_g2_i4 | 1041 | 62.717 | 1038 | 62.609 | 1035 | 62.852 | 1031 | 62.524 | 1030 |
| c36588_g2_i10 | 1041 | 62.609 | 1035 | 62.415 | 1035 | 62.852 | 1031 | 62.427 | 1030 |
| c36588_g2_i11=i1 | 1036 | 59.96 | 1033 | 59.71 | 1030 | 61.78 | 1026 | 60.1 | 1025 |
| c36588_g2_i12 | 1036 | 59.85 | 1030 | 59.52 | 1030 | 61.78 | 1026 | 60 | 1025 |
| | | c36588_g2_i1 | | c36588_g2_i4 | | c36588_g2_i10 | | c36588_g2_i11 | |
| name of seq. | length(aa) | pident (%) | length (aa) | pident (%) | length (aa) | pident (%) | length (aa) | pident (%) | length (aa) |
| chicory_S1 | 1038 | | | | | | | | |
| chicory_S2 | 1038 | | | | | | | | |
| chicory_S3 | 1034 | | | | | | | | |
| chicory_S4 | 1033 | | | | | | | | |
| c36588_g2_i1=i11 | 966 | | | | | | | | |
| c36588_g2_i4 | 1041 | 73.282 | 966 | | | | | | |
| c36588_g2_i10 | 1041 | 71.843 | 966 | 98.749 | 1039 | | | | |
| c36588_g2_i11=i1 | 1036 | 100 | 966 | 70.83 | 1033 | 69.52 | 1033 | | |
| c36588_g2_i12 | 1036 | 98.55 | 966 | 69.48 | 1033 | 70.83 | 1033 | 98.74 | 1034 |

*MIK2* was first described as *MDIS1-interacting receptor-like kinase 2 (MIK2)* in *Arabidopsis thaliana* because it was characterized to be involved in the pollen tube perception to *MDIS1*, the female signal from ovule. However, it was later claimed to be an important regulator of cell wall damage responses towards cellulose biosynthesis inhibition (Van der Does et al., 2017).

Although the function was not thoroughly clarified, it is quite clear that *MIK2* belongs to the leucine-rich repeat receptor-like protein kinase family. *MIK2* is composed of four main domains, starting with a signal domain, followed by sets of leucine-rich repeats, a transmembrane domain, and a C-terminal active domain with a binding site and an active site. Therefore, the structures of sunflower_MIK2, chicory and *Senecio* LRR-RLKs were predicted (Figure 15). Sunflower_MIK2 and all four allelic chicory LRR-RLKs were predicted to contain two transmembrane domains, which

mirrors the structure predicted on MIK2 via the same method. Following that, they all have a series of LRRs and a C-terminal kinase domain. As for the isoforms of c36588_g2 of *Senecio*, except the number of LRRs, the differences lie in the number of the transmembrane domains. *Senecio* LRR-RLKs only exhibit one transmembrane domain in the middle part of the sequence. So with the N-terminal cap, sequences from sunflower and chicory tend to form a loop with LRR as signal acceptor, while sequences from *Senecio* tend to have a loose antenna.

As all the sequences follow the normal package of the plasma-membrane-localized receptor-like kinase with extracellular leucine-rich repeats and an intracellular kinase domain, divergence among domains was analyzed (Sup 5). According to the divergence tree of the complete protein sequence (Figure 16), MIK2 and sunflower_MIK2 are more closely related to *Senecio* i4/ i10 group and chicory $S_1/S_2$ group. The LRR domains and C-terminal kinase domains all follow the same divergence pattern as the whole protein but the percentage identity varies from domains (Sup 6). Presumptive transmembrane domains display the lowest identity. LRRs, as the domain occupying most positions in the whole sequence, shares around 46% identity with MIK2, while within the LRR-RLKs of chicory and *Senecio*, the similarity reaches around 82%. C-terminal active domains are slightly more conserved with 55% identity and at least 83% within the same species. Within the C-terminal active domain, the location of the binding site and the active site are inferred from alignment with MIK2 and the sequences at both sites are conserved among all different species.

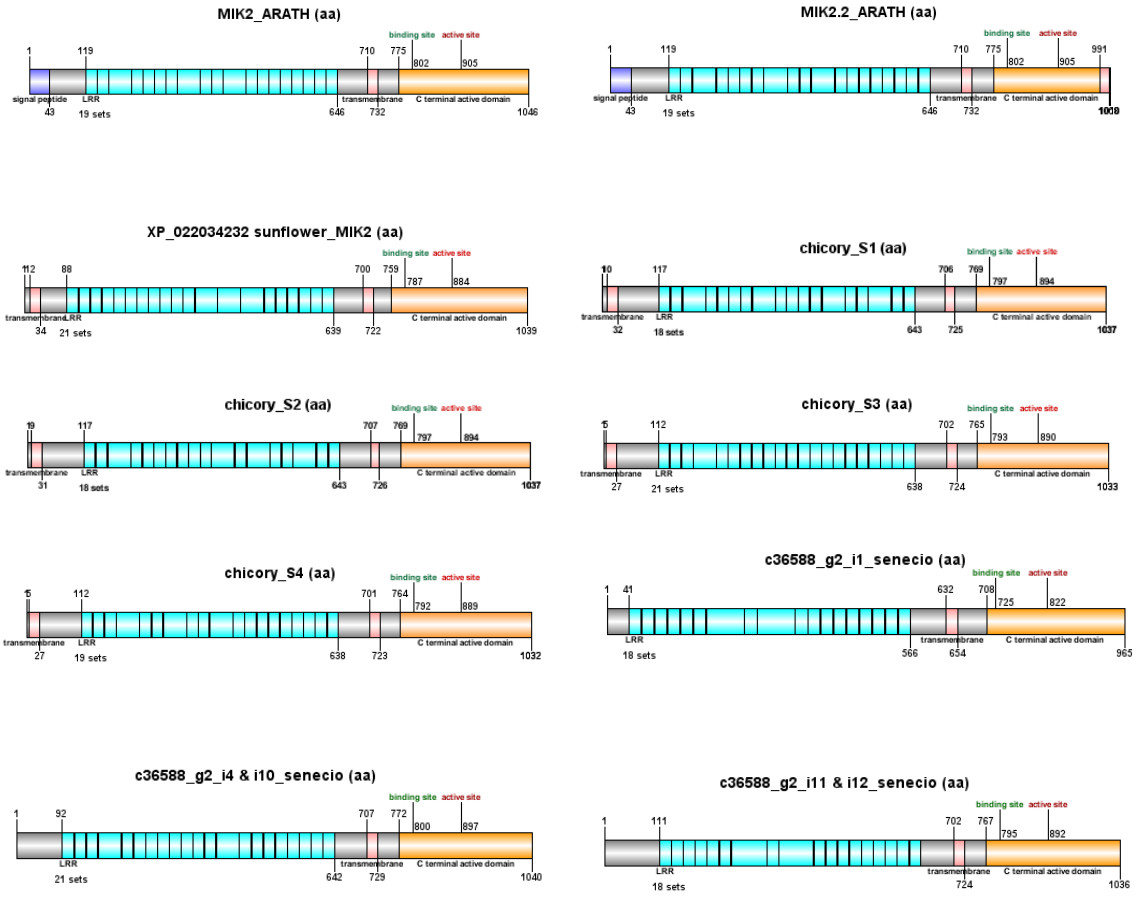



**Figure 15: Predicted gene structures of MIK2, sunflower_MIK2, LRR-RLKs in chicory and *Senecio*.** The gene is displayed as a grey band. Numbers above indicates the position (number of amino acid). Each blue square represents one leucine-rich repeat. The pink square represents the transmembrane domain. The orange bands represent active domain (LRR C-terminal). The location of the binding site and the active site are labeled in green and red separately.

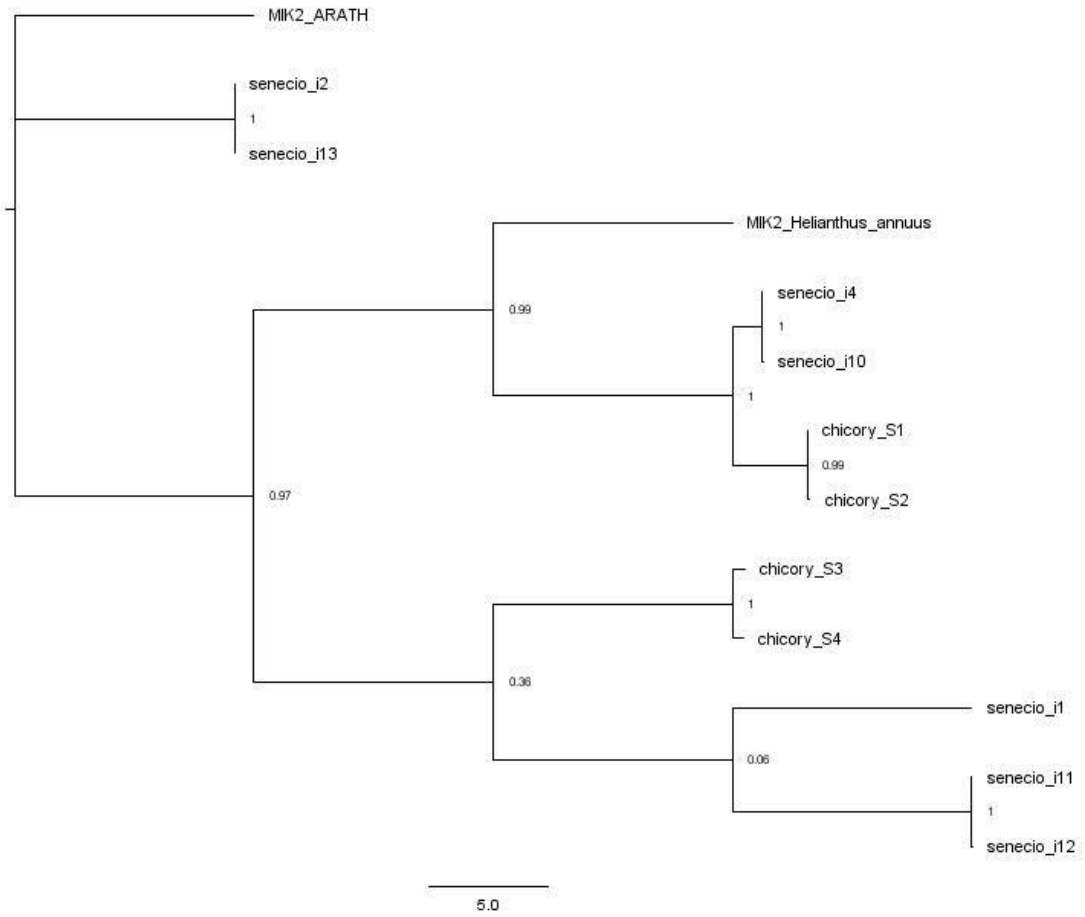



**Figure 16: Divergence tree for the protein sequence of MIK2, sunflower_MIK2, LRR-RLKs of chicory and *Senecio*.** Values at the nodes interpret the similarity between the two branches. *MIK2_Helianthus_annuss* is the top hit to LRR-RLKs of *Senecio squalidus* and chicory in the protein databank.

### 3.3.2 Molecular experiments for verifying expression pattern and polymorphism of *Senecio LRR-RLKs*

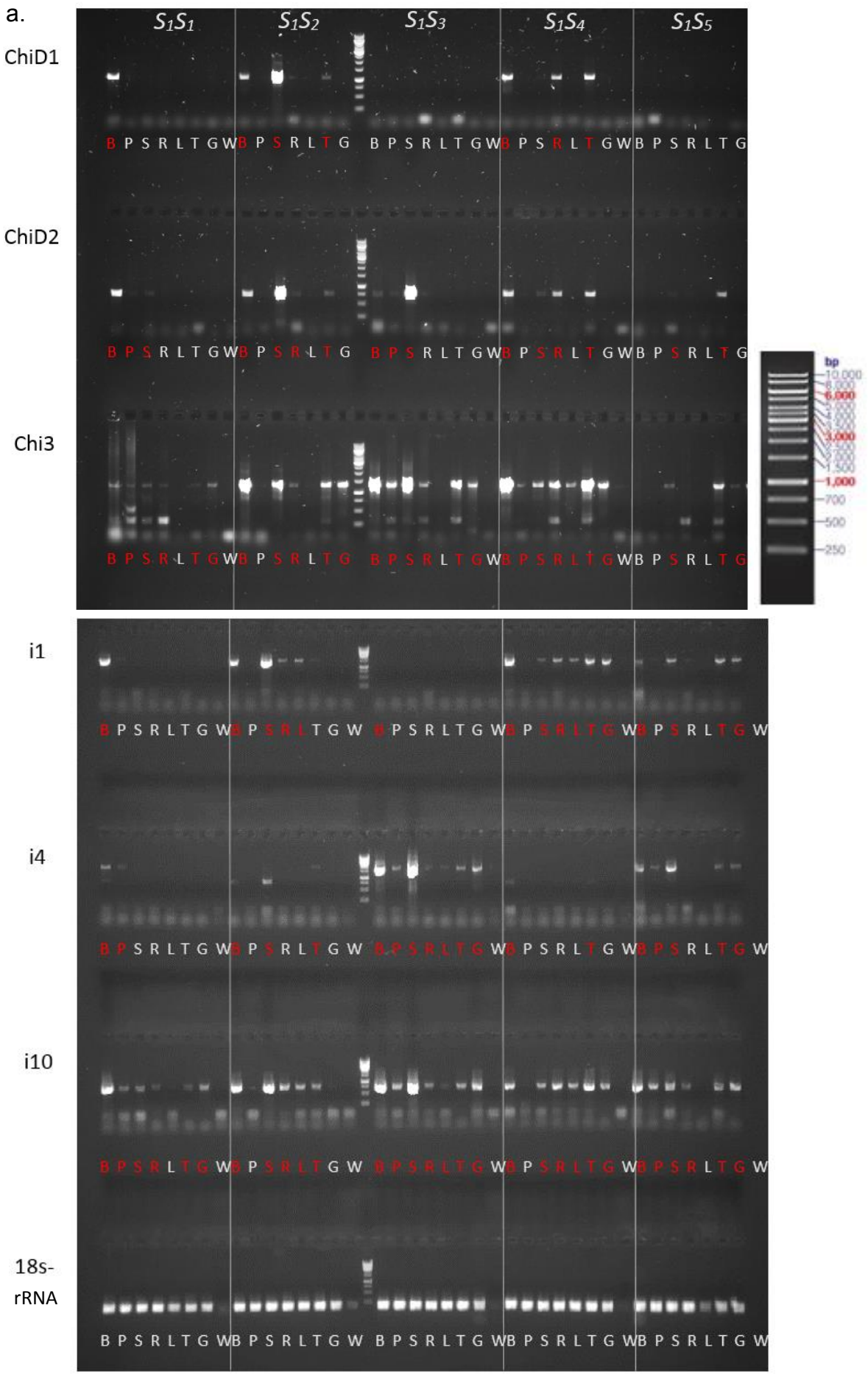
To clarify if *LRR-RLKs* of *Senecio* might act as the *S* determinant, molecular experiments were conducted to measure tissue-specific expression and to assess allelic polymorphism.

First, several sets of primers for c36588_g2 for PCR were designed (Sup 7). Considering the fragmental feature of the genome data and the abundance and

diversity of the LRR-RLK family (Gou et al., 2010), degenerate primers were also designed on the basis of the conserved region of the amino acid sequences of chicory LRR-RLKs (Sup 8) to amplify the best homologs.

None of the primers showed strict specific amplification for reproductive tissues among all the genotypes (Figure 17a). However, some primers still produced interesting expression patterns. Degenerate primer chiD1 and chiD2 both showed strong expression in $S_2$ stigmas. Compared with expression in pollen, it can be confirmed that the amplicons come from female reproductive tissues only. Meanwhile, in the $S_1$ group, the amplicon was specific to buds while in the $S_4$ group, they are present in both ray flowers and stem samples which do not contain any female reproductive tissues. ChiD2 primer mimics the general pattern of expression in $S_1$ and $S_4$ groups of ChiD1 primer. Interestingly, it is also expressed at high levels in $S_3$ stigmas. As for Chi3 primer, it amplified multiple products and its expression was spread across all tissues. The primer for i10 showed the same universal expression as the Chi3 primer. However, the primer for i1 showed exclusive expression in $S_1$ buds, but expression was also found in vegetative tissues in $S_2$, $S_4$ and $S_5$ groups, which makes the situation ambiguous.

Moreover, while the amplicons with desired length over 1,500 bp are present with primer for i4, unexpected bands less than 750 bp were also detected (Figure 17b). So based on the difference between this pair of products, specific primers were re-designed for separating them.


a.
S1S1
S1S2
S1S3
S1S4
S1S5
ChiD1
ChiD2
Chi3
i1
i4
i10
18s-rRNA
bp
10,000
6,000
3,000
1,500
1,000
700
500
250

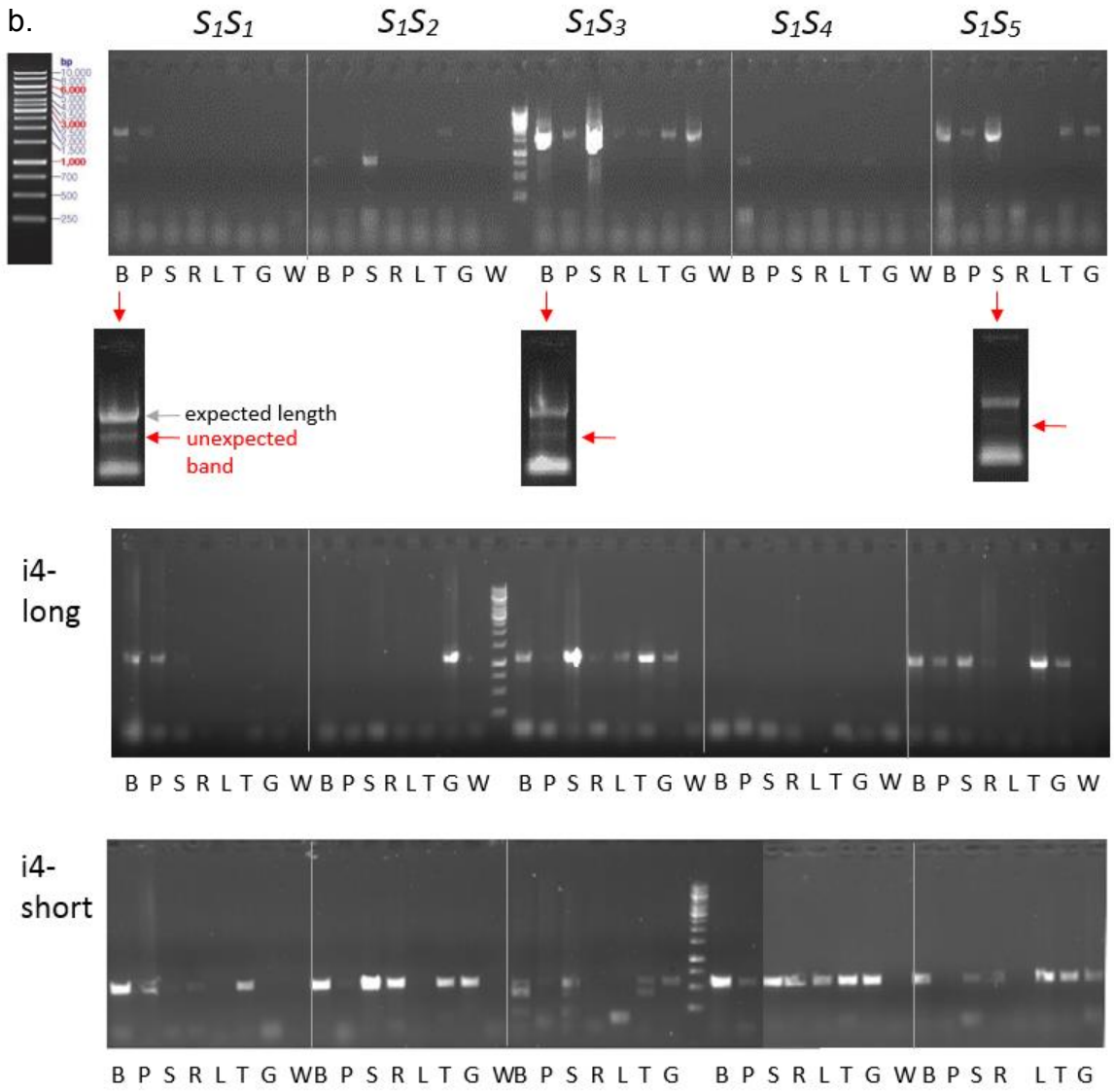



**Figure 17: Integrated pictures of 1% agarose gel results for rt-PCR.** Naming of primers are listed on the left. Templates are cDNA generated from buds (B), pollen (P), stigmas (S), ray flowers/petals (R), leaf (L), stem (T), and genomic (G) DNA extractions from individuals with defined genotype ($S_1S_1$, $S_1S_2$, $S_1S_3$, $S_1S_4$, $S_1S_5$). Positive controls were generated by 18s-rRNA primers. Negative controls were generated by using water (W) as the template. (a) Primers are listed on the left of each pannel. Tissues with positive bands under different primers are labeled in red. (b) Results for i4 primer in (a) are especially showed. Unexpected bands are marked in red. Following PCR with more specific primers were also displayed.

As the gel pictures gave an uncertain pattern of expression among reproductive and vegetative tissues, bands from different tissues or genotypes were then picked for sequencing to validate expression differences as well as to measure any sequence polymorphism. After double-checking and cleaning the Sanger sequences, it turned out that amplicons from i1 were 100% identical no matter what tissues or genotypes it came from. The same was true for i10. For amplicons from i4, longer products turned out to be identical with the i4 reference sequence, while amplicons with shorter length are around 27% different from i4 in amino acid sequence. However, no polymorphism was found between haplotypes or between reproductive tissues and vegetative tissues. Therefore, due to this lack of tissue-specificity and lack of allelic polymorphism all isoforms from c36588 of *S. squalidus* were rejected as unlikely candidates for an *S* determinant gene.

Products from degenerate primers proved to contain multiple sequences following direct Sanger sequencing of PCR products. Products from ChiD2 were therefore cloned into vectors to isolate individual sequences (as described in methods). As a result, two promising sequences were successfully identified using the degenerate primer. They shared around 77% identity with chicory *LRR-RLKs*, which is higher than the percentage identity of transcriptome contigs of *Senecio* to chicory LRR-RLKs. Unfortunately, no differences in expression could be detected between buds, petals, and stems (Figure 18).

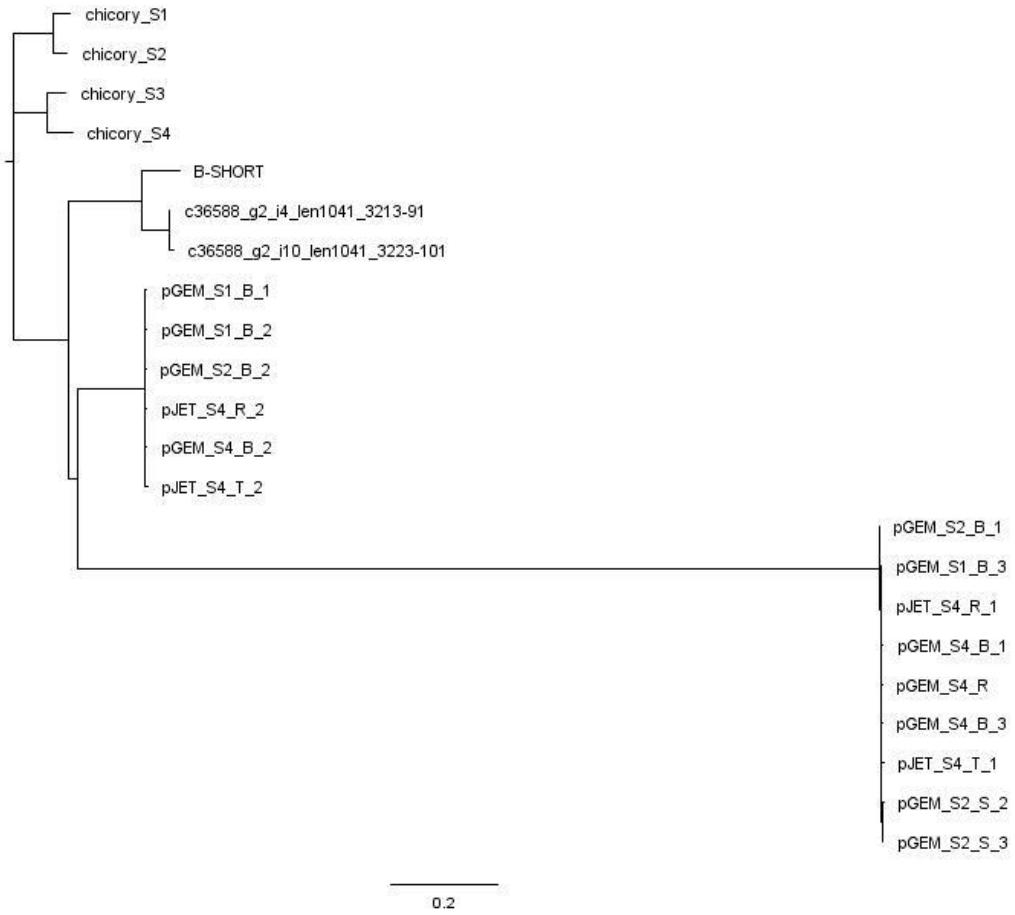



**Figure 18: Divergence relationship among amplicons from i4-p.** Amplicons from various tissues and different genotypes cluster into two group. Within each group, sequences are exactly identical to each other no matter the genotype or the tissue they come from.

## 3.4 Discussion

Bioinformatic searches and degenerate primers detected six *LRR-RLK* sequences in *Senecio squalidus* with similarity to the chicory *LRR-RLK* candidate *S* gene. By the distance of divergence with chicory *LRR-RLKs*, products from degenerate primers were most similar with 77% identity, then followed transcriptome contigs and finally the sunflower_MIK2 and *Arabidopsis* MIK2 (Figure 19).

From rt-PCR for these six *LRR-RLK* candidates, detection of expression in both reproductive tissues and vegetative tissues, indicated they are not likely to encode an *S* determinant. However, the products of reproductive tissues were at a higher density indicating these genes are expressed dominantly in reproductive tissues (Figure 17). Considering the accuracy of rt-PCR and the high sensitivity of PCR, it can be the case that *S* genes might display some leaky expression in vegetative tissues. In order to rule this possibility out and assess the true quantitative nature of expression, real-time PCR could be developed as a more precise measurement to double-confirm the expression patterns observed.

On the other hand, products from five different genotypes all gave the same sequence, indicating a lack of polymorphism between the sequences in individuals carrying different *S* alleles. This could be because that PCRs from different genotypes all amplified the recessive gene. But additionally, amplifications are also identical in different tissues, which further suggests these sequences do not encode an *S* determinant that is specific in reproductive tissues. So considering comprehensively, these *Senecio LRR-RLKs* are not likely to represent an *S* gene.

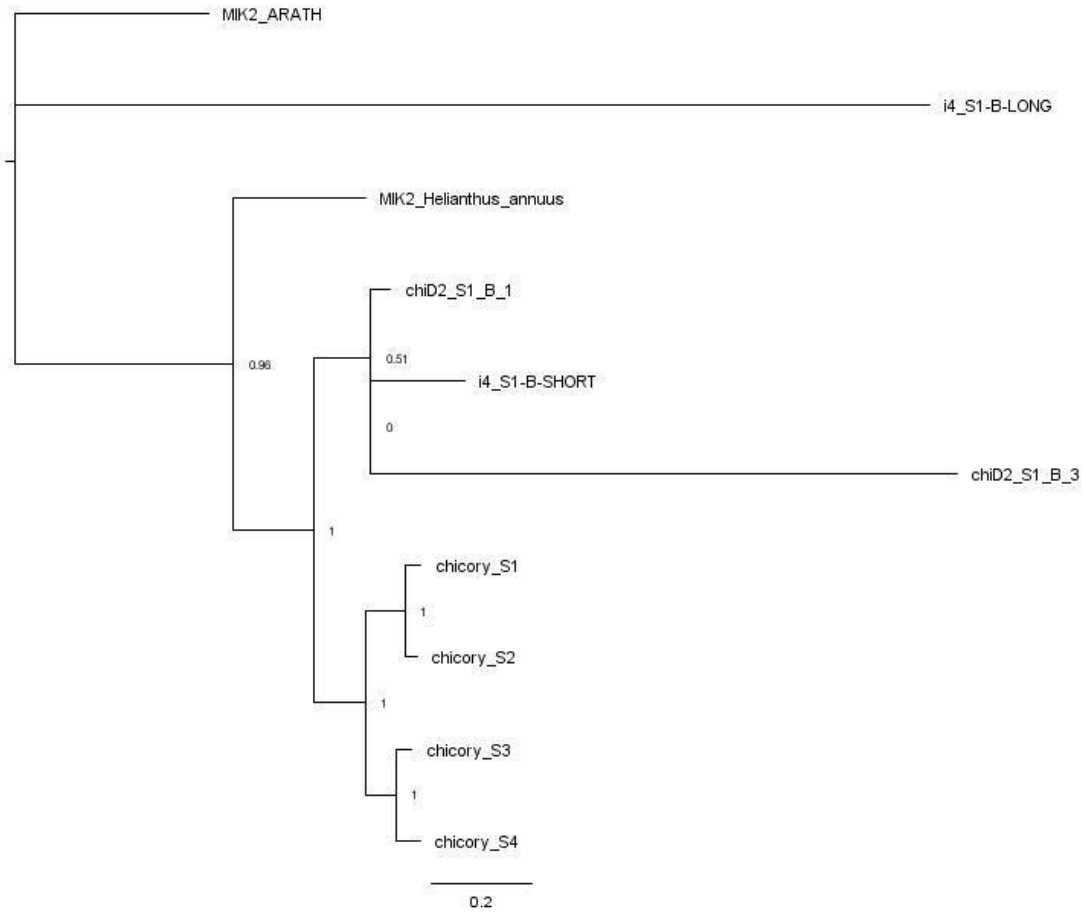



**Figure 19: Divergence tree of promising *LRR-RLKs* and reference genes.** Sequences amplified by degenerate primers (chiD2_x) show more alignment similarity with chicory *LRR-RLKs*. Together, they are relatively closer to *MIK2* of *Helianthus annuus* than to that of *Arabidopsis.*

It remains possible that the approaches employed here did not identify the true homologs/orthologues of the chicory *LRR-RLK S* candidate. This could be due to the extreme diversity and abundance of this family of proteins(Gou et al., 2010). Interestingly, the difference in the predicted structure of LRR-RLKs, mainly the number of transmembrane domains (Figure 15), can be further evidence for there not being a close relationship between chicory and *Senecio* LRR-RLKs. On the other hand, the *S* determinant gene in Brassica, *SRK* was found to be expressed at

a very low level (Stein & Nasrallah, 1993). Then it is reasonable for this potential *S* gene*, LRR-RLK* to be expressed at a so low level that was not detected by amplification experiments. So the hypothesis of chicory *LRR-RLKs* being involved in pollen-pistil recognition is still to be determined and there is a need for further studies and more direct authentication.

# Chapter IV

# Conclusion

## A. General Discussion

This thesis has revealed new insights into the dominance relationships between *S* alleles (haplotypes) in *Senecio squalidus* and so has added our understanding of the genetic basis of self-incompatibility in this model Asteraceae species. In particular, $S_4$, $S_5$ and $S_6$ alleles were found to be recessive to $S_1$ in pollen but are dominant over $S_1$ in the pistil. These new findings may help us better understand the successful colonization of *S. squalidus* from Mt Etna to the UK. More crosses following the strategy used here to generate homozygotes and more individuals with various genotypic combinations could be carried out to identify further dominance interactions.

From the comparative genomic analysis, it is useful to have a synteny map between *Tolpis* and lettuce, which gives a promising direction for the identification of the location of the *S*-locus in *Tolpis*, lettuce and chicory. Interestingly, in the list of genes predicted within *Tolpis* LG2 ---- the SC QTL and the synteny linkage group of the *S*-locus of lettuce and chicory, an *LRR-RLK* family gene is given (as annotated to AT2G07040). But it did not has alignment similarity to any of the chicory *LRR-RLKs* or hit to *Senecio* genome data. So identification can be included in the future if more *S*-locus markers and sequence information emerge.

Finally, with a small group of *LRR-RLK* sequences in *S. squalidus* tested for *S* gene characteristics, the analysis pointed to a lack of reproductive tissue-specific

expression with general expression in all tissue types investigated. In addition, no polymorphism between distinct genotypes were detected. These data demonstrate that these genes are unlikely to encode the *S* determinant gene of *S. squalidus*. However, since the divergence of the LRR family and divergence with *Senecio LRR-RLKs*, it is still possible that the chicory *LRR-RLK* is one of the *S*-genes in that species and its homolog in *Senecio* has yet to be identified. Therefore, direct verification of the expression pattern of chicory *LRR-RLKs* would be a very useful complement to this study to see that this gene is expressed in the manner predicted for an *S*-gene.

## B. Potential future work

Based on the checklist of two main criteria for *S* determinant genes: specific expression in either stigmas/pistil (female determinant) or pollen/anthers (male determinant) and allelic polymorphism, more candidate genes can be investigated in *S. squalidus* to screen for putative *S* genes.

From 174 stigma-specific genes isolated previously through SSH (suppression subtractive hybridization) experiments (Allen et al. 2011; Allen et al. 2010a; Allen et al. 2010b; Hiscock & Allen 2008), similar experiments for allelic polymorphism can be a starting point because they already present expression specificity. Gene orthologue searching could be of great help to narrow down the candidates according to whether their annotation with predicted molecular function and biological process is related to reproductive development, signaling or defense.

Candidate genes with differential expression would also be able to be recognized with the help of transcriptomes from distinct tissues (bud, floret, flower, leaf, root

and young leaf). Contigs with higher expression levels in bud, floret, and flower head than in leaf and root would be preferred candidates for the further assessments of polymorphism between different *S*-genotypes.

Recently, transcriptome sequences were obtained from 16 individuals of *S. squalidus* from different localities in the UK (B. Nevado pers. Comm.), which could be informative materials for looking for gene polymorphism. After assembling the database for each, analysis can be carried out to seek allelic sequences of candidates. There are thus some future possibilities available to identify the so far elusive *S* determinants of SSI in *Senecio* and the Asteraceae generally.

Supplementary data

Sup 1: Crossing results and deduction of phenotypes of individuals in pS2, sS3, sS4 and sS5 groups. The second row represents males during reciprocal crosses while the second column represents females. Diagonal lines show the strong self-incompatible result when conducting self-pollination checks. Marks of each pair of crosses are filled in the cell. When shaded in green, a complete compatible result is confirmed. When shaded in grey, an incompatible result is confirmed. Blank cells indicate a lack of crosses. Since *Senecio squalidus* exhibits dominance relationship of the SSI system, the phenotypes of different reproductive tissue of each are shown in the first row and the first column. Alleles previously identified with confidence are shown in bold. Question marks indicate uncertain *S* phenotype.

| Male / Female | Phenotype | S1 | S2 | S1 | S1 | S1 | S2 | S2 | S1 | **S1** | **S2** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Phenotype** | Naming | pS2-1 | pS2-2 | pS2-3 | pS2-4 | pS2-5 | pS2-6 | pS2-7 | pS2-10 | **Bill4 Self3** ***$S_1S_1$*** | **B4xOx2** ***$S_1S_2$*** | |
| S1 | pS2-1 | | 3.5 | -1 | 0 | -2 | 3 | 2 | -1 | | | -1 |
| S2 | pS2-2 | 1.5 | | 2 | | | -2 | -1.5 | 1.5 | | | -1 |
| S1 | pS2-3 | -1.5 | | | -1.5 | -1 | 1.5 | -1 | | | | -1 |
| S1 | pS2-4 | -1 | | 0 | | | 1.5 | 2.5 | | | | |
| ? | pS2-5 | -1 | | -1 | | | | 1 | 1 | | | |
| S2 | pS2-6 | 2 | | 1.5 | 2 | -1 | | -1.5 | | | | |
| S2 | pS2-7 | 2 | -1.5 | -1 | 2 | 1 | -1.5 | | 1 | | | |
| ? | pS2-10 | -1 | | | | 1 | -1.5 | 0 | | | | 1 |
| **S1** | **Bill4 Self3** ***$S_1S_1$*** | | | | | | -1 | | | | | |
| **S2** | **B4xOx2** ***$S_1S_2$*** | -1 | -1 | 0 | | | -1 | | 2 | | | |

| Male / Female | **Phenotype** | S1 | S1 | S3 | S3 | S1 | S3 | ? | ? | S3 | ? | **S3** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Phenotype** | Naming | ss3-1 | ss3-2 | ss3-3 | ss3-4 | ss3-6 | ss3-7 | ss3-8 | ss3-10 | ss3-11 | ss3-14 | **Bill4xOx4 $S_1S_3$** |
| S1 | ss3-1 | | -1.5 | -1 | 1.5 | 1 | -1 | | | 1 | | -0.5 |
| S1 | ss3-2 | -1.5 | | 1 | 2 | | 2.5 | 1 | -1.5 | 2 | | -0.5 |
| S3 | ss3-3 | 2.5 | -1 | | -1 | | | | | | | |
| S3 | ss3-4 | 1 | -1.5 | 1 | | -1 | | | -3 | | | |
| S1 | ss3-6 | -1 | -0.5 | 2 | 2 | | -1 | | | | | |
| ? | ss3-7 | | -0.5 | | | | | | 1.5 | | | -0.5 |
| S3 | ss3-8 | 1 | | | -1 | 1.5 | | | | | | |
| ? | ss3-10 | | | -1 | | | | | | 1 | | |
| S3 | ss3-11 | | 2 | | | 1.5 | -1.5 | | | | -1 | 0.5 |
| S1 | ss3-14 | | 1 | | | | | | | 2 | | 1 |
| **S3** | **Bill4xOx4 $S_1S_3$** | -0.5 | 1.5 | | | | 0.5 | | | | | |

| Male / Female | Phenotype | S1 | S1 | S1 | ? | S1 | S1 | S1 | **S1** |
|---|---|---|---|---|---|---|---|---|---|
| **Phenotype** | Naming | sS4-6 | sS4-8 | sS4-10 | sS4-14 | sS4-16 | ox6 | **Bill4xOX22 $S_1S_4$** | **Bill4 Self3 $S_1S_1$** |
| S4 | sS4-6 | | -1.5 | 4 | | 2.5 | -1 | 1 | |
| S4 | sS4-8 | | | -0.5 | -1 | -1 | 1 | 4 | 2 |
| S4 | sS4-10 | 4.5 | -1 | | -0.5 | | | -2 | |
| S4 | sS4-14 | | -1 | -0.5 | | -1 | 1.5 | -1 | |
| S4 | sS4-16 | 1 | 2.5 | | -1 | | 0.5 | -1 | |
| S4 | ox6 | 0.5 | -0.5 | | -1.5 | 1.5 | | -0.5 | 2 |
| **S4** | **Bill4xOX22 $S_1S_4$** | 3.5 | 4 | -1 | -1.5 | 1 | 0 | | |
| **S1** | **Bill4 Self3 $S_1S_1$** | | | | | | 1 | | |

| Male / Female | Phenotype | S1 | S1 | S1 | S1 | S1 | S1 | S1 | S1 | S1 | S1 | S1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Phenotype** | Naming | sS5A-1 | sS5A-2 | sS5B-1 | sS5B-2 | sS5B-3 | sS5B-4 | sS5B-5 | sS5B-6 | sS5B-9 | **B4xOX27 $S_1S_5$** | **Bill4 Self3 $S_1S_1$** |
| S5 | sS5A-1 | | -1 | 4.5 | | 0.5 | 4 | 0.5 | 3 | 3 | -1.5 | |
| S5 | sS5A-2 | -1 | | 2 | -1.5 | -1.5 | -1 | | -1 | | | |
| S5 | sS5B-1 | -1 | -1 | | 0.5 | 1.5 | -0.5 | | | 0.5 | 2 | |
| S5 | sS5B-2 | -1 | -1 | -0.5 | | -2 | -1 | -1 | | -1 | 3 | |
| S5 | sS5B-3 | 2.5 | -1.5 | 2 | -1 | | 2 | -1 | -1.5 | | 1 | |
| S5 | sS5B-4 | 4 | -1 | 4 | 1 | 3.5 | | -1.5 | -1 | | -1 | |
| ? | sS5B-5 | -1.5 | | -1 | -1 | -1.5 | -1 | | | | -1.5 | |
| S5 | sS5B-6 | 2 | -1 | | | -1 | -1 | -1.5 | | | | |
| S5 | sS5B-9 | -1.5 | 2 | -1.5 | -1.5 | -0.5 | 1 | | -0.5 | | | |
| **S5** | **B4xOX27 $S_1S_5$** | -1.5 | -1.5 | | 1.5 | -0.5 | 3 | 3 | -0.5 | -1 | | |
| **S1** | **Bill4 Self3 $S_1S_1$** | | | | | | | | | | 2.5 | |

Sup 2: Details of scaffolds and markers matched to each linkage group (LG) of *Senecio squalidus* are listed.

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_67964** | 223,323 | Sen_c1754 | 1767 | 1600 | 1 | 16.3795 |
| **ScTET64_67649** | 1,824,317 | Sen_c10630 | 1086 | 1036 | 1 | 36.3395 |
| **ScTET64_68109** | 97,574 | Sen_c3130_P | 1351 | 1358 | 1 | 39.8621 |
| **ScTET64_68371** | 130,378 | Sen_c8057 | 3158 | 1858 | 1 | 39.8621 |
| **ScTET64_429** | 99,553 | Sen_c9175_P | 2991 | 2842 | 1 | 39.8621 |
| **ScTET64_725** | 102,085 | Sen_c8 | 2942 | 2813 | 1 | 40.7755 |
| **ScTET64_49055** | 593 | Sen_c866_P | 1827 | 294 | 1 | 40.7755 |
| **ScTET64_33118** | 699 | Sen_c10234 | 2447 | 62 | 1 | 41.6270 |
| **ScTET64_8491** | 42,782 | Sen_c10234 | 2447 | 2020 | 1 | 41.6270 |
| **ScTET64_745** | 137,765 | Sen_c4065 | 2341 | 66 | 1 | 42.4786 |
| **ScTET64_67869** | 663,882 | Sen_c10453 | 2559 | 1572 | 1 | 42.4786 |
| **ScTET64_1970** | 78,195 | Sen_c1083_P | 1073 | 1118 | 1 | 42.4786 |
| **ScTET64_7317** | 63,050 | Sen_c1106 | 2158 | 2040 | 1 | 42.4786 |
| **ScTET64_68651** | 80,600 | Sen_c11170_P | 971 | 953 | 1 | 42.4786 |
| **ScTET64_24** | 467,643 | Sen_c11663 | 1469 | 988 | 1 | 42.4786 |
| **ScTET64_67808** | 64,724 | Sen_c12672 | 894 | 904 | 1 | 42.4786 |
| **ScTET64_2451** | 32,768 | Sen_c13645 | 1191 | 1211 | 1 | 42.4786 |
| **ScTET64_67831** | 290,028 | Sen_c14710 | 1246 | 1169 | 1 | 42.4786 |
| **ScTET64_67618** | 108,483 | Sen_c16388 | 1085 | 998 | 1 | 42.4786 |
| **ScTET64_3067** | 3,796 | Sen_c1662 | 1371 | 1382 | 1 | 42.4786 |
| **ScTET64_68651** | 80,600 | Sen_c1864_P | 1187 | 1030 | 1 | 42.4786 |
| **ScTET64_68108** | 24,860 | Sen_c1865 | 1600 | 1805 | 1 | 42.4786 |
| **ScTET64_66182** | 506 | Sen_c2156_P | 1379 | 118 | 1 | 42.4786 |
| **ScTET64_339** | 37,640 | Sen_c2368_P | 2227 | 1696 | 1 | 42.4786 |
| **ScTET64_68345** | 56,092 | Sen_c2926_P | 896 | 608 | 1 | 42.4786 |
| **ScTET64_2840** | 195,421 | Sen_c2999_P | 523 | 523 | 1 | 42.4786 |
| **ScTET64_68022** | 200,374 | Sen_c3021_P | 1946 | 1704 | 1 | 42.4786 |
| **ScTET64_59369** | 540 | Sen_c3378_P | 2670 | 283 | 1 | 42.4786 |
| **ScTET64_8458** | 801,173 | Sen_c3424 | 4025 | 3871 | 1 | 42.4786 |
| **ScTET64_67960** | 262,509 | Sen_c3642 | 1595 | 1607 | 1 | 42.4786 |
| **ScTET64_67869** | 663,882 | Sen_c3659 | 2178 | 1764 | 1 | 42.4786 |
| **ScTET64_2840** | 195,421 | Sen_c3936 | 2211 | 2131 | 1 | 42.4786 |
| **ScTET64_49494** | 591 | Sen_c4065 | 2341 | 186 | 1 | 42.4786 |
| **ScTET64_9354** | 24,165 | Sen_c4085_P | 1750 | 1262 | 1 | 42.4786 |
| **ScTET64_49351** | 592 | Sen_c4248 | 1795 | 276 | 1 | 42.4786 |
| **ScTET64_67915** | 31,070 | Sen_c4356_P | 1581 | 1679 | 1 | 42.4786 |
| **ScTET64_67869** | 663,882 | Sen_c4513 | 1797 | 1909 | 1 | 42.4786 |
| **ScTET64_3088** | 123,371 | Sen_c4781_P | 659 | 661 | 1 | 42.4786 |
| **ScTET64_6877** | 76,259 | Sen_c5152 | 1906 | 2079 | 1 | 42.4786 |
| **ScTET64_68574** | 34,838 | Sen_c5521_P | 2787 | 2479 | 1 | 42.4786 |
| **ScTET64_68655** | 82,642 | Sen_c5938_P | 2358 | 2108 | 1 | 42.4786 |

| **Scaffold** | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_8458** | 801,173 | Sen_c6472 | 2309 | 1176 | 1 | 42.4786 |
| **ScTET64_3088** | 123,371 | Sen_c676 | 1079 | 1082 | 1 | 42.4786 |
| **ScTET64_67995** | 56,403 | Sen_c7269 | 1360 | 1340 | 1 | 42.4786 |
| **ScTET64_10332** | 998 | Sen_c7675 | 1323 | 102 | 1 | 42.4786 |
| **ScTET64_2840** | 195,421 | Sen_c8171 | 1799 | 1780 | 1 | 42.4786 |
| **ScTET64_67808** | 64,724 | Sen_c8780_P | 831 | 838 | 1 | 42.4786 |
| **ScTET64_8940** | 103,690 | Sen_c891 | 2508 | 2383 | 1 | 42.4786 |
| **ScTET64_1045** | 7,007 | Sen_c9343 | 1235 | 1148 | 1 | 42.4786 |
| **ScTET64_7317** | 63,050 | Sen_c9584_P | 1840 | 1832 | 1 | 42.4786 |
| **ScTET64_6877** | 76,259 | Sen_c1085 | 856 | 295 | 1 | 42.4786 |
| **ScTET64_68585** | 40,461 | Sen_c15101_P | 1627 | 247 | 1 | 42.4786 |
| **ScTET64_64142** | 516 | Sen_c16388 | 1085 | 62 | 1 | 42.4786 |
| **ScTET64_27641** | 749 | Sen_c2368_P | 2227 | 369 | 1 | 42.4786 |
| **ScTET64_8458** | 801,173 | Sen_c2603 | 3644 | 1205 | 1 | 42.4786 |
| **ScTET64_68030** | 112,549 | Sen_c4065 | 2341 | 812 | 1 | 42.4786 |
| **ScTET64_59991** | 537 | Sen_c4248 | 1795 | 69 | 1 | 42.4786 |
| **ScTET64_51152** | 582 | Sen_c5521_P | 2787 | 200 | 1 | 42.4786 |
| **ScTET64_135** | 80,889 | Sen_c7675 | 1323 | 1254 | 1 | 42.4786 |
| **ScTET64_2053** | 5,916 | Sen_c9343 | 1235 | 101 | 1 | 42.4786 |
| **ScTET64_10637** | 991 | Sen_c1085 | 856 | 222 | 1 | 42.4786 |
| **ScTET64_33296** | 698 | Sen_c15101_P | 1627 | 97 | 1 | 42.4786 |
| **ScTET64_7317** | 63,050 | Sen_c2156_P | 1379 | 333 | 1 | 42.4786 |
| **ScTET64_68966** | 64,046 | Sen_c4248 | 1795 | 1339 | 1 | 42.4786 |
| **ScTET64_67649** | 1,824,317 | Sen_c1384 | 1127 | 1141 | 1 | 44.9321 |
| **ScTET64_67649** | 1,824,317 | Sen_c458 | 1662 | 1664 | 1 | 44.9321 |
| **ScTET64_68760** | 121,340 | Sen_c3586 | 1410 | 406 | 1 | 49.7585 |
| **ScTET64_68305** | 738,803 | Sen_c3586 | 1410 | 84 | 1 | 49.7585 |
| **ScTET64_1471** | 300,614 | Sen_c4521_N | 2219 | 1747 | 1 | 51.4128 |
| **ScTET64_68586** | 3,946,386 | Sen_c452 | 978 | 978 | 1 | 57.4597 |
| **ScTET64_68586** | 3,946,386 | Sen_c5512 | 1486 | 1212 | 1 | 59.9922 |
| **ScTET64_68586** | 3,946,386 | Sen_c13650 | 2196 | 2138 | 1 | 61.6184 |
| **ScTET64_68586** | 3,946,386 | Sen_c14244 | 1910 | 1861 | 1 | 63.2709 |
| **ScTET64_68586** | 3,946,386 | Sen_c3432 | 2169 | 1921 | 1 | 64.1364 |
| **ScTET64_57344** | 550 | Sen_c9233 | 1610 | 224 | 1 | 68.2693 |
| **ScTET64_1878** | 6,051 | Sen_c12140 * | 1320 | 58 | 2 | 0.0000 |
| **ScTET64_17416** | 873 | Sen_c12140 * | 1320 | 289 | 2 | 0.2000 |
| **ScTET64_30092** | 725 | Sen_c12140 * | 1320 | 174 | 2 | 0.3000 |
| **ScTET64_29364** | 733 | Sen_c1206 * | 1275 | 130 | 2 | 1.5965 |
| **ScTET64_67908** | 2,457,694 | Sen_c2047 * | 5489 | 5483 | 2 | 9.6610 |
| **ScTET64_67908** | 2,457,694 | Sen_c1265 * | 1460 | 1444 | 2 | 12.9732 |
| **ScTET64_67908** | 2,457,694 | Sen_c3198 * | 2104 | 2108 | 2 | 17.8830 |
| **ScTET64_67908** | 2,457,694 | Sen_c593 * | 1250 | 1174 | 2 | 17.8830 |
| **ScTET64_67908** | 2,457,694 | Sen_c6432_P * | 3454 | 3449 | 2 | 17.8830 |
| **ScTET64_67908** | 2,457,694 | Sen_c11833 * | 1132 | 1035 | 2 | 18.6784 |
| **ScTET64_67908** | 2,457,694 | Sen_c8848 * | 1765 | 1791 | 2 | 18.6784 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_26929** | 756 | Sen_c5342_P * | 1971 | 307 | 2 | 19.5276 |
| **ScTET64_68076** | 1,855,592 | Sen_c5612 * | 1761 | 1777 | 2 | 30.8996 |
| **ScTET64_68466** | 1,661,514 | Sen_c5586_P * | 1366 | 1074 | 2 | 33.2634 |
| **ScTET64_68466** | 1,661,514 | Sen_c262 * | 1634 | 1678 | 2 | 34.8355 |
| **ScTET64_68466** | 1,661,514 | Sen_c2715 | 1093 | 1121 | 2 | 38.8142 |
| **ScTET64_64601** | 514 | Sen_c1542 | 1971 | 73 | 2 | 45.1727 |
| **ScTET64_12811** | 950 | Sen_c4612 | 1274 | 722 | 2 | 49.1144 |
| **ScTET64_39270** | 653 | Sen_c6002 | 1527 | 635 | 2 | 52.2708 |
| **ScTET64_68076** | 1,855,592 | Sen_c11623 | 1291 | 1365 | 2 | 53.8516 |
| **ScTET64_68512** | 633,640 | Sen_c13507 | 914 | 906 | 2 | 53.8516 |
| **ScTET64_68549** | 57,771 | Sen_c3341 | 1208 | 1203 | 2 | 54.6609 |
| **ScTET64_65567** | 509 | Sen_c4101 | 2164 | 69 | 2 | 55.4703 |
| **ScTET64_30111** | 725 | Sen_c10507 | 1725 | 72 | 2 | 55.4703 |
| **ScTET64_68133** | 215,384 | Sen_c10648 | 896 | 900 | 2 | 55.4703 |
| **ScTET64_8177** | 604,683 | Sen_c10988 | 872 | 940 | 2 | 55.4703 |
| **ScTET64_448** | 248,412 | Sen_c11068 | 822 | 826 | 2 | 55.4703 |
| **ScTET64_14620** | 918 | Sen_c11250 | 1072 | 393 | 2 | 55.4703 |
| **ScTET64_6285** | 1,451,803 | Sen_c12250 | 1228 | 1233 | 2 | 55.4703 |
| **ScTET64_448** | 248,412 | Sen_c14828 | 917 | 890 | 2 | 55.4703 |
| **ScTET64_5697** | 165,683 | Sen_c1673 | 3463 | 3340 | 2 | 55.4703 |
| **ScTET64_68897** | 45,345 | Sen_c1705 | 2282 | 2168 | 2 | 55.4703 |
| **ScTET64_27362** | 751 | Sen_c1846 | 3154 | 210 | 2 | 55.4703 |
| **ScTET64_6285** | 1,451,803 | Sen_c1919 | 2152 | 1364 | 2 | 55.4703 |
| **ScTET64_8177** | 604,683 | Sen_c2070 | 2134 | 2104 | 2 | 55.4703 |
| **ScTET64_129** | 25,238 | Sen_c2182 | 2629 | 2543 | 2 | 55.4703 |
| **ScTET64_2800** | 48,245 | Sen_c241 | 2721 | 2615 | 2 | 55.4703 |
| **ScTET64_68263** | 73,382 | Sen_c2513 | 1518 | 1605 | 2 | 55.4703 |
| **ScTET64_8926** | 11,668 | Sen_c2686 | 2756 | 1799 | 2 | 55.4703 |
| **ScTET64_5112** | 114,252 | Sen_c2891 | 1970 | 1686 | 2 | 55.4703 |
| **ScTET64_215** | 196,484 | Sen_c5111 | 3041 | 2116 | 2 | 55.4703 |
| **ScTET64_6285** | 1,451,803 | Sen_c5384 | 1196 | 1093 | 2 | 55.4703 |
| **ScTET64_8177** | 604,683 | Sen_c5603 | 1319 | 886 | 2 | 55.4703 |
| **ScTET64_27378** | 751 | Sen_c5827 | 1079 | 186 | 2 | 55.4703 |
| **ScTET64_1913** | 565,163 | Sen_c6036 | 1324 | 1062 | 2 | 55.4703 |
| **ScTET64_67737** | 109,158 | Sen_c6241 | 1641 | 1505 | 2 | 55.4703 |
| **ScTET64_2972** | 4,212 | Sen_c6392 | 2691 | 1853 | 2 | 55.4703 |
| **ScTET64_68536** | 85,900 | Sen_c64 | 3498 | 3043 | 2 | 55.4703 |
| **ScTET64_68633** | 2,638,979 | Sen_c7054 | 995 | 626 | 2 | 55.4703 |
| **ScTET64_67678** | 427,320 | Sen_c737 | 2956 | 489 | 2 | 55.4703 |
| **ScTET64_68633** | 2,638,979 | Sen_c7430 | 1363 | 1084 | 2 | 55.4703 |
| **ScTET64_2524** | 156,720 | Sen_c7540 | 1319 | 1479 | 2 | 55.4703 |
| **ScTET64_154** | 1,026,361 | Sen_c7573 | 1364 | 1222 | 2 | 55.4703 |
| **ScTET64_67950** | 408,156 | Sen_c7848 | 2385 | 2402 | 2 | 55.4703 |
| **ScTET64_27506** | 750 | Sen_c7992 | 1247 | 341 | 2 | 55.4703 |
| **ScTET64_10643** | 991 | Sen_c8008 | 1781 | 221 | 2 | 55.4703 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_68866** | 955,628 | Sen_c8008 | 1781 | 1374 | 2 | 55.4703 |
| **ScTET64_6285** | 1,451,803 | Sen_c8606 | 3043 | 3101 | 2 | 55.4703 |
| **ScTET64_8177** | 604,683 | Sen_c8937 | 594 | 599 | 2 | 55.4703 |
| **ScTET64_44731** | 618 | Sen_c9178 | 1601 | 221 | 2 | 55.4703 |
| **ScTET64_57886** | 547 | Sen_c4412 | 1914 | 263 | 2 | 55.4703 |
| **ScTET64_998** | 62,713 | Sen_c5598 | 3667 | 3313 | 2 | 55.4703 |
| **ScTET64_68595** | 129,717 | Sen_c5827 | 1079 | 393 | 2 | 55.4703 |
| **ScTET64_6285** | 1,451,803 | Sen_c7992 | 1247 | 936 | 2 | 55.4703 |
| **ScTET64_68633** | 2,638,979 | Sen_c96 | 2208 | 1864 | 2 | 55.4703 |
| **ScTET64_67878** | 52,668 | Sen_c1846 | 3154 | 2349 | 2 | 55.4703 |
| **ScTET64_1913** | 565,163 | Sen_c3921_N | 5869 | 2776 | 2 | 55.4703 |
| **ScTET64_19067** | 850 | Sen_c3921_N | 5869 | 124 | 2 | 55.4703 |
| **ScTET64_45367** | 615 | Sen_c3921_N | 5869 | 77 | 2 | 55.4703 |
| **ScTET64_3114** | 44,911 | Sen_c1029 | 2123 | 723 | 2 | 55.4703 |
| **ScTET64_68018** | 282,488 | Sen_c5116 | 2739 | 2779 | 2 | 62.4685 |
| **ScTET64_448** | 248,412 | Sen_c8022 | 1950 | 2018 | 2 | 62.4685 |
| **ScTET64_6285** | 1,451,803 | Sen_c11204 | 849 | 826 | 2 | 64.8521 |
| **ScTET64_6285** | 1,451,803 | Sen_c2097 | 1210 | 1210 | 2 | 67.4539 |
| **ScTET64_47246** | 604 | Sen_c4856 | 1474 | 102 | 2 | 69.9405 |
| **ScTET64_11052** | 983 | Sen_c4856 | 1474 | 237 | 2 | 69.9405 |
| **ScTET64_35152** | 683 | Sen_c4856 | 1474 | 300 | 2 | 69.9405 |
| **ScTET64_67732** | 81,755 | Sen_c11016 | 2192 | 1959 | 2 | 77.4056 |
| **ScTET64_2461** | 3,567,568 | Sen_c10885 | 1031 | 966 | 2 | 78.1900 |
| **ScTET64_2461** | 3,567,568 | Sen_c4880 | 1966 | 1707 | 2 | 82.9946 |
| **ScTET64_2461** | 3,567,568 | Sen_c10387 | 1241 | 816 | 2 | 83.7785 |
| **ScTET64_2461** | 3,567,568 | Sen_c12194 | 3611 | 2203 | 2 | 83.7785 |
| **ScTET64_2461** | 3,567,568 | Sen_c1486 | 1729 | 1729 | 2 | 83.7785 |
| **ScTET64_2461** | 3,567,568 | Sen_c716 | 1881 | 1345 | 2 | 84.5749 |
| **ScTET64_2461** | 3,567,568 | Sen_c7461 | 1530 | 1343 | 2 | 87.7942 |
| **ScTET64_68480** | 126,539 | Sen_c11809 * | 571 | 552 | 3 | 8.8156 |
| **ScTET64_68181** | 793,708 | Sen_c7880_P * | 749 | 749 | 3 | 11.2674 |
| **ScTET64_68227** | 5,525,173 | Sen_c9365 | 1359 | 1362 | 3 | 11.2674 |
| **ScTET64_68782** | 862,966 | Sen_c1099 * | 2333 | 2098 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c12332 * | 1216 | 1316 | 3 | 12.1184 |
| **ScTET64_23605** | 792 | Sen_c1420 * | 2129 | 201 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c1617_P * | 729 | 676 | 3 | 12.1184 |
| **ScTET64_67841** | 29,104 | Sen_c1934 * | 3832 | 3891 | 3 | 12.1184 |
| **ScTET64_69** | 622,948 | Sen_c2420_P * | 987 | 948 | 3 | 12.1184 |
| **ScTET64_68066** | 5,850,706 | Sen_c3712 * | 1082 | 1092 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c373_P * | 975 | 840 | 3 | 12.1184 |
| **ScTET64_68782** | 862,966 | Sen_c4 * | 3397 | 2817 | 3 | 12.1184 |
| **ScTET64_68782** | 862,966 | Sen_c4339 * | 1099 | 1125 | 3 | 12.1184 |
| **ScTET64_68066** | 5,850,706 | Sen_c4571 * | 2177 | 1963 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c4848 * | 1115 | 1099 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c4868 * | 1885 | 1920 | 3 | 12.1184 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_68782** | 862,966 | Sen_c5720 * | 2087 | 1903 | 3 | 12.1184 |
| **ScTET64_130** | 1,242,593 | Sen_c5947_P * | 2980 | 2986 | 3 | 12.1184 |
| **ScTET64_68631** | 47,590 | Sen_c5992 * | 1837 | 1860 | 3 | 12.1184 |
| **ScTET64_170** | 15,013 | Sen_c6189 * | 1274 | 1222 | 3 | 12.1184 |
| **ScTET64_68782** | 862,966 | Sen_c6205 * | 1618 | 1435 | 3 | 12.1184 |
| **ScTET64_68782** | 862,966 | Sen_c709_P * | 1751 | 1637 | 3 | 12.1184 |
| **ScTET64_130** | 1,242,593 | Sen_c7609 * | 1248 | 1075 | 3 | 12.1184 |
| **ScTET64_68500** | 20,274 | Sen_c7666 * | 2119 | 2131 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c7826 * | 1229 | 1148 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c8307 * | 1004 | 733 | 3 | 12.1184 |
| **ScTET64_68480** | 126,539 | Sen_c8409_P * | 3219 | 3072 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c8562_P * | 1390 | 1410 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c9310 * | 1085 | 1096 | 3 | 12.1184 |
| **ScTET64_69** | 622,948 | Sen_c9620 * | 2563 | 2395 | 3 | 12.1184 |
| **ScTET64_68920** | 168,526 | Sen_c9906 * | 1994 | 1554 | 3 | 12.1184 |
| **ScTET64_68066** | 5,850,706 | Sen_c9925 * | 1570 | 1586 | 3 | 12.1184 |
| **ScTET64_68920** | 168,526 | Sen_c1420 * | 2129 | 1945 | 3 | 12.1184 |
| **ScTET64_68227** | 5,525,173 | Sen_c1543 * | 577 | 205 | 3 | 12.9030 |
| **ScTET64_68227** | 5,525,173 | Sen_c1580 * | 1813 | 1810 | 3 | 12.9030 |
| **ScTET64_68227** | 5,525,173 | Sen_c1955 * | 1872 | 1647 | 3 | 12.9030 |
| **ScTET64_68227** | 5,525,173 | Sen_c2553 * | 1496 | 1500 | 3 | 12.9030 |
| **ScTET64_68227** | 5,525,173 | Sen_c4811 * | 1178 | 1086 | 3 | 12.9030 |
| **ScTET64_68227** | 5,525,173 | Sen_c5102 * | 2496 | 1920 | 3 | 12.9030 |
| **ScTET64_68227** | 5,525,173 | Sen_c5814 * | 2471 | 2515 | 3 | 12.9030 |
| **ScTET64_68227** | 5,525,173 | Sen_c9047 * | 826 | 766 | 3 | 12.9030 |
| **ScTET64_52364** | 576 | Sen_c10607 * | 1357 | 203 | 3 | 12.9030 |
| **ScTET64_68066** | 5,850,706 | Sen_c2248 * | 995 | 992 | 3 | 14.5371 |
| **ScTET64_68066** | 5,850,706 | Sen_c1763 | 2751 | 2512 | 3 | 18.5324 |
| **ScTET64_68066** | 5,850,706 | Sen_c8394 | 1921 | 1946 | 3 | 20.2123 |
| **ScTET64_68066** | 5,850,706 | Sen_c2494 | 1731 | 1749 | 3 | 21.0631 |
| **ScTET64_68066** | 5,850,706 | Sen_c5098 | 5098 | 726 | 3 | 21.8727 |
| **ScTET64_68066** | 5,850,706 | Sen_c875 | 1945 | 1074 | 3 | 21.8727 |
| **ScTET64_68066** | 5,850,706 | Sen_c14436 | 1770 | 1779 | 3 | 22.6569 |
| **ScTET64_33928** | 693 | Sen_c8159 | 2453 | 258 | 3 | 24.2315 |
| **ScTET64_67385** | 501 | Sen_c8159 | 2453 | 104 | 3 | 24.2315 |
| **ScTET64_68066** | 5,850,706 | Sen_c8159 | 2453 | 765 | 3 | 24.2315 |
| **ScTET64_68066** | 5,850,706 | Sen_c2759 | 2398 | 2379 | 3 | 25.0282 |
| **ScTET64_68066** | 5,850,706 | Sen_c607_N | 3581 | 3321 | 3 | 25.0282 |
| **ScTET64_68066** | 5,850,706 | Sen_c10514 | 1503 | 1474 | 3 | 25.0282 |
| **ScTET64_68066** | 5,850,706 | Sen_c14398 | 976 | 644 | 3 | 26.6535 |
| **ScTET64_68066** | 5,850,706 | Sen_c1559_N | 2755 | 628 | 3 | 26.6535 |
| **ScTET64_68066** | 5,850,706 | Sen_c4762 | 913 | 875 | 3 | 26.6535 |
| **ScTET64_68066** | 5,850,706 | Sen_c3003 | 1784 | 1866 | 3 | 27.4628 |
| **ScTET64_68066** | 5,850,706 | Sen_c5047 | 1773 | 1746 | 3 | 28.2591 |
| **ScTET64_68066** | 5,850,706 | Sen_c9758 | 1590 | 327 | 3 | 28.2591 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_49832** | 589 | Sen_c9758 | 1590 | 76 | 3 | 28.2591 |
| **ScTET64_4** | 2,125,992 | Sen_c6728 | 756 | 428 | 3 | 33.8653 |
| **ScTET64_4** | 2,125,992 | Sen_c879 | 2880 | 2351 | 3 | 33.8653 |
| **ScTET64_4** | 2,125,992 | Sen_c3210 | 1163 | 1167 | 3 | 35.4378 |
| **ScTET64_4** | 2,125,992 | Sen_c3878 | 1417 | 1150 | 3 | 39.4219 |
| **ScTET64_34483** | 688 | Sen_c6740_N | 3190 | 146 | 3 | 41.0477 |
| **ScTET64_29081** | 735 | Sen_c1257 | 2782 | 82 | 3 | 65.9820 |
| **ScTET64_68692** | 1,152,996 | Sen_c12378 | 2016 | 2025 | 3 | 67.5562 |
| **ScTET64_68692** | 1,152,996 | Sen_c331 | 1416 | 1164 | 3 | 71.5431 |
| **ScTET64_36635** | 672 | Sen_c331 | 1416 | 184 | 3 | 71.5431 |
| **ScTET64_67744** | 1,268,320 | Sen_c12930 | 1567 | 932 | 3 | 82.4221 |
| **ScTET64_33248** | 698 | Sen_c1170 | 2021 | 260 | 3 | 84.0479 |
| **ScTET64_67744** | 1,268,320 | Sen_c1170 | 2021 | 1487 | 3 | 84.0479 |
| **ScTET64_67840** | 4,194,686 | Sen_c10431 * | 1723 | 1653 | 4 | 0.0000 |
| **ScTET64_68798** | 2,332,737 | Sen_c3881 * | 2997 | 3056 | 4 | 2.4238 |
| **ScTET64_68932** | 543,104 | Sen_c6735 * | 1714 | 1709 | 4 | 4.8104 |
| **ScTET64_68932** | 543,104 | Sen_c1246 * | 1269 | 1197 | 4 | 12.3664 |
| **ScTET64_68053** | 20,759 | Sen_c3183 * | 1647 | 1962 | 4 | 12.3664 |
| **ScTET64_27574** | 749 | Sen_c7529 * | 107 | 107 | 4 | 12.3664 |
| **ScTET64_68476** | 2,944,389 | Sen_c13812_P * | 958 | 807 | 4 | 13.1522 |
| **ScTET64_68476** | 2,944,389 | Sen_c16154 * | 1230 | 1229 | 4 | 13.1522 |
| **ScTET64_4238** | 205,196 | Sen_c3776 * | 2325 | 1298 | 4 | 13.1522 |
| **ScTET64_67840** | 4,194,686 | Sen_c3085 * | 2536 | 2570 | 4 | 13.9506 |
| **ScTET64_67840** | 4,194,686 | Sen_c1483 * | 1652 | 1349 | 4 | 13.9506 |
| **ScTET64_68720** | 2,345,901 | Sen_c2 * | 2887 | 2989 | 4 | 14.7365 |
| **ScTET64_67840** | 4,194,686 | Sen_c318 * | 1848 | 1885 | 4 | 14.7365 |
| **ScTET64_67840** | 4,194,686 | Sen_c3315 * | 2054 | 1939 | 4 | 14.7365 |
| **ScTET64_68720** | 2,345,901 | Sen_c3708 * | 1612 | 1613 | 4 | 14.7365 |
| **ScTET64_67840** | 4,194,686 | Sen_c5442 * | 1419 | 1424 | 4 | 14.7365 |
| **ScTET64_68720** | 2,345,901 | Sen_c6172_N * | 3969 | 3740 | 4 | 14.7365 |
| **ScTET64_68720** | 2,345,901 | Sen_c7391 * | 1297 | 1297 | 4 | 14.7365 |
| **ScTET64_68720** | 2,345,901 | Sen_c8645 * | 1327 | 1336 | 4 | 14.7365 |
| **ScTET64_67840** | 4,194,686 | Sen_c8935 * | 1405 | 1384 | 4 | 14.7365 |
| **ScTET64_38669** | 657 | Sen_c1141 * | 2328 | 232 | 4 | 15.5224 |
| **ScTET64_68720** | 2,345,901 | Sen_c1630 * | 1387 | 1370 | 4 | 15.5224 |
| **ScTET64_68720** | 2,345,901 | Sen_c1772 * | 1010 | 1001 | 4 | 15.5224 |
| **ScTET64_58** | 4,366,797 | Sen_c2075 * | 2377 | 2091 | 4 | 15.5224 |
| **ScTET64_58** | 4,366,797 | Sen_c5724_N * | 2123 | 2147 | 4 | 15.5224 |
| **ScTET64_68720** | 2,345,901 | Sen_c1141 * | 2328 | 2345 | 4 | 15.5224 |
| **ScTET64_58** | 4,366,797 | Sen_c2345 * | 1767 | 1441 | 4 | 17.1292 |
| **ScTET64_58** | 4,366,797 | Sen_c1331 * | 2632 | 2730 | 4 | 17.9275 |
| **ScTET64_58** | 4,366,797 | Sen_c2281 * | 2050 | 2064 | 4 | 17.9275 |
| **ScTET64_58** | 4,366,797 | Sen_c2700 * | 2345 | 2362 | 4 | 17.9275 |
| **ScTET64_58** | 4,366,797 | Sen_c1008 * | 608 | 515 | 4 | 18.7132 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_58** | 4,366,797 | Sen_c809_N | 2470 | 2530 | 4 | 18.7132 |
| **ScTET64_32261** | 706 | Sen_c9316 | 913 | 83 | 4 | 19.5242 |
| **ScTET64_58** | 4,366,797 | Sen_c9316 | 913 | 522 | 4 | 19.5242 |
| **ScTET64_58** | 4,366,797 | Sen_c1433 | 925 | 780 | 4 | 20.3097 |
| **ScTET64_58** | 4,366,797 | Sen_c8726 | 1353 | 1254 | 4 | 20.3097 |
| **ScTET64_58** | 4,366,797 | Sen_c12731 | 2707 | 2719 | 4 | 21.0952 |
| **ScTET64_58** | 4,366,797 | Sen_c6599 | 1775 | 1435 | 4 | 21.0952 |
| **ScTET64_58** | 4,366,797 | Sen_c498 | 1410 | 1429 | 4 | 23.4788 |
| **ScTET64_58** | 4,366,797 | Sen_c1400 | 4763 | 4813 | 4 | 29.1684 |
| **ScTET64_63853** | 517 | Sen_c568 | 4744 | 521 | 4 | 38.7938 |
| **ScTET64_42798** | 630 | Sen_c7109 | 2843 | 218 | 4 | 41.1547 |
| **ScTET64_12177** | 961 | Sen_c13519 | 2003 | 139 | 4 | 67.6552 |
| **ScTET64_68476** | 2,944,389 | Sen_c2526 | 1932 | 1897 | 4 | 68.4390 |
| **ScTET64_68476** | 2,944,389 | Sen_c10059 | 1540 | 1520 | 4 | 70.8440 |
| **ScTET64_68476** | 2,944,389 | Sen_c8558 | 1917 | 1837 | 4 | 73.2489 |
| **ScTET64_50578** | 585 | Sen_c7104 | 1440 | 351 | 4 | 74.0327 |
| **ScTET64_68476** | 2,944,389 | Sen_c138 | 1229 | 842 | 4 | 74.0327 |
| **ScTET64_28388** | 742 | Sen_c3208 | 1424 | 157 | 4 | 74.0327 |
| **ScTET64_68476** | 2,944,389 | Sen_c4622 | 1132 | 1095 | 4 | 74.0327 |
| **ScTET64_59181** | 541 | Sen_c7104 | 1440 | 374 | 4 | 74.0327 |
| **ScTET64_68476** | 2,944,389 | Sen_c3208 | 1424 | 1185 | 4 | 74.0327 |
| **ScTET64_68476** | 2,944,389 | Sen_c7104 | 1440 | 992 | 4 | 74.0327 |
| **ScTET64_39393** | 652 | Sen_c3208 | 1424 | 111 | 4 | 74.0327 |
| **ScTET64_68476** | 2,944,389 | Sen_c11109 | 2087 | 2097 | 4 | 74.8165 |
| **ScTET64_68958** | 1,521,489 | Sen_c2304 | 1367 | 964 | 4 | 74.8165 |
| **ScTET64_68476** | 2,944,389 | Sen_c8827 | 1176 | 1188 | 4 | 74.8165 |
| **ScTET64_68958** | 1,521,489 | Sen_c11321 | 1631 | 1324 | 4 | 75.6389 |
| **ScTET64_68958** | 1,521,489 | Sen_c8808 | 1739 | 1743 | 4 | 75.6389 |
| **ScTET64_68958** | 1,521,489 | Sen_c3705_N | 2857 | 2400 | 4 | 77.2113 |
| **ScTET64_68958** | 1,521,489 | Sen_c641 | 1266 | 1254 | 4 | 77.2113 |
| **ScTET64_56013** | 557 | Sen_c3705_N | 2857 | 157 | 4 | 77.2113 |
| **ScTET64_734** | 2,210,660 | Sen_c12824 | 1179 | 1216 | 4 | 77.9949 |
| **ScTET64_734** | 2,210,660 | Sen_c2165 | 1712 | 935 | 4 | 77.9949 |
| **ScTET64_734** | 2,210,660 | Sen_c6948 | 1629 | 1626 | 4 | 77.9949 |
| **ScTET64_734** | 2,210,660 | Sen_c31 | 5423 | 2205 | 4 | 77.9949 |
| **ScTET64_68958** | 1,521,489 | Sen_c3916 | 805 | 814 | 4 | 79.6166 |
| **ScTET64_1820** | 3,711,605 | Sen_c7404 | 3318 | 2041 | 5 | 0.0000 |
| **ScTET64_1820** | 3,711,605 | Sen_c13242 | 1791 | 1808 | 5 | 7.5689 |
| **ScTET64_1820** | 3,711,605 | Sen_c11199 | 1213 | 938 | 5 | 14.1578 |
| **ScTET64_1820** | 3,711,605 | Sen_c443 | 1448 | 1457 | 5 | 14.9546 |
| **ScTET64_68729** | 4,218,231 | Sen_c939 | 2237 | 1791 | 5 | 24.9395 |
| **ScTET64_68456** | 894,304 | Sen_c1237 | 1340 | 1340 | 5 | 28.9348 |
| **ScTET64_68456** | 894,304 | Sen_c17445 | 952 | 958 | 5 | 28.9348 |
| **ScTET64_54359** | 565 | Sen_c3975 | 1800 | 545 | 5 | 28.9348 |
| **ScTET64_7922** | 78,322 | Sen_c3975 | 1800 | 1313 | 5 | 28.9348 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_40161** | 647 | Sen_c3506 | 1600 | 379 | 5 | 30.6167 |
| **ScTET64_37580** | 665 | Sen_c3641_N | 1878 | 121 | 5 | 30.6167 |
| **ScTET64_67624** | 122,846 | Sen_c3797 | 4453 | 4065 | 5 | 30.6167 |
| **ScTET64_60** | 429,441 | Sen_c4094 | 1869 | 1775 | 5 | 30.6167 |
| **ScTET64_67981** | 846,468 | Sen_c5265_N | 1994 | 2017 | 5 | 30.6167 |
| **ScTET64_68787** | 95,260 | Sen_c813 | 1163 | 1166 | 5 | 30.6167 |
| **ScTET64_1321** | 275,775 | Sen_c8169 | 2871 | 2251 | 5 | 30.6167 |
| **ScTET64_4386** | 1,640,860 | Sen_c867_N | 1731 | 1757 | 5 | 30.6167 |
| **ScTET64_53421** | 570 | Sen_c12879 | 1225 | 186 | 5 | 30.6167 |
| **ScTET64_2574** | 5,589 | Sen_c3506 | 1600 | 608 | 5 | 30.6167 |
| **ScTET64_786** | 906,606 | Sen_c3641_N | 1878 | 1541 | 5 | 30.6167 |
| **ScTET64_4386** | 1,640,860 | Sen_c12879 | 1225 | 898 | 5 | 30.6167 |
| **ScTET64_68068** | 61,188 | Sen_c4573_P | 1619 | 1272 | 5 | 31.4678 |
| **ScTET64_67981** | 846,468 | Sen_c8439 | 1798 | 982 | 5 | 32.3190 |
| **ScTET64_10434** | 996 | Sen_c8439 | 1798 | 547 | 5 | 32.3190 |
| **ScTET64_68787** | 95,260 | Sen_c786 | 3313 | 3403 | 5 | 33.1289 |
| **ScTET64_44010** | 623 | Sen_c4023 | 2196 | 129 | 5 | 33.1289 |
| **ScTET64_22576** | 804 | Sen_c10292 | 3947 | 198 | 5 | 33.1289 |
| **ScTET64_67803** | 1,644,140 | Sen_c12170 | 524 | 526 | 5 | 35.6637 |
| **ScTET64_67929** | 1,533,296 | Sen_c12392 | 1583 | 1726 | 5 | 35.6637 |
| **ScTET64_67803** | 1,644,140 | Sen_c2725 | 2215 | 1891 | 5 | 35.6637 |
| **ScTET64_67929** | 1,533,296 | Sen_c5816 | 1467 | 1552 | 5 | 37.2395 |
| **ScTET64_68241** | 2,126,207 | Sen_c7833 | 1769 | 1810 | 5 | 44.7331 |
| **ScTET64_68860** | 7,789,171 | Sen_c656 | 1627 | 1517 | 6 | 0.0000 |
| **ScTET64_68860** | 7,789,171 | Sen_c870 | 1843 | 1577 | 6 | 0.0000 |
| **ScTET64_68860** | 7,789,171 | Sen_c3752 | 1061 | 992 | 6 | 0.7837 |
| **ScTET64_68860** | 7,789,171 | Sen_c3664_N | 2748 | 2587 | 6 | 3.9513 |
| **ScTET64_68860** | 7,789,171 | Sen_c8143 | 1301 | 1418 | 6 | 5.5490 |
| **ScTET64_26146** | 764 | Sen_c12358 | 1654 | 88 | 6 | 8.7719 |
| **ScTET64_41859** | 636 | Sen_c2024 | 800 | 200 | 6 | 8.7719 |
| **ScTET64_68860** | 7,789,171 | Sen_c2427 | 1978 | 2001 | 6 | 8.7719 |
| **ScTET64_68860** | 7,789,171 | Sen_c12358 | 1654 | 1610 | 6 | 8.7719 |
| **ScTET64_68860** | 7,789,171 | Sen_c2024 | 800 | 782 | 6 | 8.7719 |
| **ScTET64_68860** | 7,789,171 | Sen_c7750_N | 1472 | 1188 | 6 | 9.5682 |
| **ScTET64_68860** | 7,789,171 | Sen_c1284 | 1704 | 1475 | 6 | 21.4082 |
| **ScTET64_68860** | 7,789,171 | Sen_c7049 | 953 | 505 | 6 | 21.4082 |
| **ScTET64_18213** | 861 | Sen_c7049 | 953 | 394 | 6 | 21.4082 |
| **ScTET64_14443** | 921 | Sen_c7049 | 953 | 178 | 6 | 21.4082 |
| **ScTET64_68860** | 7,789,171 | Sen_c1087 | 1844 | 1815 | 6 | 23.7766 |
| **ScTET64_67789** | 192,225 | Sen_c1133_P | 2538 | 2540 | 6 | 35.3526 |
| **ScTET64_68802** | 52,693 | Sen_c13016_P | 1626 | 1613 | 6 | 35.3526 |
| **ScTET64_68860** | 7,789,171 | Sen_c3771 | 2140 | 1916 | 6 | 35.3526 |
| **ScTET64_68836** | 18,002 | Sen_c4353 | 3153 | 2999 | 6 | 35.3526 |
| **ScTET64_68184** | 875,752 | Sen_c6117 | 2904 | 2804 | 6 | 35.3526 |
| **ScTET64_1191** | 62,902 | Sen_c8972 | 1542 | 943 | 6 | 35.3526 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_25902** | 766 | Sen_c11331_N | 1354 | 283 | 6 | 36.2184 |
| **ScTET64_1484** | 24,429 | Sen_c11331_N | 1354 | 713 | 6 | 36.2184 |
| **ScTET64_1281** | 6,670 | Sen_c12892 | 1605 | 58 | 6 | 37.0842 |
| **ScTET64_18360** | 859 | Sen_c12892 | 1605 | 181 | 6 | 37.0842 |
| **ScTET64_68682** | 2,820,618 | Sen_c12892 | 1605 | 275 | 6 | 37.0842 |
| **ScTET64_2247** | 5,792 | Sen_c12892 | 1605 | 430 | 6 | 37.0842 |
| **ScTET64_68860** | 7,789,171 | Sen_c8200_P | 2116 | 2089 | 6 | 37.8808 |
| **ScTET64_67850** | 51,711 | Sen_c4786 | 3644 | 3665 | 6 | 38.6774 |
| **ScTET64_68404** | 3,013,363 | Sen_c12264 | 3204 | 3298 | 6 | 39.4615 |
| **ScTET64_68821** | 566,995 | Sen_c2605 | 2741 | 2590 | 6 | 39.4615 |
| **ScTET64_4744** | 1,159,436 | Sen_c3725 | 3614 | 1448 | 6 | 39.4615 |
| **ScTET64_68184** | 875,752 | Sen_c4127_P | 983 | 1022 | 6 | 39.4615 |
| **ScTET64_67801** | 79,431 | Sen_c4161_P | 960 | 979 | 6 | 39.4615 |
| **ScTET64_68184** | 875,752 | Sen_c4644 | 1830 | 112 | 6 | 39.4615 |
| **ScTET64_67937** | 122,600 | Sen_c5539 | 717 | 793 | 6 | 39.4615 |
| **ScTET64_68184** | 875,752 | Sen_c7221_P | 1239 | 1133 | 6 | 39.4615 |
| **ScTET64_4744** | 1,159,436 | Sen_c7324 | 2335 | 2341 | 6 | 39.4615 |
| **ScTET64_301** | 15,569 | Sen_c824 | 3231 | 2675 | 6 | 39.4615 |
| **ScTET64_1562** | 116,588 | Sen_c8464 | 2350 | 1964 | 6 | 39.4615 |
| **ScTET64_36950** | 670 | Sen_c2605 | 2741 | 228 | 6 | 39.4615 |
| **ScTET64_68860** | 7,789,171 | Sen_c3725 | 3614 | 1802 | 6 | 39.4615 |
| **ScTET64_68794** | 55,174 | Sen_c4185 | 995 | 480 | 6 | 39.4615 |
| **ScTET64_63032** | 521 | Sen_c4644 | 1830 | 1546 | 6 | 39.4615 |
| **ScTET64_68096** | 230,439 | Sen_c575 | 3106 | 2150 | 6 | 39.4615 |
| **ScTET64_848** | 779,428 | Sen_c4843 | 1341 | 1293 | 6 | 41.0602 |
| **ScTET64_68326** | 3,141,945 | Sen_c2849 | 1615 | 1479 | 6 | 44.3883 |
| **ScTET64_68326** | 3,141,945 | Sen_c4074 | 2687 | 2674 | 6 | 44.3883 |
| **ScTET64_68326** | 3,141,945 | Sen_c3142 | 1910 | 1905 | 6 | 45.1846 |
| **ScTET64_68326** | 3,141,945 | Sen_c5386 | 3365 | 3431 | 6 | 45.1846 |
| **ScTET64_68326** | 3,141,945 | Sen_c5410_P | 3365 | 3431 | 6 | 45.1846 |
| **ScTET64_27119** | 754 | Sen_c7607 | 884 | 305 | 6 | 46.7580 |
| **ScTET64_68326** | 3,141,945 | Sen_c7607 | 884 | 396 | 6 | 46.7580 |
| **ScTET64_67668** | 3,140,248 | Sen_c8457 | 2410 | 2455 | 6 | 69.6079 |
| **ScTET64_67668** | 3,140,248 | Sen_c11696 | 1736 | 1742 | 6 | 72.7823 |
| **ScTET64_67668** | 3,140,248 | Sen_c10807 | 870 | 881 | 6 | 73.5662 |
| **ScTET64_68146** | 4,944,594 | Sen_c9832 | 1101 | 1103 | 7 | 0.0000 |
| **ScTET64_68457** | 3,356,887 | Sen_c11208 | 1339 | 150 | 7 | 1.6515 |
| **ScTET64_67659** | 58,173 | Sen_c5487 | 2778 | 1607 | 7 | 13.0448 |
| **ScTET64_67957** | 807,823 | Sen_c10892 | 2702 | 2030 | 7 | 13.0448 |
| **ScTET64_48130** | 598 | Sen_c5487 | 2778 | 281 | 7 | 13.0448 |
| **ScTET64_8798** | 153,988 | Sen_c7145 | 2418 | 2508 | 7 | 14.7169 |
| **ScTET64_68087** | 441,253 | Sen_c9632 | 1078 | 963 | 7 | 15.5970 |
| **ScTET64_68529** | 284,269 | Sen_c6314 | 1285 | 1295 | 7 | 16.4925 |
| **ScTET64_68415** | 121,143 | Sen_c10361 | 843 | 785 | 7 | 17.3724 |
| **ScTET64_68279** | 122,095 | Sen_c7387 | 2092 | 2113 | 7 | 19.0782 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_12929** | 948 | Sen_c10194 | 857 | 208 | 7 | 20.8132 |
| **ScTET64_660** | 52,049 | Sen_c10194 | 857 | 887 | 7 | 20.8132 |
| **ScTET64_68430** | 970,663 | Sen_c10430 | 977 | 994 | 7 | 20.8132 |
| **ScTET64_68598** | 117,353 | Sen_c1052 | 2392 | 2323 | 7 | 20.8132 |
| **ScTET64_19800** | 839 | Sen_c1061_P | 2139 | 80 | 7 | 20.8132 |
| **ScTET64_36519** | 673 | Sen_c1061_P | 2139 | 269 | 7 | 20.8132 |
| **ScTET64_68317** | 55,000 | Sen_c1061_P | 2139 | 1277 | 7 | 20.8132 |
| **ScTET64_68738** | 53,650 | Sen_c10673_E | 1040 | 198 | 7 | 20.8132 |
| **ScTET64_768** | 214,749 | Sen_c10796 | 3403 | 3245 | 7 | 20.8132 |
| **ScTET64_457** | 103,614 | Sen_c10886 | 680 | 689 | 7 | 20.8132 |
| **ScTET64_68036** | 36,505 | Sen_c11706 | 1077 | 1035 | 7 | 20.8132 |
| **ScTET64_67957** | 807,823 | Sen_c11926 | 1781 | 1985 | 7 | 20.8132 |
| **ScTET64_67893** | 142,245 | Sen_c12132 | 1563 | 1569 | 7 | 20.8132 |
| **ScTET64_68864** | 990,603 | Sen_c12297_P | 1534 | 1224 | 7 | 20.8132 |
| **ScTET64_68279** | 122,095 | Sen_c1234 | 1109 | 981 | 7 | 20.8132 |
| **ScTET64_5622** | 239,663 | Sen_c12406 | 1396 | 487 | 7 | 20.8132 |
| **ScTET64_26217** | 763 | Sen_c12469 | 2235 | 171 | 7 | 20.8132 |
| **ScTET64_8602** | 43,936 | Sen_c13366 | 853 | 803 | 7 | 20.8132 |
| **ScTET64_67957** | 807,823 | Sen_c13532 | 1295 | 1152 | 7 | 20.8132 |
| **ScTET64_67738** | 102,972 | Sen_c1362 | 3499 | 3520 | 7 | 20.8132 |
| **ScTET64_31654** | 712 | Sen_c13854 | 1207 | 172 | 7 | 20.8132 |
| **ScTET64_68519** | 79,571 | Sen_c152 | 1526 | 1342 | 7 | 20.8132 |
| **ScTET64_4556** | 48,805 | Sen_c1805 | 3594 | 3703 | 7 | 20.8132 |
| **ScTET64_9246** | 547,461 | Sen_c1893 | 1806 | 1835 | 7 | 20.8132 |
| **ScTET64_68430** | 970,663 | Sen_c2014 | 1635 | 1239 | 7 | 20.8132 |
| **ScTET64_68777** | 36,633 | Sen_c2028 | 2149 | 1730 | 7 | 20.8132 |
| **ScTET64_68864** | 990,603 | Sen_c2234 | 1663 | 1683 | 7 | 20.8132 |
| **ScTET64_68430** | 970,663 | Sen_c2534 | 3513 | 3243 | 7 | 20.8132 |
| **ScTET64_67966** | 203,279 | Sen_c4040 | 1930 | 1563 | 7 | 20.8132 |
| **ScTET64_872** | 78,103 | Sen_c4439 | 2599 | 2521 | 7 | 20.8132 |
| **ScTET64_8602** | 43,936 | Sen_c4655 | 3651 | 3465 | 7 | 20.8132 |
| **ScTET64_603** | 75,627 | Sen_c4858 | 3755 | 3521 | 7 | 20.8132 |
| **ScTET64_68492** | 71,956 | Sen_c5236 | 1385 | 1395 | 7 | 20.8132 |
| **ScTET64_2510** | 5,634 | Sen_c5245 | 537 | 77 | 7 | 20.8132 |
| **ScTET64_3096** | 3,682 | Sen_c5478 | 1682 | 1061 | 7 | 20.8132 |
| **ScTET64_8325** | 1,151 | Sen_c6383 | 1582 | 473 | 7 | 20.8132 |
| **ScTET64_5075** | 22,920 | Sen_c6430 | 3004 | 3105 | 7 | 20.8132 |
| **ScTET64_6826** | 41,696 | Sen_c6855 | 1721 | 1711 | 7 | 20.8132 |
| **ScTET64_660** | 52,049 | Sen_c713 | 1512 | 1458 | 7 | 20.8132 |
| **ScTET64_872** | 78,103 | Sen_c7290 | 1121 | 1123 | 7 | 20.8132 |
| **ScTET64_67766** | 108,052 | Sen_c7583 | 2489 | 2320 | 7 | 20.8132 |
| **ScTET64_67785** | 327,025 | Sen_c7966 | 2516 | 1103 | 7 | 20.8132 |
| **ScTET64_3016** | 3,998 | Sen_c8353 | 2209 | 1691 | 7 | 20.8132 |
| **ScTET64_67809** | 61,190 | Sen_c8440 | 562 | 529 | 7 | 20.8132 |
| **ScTET64_5622** | 239,663 | Sen_c8811 | 1479 | 1482 | 7 | 20.8132 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_68123** | 34,128 | Sen_c975 | 1064 | 1064 | 7 | 20.8132 |
| **ScTET64_7646** | 1,222 | Sen_c10673_E | 1040 | 351 | 7 | 20.8132 |
| **ScTET64_53100** | 572 | Sen_c10796 | 3403 | 181 | 7 | 20.8132 |
| **ScTET64_68461** | 94,286 | Sen_c12469 | 2235 | 2077 | 7 | 20.8132 |
| **ScTET64_8602** | 43,936 | Sen_c13854 | 1207 | 444 | 7 | 20.8132 |
| **ScTET64_67893** | 142,245 | Sen_c14698 | 1517 | 1096 | 7 | 20.8132 |
| **ScTET64_32309** | 706 | Sen_c2534 | 3513 | 333 | 7 | 20.8132 |
| **ScTET64_67775** | 46,224 | Sen_c3734 | 2124 | 1426 | 7 | 20.8132 |
| **ScTET64_1998** | 45,828 | Sen_c5478 | 1682 | 1402 | 7 | 20.8132 |
| **ScTET64_2875** | 4,763 | Sen_c6383 | 1582 | 1282 | 7 | 20.8132 |
| **ScTET64_818** | 7,509 | Sen_c8353 | 2209 | 324 | 7 | 20.8132 |
| **ScTET64_940** | 7,211 | Sen_c6383 | 1582 | 204 | 7 | 20.8132 |
| **ScTET64_1939** | 1,267,191 | Sen_c219 | 1634 | 1735 | 7 | 25.5600 |
| **ScTET64_1939** | 1,267,191 | Sen_c3135 | 2920 | 2973 | 7 | 25.5600 |
| **ScTET64_1939** | 1,267,191 | Sen_c8381 | 1327 | 1259 | 7 | 25.5600 |
| **ScTET64_68146** | 4,944,594 | Sen_c8211 | 1943 | 1947 | 7 | 29.5050 |
| **ScTET64_68146** | 4,944,594 | Sen_c6441 | 3347 | 3357 | 7 | 31.0796 |
| **ScTET64_68146** | 4,944,594 | Sen_c6003 | 2681 | 2473 | 7 | 32.6792 |
| **ScTET64_68146** | 4,944,594 | Sen_c8004 | 2086 | 2099 | 7 | 32.6792 |
| **ScTET64_1194** | 257,634 | Sen_c9077 | 2020 | 1663 | 7 | 32.6792 |
| **ScTET64_1194** | 257,634 | Sen_c95 | 1614 | 1604 | 7 | 32.6792 |
| **ScTET64_2439** | 5,669 | Sen_c9077 | 2020 | 273 | 7 | 32.6792 |
| **ScTET64_39987** | 648 | Sen_c9077 | 2020 | 114 | 7 | 32.6792 |
| **ScTET64_68146** | 4,944,594 | Sen_c5441 | 1399 | 1406 | 7 | 36.6661 |
| **ScTET64_68146** | 4,944,594 | Sen_c5341 | 943 | 943 | 7 | 37.4501 |
| **ScTET64_68146** | 4,944,594 | Sen_c11362 | 1442 | 1635 | 7 | 38.2466 |
| **ScTET64_68146** | 4,944,594 | Sen_c5278 | 1267 | 1237 | 7 | 38.2466 |
| **ScTET64_68678** | 2,146,070 | Sen_c2780 | 950 | 860 | 7 | 42.2307 |
| **ScTET64_68678** | 2,146,070 | Sen_c4610 | 3415 | 3327 | 7 | 42.2307 |
| **ScTET64_18520** | 857 | Sen_c2146 | 861 | 345 | 7 | 63.2679 |
| **ScTET64_67844** | 159,740 | Sen_c10310 | 1301 | 1328 | 7 | 76.6695 |
| **ScTET64_68457** | 3,356,887 | Sen_c4169 | 642 | 265 | 7 | 76.6695 |
| **ScTET64_68457** | 3,356,887 | Sen_c6384 | 1165 | 1229 | 7 | 76.6695 |
| **ScTET64_68457** | 3,356,887 | Sen_c1035 | 2155 | 2155 | 7 | 77.4790 |
| **ScTET64_68457** | 3,356,887 | Sen_c11349 | 5179 | 5200 | 7 | 77.4790 |
| **ScTET64_68457** | 3,356,887 | Sen_c1104 | 1818 | 1739 | 7 | 79.8909 |
| **ScTET64_68457** | 3,356,887 | Sen_c6552 | 2651 | 2911 | 7 | 79.8909 |
| **ScTET64_68457** | 3,356,887 | Sen_c9720 | 4847 | 4748 | 7 | 79.8909 |
| **ScTET64_67967** | 7,944,149 | Sen_c12679 | 1250 | 1281 | 7 | 83.0739 |
| **ScTET64_67967** | 7,944,149 | Sen_c9503 | 664 | 664 | 7 | 83.9249 |
| **ScTET64_67967** | 7,944,149 | Sen_c1058 | 1276 | 1124 | 7 | 84.7759 |
| **ScTET64_67967** | 7,944,149 | Sen_c2218 | 1620 | 1693 | 7 | 84.7759 |
| **ScTET64_67967** | 7,944,149 | Sen_c3068 | 1420 | 180 | 7 | 84.7759 |
| **ScTET64_67967** | 7,944,149 | Sen_c7361 | 3430 | 2072 | 7 | 84.7759 |
| **ScTET64_33746** | 694 | Sen_c5173_N | 2905 | 306 | 7 | 85.5859 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_67967** | 7,944,149 | Sen_c5173_N | 2905 | 1758 | 7 | 85.5859 |
| **ScTET64_67967** | 7,944,149 | Sen_c11810 | 3500 | 3443 | 7 | 87.2135 |
| **ScTET64_67967** | 7,944,149 | Sen_c13684 | 1468 | 1324 | 7 | 87.2135 |
| **ScTET64_67967** | 7,944,149 | Sen_c11075 | 1463 | 634 | 7 | 88.0105 |
| **ScTET64_67967** | 7,944,149 | Sen_c5532 | 1548 | 1562 | 7 | 89.5868 |
| **ScTET64_67967** | 7,944,149 | Sen_c10549 | 1276 | 1119 | 7 | 91.1630 |
| **ScTET64_67967** | 7,944,149 | Sen_c3884 | 2875 | 2490 | 7 | 92.7397 |
| **ScTET64_67967** | 7,944,149 | Sen_c6569 | 1298 | 1182 | 7 | 92.7397 |
| **ScTET64_67967** | 7,944,149 | Sen_c9743 | 1687 | 1714 | 7 | 92.7397 |
| **ScTET64_33032** | 700 | Sen_c3884 | 2875 | 418 | 7 | 92.7397 |
| **ScTET64_67967** | 7,944,149 | Sen_c5096 | 1066 | 775 | 7 | 94.3159 |
| **ScTET64_67967** | 7,944,149 | Sen_c67 | 2610 | 1902 | 7 | 94.3159 |
| **ScTET64_67967** | 7,944,149 | Sen_c12630 | 1036 | 385 | 7 | 95.8925 |
| **ScTET64_67967** | 7,944,149 | Sen_c2098 | 1880 | 1822 | 7 | 95.8925 |
| **ScTET64_67967** | 7,944,149 | Sen_c3302 | 1183 | 833 | 7 | 95.8925 |
| **ScTET64_67967** | 7,944,149 | Sen_c4110 | 1579 | 1611 | 7 | 95.8925 |
| **ScTET64_67967** | 7,944,149 | Sen_c5054 | 1911 | 1943 | 7 | 95.8925 |
| **ScTET64_67967** | 7,944,149 | Sen_c852 | 1806 | 1684 | 7 | 95.8925 |
| **ScTET64_67967** | 7,944,149 | Sen_c9859_P | 1728 | 1781 | 7 | 95.8925 |
| **ScTET64_67795** | 2,767,170 | Sen_c4300 | 1142 | 721 | 7 | 98.2685 |
| **ScTET64_35407** | 681 | Sen_c9481 | 2274 | 228 | 7 | 98.2685 |
| **ScTET64_67795** | 2,767,170 | Sen_c9481 | 2274 | 2106 | 7 | 98.2685 |
| **ScTET64_67795** | 2,767,170 | Sen_c3121 | 3455 | 3495 | 7 | 99.0783 |
| **ScTET64_67795** | 2,767,170 | Sen_c3704 | 996 | 920 | 7 | 99.8753 |
| **ScTET64_67795** | 2,767,170 | Sen_c10866 | 1827 | 1394 | 7 | 100.6854 |
| **ScTET64_67795** | 2,767,170 | Sen_c2839 | 1544 | 1419 | 7 | 101.4955 |
| **ScTET64_67795** | 2,767,170 | Sen_c427 | 3791 | 3465 | 7 | 101.4955 |
| **ScTET64_67795** | 2,767,170 | Sen_c460 | 798 | 814 | 7 | 101.4955 |
| **ScTET64_67795** | 2,767,170 | Sen_c4912 | 1186 | 1126 | 7 | 101.4955 |
| **ScTET64_67795** | 2,767,170 | Sen_c6399_N | 2177 | 1641 | 7 | 101.4955 |
| **ScTET64_67795** | 2,767,170 | Sen_c8505 | 1464 | 1095 | 7 | 101.4955 |
| **ScTET64_67795** | 2,767,170 | Sen_c1817 | 1515 | 1529 | 7 | 102.2802 |
| **ScTET64_67795** | 2,767,170 | Sen_c3102 | 1440 | 1458 | 7 | 102.2802 |
| **ScTET64_67795** | 2,767,170 | Sen_c3243 | 2499 | 2508 | 7 | 102.2802 |
| **ScTET64_67795** | 2,767,170 | Sen_c4597 | 1864 | 1647 | 7 | 102.2802 |
| **ScTET64_67795** | 2,767,170 | Sen_c4665 | 1314 | 1304 | 7 | 102.2802 |
| **ScTET64_67795** | 2,767,170 | Sen_c6772 | 1706 | 1708 | 7 | 102.2802 |
| **ScTET64_2221** | 5,804 | Sen_c8348_P | 965 | 256 | 7 | 102.2802 |
| **ScTET64_67795** | 2,767,170 | Sen_c8348_P | 965 | 474 | 7 | 102.2802 |
| **ScTET64_67795** | 2,767,170 | Sen_c1228_P | 931 | 912 | 7 | 103.9621 |
| **ScTET64_67795** | 2,767,170 | Sen_c7942 | 3258 | 1162 | 7 | 109.2949 |
| **ScTET64_67996** | 18,540 | Sen_c4607 | 2468 | 2509 | 8 | 0.7839 |
| **ScTET64_6583** | 1,930,688 | Sen_c10440 | 1832 | 1835 | 8 | 1.5678 |
| **ScTET64_32483** | 705 | Sen_c5012 | 892 | 145 | 8 | 2.3515 |
| **ScTET64_22928** | 800 | Sen_c1780 | 2705 | 91 | 8 | 3.1604 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_66135** | 506 | Sen_c2861 | 2516 | 296 | 8 | 18.2442 |
| **ScTET64_28344** | 742 | Sen_c9018 | 1762 | 393 | 8 | 41.5108 |
| **ScTET64_49747** | 589 | Sen_c9018 | 1762 | 208 | 8 | 41.5108 |
| **ScTET64_2505** | 1,697,774 | Sen_c957 | 1415 | 1413 | 8 | 43.0854 |
| **ScTET64_2505** | 1,697,774 | Sen_c6632 | 1918 | 1575 | 8 | 43.8697 |
| **ScTET64_8882** | 6,191,619 | Sen_c5740 | 2787 | 2670 | 8 | 60.2084 |
| **ScTET64_8882** | 6,191,619 | Sen_c6326 | 1158 | 1171 | 8 | 60.9931 |
| **ScTET64_8882** | 6,191,619 | Sen_c1730 | 1917 | 1927 | 8 | 61.7903 |
| **ScTET64_8882** | 6,191,619 | Sen_c10162 | 2515 | 2344 | 8 | 62.6136 |
| **ScTET64_18691** | 855 | Sen_c10162 | 2515 | 79 | 8 | 62.6136 |
| **ScTET64_1** | 1,629,908 | Sen_c4209 | 1370 | 1375 | 9 | 7.8889 |
| **ScTET64_66474** | 505 | Sen_c1321 | 1112 | 113 | 9 | 9.4639 |
| **ScTET64_57906** | 547 | Sen_c1321 | 1112 | 153 | 9 | 9.4639 |
| **ScTET64_67686** | 61,324 | Sen_c1321 | 1112 | 118 | 9 | 9.4639 |
| **ScTET64_67807** | 19,227 | Sen_c3220 | 746 | 734 | 9 | 14.2224 |
| **ScTET64_33931** | 693 | Sen_c1513 | 1954 | 73 | 9 | 23.2458 |
| **ScTET64_5387** | 1,630 | Sen_c514_N | 1195 | 824 | 9 | 30.4006 |
| **ScTET64_198** | 49,911 | Sen_c12615 | 976 | 976 | 9 | 35.3555 |
| **ScTET64_68572** | 22,884 | Sen_c10153 | 2551 | 2648 | 9 | 37.0426 |
| **ScTET64_68824** |  | Sen_c2023 | 2465 | 2381 | 9 | 37.0426 |
| **ScTET64_141** | 47,506 | Sen_c14206 | 807 | 536 | 9 | 37.0426 |
| **ScTET64_68692** | 1,152,996 | Sen_c7777 | 1804 | 440 | 9 | 39.5311 |
| **ScTET64_63** | 66,166 | Sen_c11778 | 1549 | 1424 | 9 | 39.5311 |
| **ScTET64_68335** | 65,734 | Sen_c9413 | 1989 | 1138 | 9 | 39.5311 |
| **ScTET64_1983** | 5,960 | Sen_c9615 | 1616 | 245 | 9 | 39.5311 |
| **ScTET64_29219** | 734 | Sen_c7300 | 2453 | 53 | 9 | 40.3277 |
| **ScTET64_35617** | 679 | Sen_c10180_P | 1036 | 114 | 9 | 40.3277 |
| **ScTET64_68638** | 725,398 | Sen_c10207 | 1904 | 2455 | 9 | 40.3277 |
| **ScTET64_68824** | 262,210 | Sen_c10235 | 1916 | 1923 | 9 | 40.3277 |
| **ScTET64_20718** | 827 | Sen_c1202 | 1067 | 173 | 9 | 40.3277 |
| **ScTET64_59353** | 540 | Sen_c1231 | 782 | 294 | 9 | 40.3277 |
| **ScTET64_1568** | 49,675 | Sen_c12948 | 850 | 858 | 9 | 40.3277 |
| **ScTET64_145** | 61,039 | Sen_c13192 | 852 | 541 | 9 | 40.3277 |
| **ScTET64_68151** | 135,857 | Sen_c1326 | 2223 | 1617 | 9 | 40.3277 |
| **ScTET64_145** | 61,039 | Sen_c1600 | 2662 | 2733 | 9 | 40.3277 |
| **ScTET64_67823** | 51,730 | Sen_c1997 | 1477 | 1497 | 9 | 40.3277 |
| **ScTET64_67848** | 463,067 | Sen_c2362 | 1874 | 2099 | 9 | 40.3277 |
| **ScTET64_67627** | 90,596 | Sen_c2529 | 746 | 202 | 9 | 40.3277 |
| **ScTET64_67665** | 97,337 | Sen_c291 | 2239 | 2285 | 9 | 40.3277 |
| **ScTET64_68745** | 197,270 | Sen_c2958 | 6150 | 6146 | 9 | 40.3277 |
| **ScTET64_216** | 14,262 | Sen_c3040 | 1462 | 1132 | 9 | 40.3277 |
| **ScTET64_68884** | 49,548 | Sen_c3094 | 2806 | 2587 | 9 | 40.3277 |
| **ScTET64_68373** | 103,396 | Sen_c3197_N | 941 | 583 | 9 | 40.3277 |
| **ScTET64_68179** | 75,385 | Sen_c3603 | 1824 | 1776 | 9 | 40.3277 |
| **ScTET64_1221** | 68,729 | Sen_c3810 | 897 | 900 | 9 | 40.3277 |

| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_68745** | 197,270 | Sen_c386_P | 1527 | 1436 | 9 | 40.3277 |
| **ScTET64_650** | 826,423 | Sen_c4206 | 1550 | 1546 | 9 | 40.3277 |
| **ScTET64_2375** | 76,214 | Sen_c4384 | 1193 | 835 | 9 | 40.3277 |
| **ScTET64_739** | 129,637 | Sen_c4775 | 1001 | 1002 | 9 | 40.3277 |
| **ScTET64_14** | 39,855 | Sen_c5508 | 2388 | 1591 | 9 | 40.3277 |
| **ScTET64_68252** | 996,268 | Sen_c5610 | 1771 | 1728 | 9 | 40.3277 |
| **ScTET64_739** | 129,637 | Sen_c5857 | 2343 | 1936 | 9 | 40.3277 |
| **ScTET64_68652** | 53,497 | Sen_c606 | 1879 | 1760 | 9 | 40.3277 |
| **ScTET64_26286** | 763 | Sen_c6120 | 2086 | 62 | 9 | 40.3277 |
| **ScTET64_68667** | 811,928 | Sen_c684 | 911 | 917 | 9 | 40.3277 |
| **ScTET64_7608** | 1,227 | Sen_c7225 | 1233 | 180 | 9 | 40.3277 |
| **ScTET64_67848** | 463,067 | Sen_c7716 | 1607 | 1642 | 9 | 40.3277 |
| **ScTET64_68031** | 50,236 | Sen_c8237 | 1660 | 1617 | 9 | 40.3277 |
| **ScTET64_67989** | 39,592 | Sen_c9245 | 1265 | 328 | 9 | 40.3277 |
| **ScTET64_67848** | 463,067 | Sen_c9356 | 2785 | 2794 | 9 | 40.3277 |
| **ScTET64_1221** | 68,729 | Sen_c9806 | 2236 | 2260 | 9 | 40.3277 |
| **ScTET64_20737** | 827 | Sen_c10180_P | 1036 | 103 | 9 | 40.3277 |
| **ScTET64_1221** | 68,729 | Sen_c1202 | 1067 | 908 | 9 | 40.3277 |
| **ScTET64_353** | 21,364 | Sen_c2647 | 5405 | 4483 | 9 | 40.3277 |
| **ScTET64_67941** | 78,087 | Sen_c2703 | 3063 | 2183 | 9 | 40.3277 |
| **ScTET64_29000** | 736 | Sen_c3807 | 1594 | 520 | 9 | 40.3277 |
| **ScTET64_67939** | 38,480 | Sen_c438 | 3258 | 2442 | 9 | 40.3277 |
| **ScTET64_38471** | 659 | Sen_c4384 | 1193 | 251 | 9 | 40.3277 |
| **ScTET64_16845** | 881 | Sen_c5857 | 1079 | 91 | 9 | 40.3277 |
| **ScTET64_68491** | 49,504 | Sen_c7225 | 1233 | 858 | 9 | 40.3277 |
| **ScTET64_353** | 21,364 | Sen_c9245 | 1265 | 641 | 9 | 40.3277 |
| **ScTET64_24481** | 782 | Sen_c9806 | 2236 | 126 | 9 | 40.3277 |
| **ScTET64_141** | 47,506 | Sen_c10180_P | 1036 | 500 | 9 | 40.3277 |
| **ScTET64_68638** | 725,398 | Sen_c235 | 4326 | 3915 | 9 | 44.8227 |
| **ScTET64_18559** | 857 | Sen_c235 | 4326 | 128 | 9 | 44.8227 |
| **ScTET64_67903** | 54,324 | Sen_c146 | 758 | 758 | 9 | 48.9312 |
| **ScTET64_68209** | 1,351,264 | Sen_c11375 | 933 | 944 | 9 | 50.5058 |
| **ScTET64_42904** | 630 | Sen_c15266 | 1587 | 438 | 9 | 56.1735 |
| **ScTET64_27068** | 754 | Sen_c1979 | 3075 | 430 | 9 | 60.5711 |
| **ScTET64_17372** | 873 | Sen_c6760 | 664 | 162 | 9 | 61.3674 |
| **ScTET64_16107** | 894 | Sen_c6017 | 1681 | 292 | 9 | 63.7359 |
| **ScTET64_5168** | 1,706 | Sen_c3588 | 2793 | 82 | 9 | 68.5165 |
| **ScTET64_12645** | 953 | Sen_c5696 | 1736 | 96 | 9 | 79.2634 |
| **ScTET64_68510** | 3,137,168 | Sen_c925_P | 1711 | 1473 | 9 | 80.0466 |
| **ScTET64_68510** | 3,137,168 | Sen_c2641_P | 622 | 629 | 9 | 81.6171 |
| **ScTET64_68510** | 3,137,168 | Sen_c2875 | 1740 | 1579 | 9 | 81.6171 |
| **ScTET64_68510** | 3,137,168 | Sen_c3236 | 1539 | 1396 | 9 | 84.0565 |
| **ScTET64_68510** | 3,137,168 | Sen_c3439 | 2140 | 865 | 9 | 84.8653 |
| **ScTET64_33731** | 694 | Sen_c5348_N | 2667 | 236 | 9 | 85.6488 |
| **ScTET64_2550** | 102,414 | Sen_c5348_N | 2667 | 1815 | 9 | 85.6488 |

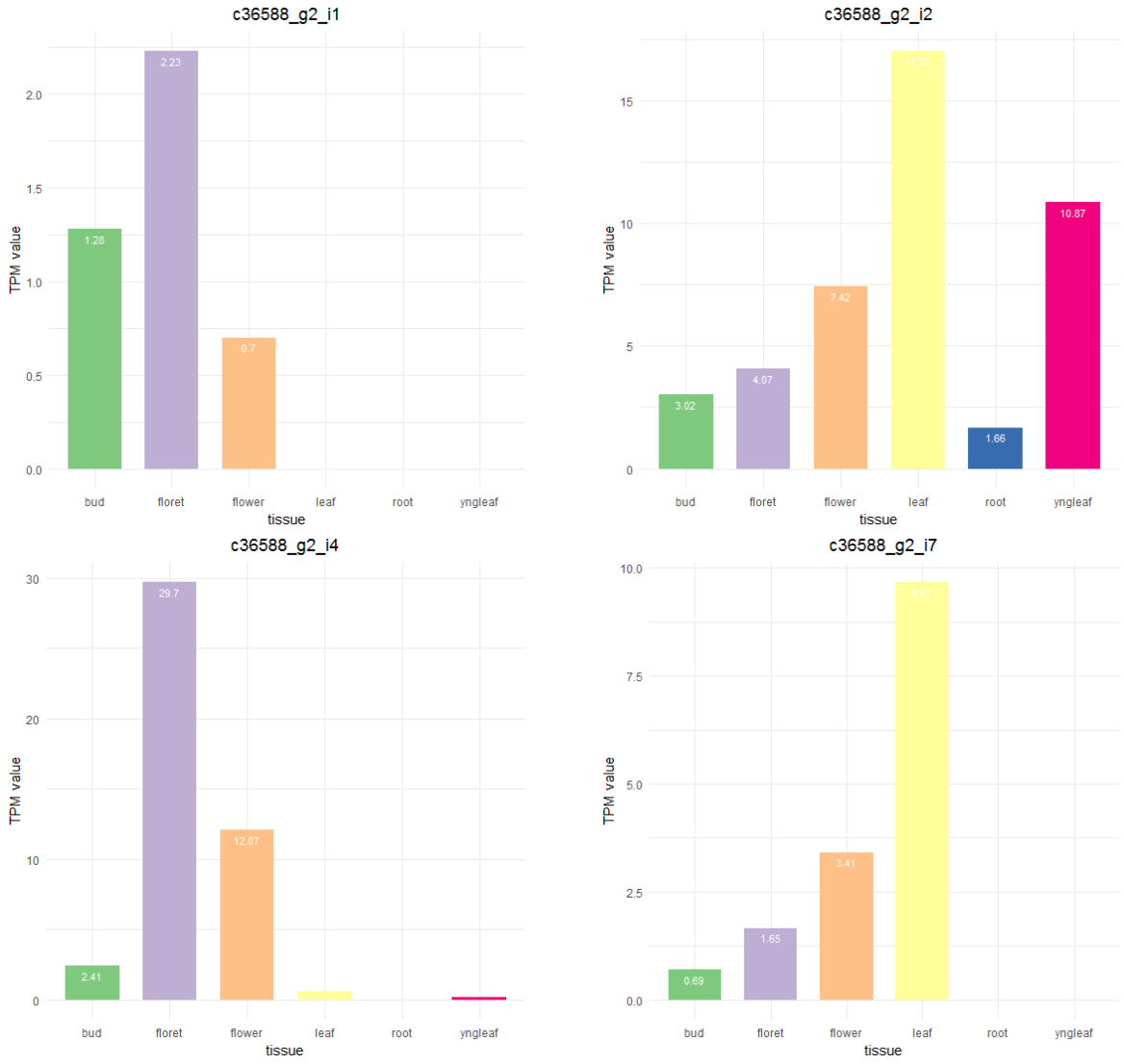
| Scaffold | Scaff_size | Hit_marker | Marker_len | Hit_len | LG | Position (cM) |
|---|---|---|---|---|---|---|
| **ScTET64_5192** | 3,606,217 | Sen_c2459 | 1703 | 1721 | 10 | 0.0000 |
| **ScTET64_5192** | 3,606,217 | Sen_c3428 | 967 | 491 | 10 | 2.3779 |
| **ScTET64_5192** | 3,606,217 | Sen_c4706 | 2181 | 2087 | 10 | 4.0059 |
| **ScTET64_5192** | 3,606,217 | Sen_c10902 | 2876 | 2884 | 10 | 5.6340 |
| **ScTET64_5192** | 3,606,217 | Sen_c1515 | 2510 | 2360 | 10 | 8.0119 |
| **ScTET64_68089** | 3,713,004 | Sen_c10575 | 3054 | 3081 | 10 | 24.1351 |
| **ScTET64_67783** | 63,327 | Sen_c10611 | 1495 | 1155 | 10 | 24.1351 |
| **ScTET64_67743** | 151,859 | Sen_c1359 | 4275 | 4334 | 10 | 24.1351 |
| **ScTET64_32237** | 707 | Sen_c2825 | 1112 | 313 | 10 | 24.1351 |
| **ScTET64_68056** | 669,265 | Sen_c3022_P | 2956 | 3020 | 10 | 24.1351 |
| **ScTET64_68089** | 3,713,004 | Sen_c3144_P | 1273 | 67 | 10 | 24.1351 |
| **ScTET64_38597** | 658 | Sen_c3853_P | 1373 | 98 | 10 | 24.1351 |
| **ScTET64_37961** | 662 | Sen_c3979 | 937 | 208 | 10 | 24.1351 |
| **ScTET64_68089** | 3,713,004 | Sen_c4331 | 3646 | 3432 | 10 | 24.1351 |
| **ScTET64_44660** | 619 | Sen_c4436 | 1636 | 236 | 10 | 24.1351 |
| **ScTET64_67703** | 33,696 | Sen_c4968 | 1913 | 1751 | 10 | 24.1351 |
| **ScTET64_6368** | 1,407 | Sen_c5496_P | 1883 | 360 | 10 | 24.1351 |
| **ScTET64_68037** | 150,233 | Sen_c573 | 970 | 652 | 10 | 24.1351 |
| **ScTET64_8107** | 36,430 | Sen_c7308 | 1295 | 449 | 10 | 24.1351 |
| **ScTET64_4096** | 78,224 | Sen_c7357 | 2915 | 2789 | 10 | 24.1351 |
| **ScTET64_480** | 454,307 | Sen_c7656 | 1185 | 823 | 10 | 24.1351 |
| **ScTET64_58987** | 542 | Sen_c7965 | 1148 | 50 | 10 | 24.1351 |
| **ScTET64_67743** | 151,859 | Sen_c9266 | 2895 | 2897 | 10 | 24.1351 |
| **ScTET64_13785** | 932 | Sen_c973 | 1542 | 313 | 10 | 24.1351 |
| **ScTET64_68830** | 68,457 | Sen_c2825 | 1112 | 786 | 10 | 24.1351 |
| **ScTET64_730** | 25,861 | Sen_c3144_P | 1273 | 1444 | 10 | 24.1351 |
| **ScTET64_58503** | 544 | Sen_c3979 | 937 | 439 | 10 | 24.1351 |
| **ScTET64_83** | 73,788 | Sen_c4436 | 1636 | 1461 | 10 | 24.1351 |
| **ScTET64_4806** | 114,834 | Sen_c5496_P | 1883 | 1508 | 10 | 24.1351 |
| **ScTET64_46393** | 609 | Sen_c2825 | 1112 | 210 | 10 | 24.1351 |
| **ScTET64_68056** | 669,265 | Sen_c4538 | 2020 | 2019 | 10 | 27.3725 |
| **ScTET64_21997** | 811 | Sen_c359 | 3418 | 102 | 10 | 54.4226 |
| **ScTET64_40301** | 646 | Sen_c4311_N | 1810 | 145 | 10 | 68.4969 |

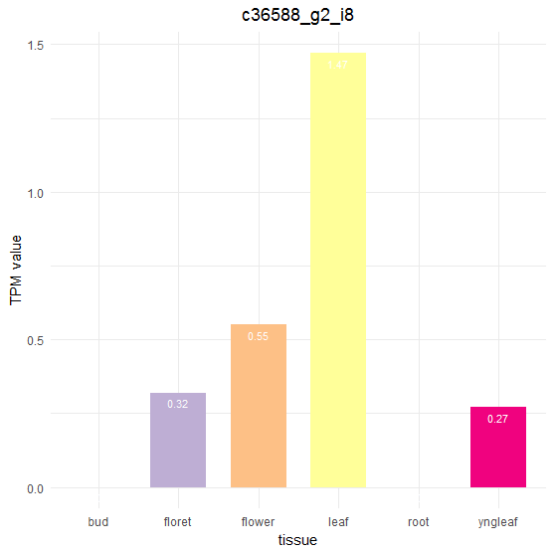
Sup 3: Details on the linkage group and size of aligned scaffolds from comparative genome analysis.

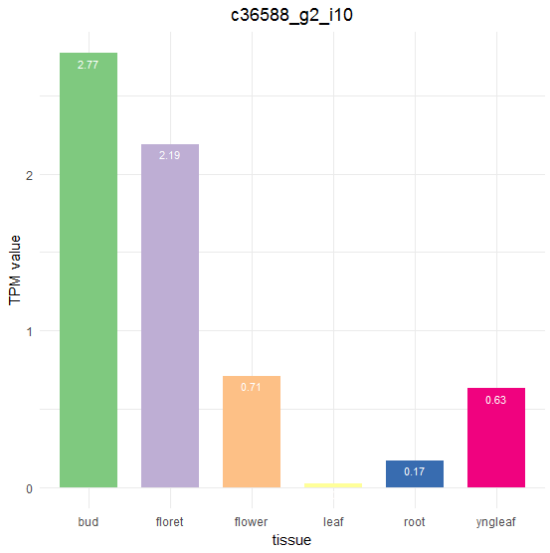
| QTL | *Senecio* scaffolds | *Senecio* LG | position | Scaffold size (bp) |
|---|---|---|---|---|
| ***Tolpis* LG2** | ScTET64_67848 | 9 | 40.32773 | 463,067 |
| | ScTET64_5192 | 10 | 0 | 3,606,217 |
| | ScTET64_103 | | | / |
| | ScTET64_67998 | | | / |
| | ScTET64_68056 | | | / |
| | ScTET64_68644 | | | / |
| **Sunflower LG17** | ScTET64_2840 | 1 | 42.47855 | 195,421 |
| | ScTET64_67649 | 1 | 36.33946 | 1,824,317 |
| | ScTET64_67869 | 1 | 42.47855 | 663,882 |
| | ScTET64_8458 | 1 | 42.47855 | 801,173 |
| | ScTET64_130 | 3 | 12.11837 | 1,242,593 |
| | ScTET64_4 | 3 | 33.86531 | 2,125,992 |
| | ScTET64_68227 | 3 | 11.26736 | 5,525,173 |
| | ScTET64_69 | 3 | 12.11837 | 622,948 |
| | ScTET64_58 | 4 | 15.52237 | 4,366,797 |
| | ScTET64_67840 | 4 | 0 | 4,194,686 |
| | ScTET64_1820 | 5 | 0 | 3,711,605 |
| | ScTET64_67803 | 5 | 35.66366 | 1,644,140 |
| | ScTET64_68456 | 5 | 28.93479 | 894,304 |
| | ScTET64_68729 | 5 | 24.93951 | 4,218,231 |
| | ScTET64_67795 | 7 | 98.26851 | 2,767,170 |
| | ScTET64_68146 | 7 | 0 | 4,944,594 |
| | ScTET64_5192 | 10 | 0 | 3,606,217 |
| | ScTET64_10 | | | / |
| | ScTET64_1294 | | | / |
| | ScTET64_2156 | | | / |
| | ScTET64_5930 | | | / |
| | ScTET64_67769 | | | / |
| | ScTET64_67888 | | | / |
| | ScTET64_67898 | | | / |
| | ScTET64_67946 | | | / |
| | ScTET64_67998 | | | / |
| | ScTET64_67999 | | | / |
| | ScTET64_68257 | | | / |
| | ScTET64_68436 | | | / |
| | ScTET64_68570 | | | / |

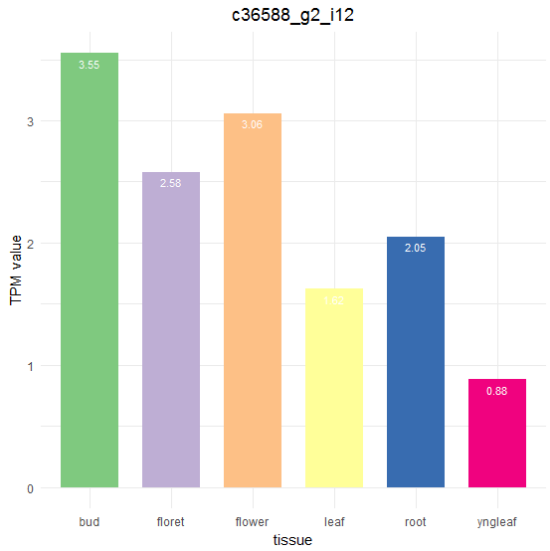
| QTL | *Senecio* scaffolds | *Senecio* LG | position | Scaffold size (bp) |
|---|---|---|---|---|
| **Lettuce LG1** | ScTET64_68586 | 1 | 57.45974 | 3,946,386 |
| | ScTET64_67908 | 2 | 9.660952 | 2,457,694 |
| | ScTET64_58 | 4 | 15.52237 | 4,366,797 |
| | ScTET64_68798 | 4 | 2.423848 | 2,332,737 |
| | ScTET64_68241 | 5 | 44.73311 | 2,126,207 |
| | ScTET64_67668 | 6 | 69.60791 | 3,140,248 |
| | ScTET64_68860 | 6 | 0 | 7,789,171 |
| | ScTET64_68678 | 7 | 42.23068 | 2,146,070 |
| | ScTET64_2505 | 8 | 43.08539 | 1,697,774 |
| | ScTET64_8882 | 8 | 60.20839 | 6,191,619 |
| | ScTET64_1 | 9 | 7.888913 | 1,629,908 |
| | ScTET64_67848 | 9 | 40.32773 | 463,067 |
| | ScTET64_67939 | 9 | 40.32773 | 38,480 |
| | ScTET64_68510 | 9 | 80.04662 | 3,137,168 |
| | ScTET64_68667 | 9 | 40.32773 | 811,928 |
| | ScTET64_68745 | 9 | 40.32773 | 197,270 |
| | ScTET64_68824 | 9 | 37.04264 | 262,210 |
| | ScTET64_5192 | 10 | 0 | 3,606,217 |
| | ScTET64_68056 | 10 | 24.13515 | 669,265 |
| | ScTET64_68089 | 10 | 24.13515 | 3,713,004 |
| | ScTET64_103 | | | / |
| | ScTET64_1294 | | | / |
| | ScTET64_2122 | | | / |
| | ScTET64_226 | | | / |
| | ScTET64_3631 | | | / |
| | ScTET64_4705 | | | / |
| | ScTET64_67770 | | | / |
| | ScTET64_67857 | | | / |
| | ScTET64_67998 | | | / |
| | ScTET64_68465 | | | / |
| | ScTET64_68515 | | | / |
| | ScTET64_68568 | | | / |
| | ScTET64_68570 | | | / |
| | ScTET64_68644 | | | / |
| | ScTET64_68665 | | | / |
| | ScTET64_68856 | | | / |
| | ScTET64_68891 | | | / |
| | ScTET64_68908 | | | / |
| | ScTET64_68911 | | | / |
| | ScTET64_68954 | | | / |
| | ScTET64_68957 | | | / |
| | ScTET64_8168 | | | / |
| | ScTET64_9 | | | / |
| | ScTET64_9404 | | | / |

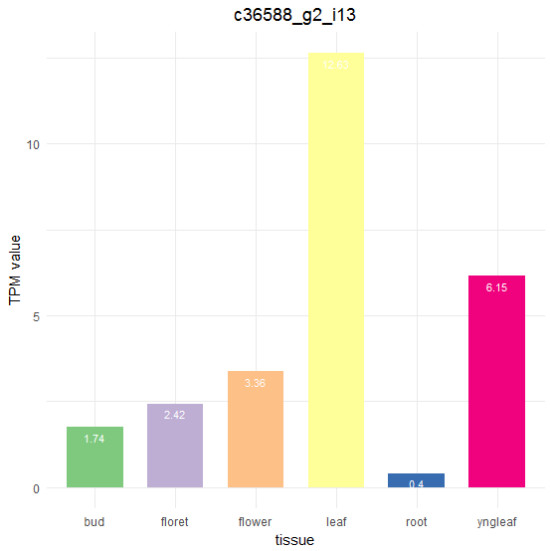
Sup 4: Calculated TPM (transcriptome per million) value of all homolog isoforms of *LRR-RLK* (c36588, c36143, c37873, c2375 and c4096) for different tissues (bud, floret, flower, leaf, root and young leaf). Expression pattern of each isoform is shown.

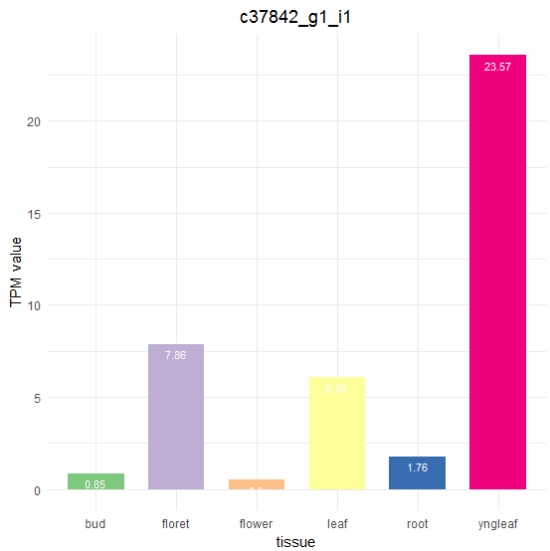


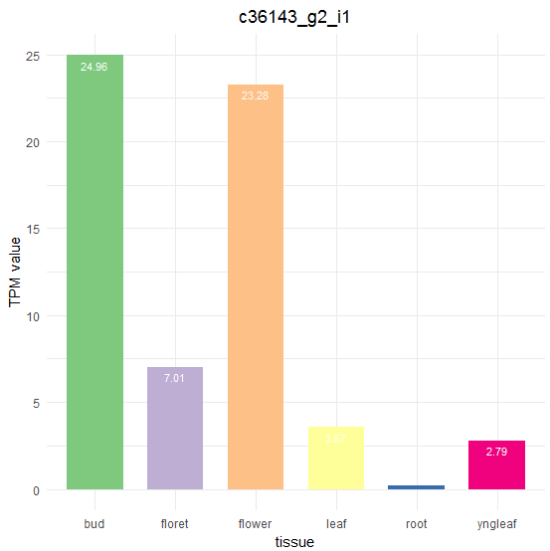

c36588_g2_i8
TPM value
1.5
1.0
0.5
0.0
0.32
0.55
1.47
0.27
bud
floret
flower
leaf
root
yngleaf
tissue

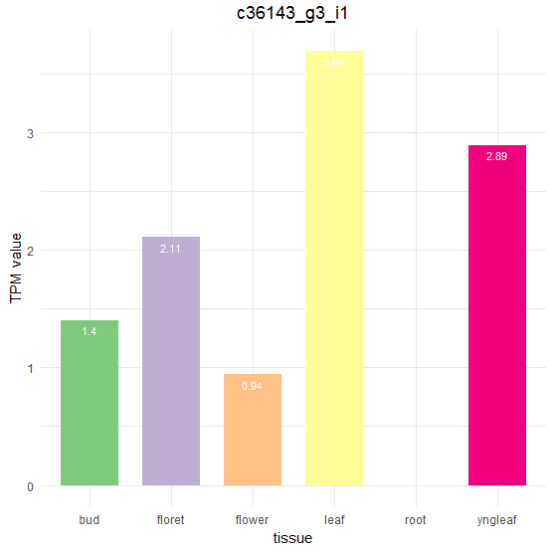

c36588_g2_i10
TPM value
2
1
0
2.77
2.19
0.71
0.17
0.63
bud
floret
flower
leaf
root
yngleaf
tissue

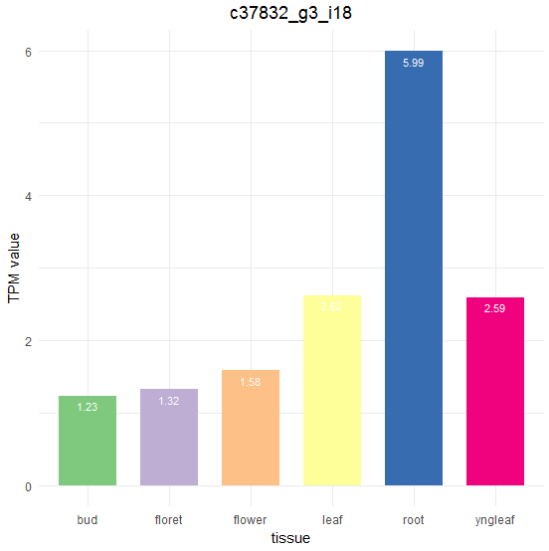

c36588_g2_i12
TPM value
3
2
1
0
3.55
2.58
3.06
1.62
2.05
0.88
bud
floret
flower
leaf
root
yngleaf
tissue

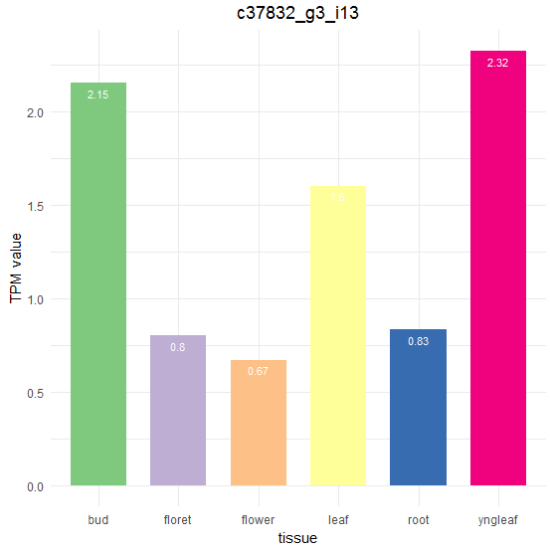

c36588_g2_i13
TPM value
10
5
0
1.74
2.42
3.36
12.60
0.4
6.15
bud
floret
flower
leaf
root
yngleaf
tissue

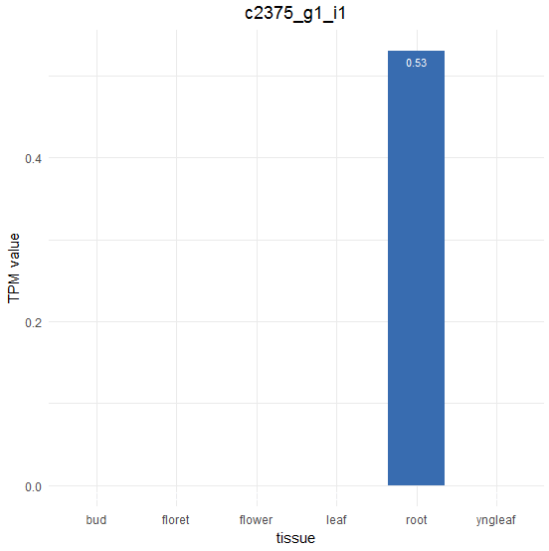

c37842_g1_i1
TPM value
20
15
10
5
0
0.85
7.86
6.06
1.76
23.57
bud
floret
flower
leaf
root
yngleaf
tissue

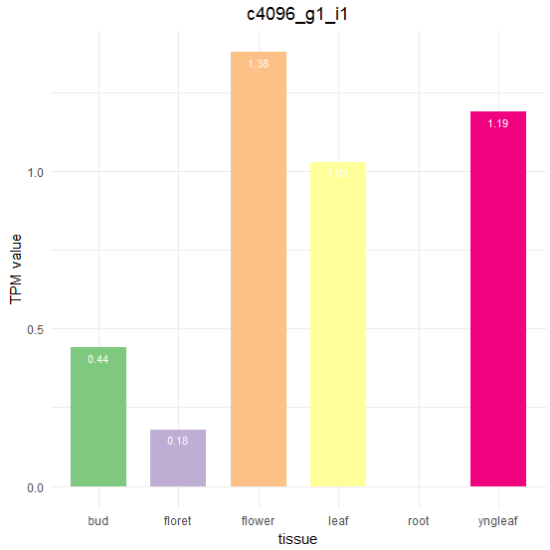

c36143_g2_i1
TPM value
25
20
15
10
5
0
24.96
7.01
23.28
3.57
2.79
bud
floret
flower
leaf
root
yngleaf
tissue


c36143_g3_i1
TPM value
tissue
bud
floret
flower
leaf
root
yngleaf
1.4
2.11
0.94
3.69
2.89

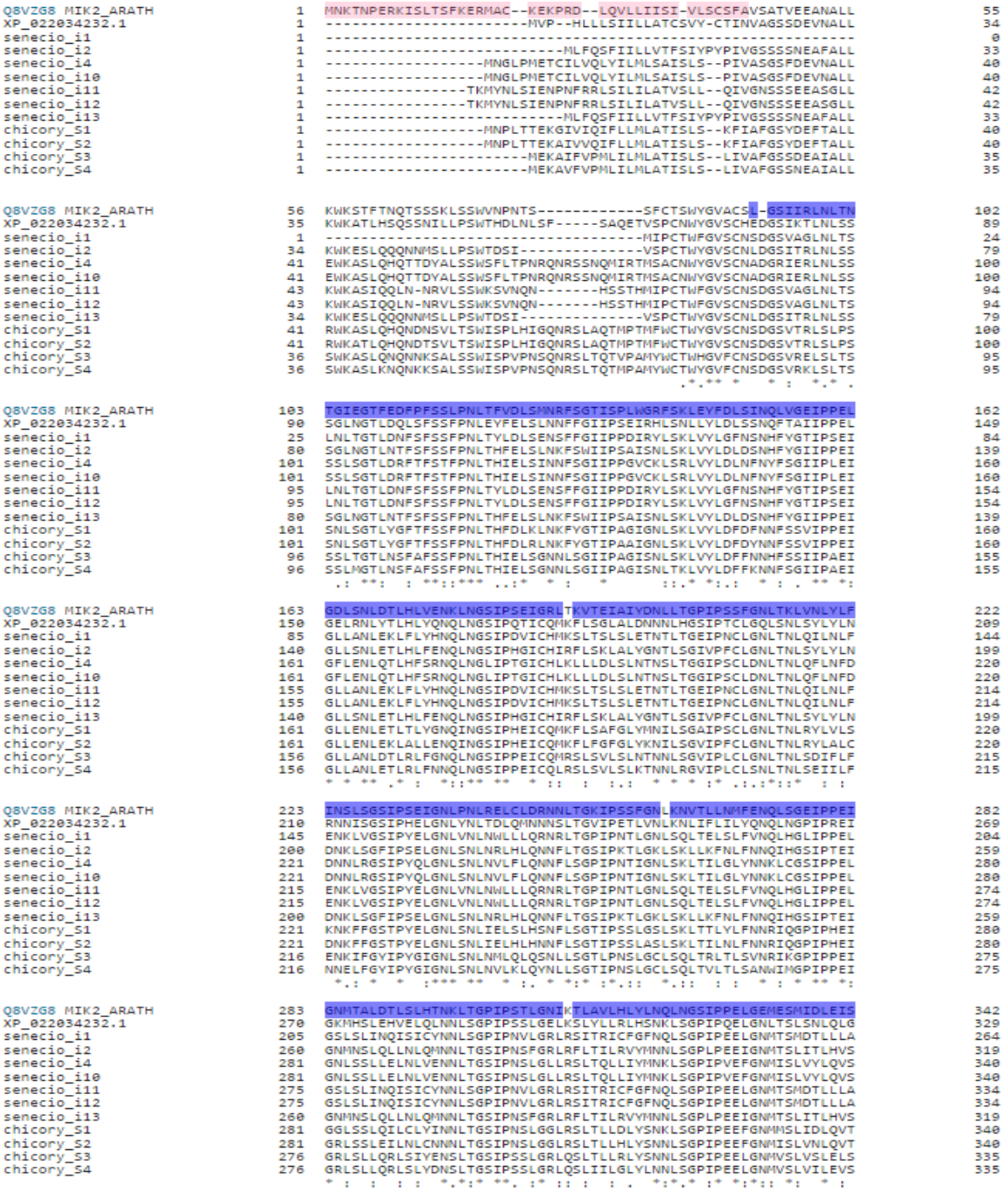

c37832_g3_i18
TPM value
tissue
bud
floret
flower
leaf
root
yngleaf
1.23
1.32
1.58
2.62
5.99
2.59

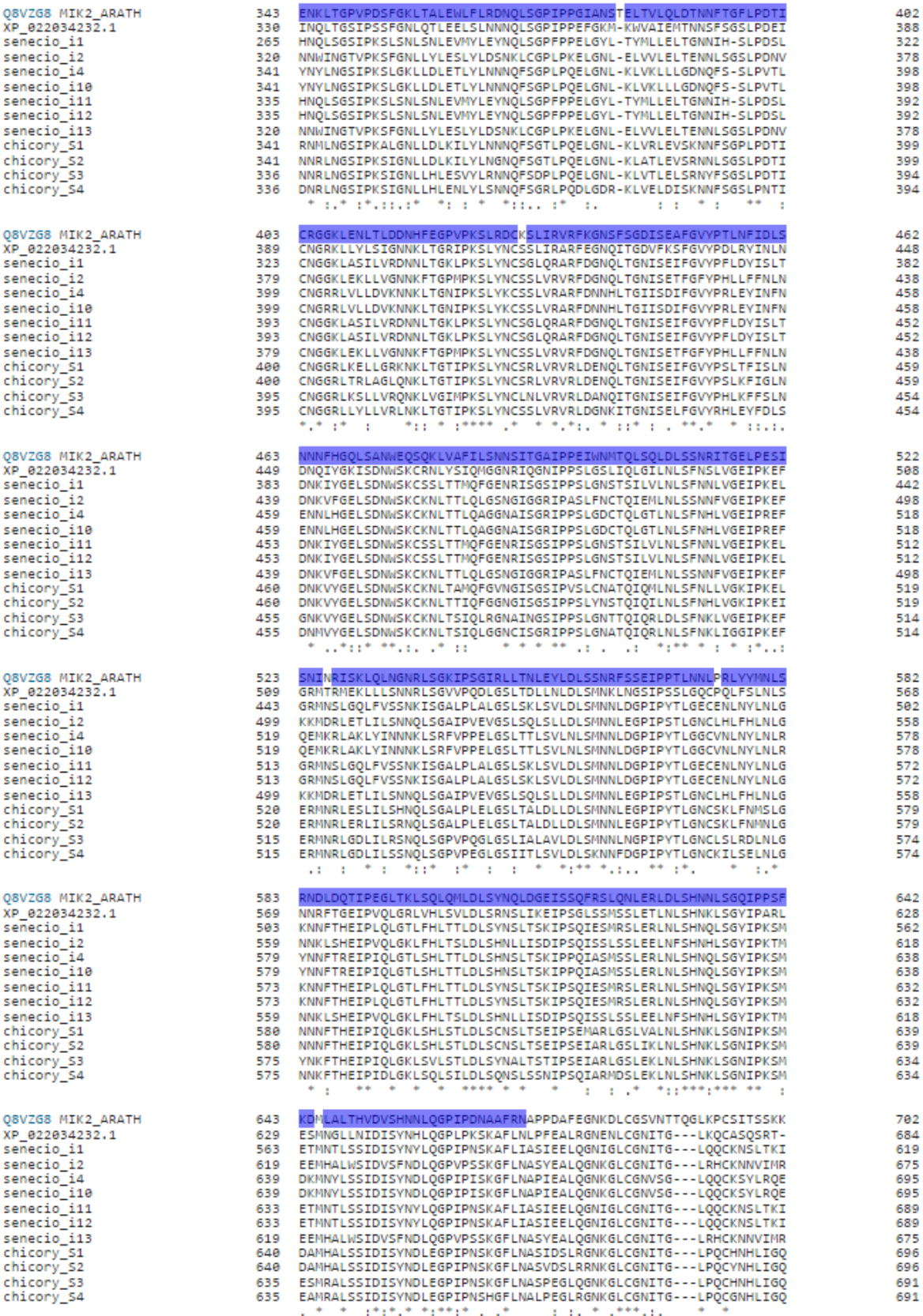

c37832_g3_i13
TPM value
tissue
bud
floret
flower
leaf
root
yngleaf
2.15
0.8
0.67
1.6
0.83
2.32

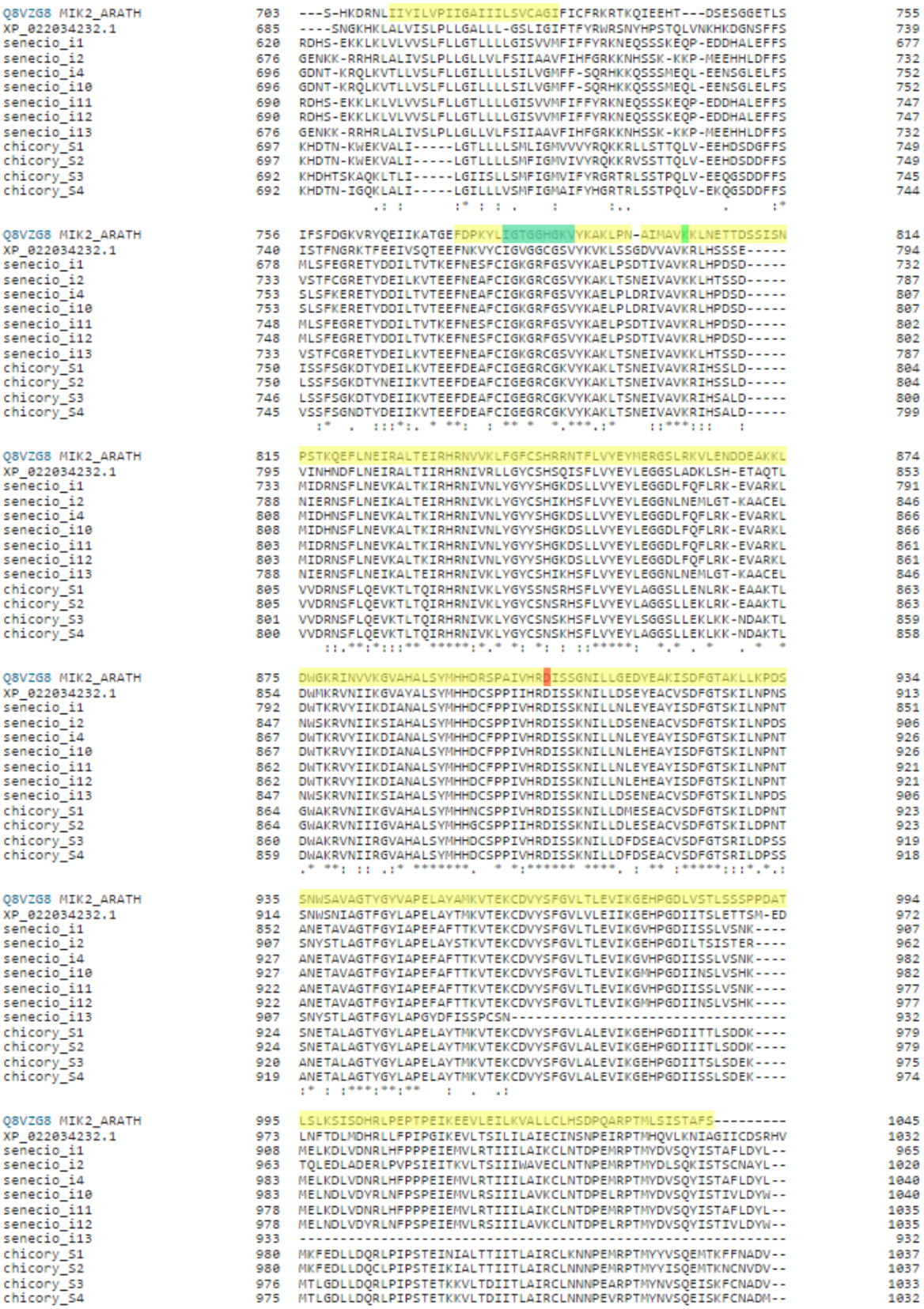

c2375_g1_i1
TPM value
tissue
bud
floret
flower
leaf
root
yngleaf
0.53


c4096_g1_i1
TPM value
tissue
bud
floret
flower
leaf
root
yngleaf
0.44
0.18
1.38
1.03
1.19

Sup 5: Alignments between MIK2 of *Arabidopsis thaliana*, sunflower_MIK2 (XP_022034232.1), LRR-RLKs of chicory and *Senecio*. Highlights in blue indicate LRR repeat domain, yellow means transmembrane domain, sky blue indicates nucleotide-binding region, green indicates binding site, and red indicates active site. An * (asterisk) indicates positions which have a single, fully conserved residue. A : (colon) indicates conservation between groups of strongly similar properties, scoring >0.5 in the Gonnet PAM 250 matrix. A . (period) indicates conservation between groups of weakly similar properties, scoring +< 0.5 in the Gonnet PAM 250 matrix.


Q8VZG8 MIK2_ARATH
XP_022034232.1
senecio_i1
senecio_i2
senecio_i4
senecio_i10
senecio_i11
senecio_i12
senecio_i13
chicory_S1
chicory_S2
chicory_S3
chicory_S4

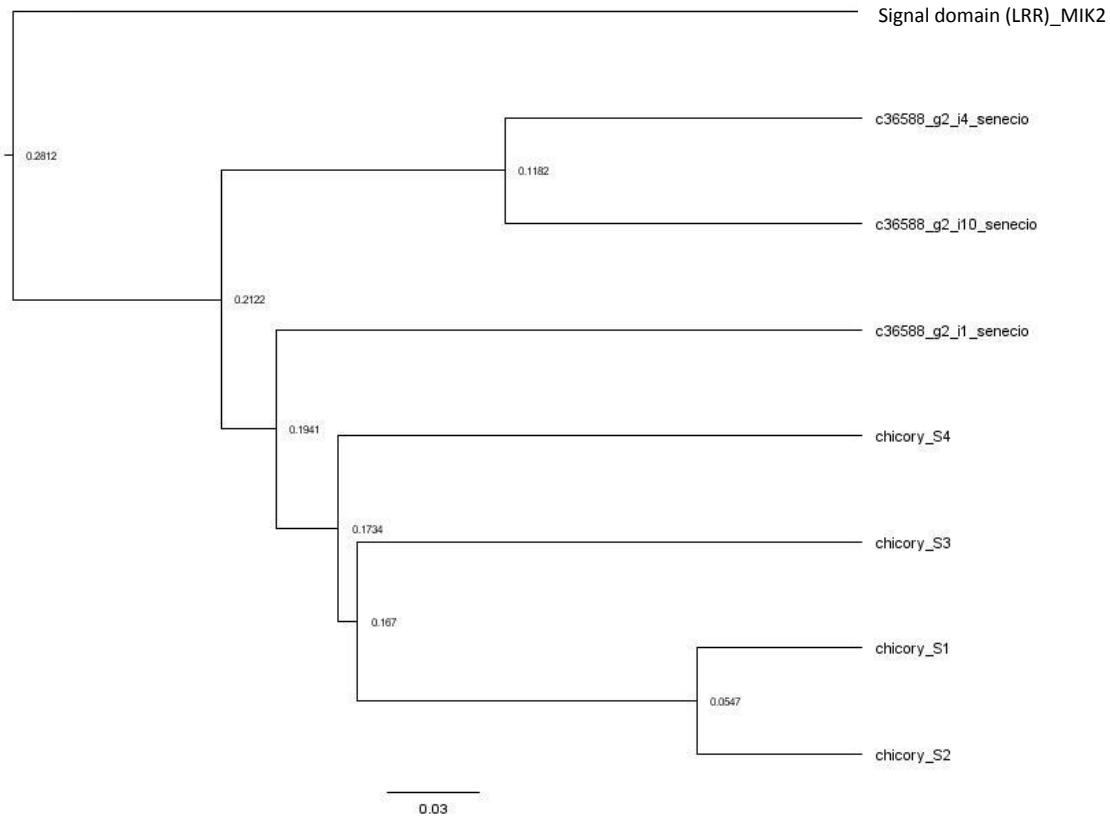

Q8VZG8 MIK2_ARATH
XP_022034232.1
senecio_i1
senecio_i2
senecio_i4
senecio_i10
senecio_i11
senecio_i12
senecio_i13
chicory_S1
chicory_S2
chicory_S3
chicory_S4


Q8VZG8 MIK2_ARATH 703 ---S-HKDRNLIIYILVPIIGAIIILSVCAGIFICFRKRTKQIEEHT---DSESGGETLS 755
XP_022034232.1 685 ----SNGKHKLALVISLPLLGALLL-GSLIGIFTFYRWRSNYHPSTQLVNKHKDGNSFFS 739
senecio_i1 620 RDHS-EKKLKLVLVVSLFLLGTLLLLGISVVMFIFFYRKNEQSSSKEQP-EDDHALEFFS 677
senecio_i2 676 GENKK-RRHRLALIVSLPLLGLLVLFSIIAAVFIHFGRKKNHSSK-KKP-MEEHHLDFFS 732
senecio_i4 696 GDNT-KRQLKVTLLVSLFLLGILLLLSILVGMFF-SQRHKKQSSSMEQL-EENSGLELFS 752
senecio_i10 696 GDNT-KRQLKVTLLVSLFLLGILLLLSILVGMFF-SQRHKKQSSSMEQL-EENSGLELFS 752
senecio_i11 690 RDHS-EKKLKLVLVVSLFLLGTLLLLGISVVMFIFFYRKNEQSSSKEQP-EDDHALEFFS 747
senecio_i12 690 RDHS-EKKLKLVLVVSLFLLGTLLLLGISVVMFIFFYRKNEQSSSKEQP-EDDHALEFFS 747
senecio_i13 676 GENKK-RRHRLALIVSLPLLGLLVLFSIIAAVFIHFGRKKNHSSK-KKP-MEEHHLDFFS 732
chicory_S1 697 KHDTN-KWEKVALI-----LGTLLLLSMLIGMVVVYRQKKRLLSTTQLV-EEHDSDGFFS 749
chicory_S2 697 KHDTN-KWEKVALI-----LGTLLLLSMFIGMVIVYRQKKRVSSTTQLV-EEHDSDDFFS 749
chicory_S3 692 KHDHTSKAQKLTLI-----LGIISLLSMFIGMVIFYRGRTRLSSTPQLV-EEQGSDDFFS 745
chicory_S4 692 KHDTN-IGQKLALI-----LGILLLVSMFIGMAIFYHGRTRLSSTPQLV-EKQGSDDFFS 744
Q8VZG8 MIK2_ARATH 756 IFSFDGKVRYQEIIKATGEFDPKYLIGTGGHGKVYKAKLPN-AIMAVKKLNETTDSSISN 814
XP_022034232.1 740 ISTFNGRKTFEEIVSQTEEFNKVYCIGVGGCGSVYKVKLSSGDVVAVKRLHSSSE----- 794
senecio_i1 678 MLSFEGRETYDDILTVTKEFNESFCIGKGRFGSVYKAELPSDTIVAVKRLHPDSD----- 732
senecio_i2 733 VSTFCGRETYDEILKVTEEFNEAFCIGKGRCGSVYKAKLTSNEIVAVKKLHTSSD----- 787
senecio_i4 753 SLSFKERETYDDILTVTEEFNEAFCIGKGRFGSVYKAELPLDRIVAVKRLHPDSD----- 807
senecio_i10 753 SLSFKERETYDDILTVTEEFNEAFCIGKGRFGSVYKAELPLDRIVAVKRLHPDSD----- 807
senecio_i11 748 MLSFEGRETYDDILTVTKEFNESFCIGKGRFGSVYKAELPSDTIVAVKRLHPDSD----- 802
senecio_i12 748 MLSFEGRETYDDILTVTKEFNESFCIGKGRFGSVYKAELPSDTIVAVKRLHPDSD----- 802
senecio_i13 733 VSTFCGRETYDEILKVTEEFNEAFCIGKGRCGSVYKAKLTSNEIVAVKKLHTSSD----- 787
chicory_S1 750 ISSFSGKDTYDEILKVTEEFDEAFCIGEGRCGKVYKAKLTSNEIVAVKRIHSSLD----- 804
chicory_S2 750 LSSFSGKDTYNEIIKVTEEFDEAFCIGEGRCGKVYKAKLTSNEIVAVKRIHSSLD----- 804
chicory_S3 746 LSSFSGKDTYDEIIKVTEEFDEAFCIGEGRCGKVYKAKLTSNEIVAVKRIHSALD----- 800
chicory_S4 745 VSSFSGNDTYDEIIKVTEEFDEAFCIGEGRCGKVYKAKLTSNEIVAVKRIHSALD----- 799
Q8VZG8 MIK2_ARATH 815 PSTKQEFLNEIRALTEIRHRNVVKLFGFCSHRRNTFLVYEYMERGSLRKVLENDDEAKKL 874
XP_022034232.1 795 VINHNDFLNEIRALTIIRHRNIVRLLGYCSHSQISFLVYEYLEGGSLADKLSH-ETAQTL 853
senecio_i1 733 MIDRNSFLNEVKALTKIRHRNIVNLYGYYSHGKDSLLVYEYLEGGDLFQFLRK-EVARKL 791
senecio_i2 788 NIERNSFLNEIKALTEIRHRNIVKLYGYCSHIKHSFLVYEYLEGGNLNEMLGT-KAACEL 846
senecio_i4 808 MIDHNSFLNEVKALTKIRHRNIVNLYGYYSHGKDSLLVYEYLEGGDLFQFLRK-EVARKL 866
senecio_i10 808 MIDHNSFLNEVKALTKIRHRNIVNLYGYYSHGKDSLLVYEYLEGGDLFQFLRK-EVARKL 866
senecio_i11 803 MIDRNSFLNEVKALTKIRHRNIVNLYGYYSHGKDSLLVYEYLEGGDLFQFLRK-EVARKL 861
senecio_i12 803 MIDRNSFLNEVKALTKIRHRNIVNLYGYYSHGKDSLLVYEYLEGGDLFQFLRK-EVARKL 861
senecio_i13 788 NIERNSFLNEIKALTEIRHRNIVKLYGYCSHIKHSFLVYEYLEGGNLNEMLGT-KAACEL 846
chicory_S1 805 VVDRNSFLQEVKTLTQIRHRNIVKLYGYSSNSRHSFLVYEYLAGGSLLENLRK-EAAKTL 863
chicory_S2 805 VVDRNSFLQEVKTLTQIRHRNIVKLYGYCSNSRHSFLVYEYLAGGSLLEKLRK-EAAKTL 863
chicory_S3 801 VVDRNSFLQEVKTLTQIRHRNIVKLYGYCSNSKHSFLVYEYLSGGSLLEKLKK-NDAKTL 859
chicory_S4 800 VVDRNSFLQEVKTLTQIRHRNIVKLYGYCSNSKHSFLVYEYLAGGSLLEKLKK-NDAKTL 858
Q8VZG8 MIK2_ARATH 875 DWGKRINVVKGVAHALSYMHHDRSPAIVHRDISSGNILLGEDYEAKISDFGTAKLLKPDS 934
XP_022034232.1 854 DWMKRVNIIKGVAYALSYMHHDCSPPIIHRDISSKNILLDSEYEACVSDFGTSKILNPNS 913
senecio_i1 792 DWTKRVYIIKDIANALSYMHHDCFPPIVHRDISSKNILLNLEYEAYISDFGTSKILNPNT 851
senecio_i2 847 NWSKRVNIIKSIAHALSYMHHDCSPPIVHRDISSKNILLDSENEACVSDFGTSKILNPDS 906
senecio_i4 867 DWTKRVYIIKDIANALSYMHHDCFPPIVHRDISSKNILLNLEYEAYISDFGTSKILNPNT 926
senecio_i10 867 DWTKRVYIIKDIANALSYMHHDCFPPIVHRDISSKNILLNLEHEAYISDFGTSKILNPNT 926
senecio_i11 862 DWTKRVYIIKDIANALSYMHHDCFPPIVHRDISSKNILLNLEYEAYISDFGTSKILNPNT 921
senecio_i12 862 DWTKRVYIIKDIANALSYMHHDCFPPIVHRDISSKNILLNLEHEAYISDFGTSKILNPNT 921
senecio_i13 847 NWSKRVNIIKSIAHALSYMHHDCSPPIVHRDISSKNILLDSENEACVSDFGTSKILNPDS 906
chicory_S1 864 GWAKRVNIIKGVAHALSYMHHNCSPPIVHRDISSKNILLDMESEACVSDFGTSKILDPNT 923
chicory_S2 864 GWAKRVNIIIGVAHALSYMHHGCSPPIIHRDISSKNILLDLESEACVSDFGTSKILDPNT 923
chicory_S3 860 DWAKRVNIIRGVAHALSYMHHDCSPPIVHRDISSKNILLDFDSEACVSDFGTSRILDPSS 919
chicory_S4 859 DWAKRVNIIRGVAHALSYMHHDCSPPIVHRDISSKNILLDFDSEACVSDFGTSRILDPSS 918
Q8VZG8 MIK2_ARATH 935 SNWSAVAGTYGYVAPELAYAMKVTEKCDVYSFGVLTLEVIKGEHPGDLVSTLSSSPPDAT 994
XP_022034232.1 914 SNWSNIAGTFGYLAPELAYTMKVTEKCDVYSFGVLVLEIIKGEHPGDIITSLETTSM-ED 972
senecio_i1 852 ANETAVAGTFGYIAPEFAFTTKVTEKCDVYSFGVLTLEVIKGVHPGDIISSLVSNK---- 907
senecio_i2 907 SNYSTLAGTFGYLAPELAYSTKVTEKCDVYSFGVLTLEVIKGEHPGDILTSISTER---- 962
senecio_i4 927 ANETAVAGTFGYIAPEFAFTTKVTEKCDVYSFGVLTLEVIKGVHPGDIISSLVSNK---- 982
senecio_i10 927 ANETAVAGTFGYIAPEFAFTTKVTEKCDVYSFGVLTLEVIKGMHPGDIINSLVSHK---- 982
senecio_i11 922 ANETAVAGTFGYIAPEFAFTTKVTEKCDVYSFGVLTLEVIKGVHPGDIISSLVSNK---- 977
senecio_i12 922 ANETAVAGTFGYIAPEFAFTTKVTEKCDVYSFGVLTLEVIKGMHPGDIINSLVSHK---- 977
senecio_i13 907 SNYSTLAGTFGYLAPGYDFISSPCSN---------- 932
chicory_S1 924 SNETALAGTYGYLAPELAYTMKVTEKCDVYSFGVLALEVIKGEHPGDIITTLSDDK---- 979
chicory_S2 924 SNETALAGTYGYLAPELAYTMKVTEKCDVYSFGVLALEVIKGEHPGDIIITLSDDK---- 979
chicory_S3 920 ANETALAGTYGYLAPELAYTMKVTEKCDVYSFGVLALEVIKGEHPGDIITSLSDEK---- 975
chicory_S4 919 ANETALAGTYGYLAPELAYTMKVTEKCDVYSFGVLALEVIKGEHPGDIISSLSDEK---- 974
Q8VZG8 MIK2_ARATH 995 LSLKSISDHRLPEPTPEIKEEVLEILKVALLCLHSDPQARPTMLSISTAFS--------- 1045
XP_022034232.1 973 LNFTDLMDHRLLFPIPGIKEVLTSILILAIECINSNPEIRPTMHQVLKNIAGIICDSRHV 1032
senecio_i1 908 MELKDLVDNRLHFPPPEIEMVLRTIIILAIKCLNTDPEMRPTMYDVSQYISTAFLDYL-- 965
senecio_i2 963 TQLEDLADERLPVPSIEITKVLTSIIIWAVECLNTNPEMRPTMYDLSQKISTSCNAYL-- 1020
senecio_i4 983 MELKDLVDNRLHFPPPEIEMVLRTIIILAIKCLNTDPEMRPTMYDVSQYISTAFLDYL-- 1040
senecio_i10 983 MELNDLVDYRLNFPSPEIEMVLRSIIILAVKCLNTDPELRPTMYDVSQYISTIVLDYW-- 1040
senecio_i11 978 MELKDLVDNRLHFPPPEIEMVLRTIIILAIKCLNTDPEMRPTMYDVSQYISTAFLDYL-- 1035
senecio_i12 978 MELNDLVDYRLNFPSPEIEMVLRSIIILAVKCLNTDPELRPTMYDVSQYISTIVLDYW-- 1035
senecio_i13 933 ---------- 932
chicory_S1 980 MKFEDLLDQRLPIPSTEINIALTTIITLAIRCLKNNPEMRPTMYYVSQEMTKFFNADV-- 1037
chicory_S2 980 MKFEDLLDQCLPIPSTEIKIALTTIITLAIRCLNNNPEMRPTMYYISQEMTKNCNVDV-- 1037
chicory_S3 976 MTLGDLLDQRLPIPSTETKKVLTDIITLAIRCLNNNPEARPTMYNVSQEISKFCNADV-- 1033
chicory_S4 975 MTLGDLLDQRLPIPSTETKKVLTDIITLAIRCLNNNPEVRPTMYNVSQEISKFCNADM-- 1032

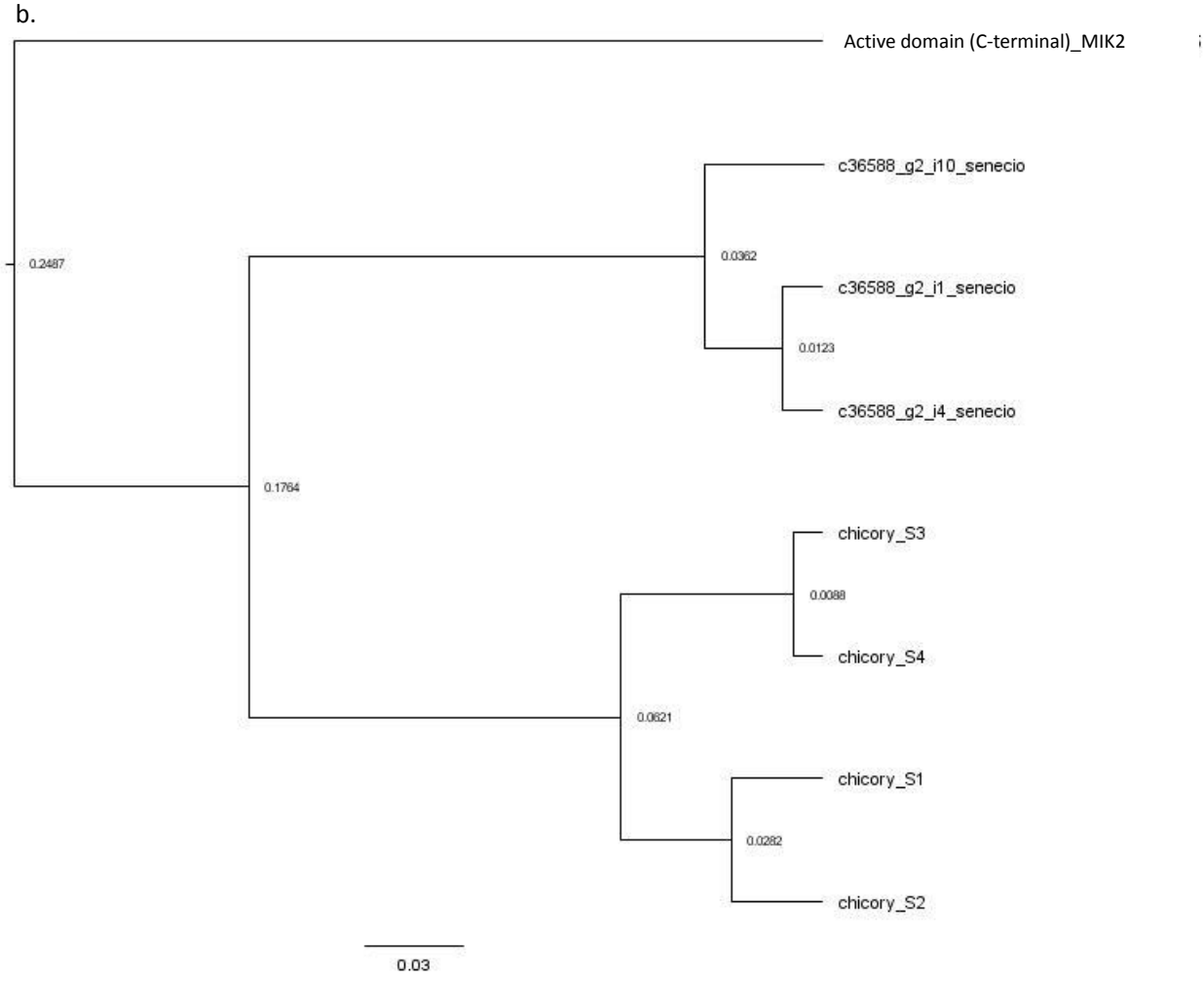
Sup 6: Divergence of the whole protein sequence and separate domains among MIK2 and others. Sunflower_MIK2 is shadowed in green. The average divergence of chicory or *Senecio* is calculated. Especially, divergence trees of (a) siganal domain and (b) active domain among top homolog sequences are showed. Values at the nodes interpret the divergence between the two branches.

| query | start of whole seq | of whole seq | start for blast | end for blast | lengt h (aa) | subject | | % identity | alignment length | mismatches | gap open | q start | q end | s start | s end | e value | bit score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| gi\|2811854\|sp\|Q8VZG8.3\|MIK2_ARATH | 1 | 1045 | 1 | 1045 | 1045 | | senecio_i2 | 48.235 | 1020 | 505 | 10 | 36 | 1045 | 7 | 1013 | 0 | 902 |
| | | | | | | | senecio_i13 | 47.835 | 924 | 463 | 9 | 36 | 949 | 7 | 921 | 0 | 801 |
| | | | | | | | chicory_S3 | 46.957 | 1035 | 511 | 13 | 30 | 1045 | 11 | 1026 | 0 | 893 |
| | | | | | | | chicory_S4 | 46.654 | 1031 | 519 | 10 | 30 | 1045 | 11 | 1025 | 0 | 864 |
| | | | | | | | XP_022034232.1 | 46.439 | 1025 | 518 | 11 | 28 | 1037 | 7 | 1015 | 0 | 897 |
| | | | | | | | chicory_S2 | 46.021 | 1043 | 516 | 15 | 23 | 1041 | 7 | 1026 | 0 | 872 |
| | | | | | | | senecio_i1 | 45.932 | 971 | 504 | 10 | 82 | 1044 | 4 | 961 | 0 | 798 |
| | | | | | | | chicory_S1 | 45.733 | 1043 | 519 | 14 | 23 | 1041 | 7 | 1026 | 0 | 853 |
| | | | | | | | senecio_i4 | 45.673 | 1040 | 525 | 15 | 28 | 1044 | 14 | 1036 | 0 | 820 |
| | | | | | | | senecio_i11 | 45.305 | 1033 | 536 | 14 | 26 | 1044 | 14 | 1031 | 0 | 820 |
| | | | | | | | senecio_i10 | 45.227 | 1037 | 532 | 14 | 28 | 1045 | 14 | 1033 | 0 | 814 |
| | | | | | | | senecio_i12 | 44.854 | 1030 | 543 | 13 | 26 | 1045 | 14 | 1028 | 0 | 815 |
| | | | | | | average % identity with chicory | | 46.34125 | | | | | | | | | |
| | | | | | | average % identity with senecio | | 46.15157143 | | | | | | | | | |
| signal_peptide | 1 | 43 | 1 | 79 | 79 | subject | | % identity | alignment length | mismatches | gap open | q start | q end | s start | s end | e value | bit score |
| | | | | | | | chicory_S2 | 36.364 | 55 | 34 | 1 | 23 | 76 | 7 | 61 | 8.47E-11 | 40.8 |
| | | | | | | | chicory_S1 | 34.545 | 55 | 35 | 1 | 23 | 76 | 7 | 61 | 3.4E-10 | 39.3 |
| | | | | | | | senecio_i12 | 35.849 | 53 | 30 | 3 | 23 | 73 | 9 | 59 | 0.000000962 | 28.1 |
| | | | | | | | senecio_i11 | 35.849 | 53 | 30 | 3 | 23 | 73 | 9 | 59 | 0.000000962 | 28.1 |
| | | | | | | | chicory_S3 | 42.308 | 52 | 27 | 1 | 25 | 76 | 8 | 56 | 5.28E-10 | 38.9 |
| | | | | | | | chicory_S4 | 42.308 | 52 | 27 | 1 | 25 | 76 | 8 | 56 | 1.99E-09 | 37.4 |
| | | | | | | | XP_022034232.1 | 37.5 | 48 | 30 | 0 | 28 | 75 | 7 | 54 | 2.62E-11 | 39.7 |
| | | | | | | | senecio_i10 | 41.304 | 46 | 26 | 1 | 28 | 73 | 14 | 58 | 2.32E-09 | 37.4 |
| | | | | | | | senecio_i4 | 41.304 | 46 | 26 | 1 | 28 | 73 | 14 | 58 | 2.32E-09 | 37.4 |
| | | | | | | | senecio_i13 | 34.783 | 46 | 30 | 0 | 30 | 75 | 8 | 53 | 0.00000311 | 29.3 |
| | | | | | | | senecio_i2 | 34.783 | 46 | 30 | 0 | 30 | 75 | 8 | 53 | 0.00000311 | 29.3 |
| | | | | | | | senecio_i1 | 0 | | | | | | | | | |
| | | | | | | average % identity with chicory | | 38.88125 | | | | | | | | | |
| | | | | | | average % identity with senecio | | 37.312 | | | | | | | | | |
| leucine-rich_repeats | 92 | 670 | 80 | 704 | 624 | subject | | % identity | alignment length | mismatches | gap open | q start | q end | s start | s end | e value | bit score |
| | | | | | | | senecio_i13 | 49.34 | 606 | 304 | 3 | 6 | 610 | 5 | 608 | 0 | 542 |
| | | | | | | | senecio_i2 | 49.34 | 606 | 304 | 3 | 6 | 610 | 5 | 608 | 0 | 542 |
| | | | | | | | chicory_S4 | 46.962 | 607 | 319 | 3 | 5 | 610 | 18 | 622 | 1.29E-174 | 501 |
| | | | | | | | chicory_S3 | 46.623 | 607 | 321 | 3 | 5 | 610 | 18 | 622 | 7.61E-180 | 514 |
| | | | | | | | chicory_S1 | 46.458 | 607 | 323 | 2 | 5 | 610 | 17 | 622 | 5.1E-177 | 507 |
| | | | | | | | chicory_S2 | 46.293 | 607 | 324 | 2 | 5 | 610 | 17 | 622 | 0 | 522 |
| | | | | | | | XP_022034232.1 | 46.266 | 616 | 326 | 3 | 85 | 699 | 71 | 682 | 0 | 526 |
| | | | | | | | senecio_i10 | 45.902 | 610 | 326 | 4 | 2 | 610 | 15 | 621 | 1.47E-165 | 478 |
| | | | | | | | senecio_i4 | 45.902 | 610 | 326 | 4 | 2 | 610 | 15 | 621 | 1.47E-165 | 478 |
| | | | | | | | senecio_i12 | 45.545 | 606 | 326 | 4 | 6 | 610 | 12 | 614 | 6.19E-169 | 486 |
| | | | | | | | senecio_i11 | 45.545 | 606 | 326 | 4 | 6 | 610 | 12 | 614 | 6.19E-169 | 486 |
| | | | | | | | senecio_i1 | 45.545 | 606 | 326 | 4 | 6 | 610 | 4 | 606 | 1.16E-168 | 485 |
| | | | | | | average % identity with chicory | | 46.5815 | | | | | | | | | |
| | | | | | | average % identity with senecio | | 46.73128571 | | | | | | | | | |
| transmembrane_domain | 710 | 730 | 705 | 760 | 55 | subject | | % identity | alignment length | mismatches | gap open | q start | q end | s start | s end | e value | bit score |
| | | | | | | | chicory_S3 | 42.308 | 26 | 15 | 0 | 26 | 51 | 22 | 47 | 0.000303 | 23.1 |
| | | | | | | | senecio_i13 | 38.462 | 13 | 8 | 0 | 34 | 46 | 34 | 46 | 0.49 | 15 |
| | | | | | | | senecio_i2 | 38.462 | 13 | 8 | 0 | 34 | 46 | 34 | 46 | 0.49 | 15 |
| | | | | | | | XP_022034232.1 | 31.915 | 47 | 25 | 2 | 709 | 749 | 691 | 736 | 1.3 | 112 |
| | | | | | | subject | | % identity | alignment length | mismatches | gap open | q start | q end | s start | s end | e value | bit score |
| C_terminal_active_domain | 775 | 1045 | 761 | 1045 | 284 | | chicory_S3 | 56.997 | 293 | 115 | 4 | 12 | 303 | 13 | 295 | 2.53E-120 | 338 |
| | | | | | | | senecio_i13 | 56.853 | 197 | 78 | 3 | 12 | 207 | 12 | 202 | 1.03E-78 | 229 |
| | | | | | | | XP_022034232.1 | 56.835 | 278 | 112 | 4 | 761 | 1037 | 745 | 1015 | 6.85E-118 | 328 |
| | | | | | | | chicory_S1 | 56.701 | 291 | 111 | 5 | 12 | 299 | 13 | 291 | 3.03E-114 | 322 |
| | | | | | | | chicory_S2 | 56.401 | 289 | 115 | 4 | 12 | 299 | 13 | 291 | 1.28E-113 | 321 |
| | | | | | | | senecio_i2 | 55.29 | 293 | 120 | 4 | 12 | 303 | 12 | 294 | 1.04E-116 | 328 |
| | | | | | | | chicory_S4 | 55.15 | 301 | 124 | 4 | 4 | 303 | 5 | 295 | 2.22E-119 | 335 |
| | | | | | | | senecio_i4 | 52.443 | 307 | 131 | 5 | 1 | 302 | 2 | 298 | 2.81E-109 | 310 |
| | | | | | | | senecio_i11 | 52.117 | 307 | 132 | 5 | 1 | 302 | 2 | 298 | 1.76E-110 | 313 |
| | | | | | | | senecio_i1 | 52.117 | 307 | 132 | 5 | 1 | 302 | 2 | 298 | 1.76E-110 | 313 |
| | | | | | | | senecio_i10 | 50.987 | 304 | 138 | 4 | 1 | 303 | 2 | 295 | 3.05E-107 | 305 |
| | | | | | | | senecio_i12 | 50.658 | 304 | 139 | 4 | 1 | 303 | 2 | 295 | 2.46E-108 | 307 |
| | | | | | | average % identity with chicory | | 56.31225 | | | | | | | | | |
| | | | | | | average % identity with senecio | | 52.92357143 | | | | | | | | | |

a.


Signal domain (LRR)_MIK2
c36588_g2_i4_senecio
c36588_g2_i10_senecio
c36588_g2_i1_senecio
chicory_S4
chicory_S3
chicory_S1
chicory_S2
0.2812
0.1182
0.2122
0.1941
0.1734
0.167
0.0547
0.03

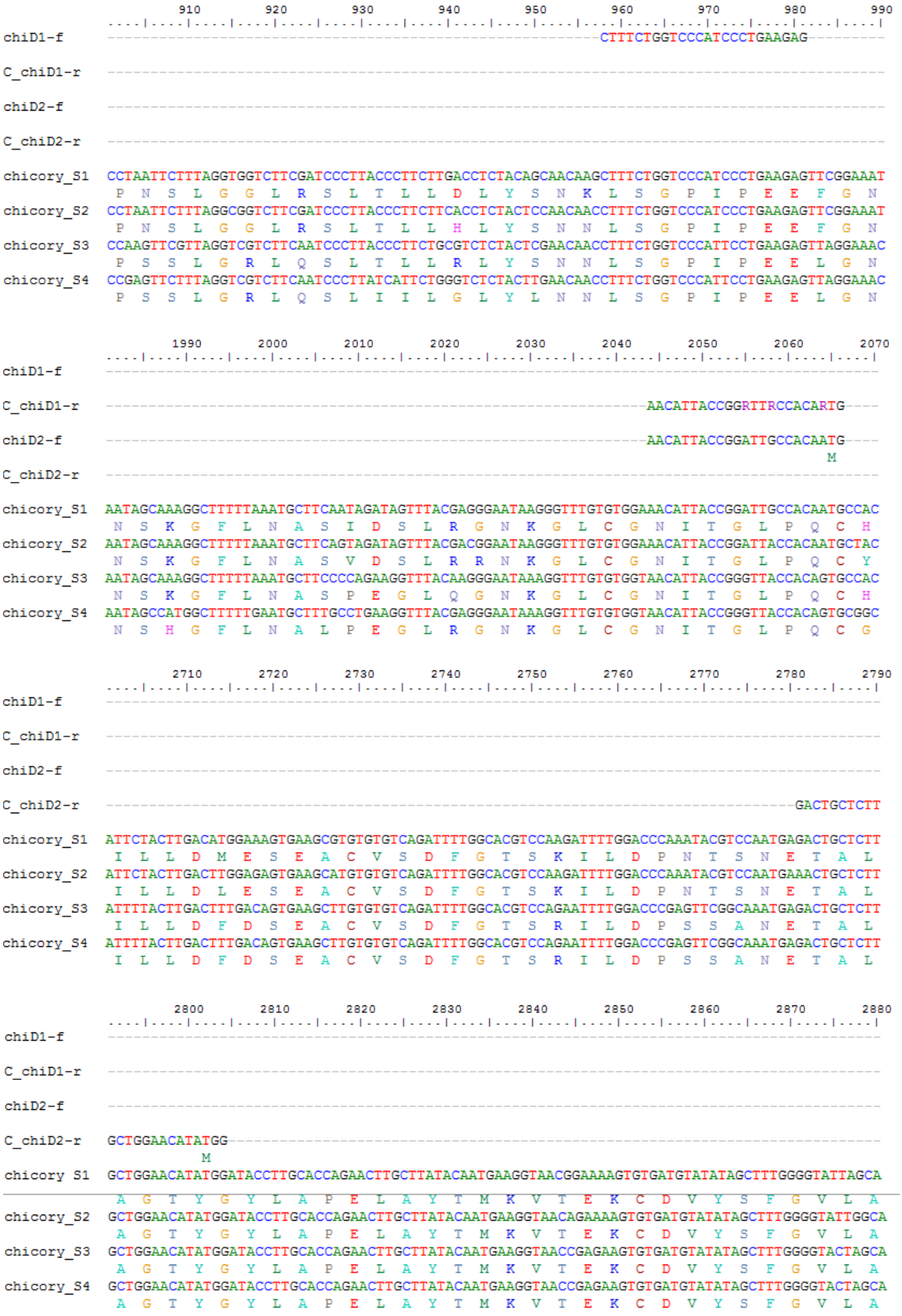
b.


Active domain (C-terminal)_MIK2
c36588_g2_i10_senecio
c36588_g2_i1_senecio
c36588_g2_i4_senecio
chicory_S3
chicory_S4
chicory_S1
chicory_S2
0.2487
0.0362
0.0123
0.1764
0.0088
0.0821
0.0282
0.03

Sup 7: List of primers used for molecular experiments.

| Name of primer | Sequence | Length (bp) | Start_pos. | End_pos. | Length of the amplicon (bp) | Specificity |
|---|---|---|---|---|---|---|
| **i1-pf** | TGAAGTCTCTAACCTCGCTTTC | 22 | 559 | | 1503 | work on i1/i11+i12 |
| **i1-pr** | ACTGCTGTAACCCGGTAATG | 20 | | 2062 | | |
| **i4-pf** | CTTTCTGGTCCCATTCCTGTAG | 22 | 957 | | 1779 | specific on i4 |
| **i4-pr** | GGCCTCGTACTCCAAGTTAAG | 21 | | 2736 | | |
| **i10-pf** | GTGGTAACGTCAGCGGGTTA | 20 | 2035 | | 753 | work on i4+i10 |
| **i10-pr** | CGTTGGCCGTGTTTGGATTC | 20 | | 2788 | | |
| **chiD1-f** | CTTTCTGGTCCCATCCCTGAAGAG | 24 | 958 | | 1108 | / |
| **chiD1-r** | CAYTGTGGYAAYCCGGTAATGTT | 23 | | 2066 | | |
| **chiD2-f** | AACATTACCGGATTGCCACAATG | 23 | 2044 | | 789 | / |
| **chiD2-r** | CCATATGTTCCAGCAAGAGCAGTC | 24 | | 2801 | | |
| **chi3-f** | GTWTATGGRGAACTTTCTGACAAC | 24 | 1387 | | 1306 | / |
| **chi3-r** | TTGCTTGATATGTCTCGATG | 20 | | 2693 | | |
| **i4-long-f** | GACCTGCATACTCGTTCAA | 19 | 21 | | 1139 | specific on the i4 long band |
| **i4-long-r** | CCCAGAAGAAGTTTAACTAGCTTC | 24 | | 1160 | | |
| **i4-short-f** | AACTCTTTGACAAAAATAAGGG | 22 | 62 | | 591 | specific on the i4 short band |
| **i4-short-r** | GCATATACGACAAAGCATTAGC | 22 | | 653 | | |

Sup 8: Regions of sequences where degenerate primers sit.



References

Abbott, R. J., Brennan, A. C., James, J. K., Forbes, D. G., Hegarty, M. J., & Hiscock, S. J. (2009). Recent hybrid origin and invasion of the British Isles by a self-incompatible species, Oxford ragwort (Senecio squalidus L., Asteraceae). *Biological Invasions*, *11*(5), 1145–1158. https://doi.org/10.1007/s10530-008-9382-3

Abbott, R. J., James, J. K., Milne, R. I., & Gillies, A. C. M. (2003). Plant introductions, hybridization and gene flow. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *358*(1434), 1123–32. https://doi.org/10.1098/rstb.2003.1289

Acquadro, A., Barchi, L., Portis, E., Mangino, G., Valentino, D., Mauromicale, G., & Lanteri, S. (2017). Genome reconstruction in Cynara cardunculus taxa gains access to chromosome-scale DNA variation. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-05085-7

Allen, A. M., & Hiscock, S. J. (2008). Evolution and phylogeny of self-incompatibility systems in angiosperms. *Self-Incompatibility in Flowering Plants: Evolution, Diversity, and Mechanisms*, 73–101. https://doi.org/10.1007/978-3-540-68486-2_4

Allen, A. M., Lexer, C., & Hiscock, S. J. (2010). Characterisation of sunflower-21 (SF21) genes expressed in pollen and pistil of Senecio squalidus (Asteraceae) and their relationship with other members of the SF21 gene family. *Sexual Plant Reproduction*, *23*(3), 173–186. https://doi.org/10.1007/s00497-010-0137-9

Allen, A. M., Lexer, C., & Hiscock, S. J. (2010). Comparative Analysis of Pistil Transcriptomes Reveals Conserved and Novel Genes Expressed in Dry, Wet, and Semidry Stigmas. *Plant Physiology*, *154*(3), 1347–1360. https://doi.org/10.1104/pp.110.162172

Allen, A. M., Thorogood, C. J., Hegarty, M. J., Lexer, C., & Hiscock, S. J. (2011). Pollenpistil interactions and self-incompatibility in the Asteraceae: New insights from studies of Senecio squalidus (Oxford ragwort). *Annals of Botany*, *108*(4), 687–698. https://doi.org/10.1093/aob/mcr147

Antolín-Llovera, M., Petutsching, E. K., Ried, M. K., Lipka, V., Nürnberger, T., Robatzek, S., & Parniske, M. (2014). Knowing your friends and foes - plant receptor-like kinases as initiators of symbiosis or defence. *New Phytologist*. https://doi.org/10.1111/nph.13117

Badouin, H., Gouzy, J., Grassa, C. J., Murat, F., Staton, S. E., Cottret, L., … Langlade, N. B. (2017). The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution. *Nature*, *546*(7656), 148–152. https://doi.org/10.1038/nature22380

Barrett, S. (2013). The evolution of plant reproductive systems: how often are transitions irreversible? *Proceedings. Biological Sciences / The Royal Society*, *280*(1765), 20130913. https://doi.org/10.1098/rspb.2013.0913

Barrett, S. C. H. (2002). The evolution of plant sexual diversity. *Nature Reviews Genetics*, *3*(4), 274–284. https://doi.org/10.1038/nrg776

Barrett, S. C. H. (2010). Understanding plant reproductive diversity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1537), 99–109. https://doi.org/10.1098/rstb.2009.0199

Barrett, S. C. H. (2013). Evolution of Mating Systems : Outcrossing versus Selfing. *The Princeton Guide to Evolution*, 356–362.

Barrett, S. C. H., Colautti, R. I., & Eckert, C. G. (2008). Plant reproductive systems and evolution during biological invasion. *Molecular Ecology*, *17*(1), 373–383. https://doi.org/10.1111/j.1365-294X.2007.03503.x

Brennan, a C., Harris, S. a, Tabah, D. a, & Hiscock, S. J. (2002). The population genetics of sporophytic self-incompatibility in Senecio squalidus L. (Asteraceae) I: S allele diversity in a natural population. *Heredity*, *89*, 430–438. https://doi.org/10.1038/sj.hdy.6800159

Brennan, a C., Tabah, D. a, Harris, S. a, & Hiscock, S. J. (2011). Sporophytic self-incompatibility in Senecio squalidus (Asteraceae): S allele dominance interactions and modifiers of cross-compatibility and selfing rates. *Heredity*, *106*(1), 113–23. https://doi.org/10.1038/hdy.2010.29

Brennan, A. C., Harris, S. a, & Hiscock, S. J. (2006). The population genetics of

sporophytic self-incompatibility in L. (Asteraceae): avoidance of mating constraints imposed by low S Senecio -allele number. *Evolution; International Journal of Organic Evolution*, *60*(May), 213–224. https://doi.org/10.1111/j.0014-3820.2006.tb01100.x

Brennan, A. C., & Hiscock, S. J. (2010). Expression and inheritance of sporophytic self-incompatibility in synthetic allohexaploid Senecio cambrensis (Asteraceae). *New Phytologist*, *186*(1), 251–261. https://doi.org/10.1111/j.1469-8137.2009.03082.x

Cadalen, T., Mörchen, M., Blassiau, C., Clabaut, A., Scheer, I., Hilbert, J. L., … Quillet, M. C. (2010). Development of SSR markers and construction of a consensus genetic map for chicory (Cichorium intybus L.). *Molecular Breeding*, *25*(4), 699–722. https://doi.org/10.1007/s11032-009-9369-5

Chapman, M. A., Hiscock, S. J., & Filatov, D. A. (2016). The genomic bases of morphological divergence and reproductive isolation driven by ecological speciation in Senecio (Asteraceae). *Journal of Evolutionary Biology*, *29*(1), 98–113. https://doi.org/10.1111/jeb.12765

Charlesworth, D. (2002). Self-incompatibility: How to stay incompatible. *Current Biology*, *12*(12), 424–426. https://doi.org/10.1016/S0960-9822(02)00915-6

Charlesworth, D. (2006). Evolution of Plant Breeding Systems. *Current Biology*, *16*(17), 726–735. https://doi.org/10.1016/j.cub.2006.07.068

Cheptou, P. O. (2012). Clarifying bakers law. *Annals of Botany*, *109*(3), 633–641. https://doi.org/10.1093/aob/mcr127

Dangl, J. L., & Jones, J. D. G. (2001). defence responses to infection, *411*(June).

Franklin-tong, B. A. M. V. (2006). Gametophytic self-incompatibility : understanding the cellular mechanisms involved in “ self ” pollen tube inhibition, 233–245. https://doi.org/10.1007/s00425-006-0284-2

Fujii, S., Kubo, K., & Takayama, S. (2016). Non-self- and self-recognition models in plant self-incompatibility. *Nature Plants*, *2*(September), 16130. https://doi.org/10.1038/nplants.2016.130

Gandhi, S. D., Heesacker, A. F., Freeman, C. A., Argyris, J., Bradford, K., & Knapp,

S. J. (2005). The self-incompatibility locus (S) and quantitative trait loci for self-pollination and seed dormancy in sunflower. *Theoretical and Applied Genetics*, *111*(4), 619–629. https://doi.org/10.1007/s00122-005-1934-7

Gonthier, L., Blassiau, C., Mörchen, M., Cadalen, T., Poiret, M., Hendriks, T., & Quillet, M. C. (2013). High-density genetic maps for loci involved in nuclear male sterility (NMS1) and sporophytic self-incompatibility (S-locus) in chicory (Cichorium intybus L., Asteraceae). *Theoretical and Applied Genetics*, *126*(8), 2103–2121. https://doi.org/10.1007/s00122-013-2122-9

Gou, X., He, K., Yang, H., Yuan, T., Lin, H., Clouse, S. D., & Li, J. (2010). Genome-wide cloning and sequence analysis of leucine-rich repeat receptor-like protein kinase genes in Arabidopsis thaliana.

Graham, L. A., Besser, K., Blumer, S., Branigan, C. A., Czechowski, T., Elias, L., … Bowles, D. (2010). The genetic map of Artemisia annua L identifies loci affecting yield of the antimalarial drug artemisinin. *Science*, *327*(5963), 328–331. https://doi.org/10.1126/science.1182612

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Philip, D., Bowden, J., … Pochet, N. (2013). De novo transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. *Nat Protocols*, *8*(8), 1494–1512. https://doi.org/10.1038/nprot.2013.084.De

Harris, S. (2002). Introduction of Oxford ragwort, Senecio squalidus L.(Asteraceae), to the United Kingdom. *Watsonia*, *24*(1), 31–43. Retrieved from http://kbd.kew.org/kbd/detailedresult.do?id=346363

Hiscock, S. . (2000). Self-incompatibility in Senecio squalidus L. (Asteraceae). *Annals of Botany*, *85*, 181–190. https://doi.org/10.1006/anbo.1999.1058

Hiscock, S. J. (2000). Genetic control of self-incompatibility in Senecio squalidus L. (Asteraceae): A successful colonizing species. *Heredity*, *85*(1), 10–19. https://doi.org/10.1046/j.1365-2540.2000.00692.x

Hiscock, S. J. (2002). Pollen recognition during the self-incompatibility response in plants. *Genome Biology*, *3*(2), REVIEWS1004.

Hiscock, S. J., & Allen, A. M. (2008). Diverse cell signalling pathways regulate

pollen-stigma interactions: The search for consensus. *New Phytologist*, *179*(2), 286–317. https://doi.org/10.1111/j.1469-8137.2008.02457.x

Hiscock, S. J., & McInnis, S. M. (2003). The diversity of self-incompatibility systems in flowering plants. *Plant Biology*, *5*(1), 23–32. https://doi.org/10.1055/s-2003-37981

Hiscock, S. J., McInnis, S. M., Tabah, D. A., Henderson, C. A., & Brennan, A. C. (2003). Sporophytic self-incompatibility in Senecio squalidus L. (Asteraceae) - The search for S. *Journal of Experimental Botany*, *54*(380), 169–174. https://doi.org/10.1093/jxb/54.380.169

Igic, B., Lande, R., & Kohn, J. R. (2008). Loss of Self-Incompatibility and Its Evolutionary Consequences. *International Journal of Plant Sciences*, *169*(1), 93–104. https://doi.org/10.1086/523362

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F., & Donoghue, M. J. (2008). Plant Systematics: A Phylogenetic Approach, Sinauer Associates, Sunderland, Mass, USA, 3rd edition. *International Journal of Sustainable Development & World Ecology*. https://doi.org/10.1080/13504501003788180

Kim, M., Cui, M. L., Cubas, P., Gillies, A., Lee, K., Chapman, M. A., … Coen, E. (2008). Regulatory genes control a key morphological and ecological trait transferred between species. *Science*, *322*(5904), 1116–1119. https://doi.org/10.1126/science.1164371

Koseva, B., Crawford, D. J., Brown, K. E., Mort, M. E., & Kelly, J. K. (2017). The genetic breakdown of sporophytic self-incompatibility in Tolpis coronopifolia (Asteraceae). *New Phytologist*, *216*(4), 1256–1267. https://doi.org/10.1111/nph.14759

Levin, S. A. (2001). Encyclopedia of biodiversity (No. Sirsi) i9780122268656.

Lewis, D., Verma, S. C., & Zuberi, M. I. (1988). Gametophytic-sporophytic incompatibility in the cruciferae-raphanus sativus. *Heredity*, *61*(3), 355–366. https://doi.org/10.1038/hdy.1988.126

Mable, B. K., Schierup, M. H., & Charlesworth, D. (2003). Estimating the number, frequency, and dominance of S-alleles in a natural population of Arabidopsis

lyrata (Brassicaceae) with sporophytic control of self-incompatibility. *Heredity*, *90*(6), 422–431. https://doi.org/10.1038/sj.hdy.6800261

McInnis, S. M., Costa, L. M., Gutiérrez-Marcos, J. F., Henderson, C. A., & Hiscock, S. J. (2005). Isolation and characterization of a polymorphic stigma-specific class III peroxidase gene from Senecio squalidus L. (Asteraceae). *Plant Molecular Biology*, *57*(5), 659–677. https://doi.org/10.1007/s11103-005-1426-9

McInnis, S. M., Emery, D. C., Porter, R., Desikan, R., Hancock, J. T., & Hiscock, S. J. (2006). The role of stigma peroxidases in flowering plants: Insights from further characterization of a stigma-specific peroxidase (SSP) from Senecio squalidus (Asteraceae). *Journal of Experimental Botany*, *57*(8), 1835–1846. https://doi.org/10.1093/jxb/erj182

Möller, S., Croning, M. D. R., & Apweiler, R. (2001). Evaluation of methods for the prediction of membrane spanning regions. *Bioinformatics*. https://doi.org/10.1093/bioinformatics/17.7.646

Nasrallah, J. B. (2017). Plant mating systems: self-incompatibility and evolutionary transitions to self-fertility in the mustard family. *Current Opinion in Genetics and Development*, *47*, 54–60. https://doi.org/10.1016/j.gde.2017.08.005

Nieuwenhuis, B. P. S., & Immler, S. (2016). The evolution of mating-type switching for reproductive assurance. *BioEssays*, *38*(11), 1141–1149. https://doi.org/10.1002/bies.201600139

Paterson, T., & Law, A. (2013). ArkMAP: Integrating genomic maps across species and data sources. *BMC Bioinformatics*. https://doi.org/10.1186/1471-2105-14-246

Reyes-Chin-Wo, S., Wang, Z., Yang, X., Kozik, A., Arikit, S., Song, C., … Michelmore, R. W. (2017). Genome assembly with in vitro proximity ligation data and whole-genome triplication in lettuce. *Nature Communications*, *8*. https://doi.org/10.1038/ncomms14953

Richards, A. J. (1997). *Plant breeding systems*. Chapman & Hall. Retrieved from https://books.google.co.uk/books?hl=en&lr=&id=isLXD8tZ5GMC&oi=fnd&pg=

PR9&dq=Plant+breeding+systems.&ots=yk02-_z15t&sig=H1ccQS5i2bySrIMlI0JegiZhGZQ#v=onepage&q=Plant breeding systems.&f=false

Richard M. Amasino, Alice Y. Cheung, Thomas Dresselhaus, Cris Kuhlemeier. (2017) Focus on Flowering and Reproduction. *Plant Physiology, 173* (1) 1-4; DOI: 10.1104/pp.16.01867

Sanabria, N., Goring, D., Nürnberger, T., Dubery, I., The, S., Phytologist, N., … Dubery, I. (2017). Self / Nonself Perception and Recognition Mechanisms in Plants : A Comparison of Self- Incompatibility and Innate Immunity Linked references are available on JSTOR for this article : Remve Research review Self / nonself perception and recognition mechanis, *178*(3), 503–514. https://doi.org/10.1111/j.1469-8137.2008.02403.x

Sassa, H. (2016). Molecular mechanism of the S-RNase-based gametophytic self-incompatibility in fruit trees of Rosaceae. *Breeding Science*, *66*(1), 116–121. https://doi.org/10.1270/jsbbs.66.116

Schierup, M. H., Vekemans, X., & Christiansen, F. B. (1997). Evolutionary dynamics of sporophytic self-incompatibility alleles in plants. *Genetics*, *147*(2), 835–46. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9335618%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1208203

Shimizu, K. K., & Tsuchimatsu, T. (2015). Evolution of Selfing: Recurrent Patterns in Molecular Adaptation. *Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 593–622. https://doi.org/10.1146/annurev-ecolsys-112414-054249

Soderlund, C., Bomhoff, M., & Nelson, W. M. (2011). SyMAP v3.4: A turnkey synteny system with application to plant genomes. *Nucleic Acids Research*. https://doi.org/10.1093/nar/gkr123

Stein, J. C., & Nasrallah, J. B. (1993). A plant receptor-like gene, the S-locus receptor kinase of Brassica oleracea L., encodes a functional serine/threonine kinase. *Plant Physiology*, *101*(3), 1103–6.

https://doi.org/10.1104/PP.101.3.1103

Stone, S. L., & Goring, D. R. (2001). The molecular biology of self-incompatibility systems in flowering plants. *Plant Cell, Tissue and Organ Culture*, *67*(2), 93–114. https://doi.org/10.1023/A:1011980210048

Tabah, D. A., McInnis, S. M., & Hiscock, S. J. (2004). Members of the S-receptor kinase multigene family in Senecio squalidus L. (Asteraceae), a species with sporophytic self-incompatibility. *Sexual Plant Reproduction*, *17*(3), 131–140. https://doi.org/10.1007/s00497-004-0222-z

Takayama, S., & Isogai, A. (2005). Self-Incompatibility in Plants. *Annual Review of Plant Biology*, *56*(1), 467–489. https://doi.org/10.1146/annurev.arplant.56.032604.144249

Van der Does, D., Boutrot, F., Engelsdorf, T., Rhodes, J., McKenna, J. F., Vernhettes, S., … Zipfel, C. (2017). The Arabidopsis leucine-rich repeat receptor kinase MIK2/LRR-KISS connects cell wall integrity sensing, root growth and response to abiotic and biotic stresses. *PLoS Genetics*, *13*(6), 1–27. https://doi.org/10.1371/journal.pgen.1006832

Voillemot, M., & Pannell, J. R. (2017). Maintenance of mixed mating after the loss of self-incompatibility in a long-lived perennial herb. *Annals of Botany*, *119*(1), 177–190. https://doi.org/10.1093/aob/mcw203

Zuberi, M. I., & Lewis, D. (1988). Gametophytic-sporophytic incompatibility in the cruciferae -brassica campestris. *Heredity*, *61*(3), 367–377. https://doi.org/10.1038/hdy.1988.127