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Genetic and genomic characterization of the LOP locus from *Pilosella*

piloselloides subsp. Praealta

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Master of Science

at

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by

Marion Eliane Annie Gaillard

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Abstract of a thesis submitted in partial fulfilment of the requirements

for the Degree of Master of Science

The Genetic and genomic characterization of the LOP locus from *Pilosella piloselloides* subsp. Praealta

by

Marion Eliane Annie Gaillard

Apomixis is an asexual way of reproduction leading to the creation of clones of the mother plant. It is controlled by two independent loci in *Pilosella piloselloides subsp. Praealta* (Hawkweed): LOA (Loss of apomeiosis) and LOP (Loss of parthenogenesis), which are dominant. Apomictic plants develop one extremely competitive genotype and spread it in a colonial strategy. Only a few crops are apomictic; however, the natural creation of clones could considerably speed up the process of plant breeding and quickly stabilize new varieties adapted to climate change. *Pilosella* is one of the model plants to study apomixis. The purpose of this work was to investigate the LOP locus on a genetic and genomic level to better understand the mechanism of apomictic plants in the hope of applying it to crops. Using a panel of deletion mutants, this study made it possible to define the size of the LOP locus to about 650kb. A sequence of acceptable quality was checked for this locus using BAC isolates. Finally, the PAR gene contained in the locus for parthenogenesis in *Taraxacum* (dandelion) (Underwood *et al.*, 2020) was sequenced in *Pilosella* and several species from the Asteraceae family, indicating the level of conservation of the dominant allele compared to the recessive alleles.

Keywords: Apomixis, asexual reproduction, apospory, parthenogenesis, LOP, amplicon sequencing

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Chapter 1 Introduction to apomixis

Apomixis (Apo- = "free from"; mixis = "mixing" in Greek) is a term that was introduced by Winkler in 1908 to describe a "substitution of sexual reproduction by an asexual multiplication process without nucleus and cell fusion." [Translated from German] (Winkler, 1908; as cited in Bicknell et Koltunow, 2004).

It is believed that the first time apomixis was witnessed and described was in 1841 by John Smith. He noted that plants he imported from Australia to England had only female flowers, but they produced perfect seeds despite the absence of any male organ. In other words, the female flowers produced seeds without intervention of pollen. He was not able to precisely establish the name of the characterized plant at the time, but it was later described as *Alchornea ilicifolia* (syn. *Caelebogyne ilicifolia*).

This definition of apomixis has been refined with time to describe an alternative to sexual reproduction, which does not involve meiosis or fertilization, but results in the clonal production of a mother plant through seed. In sexual reproduction, germ cells (2n,4c) first undergo meiosis, a two-step division process that results in haploid gametes (1n,1c). The crossing over and different assortments of the homologous chromosomes occurring during meiosis lead to a unique set of DNA. Each of the male and female gametes then fuses, resulting in a zygote (2n) that is genetically different from the parents.

In apomixis, embryos are directly formed from unreduced egg cells or maternal tissues of the ovary. The chromosomal material transmitted to the progeny does not undergo any recombination event and is identical to the maternal plant (Bicknell et al., 2016). Apomixis, therefore, results in uniform populations and in cases where it can occur without a pollination partner, apomixis can facilitate the rapid colonization of new environments. Many apomictic species are known as weeds. Some examples are *Pilosella* (hawkweed), *Taraxacum* (dandelion) and *Opuntia* (prickly pear).

Apomixis has been witnessed in more than 400 species within 300 genera and 40 plant families of Angiosperms (Carman, 1997) including *Pilosella piloselloides subsp. Praealta*. A common theory suggests apomixis evolved out of sexual ancestors (Holsinger, 2000) as a result of mutations in genes involved in the key stages of the sexual development program, resulting in a "short circuit of the sexual pathway" (Spillane et al., 2001). Apomixis has evolved independently many times across the different angiosperm plant families (Bicknell, 2004).

It was initially suggested that it should lead to an evolutionary 'dead end' (Darlington, 1939; Grant, 1981) because the loss of genotype heterogeneity would make these apomictic populations unable to adapt to environmental change.

Although it is counterintuitive, this expectation does not meet the reality in nature. One reason for this is that apomixis is seldom expressed as the only mechanism of reproduction. Often sexual and asexual reproduction coexist independently, either in different individuals within a population or both can occur in the same plant, either side by side or one is favoured over the other. In *Pilosella*, both sexual and asexual seeds form on the same plant, with the asexual seeds typically representing more than 98% of the total (Bicknell et al., 2003).

Table 1.1 Advantages and disadvantages of apomixis vs sexual reproduction.

In green the advantages, in red the disadvantages.

| Only the existing, elite genotype is multiplied Uniparental reproduction avoids the cost of maintaining males and provides an advantage in colonization (Baker, 1959 ; Baker et Stebbins, 1965). | Energy is allocated to producing offspring that may be unfit to its environment The mother plant always needs a mating partner A high amount of recombination occurs during meiosis |
|---|---|
| Deleterious mutations accumulate through generations The multiplied genotype is only performant in a stable environment | Sexual reproduction creates genetic diversity Genetic variability provides opportunities to adapt to environmental changes |

Apomixis

Sexual reproduction

As shown in the previous table, the advantages of one reproductive pathway are often the disadvantages of the alternative one and vice versa. This could explain why apomixis and sexual reproduction often coexist in nature. One theory suggests there may be a sex/apomixis switch that responds to environmental conditions in some species. Environment factors would then modify the penetrance of the traits. Indeed, an increase in the frequency of sexual ovule development (meiosis instead of apomeiosis) under stress has been observed in some species of the genus *Boechera* (Mateo de Arias, 2015).

1.1 Types of apomixis

The different forms of apomixis are distinguished according to the cell type involved in the creation of the unreduced embryo sac.

1.1.1 Sporophytic apomixis

In this type of apomixis, the female gametophyte phase is entirely bypassed (Leblanc and Mazzucato, 2001). The embryo forms directly from a somatic cell of the ovule (Fei et al., 2019).

This form of apomixis typically requires the sexual formation of an endosperm tissue, so pollination is still a requirement for seed formation. The fertilization of the meiotic embryo sac is, therefore, still required for the development of the clonal embryo.

1.1.2 Gametophytic apomixis

When we talk about apomixis, we usually refer to gametophytic apomixis. The embryo comes from an unreduced egg cell, which means an egg cell that didn't undergo meiosis (was not reduced). This process is called "apomeiosis". The unreduced egg cell then differentiates to form an embryo sac by mitosis. Within this structure, an unreduced egg cell forms. The subsequent development of an embryo from this egg cell without fertilization is a process called "parthenogenesis" (Bicknell and Catanach, 2015). Gametophytic apomicts are sub-divided into two categories according to the mechanism used to avoid meiosis. These two mechanisms probably evolved independently between *Hieracium* and *Pilosella* (Mráz and Zdvořák, 2019).

Diplospory

The diploid megaspore mother cell avoids meiosis to develop an embryo sac (Tucker and Koltunow, 2009). The interruption of the meiosis is due to a failure in chromosome pairing. It leads to a mitosis-like division and the formation of an unreduced megaspore (with the same ploidy as the maternal plant). This unreduced megaspore divides, leading to the formation of an unreduced embryo sac and then an unreduced egg. Plants in the genus *Hieracium* use this type of apomixis.

Apospory

Meiosis is completely bypassed. Somatic cells of the ovule near the developing megaspore enlarge and differentiate to form aposporous initial cells. These have the maternal genotype and act as megaspore-like cells (Bicknell and Catanach, 2015). The Al's then develop into embryo sacs without the intervention of any external material (Okada et al., 2013). Plants in the genus *Pilosella* use this type of apomixis.

Aposporous apomicts may or may not require pollination for endosperm development (a sperm cell from the male gametophyte fuses selectively with an unreduced polar nucleus). *Pilosella* species typically do not require pollination and are therefore called 'autonomous apomicts' (Koltunow, 1993).

1.2 Apomixis in Pilosella praealta

Gregor Mendel was one of the first geneticists to be interested in *Pilosella* (formerly *Hieracium*). Mendel chose this plant to retry the experiments he conducted on other species to validate his hypothesis on the particulate nature of inheritance. He didn't know that this plant rarely uses sexual reproduction and therefore *Pilosella* didn't show the inheritance pattern that Mendel previously found on peas. Mendel remained convinced of his findings but died with the idea that the patterns he found were only applicable to some species and could not be generalized. We now know that, unfortunately, *Pilosella* was a poor model system for Mendel's work on sexual inheritance. It has, however, recently been found that *P.praealta* possesses several useful features for the molecular study of apomixis (Bicknell et al., 2016).

Most of the species in the genus *Pilosella* are apomictic, but some sexual species exist and some sexual individuals do occur within typically apomictic species. As both asexual and sexual seed form in this system, *Pilosella* is called a "facultative apomict" (Mráz and Zdvořák, 2019). Estimates of the level of facultativeness vary between species and variety, but they are typically above 90% asexual (93%: Mráz and Zdvořák, 2019, 97%: Bicknell et al., 2003). In some species, the sexual female gametophyte development is not affected. Meiotically reduced and unreduced aposporous gametophytes co-exist in the same ovule (Drews and Koltunow, 2011). However, the initiation of apomixis in *Pilosella* usually interrupts the sexual process (Tucker et al., 2003). The adjacent sexual megaspores then degenerate during aposporous embryo sac formation.

1.2.1 Pilosella praealta as a model plant

Pilosella praealta is a good model plant to study apomixis because:

- It is easily cultivated
- It is easily grown and manipulated in vitro
- It has a small stature,
- It has a short generation time, and abundant seed set to facilitate rapid turnover of experimental populations.

- Both sexual and apomictic biotypes are available.
- It uses autonomous endospermy to avoid difficulties associated with pseudogamy.
- Male meiosis and pollen formation are functional in most apomictic biotypes (for transfer to sexual recipients)
- Apomixis is easily assessed and quantified in these plants

(Bicknell, 1994)

1.2.2 The genetics of apomixis in Pilosella

Apomixis in *Pilosella praealta* is controlled by two genetic loci (*LOA* and *LOP*). They were first described in a wild type individual called R35 through the use of deletion mutants (Catanach et al., 2006; Koltunow et al., 2011). Thus, the loci were named after the phenotypes of the mutants presenting a defect in the so-called loci.

The LOSS OF APOMEIOSIS Locus (LOA/loa). This locus is associated with the avoidance of meiosis and the differentiation of aposporous initial cells. The inheritance of the dominant *LOA* allele confers apomeiosis (meiosis does not occur). Homozygous recessive genotypes carrying *loa* alleles have the sexual biotype using meiotic egg cell formation.

The LOSS OF PARTHENOGENESIS locus (LOP/lop). This locus is associated with the avoidance of fertilization. It controls the development of the unreduced embryo and the endosperm. The LOP locus exhibits suppressed recombination (Catanach et al., 2006). Inheritance of the dominant LOP allele confers parthenogenesis (embryo development occurs spontaneously). Homozygous recessive genotypes carrying *lop* alleles have the sexual biotype requiring fertilization to initiate embryo formation.

In both cases, the dominant alleles at these loci act to overcome a specific developmental checkpoint. In the case of *LOA*, the dominant allele enables cells adjacent to the megaspore mother cell to assume a fate similar to the megaspore. They then divide, and the products undergo the specific developmental pathway of mega-gametogenesis, leading to the formation of an embryo sac.

In the case of *LOP*, the dominant allele enables the egg cell to overcome quiescence and to divide without the stimulus of fertilization.

A third locus controls the development of the endosperm, called the **LOSS OF AUTONOMOUS ENDOSPERMY** locus. There is some data to suggest that this is in linkage with *LOP*, but very little is currently understood about the structure or function of this locus in *Pilosella*.

1.2.3 Identification of apomixis genes in other systems

Pennisetum

Many of the species in the genus *Pennisetum* reproduce by apospory. Only one dominant locus is involved, but it always exists in the heterozygous state in an apomictic parent (Ozias-Akins et al., 2003). In *Pennisetum squamulatum* this locus is a large, dominant sequence called the Apospory-Specific Genomic Region (ASGR), which confers both apomeiosis and parthenogenesis (Ozias-Akins et al., 1998). *BabyBooM-Like* (*PsASGR-BBML*) genes located within this locus have been discovered and present similarity to *BABY BOOM* (*BBM*) genes of Arabidopsis and Brassica, which are involved in embryogenesis (Boutilier et al., 2002).

A study showed that the expression of one of these genes called *BBM1* is initially specific to the male allele but is subsequently biparental. However, in rice (*Oryza sativa*), this gene induces embryo formation without fertilization when it is only expressed in the egg cell. This study also claimed that "asexual propagation without genetic segregation could be engineered in a sexually reproducing plant", although this was not actually achieved. (Khanday et al., 2019).

Taraxacum

Apomixis in *Taraxacum* is obligate meiotic diplospory, which has been described as "the type that is most similar to sexual reproduction among apomixis systems" (Asker and Jerling, 1992; as cited in Majeský et al., 2012). Two unlinked dominant apomixis loci have been described, controlling: 1. The avoidance of meiotic reduction (*DIPLOSPOROUS-Dip*) and 2. Parthenogenesis of the embryo (*PARTHENOGENESIS-Par*)" (Van Dijk et al., 2009).

Boechera

Boechera is the model plant for studying apomixis in the Brassicaceae family. Apomixis in *Boechera* is gametophytic apomixis (Böcher, 1951) where both apospory and diplospory can occur (Carman et al., 2019). One candidate apomixis gene has been identified in *Boechera* and is called APOLLO (apomixis-linked locus). This gene shows increased expression in apomictic ovaries compared to sexual ovaries (Corral et al., 2013).

Poa

In the case of *Poa*, there is evidence that parthenogenesis can segregate independently of aposporous embryo sac development (Albertini et al., 2001).

A complex model has been described for *Poa pratensis* that includes five single, unlinked genes: the Apospory initiator (*Ait*) gene, the Apospory preventer (*Apv*) gene, a Megaspore development (*Mdv*) gene, the Parthenogenesis initiator (*Pit*) gene, and the Parthenogenesis preventer (*Ppv*) gene (Matzk et al., 2005).

1.3 Use of apomixis in plant breeding

Apomixis has long been avoided by plant breeders in a quest for new cultivars as it resulted in no change between generations, so it opposed genetic gain. Now it is realized that it could be very valuable in crops if it could be controlled.

Every year, farmers around the world buy new seeds whose valuable characteristics (resistance to a pathogen, resistance to drought, high productivity...) have been stabilized using F1 hybrid technology. Unfortunately, this genetic work is compromised with each new generation because the events of meiosis (division of genetic material) and fertilization (supply of external genetic material) generate new genotypes. As a result, F2 generations tend to be much more variable and to have lower average yields than F1 generations, so farmers are encouraged to buy more F1 hybrid seed rather than save seed from a previous crop.

One of the reasons why we study apomixis is that if we applied this mechanism to crops, during the breeding process, the avoidance of recombination would mean that valuable traits would be retained through the generations. This would empower poor farmers to raise their own high yielding hybrid seeds, increasing production levels in countries that currently do not have the resources to buy hybrid seeds every year. It may also reduce the cost and time of crop breeding as it does not require inbred line production or mechanisms of pollination control such as cytoplasmic sterility and fertility restoration.

Reducing the cost and the time needed for plant breeding will also help breeders to respond more rapidly to changing ecological circumstances.

The idea of a "conditional apomixis" has emerged in the last decades where reproduction could be temporally switched to sexual reproduction for breeding, then back to apomixis for seed multiplication (Spillane et al., 2004). To achieve this, apomictic crops would need to retain an ability to reproduce sexually so that they can be bred, and some form of inducible gene action would be required. Second, ideally, male organ development would be blocked to prevent the horizontal transfer of apomixis genes into wild relatives. Finally, these crops should have autonomous endosperm development so pollination is not essential for crop production (Kandemirand Saygili, 2015).

Table 1.2 Advantages and disadvantages of apomictic crops.

In green the advantages, in red the disadvantages

| Advantages | Disadvantages | | | | |
|------------------------------------|--|--|--|--|--|
| Reduced cost of breeding | Sexual reproduction is still needed for breeding | | | | |
| Reduced time per breeding cycle | Horizontal transfer to weeds | | | | |
| Elite genotypes in two generations | Require endosperm development | | | | |
| Hybrid seeds in almost all crops | Reduction of genetic diversity | | | | |

1.3.1 Conversion of sexual crops to apomictic crops

Wide crosses with apomictic wild relatives

Apomixis is present in some wild species but rarely in crops. Only some crops species such as Citrus, berries, guayule and several forage grasses have been observed to use apomixis as the main method of reproduction. There is, however, evidence of apomixis in several wild relatives to the cultivated crops (Hanna, 1991). Traces of apomixis are also believed to remain in grain sorghum and pearl millet (Bashaw, 1980). The transfer of apomixis from wild relatives may therefore be possible for some crops, but even in cases where this occurs, attempts at the transfer of apomixis have not been successful so far (Fiaz et al., 2021).

Mutations in apomixis related genes

Mutants capable of meiosis-independent embryo sacs development

Two mechanisms have been reported to artificially induce apomeiosis by mutations that convert meiosis into a mitosis-like division. The first mechanism involves the mutation of the gene *Osd1* which ultimately results in the elimination of the second division in meiosis in *Arabidopsis*. When associated with two other mutations that eliminate recombination and modify chromatid segregations, the triple mutant genotype is called *MiMe*. (Fiaz et al., 2021; Kandemirand Saygili, 2015). In the second mechanism, a mutation in the *Arabidopsis SWI1* gene leads to apomeiosis and diploid egg formation, but only at a very low level (Ravi et al., 2008). Both of these mechanisms need a way of eliminating the paternal chromosome since the seeds they produce have higher ploidy levels than the species.

Mutants capable of fertilization-independent embryo development

Some apomixis candidate genes have been described to be implied in the control of embryo development, but only a few are promising.

<u>The PsASGR-BabyBoom-Like Gene</u>. The transfer of the *PsASGR-BABYBOOM*-like gene (*PsASGR-BBML*) enables parthenogenesis in the monocotyledonous crops maize and rice (Conner et al., 2017) and the dicotyledonous species tobacco (Zhang et al., 2020).

<u>The "Salmon System" in Wheat</u>. The transfer of the nucleus from the sexual wheat line *Salmon* into the cytoplasm of two varieties of *Aegilops* resulted in male sterile plants with the ability for autonomous embryo development (Matzk et al., 1995).

<u>The RWP-RK Domain (*RKD*)</u> is responsible for keeping the egg cell quiescent in the absence of fertilization. The knockdown of this single gene domain leads to the egg cell division without fertilization (Rövekamp et al., 2016).

Genetic transformation

Genetic transformation is a potential approach for the transfer and introgression of apomixis into crops. However, induction of apomixis with gene-editing strategies requires prior in-depth knowledge of apomictic genes and genetic variation that naturally exist in apomictic populations.

The cloning of the LOA (Kotani et al., 2014) and LOP (current studies) loci in *P.praealta* is progressing.

Henderson et al. (2020) have introduced a *Cas9* construct in an apomictic *Pilosella* (formerly *Hieracium*) species via *Agrobacterium*-mediated leaf disk transformation. The efficiency of CRISPR/Cas9 editing to target the endogenous *PHYTOENE DESATURASE* (*PDS*) gene was evaluated and gave promising results, opening a way for the use of gene editing in apomixis research and identification of apomixis genes.

AIMS OF THE THESIS STUDY

If we knew more about the genes that control apomixis in a model system we would be better equipped to engineer it into crops. The *LOA* and *LOP* loci of *Pilosella* have been mapped through ESTs to chromosomal regions, and the structure of *LOA* has been reported.

- 1. The first aim was to extend a BAC tiling path within the LOP locus
- 2. The second aim was to fine map the *LOP* region. Using the sequenced BAC tiling path and a genome draft, markers were designed and tested to define the region of suppressed recombination around the *LOP* locus.
- 3. The third aim was to examine whether the different forms of reproduction conferred by different alleles at *LOP* (sexual and parthenogenic) influence patterns of allelic diversity in the genus *Pilosella*.

Chapter 2 General Materials and Methods

2.1 A note on taxonomy

Devising a taxonomic scheme that adequately describes and categorizes the diversity present within an apomictic complex is a difficult task. The genus *Hieracium*, as instated by Linnaeus in 1753, has been the subject of several major revisions. At the time of Mendel's work (Mendel, 1869), all species were included within the genus Hieracium, but subsequently, the genus was subdivided into two genera; *Hieracium* and *Pilosella*. Most of the plants used in the current study are now assigned to the genus *Pilosella*. At different times individual clones have also been renamed. The model plant used in this study, R35, has previously been named *Hieracium caespitosum* (Catanach et al., 2006), *Hieracium praealtum* (Bicknell et al., 2016) and *Pilosella praealta* (Bräuning et al., 2018) in accordance with previous naming conventions. The Global Compositae Database currently lists it under the name *Pilosella piloselloides subsp. Praealta*. For simplification the name *Pilosella praealta* is used in the text.

2.2 Plant material

2.2.1 Wild types and mutants

The plant material used for this study were generated for previous studies (Catanach et al., 2016) and maintained throughout the years. The wild type tetraploid "R35" was extensively used throughout the study. The diploid apomict "PH70" was derived from R35 as a meiotic product, so it just contains alleles from R35 but has half the nuclear DNA. This individual PH70 has both the *LOA* and *LOP* alleles at the appropriate loci and acts as an apomict like the wild type. A genome sequence based on PH70 DNA was used for the mapping work. Mutants of R35 lacking the *LOP* locus were also used in the allelic diversity study along with wild types and mutants of other species of *Pilosella* and *Hieracium* (see chapter 5).

2.2.2 Detection of apomixis

The detection of apomixis for this study was performed by Dr Ross Bicknell. "Detection of apomixis on the basis of morphological indicators requires careful observation of the progeny of individual plants by a person who is thoroughly familiar with the mode of pollination of the parent and the variability expected among its sexually produced offspring" (Bashaw, 1980). In *P. praealta,* apomixis can be demonstrated by severing the top off an immature floral bud. This removes the anthers and stigmas but leaves the ovaries intact. If seed still forms, then the plant has elements of apomixis. It is recommended that the seed be sown and the progeny tested for ploidy as this further qualifies the degree of apomixis, separating plants that have full apomixis (for example a

tetraploid will yield tetraploid seedlings) from those that have only a functional copy of *LOP* but not *LOA* (in which a tetraploid would yield diploid seedlings). This method is simple, quick, repeatable and quantifiable.

2.3 DNA extraction

Approximatively 100mg of fresh leaves were harvested from each plant and placed in a test tube containing glass beads and snap-frozen using liquid nitrogen. Each tube was vortexed for 6 seconds using a Silamat S5 shaker to grind the plant material to a fine powder. DNA was extracted using the reagents and manufacturer's protocol in the DNeasy[®] Plant Mini Kit 15 (Qiagen, Hilden, Germany).

Genomic DNA was quantified using an Invitrogen Qubit[™] 4 Fluorometer.

2.4 Sequencing

The region containing the *LOP* locus has been sequenced using Oxford Nanopore and Illumina technologies. The data were combined using different assembly tools to polish the data and create the most accurate sequence.

2.4.1 3.4.1 Illumina

Illumina is a next-generation technology where DNA is synthesized using a modified DNA polymerase that incorporates fluorescently labelled dNTPs into a DNA template.

Illumina sequencing was performed by Dr Ross Bicknell's team prior to this study. This technology provided **short reads** of 25-100bp that were be assembled into contigs to create a map of the genome of the apomict *P. praealta* individual PH70. The level of base-calling accuracy of Illumina technology is high (**99.9%**). However, the sequences coming from this technology were subject to random errors, especially at the overlapping regions of the contigs, and assembly was complicated in regions rich in repeat sequences.

2.4.2 Oxford Nanopore

Oxford Nanopore Technology is a fourth-generation DNA sequencing technology based on ion current variations caused by the passage of single strands of DNA molecules through a tiny protein channel (Nanopore).

This high throughput technology produced **long reads** that offered a better coverage of the LOP locus. The samples were processed rapidly, and the results were displayed in real-time. However, the accuracy is lower than Illumina (**98%**), and the data were subject to systemic errors.

2.5 Genome assembly and identification of the LOP locus in P. praealta

Following the sequencing, assembly programs were used to reconstruct the *P. praealta* PH70 genome. Two assemblers were used: SHASTA and FLYE. Both of these assemblers create a 'hybrid assembly' merging the data from the two alleles at LOP within the diploid PH70. Consequently, the assembly used was not accurate for either allele, but it was valuable for developing markers and estimating genetic distances. This work was conducted by my supervisor Dr Chris Winefield in support of this study.

2.5.1 SHASTA/FLYE

The strength of two technologies, SHASTA and FLYE, were combined to create a more accurate assembly. SHASTA used the Oxford Nanopore data, and FLYE used Illumina data.

One contig within the SHASTA assembly ("1486 BLAST hit") aligned to the known BAC sequence at the dominant allele of *LOP*. Contig 1486 SHASTA was BLAST back against the FLYE assembly with a 100% match).

This contig was used for mapping the deletion mutants (Catanach et al., 2016) and for the research of allelic diversity in this study.

Chapter 3 BAC library screening: BAC walking

3.1 Introduction

One of the aims of this study was the genetic characterization of the *LOP* locus of *P.praealta*. For this to succeed, the mapping data needed to be as precise as possible so that studies of genome structure, organization and function on which it was to be based were also accurate. Sequence quality in particular, has a significant impact on downstream analysis.

The original genome map for *P. praealta* was built mainly from short-read data from next-generation sequencing technologies. The read length was around 50 - 300 bp. Although NGS technologies have the advantage of a much higher throughput with dramatically lower cost, they also have the disadvantages of higher error rates compared to Sanger sequencing. The first errors appear at the nucleotide level with the base calling (substitutions, insertions or deletions). Each base is assigned a phred-like quality score as a by-product. At the technology level, there are also specific error patterns associated with each genome sequencer (Victoria Wang et al., 2012). At the assembly level, mistakes happen during the computation in genome assembly: missed overlaps leave gaps in the assembly, while false overlaps create ambiguous maps or create connections between regions that should not be associated (Palmer et al., 2010).

To improve the quality of the sequence, a genetic map can be integrated with a physical map. Bacterial Artificial Chromosomes (BACs) accept inserts up to 300 Kb in length. The use of BACs containing genes of interest is an advantageous method for obtaining sequences from specific regions of interest. One of the inserts from a BAC can cover several sequencing reads and thus polish the sequence to facilitate the construction of DNA libraries to analyze genomic structure.

The bacterial artificial chromosome (BAC) cloning vector is a circular plasmid based on the E. coli fertility factor (F-factor) replicon. It is difficult to clone and maintain large intact DNA in bacteria. However, the F factor controls its own replication. It contains oriS and repE genes, which allow unidirectional replication, and parA and parB genes, which maintain its low copy number (Shizuya et al., 1992). The F-factor also has genes that regulate DNA synthesis so that its copy number is kept at a low level; and genes that regulate the partition into the daughter cells after E. coli divides.

| advantages | disadvantages |
|---|--|
| Stability of insert propagation over multiple generations | Unanticipated changes in the cell phenotype Recombinant BAC construct can cost time |
| Large inserts | More easily degraded |
| Handy manipulation | |
| Accurate endogenous gene expression | |

 Table 3.1 Advantages and disadvantages of BAC system over YAC or mammalian artificial chromosomes

This chapter is about the identification of specific BAC clones corresponding to genomic sequences of the *LOP* locus. One BAC clone was identified in a previous study, but it didn't cover the entire *LOP* locus. The aim of the library screening was to find clones corresponding to two marker probes, originally designed against lettuce genome sequence.

3.1.1 BAC library

In a previous study, a BAC (Bacterial Artificial Chromosome) library was created by Dr Ross Bicknell's team from DNA of the wild type tetraploid apomict R35 (unpublished data). The library contains approximately 258,048 colonies (672 Petri dishes containing 16x24=384 colonies), each carrying one piece of *Pilosella piloselloides var praealta* wild type "R35" DNA. The average insert size in the library is approximately 141kb. BAC libraries usually have several times coverage of the genome (Shizuya et al., 1992). The fold genome coverage is inversely proportional to the average size of the insert. The larger the size of the insert, the fewer clones that are required. The higher the coverage, the better the data quality. In this study, the whole genome of R35 has a 5-fold coverage.

3.1.2 BAC pooling

For a faster identification of the BAC colonies of interest, a compression of the 258,048 colonies into one super-pool was done in a previous study (unpublished data) according to this process:

The library consisted of 672, 384-well plates. Each plate contains 384 x 141kb = 54Mbp (0.75% of 4x).

The 672 384-well plates were pooled into seven 96-well plates, where each well contained the equivalent of a 384 BAC clones. These were referred to as the 'pool plates'. Each pool plate contains 54Mbp x 96 = 5,184Mbp (72% of 4x) Seven pool plates were then reunited in 7 rows of a new super-pool plate. In this unique plate, each horizontal row contained the equivalent of one of the 7 pool plates (see figure 1). Each row of the super pool well contains 12×54 Mbp = 1,512Mbp (9% of 4x) and each column contains 8×54 Mbp = 432Mbp (6% of 4x).

The position of a well in a super-pool plate gave an equivalence of one row of one of the 7 pool plates, and the position of a well on a pool plate gave the address of the source BAC colony plate.



Figure 3.1 Schematic display of the BAC pooling

3.1.3 Bac screening

The approach used was PCR-based screening, going back from the super-pool to the clone containing the DNA sequence of interest. All PCR amplifications were performed on a Bio-Rad T100 Thermal Cycler. Once pooled, clones containing sequences of interest were located by identifying the subset of pools containing the corresponding markers. The pooling technique saved both time and materials. Instead of doing a PCR for each of the 258,048 colonies, the best candidate BAC colony plates were identified with 180 PCR reactions (84 super-pool reactions followed by 96 pool column reactions). An electrophoresis gel was run to check if the amplification was successful and identify the candidate BAC colony plates. A DNA fragment analyzer partially automated the process, and it also allowed discernment of different bands that varied by only a few base pairs. This was particularly valuable for the markers with allelic product size differences that would not have been apparent on a gel. Twelve super pools for each primer were chosen to go back to the seven pool plates. The electrophoresis gel identified 3 to 4 candidate column pools, which was confirmed by the fragment analyzer. The numbers of the plates and the columns gave an equivalence for each of the 672 BAC library plates. Once a BAC library plate was identified it was replicated onto an agar plate. Colonies were then picked using pipette tips. To avoid contamination, the columns were split into two halves (A-H and I-P).



Figure 3.2 Plan showing the process to collect pools of 8 BAC colonies (half columns).

Picked colonies were placed in a template plate with 50uL of water, boiled up at 95 degrees for 10 minutes, cooled to 4 degrees then spun for 2 minutes at 4000 rpm. Two microliters of this supernatant was used as a template in the subsequent PCR reaction. This final step typically required a further 40 reactions (24 column streaks and 16 well reactions) to test and identify the colony of interest. Some savings were made because several candidates often resulted from the first set of 84 super-pool reactions, and this step only needed to be conducted once for each primer set.

Markers

The super-pool plates were tested using primers to the markers Lsat46 and 44/45 (Table 4). Genomic DNA of the wild type R35 was used as the positive control. The marker Lsat46 is co-dominant, identifying 3 of the 4 alleles at *LOP* in R35. Allele 1 is the dominant allele at LOP that confers parthenogenesis. Alleles 2, 3 and 4 are recessive alleles that encode for the *lop* alleles. The Lsat46 primers clearly identified alleles 1 and 4 by product size differences (177bp for allele 1 and 174 for allele 4), while products of alleles 2 and 3 are of a similar size (176bp) and could not be separated for this marker (see figure 3.3). Marker 44/45 is a dominant marker with a 330bp product for allele 1 at LOP. For both markers, the PCR conditions were:

Step 1: 95°C: 3 minutes Step 2: 95°C: 30 seconds Step 3: 60°C: 30 seconds Step 4: 72°C: 1 minute Repeat steps 2-4 a total of 40 times Step 5: 72°C: 5 minutes Hold: 8°C:

Table 3.2 Details of the two markers used for the BAC screening

| Marker | Forward primer | Reverse primer | Predicted allele product sizes* |
|---------|--------------------------|--------------------------|------------------------------------|
| Lsat 46 | GAGAGAAGATTGCCATCAGAACC | GAATTAAATAGYGGATGGAAGAGG | 177 (1), 176 (2 & 3), 174 (4) |
| 44/45 | TCTTGGCCACCGCCAACAAGGTGA | ACTGGCCCG CGCGGATGTCT | 330 (1), 327 (2&3), 339 (4) |

* Alleles in parentheses



Figure 3.3 Fragment analyzer data for the marker 44/45 with the wild type R35 as a positive control and \$\square{136}\$ as a negative control, used to assess the candidates

The dominant allele (called allele 1) can be found using deduction: R35 is tetraploid and possess 4 alleles (One dominant allele 1 + alleles 2 and 3 on top of each other and allele 4). y136 is a deletion mutant that only possesses recessive alleles (2 or 3, and 4)



Figure 3.4 Fragment analyzer data for the marker Lsat46 with the wild type R35 and the polyploid PH70 as positive controls and y136 as a negative control.

The dominant allele (called allele 1) can be found using deduction: R35 is tetraploid and possess 4 alleles (One dominant allele 1 + alleles 2 and 3 on top of each other and allele 4). Allele 1 is only expressed as a shoulder in R35 but is a clear peak in its diploid derivative PH70. γ 136 is a deletion mutant that only possess recessive alleles (2 or 3, and 4)

3.2 Results and discussion

3.2.1 Lsat46: Co-dominant Marker (for Alleles 1, 2&3, 4) at LOP

Step 1 – super-pool

84 reactions were necessary to amplify each well of the super-pool. The remaining 12 wells left in the 94-well PCR plate were used for controls: R35 (alleles 1,2,3,4), PH70 (1,3), y179 (2,3,4), y115 (2,3,4), y138 (2,3,4), y136 (2,3,4), PH511 (1,2), PH544 (1,3), PH410 (1,3), PH191 (1,2), PH325 (1,2) and No Template Control.



Figure 3.5 Electrophoresis gel of the PCR products from the super-pool with the marker Lsat46: Plate/column pools

Each well was identified with the number of plate and column from which they came from. P: plate; C: column A strong and compact band around 200bp is an indicator of the potential presence of the desired insert

Super-pools with BAC colonies that contain the sequence of interest gave an intense amplification. Strong amplifications were seen in wells P7C1, P7C3, P3C10, P2C12, P3C11, P7C12. Analysis by the fragment analyzer was used to confirm the PCR product size carried by each of the candidate column pools, which might contain a BAC colony of interest. From this point, attempts to isolate BACs for each allele were treated separately (except alleles 2 and 3 which could not be separated).

| | L | | | | | | | | | | | |
|------------|-----------|--|-------|------|------|------|------|------|------|-------|-------|-------|
| Allele 1 | P1C1 | P1C2 | P1C3 | P1C4 | P1C5 | P1C6 | P1C7 | P1C8 | P1C9 | P1C10 | P1C11 | P1C12 |
| | P2C1 | P2C2 | P2C3 | P2C4 | P2C5 | P2C6 | P2C7 | P2C8 | P2C9 | P2C10 | P2C11 | P2C12 |
| | P3C1 | P3C2 | P3C3 | P3C4 | P3C5 | P3C6 | P3C7 | P3C8 | P3C9 | P3C10 | P3C11 | P3C12 |
| | P4C1 | P4C2 | P4C3 | P4C4 | P4C5 | P4C6 | P4C7 | P4C8 | P4C9 | P4C10 | P4C11 | P4C12 |
| | P5C1 | P5C2 | P5C3 | P5C4 | P5C5 | P5C6 | P5C7 | P5C8 | P5C9 | P5C10 | P5C11 | P5C12 |
| | P6C1 | P6C2 | P6C3 | P6C4 | P6C5 | P6C6 | P6C7 | P6C8 | P6C9 | P6C10 | P6C11 | P6C12 |
| | P7C1 | P7C2 | P7C3 | P7C4 | P7C5 | P7C6 | P7C7 | P7C8 | P7C9 | P7C10 | P7C11 | P7C12 |
| | 1.1.1.1.1 | 1 | · · · | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | 1 | | | | | | |
| Allele 2/3 | P1C1 | P1C2 | P1C3 | P1C4 | P1C5 | P1C6 | P1C7 | P1C8 | P1C9 | P1C10 | P1C11 | P1C12 |
| | P2C1 | P2C2 | P2C3 | P2C4 | P2C5 | P2C6 | P2C7 | P2C8 | P2C9 | P2C10 | P2C11 | P2C12 |
| | P3C1 | P3C2 | P3C3 | P3C4 | P3C5 | P3C6 | P3C7 | P3C8 | P3C9 | P3C10 | P3C11 | P3C12 |
| | P4C1 | P4C2 | P4C3 | P4C4 | P4C5 | P4C6 | P4C7 | P4C8 | P4C9 | P4C10 | P4C11 | P4C12 |
| | P5C1 | P5C2 | P5C3 | P5C4 | P5C5 | P5C6 | P5C7 | P5C8 | P5C9 | P5C10 | P5C11 | P5C12 |
| | P6C1 | P6C2 | P6C3 | P6C4 | P6C5 | P6C6 | P6C7 | P6C8 | P6C9 | P6C10 | P6C11 | P6C12 |
| | P7C1 | P7C2 | P7C3 | P7C4 | P7C5 | P7C6 | P7C7 | P7C8 | P7C9 | P7C10 | P7C11 | P7C12 |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | . | | - | | - | - | - | | | | |
| Allele 4 | P1C1 | P1C2 | P1C3 | P1C4 | P1C5 | P1C6 | P1C7 | P1C8 | P1C9 | P1C10 | P1C11 | P1C12 |
| | P2C1 | P2C2 | P2C3 | P2C4 | P2C5 | P2C6 | P2C7 | P2C8 | P2C9 | P2C10 | P2C11 | P2C12 |
| | P3C1 | P3C2 | P3C3 | P3C4 | P3C5 | P3C6 | P3C7 | P3C8 | P3C9 | P3C10 | P3C11 | P3C12 |
| | P4C1 | P4C2 | P4C3 | P4C4 | P4C5 | P4C6 | P4C7 | P4C8 | P4C9 | P4C10 | P4C11 | P4C12 |
| | P5C1 | P5C2 | P5C3 | P5C4 | P5C5 | P5C6 | P5C7 | P5C8 | P5C9 | P5C10 | P5C11 | P5C12 |
| | P6C1 | P6C2 | P6C3 | P6C4 | P6C5 | P6C6 | P6C7 | P6C8 | P6C9 | P6C10 | P6C11 | P6C12 |
| | P7C1 | P7C2 | P7C3 | P7C4 | P7C5 | P7C6 | P7C7 | P7C8 | P7C9 | P7C10 | P7C11 | P7C12 |
| | | | | | | | | | | | | |

Figure 3.6 Identification of the potential pool candidates for each allele with the marker Lsat46: Plate/column pools

Colored squares represent positive results. Saturated toned squares for allele 1 and 2/3 are the results with the highest confidence. For allele 4, all the results are supposedly reliable.

Step 2 – pools

For each of the 8 super pools for allele 1 and alleles 4, 7 pool plates were tested. A well a the pool plate corresponds to a BAC library plate.



Figure 3.7 Electrophoresis gel of the PCR products from the pool plates with the marker Lsat46.

The four candidates that have been found are marked in yellow. As other candidates did not contain the insert, it is likely that primer dimers formed during the PCR leading to the lower bands on the electrophoresis gel.

Four BAC plate candidates were identified, two for allele 1 and two for allele 4, corresponding to plates 263, 661, 168, 648.

| Allele 1 Allele 4 | | | | | | | | | | | |
|-------------------|---------|---------|--------|--------|--------|---------|---------|--------|--------|--------|---------|
| l | | | | | | | | | | | |
| | | | | | | [| | | | | 1 |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| P2C3_A | P3C10_A | P3C11_A | P6C4_A | P7C1_A | P7C2_A | P2C11_A | P2C12_A | P3C3_A | P4C7_A | P4C9_A | P7C12_A |
| P2C3_B | P3C10_B | P3C11_B | P6C4_B | P7C1_B | P7C2_B | P2C11_B | P2C12_B | P3C3_B | P4C7_B | P4C9_B | P7C12_B |
| P2C3_C | P3C10_C | P3C11_C | P6C4_C | P7C1_C | P7C2_C | P2C11_C | P2C12_C | P3C3_C | P4C7_C | P4C9_C | P7C12_C |
| P2C3_D | P3C10_D | P3C11_D | P6C4_D | P7C1_D | P7C2_D | P2C11_D | P2C12_D | P3C3_D | P4C7_D | P4C9_D | P7C12_D |
| P2C3_E | P3C10_E | P3C11_E | P6C4_E | P7C1_E | P7C2_E | P2C11_E | P2C12_E | P3C3_E | P4C7_E | P4C9_E | P7C12_E |
| P2C3_F | P3C10_F | P3C11_F | P6C4_F | P7C1_F | P7C2_F | P2C11_F | P2C12_F | P3C3_F | P4C7_F | P4C9_F | P7C12_F |
| P2C3_G | P3C10_G | P3C11_G | P6C4_G | P7C1_G | P7C2_G | P2C11_G | P2C12_G | P3C3_G | P4C7_G | P4C9_G | P7C12_G |
| P2C3_H | P3C10_H | P3C11_H | P6C4_H | P7C1_H | P7C2_H | P2C11_H | P2C12_H | P3C3_H | P4C7_H | P4C9_H | P7C12_H |

Figure 3.8 Identification of the potential pool candidates for each allele with marker Lsat46 *Saturated toned squares are the results with the highest confidence.*

| Address | allele | plate |
|---------|--------|-------|
| P3C11_F | 1 | 263 |
| P7C1_H | 1 | 661 |
| P2C12_F | 4 | 168 |
| P7C12_F | 4 | 648 |

 Table 3.3 Detail of the four candidates BAC plates identified for the marker Lsat46

Step 3 - Petri dishes

For each positive pool result, the colonies on the corresponding BAC plate were tested to isolate the colony containing the identified sequence. The Petri dishes 263 and 168 did not give any result. One BAC colony of interest was identified in plate 661, half column (A to H) number 3. One BAC colony of interest was potentially identified in plate 648 in two positions, respectively half column (I to P) number 13 or half column (A-H) number 22. As only one BAC can be present in one plate, one or the other must be due to a contamination.



Figure 3.9 Electrophoresis gel of the PCR products from the tested BAC colonies with the marker Lsat46. *A strong band around 200bp is likely to represent the desired insert while lower weak bands are likely to represent primer dimers.*

Each of these 3 candidates were investigated. A PCR plate was set with 3 x 8 colonies.



Figure 3.10 Electrophoresis gel of the PCR products from the targeted half columns of the Petri dish with the marker Lsat46.

The plate 661 did not give any amplification. However, a BAC colony of interest was successfully identified in the plate 648 despite a contamination.

Final confirmation

At the final step, the three candidates which remained that could contain the marker Lsat46 were tested using the genetic analyzer. R35 was used as a positive control. The colony 648-13-P (Petri dish n° 648, column n°13, line P) contained a DNA fragment that corresponded to the Lsat46 marker at allele 4. Considering the low level of amplification (see figure 3.11) of the two other colonies, the results appear to be due to contamination.



Figure 3.11 Detail of the fragment analyzer data for 3 BAC candidates and R35 as a positive control The allele 1 is represented by a "shoulder" next to allele 2/3

The level of amplification can be seen on the y axis and reveal that only the candidate 648_13_P is relevant while the two others must be due to contamination.

This figure also indicates that the insert contained in the candidate 648-13-P is likely to correspond to the allele 4
3.2.2 Marker 44/45: Co-dominant Marker (for Alleles 1, 2&3, 4) at LOP

Step 1 – super-pool

84 reactions were necessary to amplify each well of the super-pool. The remaining 12 wells left in the 94-well PCR plate were used for controls: R35 (alleles 1,2,3,4), PH70 (1,3), γ 179 (2,3,4), γ 115 (2,3,4), γ 138 (2,3,4), γ 136 (2,3,4), PH511 (1,2), PH544 (1,3), PH410 (1,3), PH191 (1,2), PH325 (1,2) and No Template Control.



Figure 3.12 Electrophoresis gel of the PCR products from the super-pool with the marker 44/45.

Each well was identified with the number of the plate and column from which they came from. P: plate; C: column A strong and compact band around 200bp is an indicator of the potential presence of the desired insert

Here we can clearly identify P7C1, P7C2, P2C3, P7C3, P2C12, P3C11, P5C11, P7C12 as amplifying very well. This result was then confirmed by the fragment analyzer and the alleles involved were identified.

| All | e | e | 1 |
|-----|---|---|---|
| | | | |

| P1C1 | P1C2 | P1C3 | P1C4 | P1C5 | P1C6 | P1C7; | P1C8 | P1C9 | P1C10 | P1C11 | P1C12 |
|-------|------|------|------|------|------|-------|------|------|-------|-------|-------|
| P2C1 | P2C2 | P2C3 | P2C4 | P2C5 | P2C6 | P2C7 | P2C8 | P2C9 | P2C10 | P2C11 | P2C12 |
| P3C1 | P3C2 | P3C3 | P3C4 | P3C5 | P3C6 | P3C7 | P3C8 | P3C9 | P3C10 | P3C11 | P3C12 |
| P4C1 | P4C2 | P4C3 | P4C4 | P4C5 | P4C6 | P4C7 | P4C8 | P4C9 | P4C10 | P4C11 | P4C12 |
| P5C1 | P5C2 | P5C3 | P5C4 | P5C5 | P5C6 | P5C7 | P5C8 | P5C9 | P5C10 | P5C11 | P5C12 |
| P6C1 | P6C2 | P6C3 | P6C4 | P6C5 | P6C6 | P6C7 | P6C8 | P6C9 | P6C10 | P6C11 | P6C12 |
| P7C1 | P7C2 | P7C3 | P7C4 | P7C5 | P7C6 | P7C7 | P7C8 | P7C9 | P7C10 | P7C11 | P7C12 |
| 1.1.1 | 1 | 4.5 | | | | | | | | | |

Allele 2/

| e 2/3 | P1C1 | P1C2 | P1C3 | P1C4 | P1C5 | P1C6 | P1C7 | P1C8 | P1C9 | P1C10 | P1C11 | P1C12 |
|-------|------|------|------|------|------|------|------|------|-----------|-------|-------|-------|
| ,- | P2C1 | P2C2 | P2C3 | P2C4 | P2C5 | P2C6 | P2C7 | P2C8 | P2C9 | P2C10 | P2C11 | P2C12 |
| | P3C1 | P3C2 | P3C3 | P3C4 | P3C5 | P3C6 | P3C7 | P3C8 | P3C9 | P3C10 | P3C11 | P3C12 |
| | P4C1 | P4C2 | P4C3 | P4C4 | P4C5 | P4C6 | P4C7 | P4C8 | P4C9 | P4C10 | P4C11 | P4C12 |
| | P5C1 | P5C2 | P5C3 | P5C4 | P5C5 | P5C6 | P5C7 | P5C8 | P5C9 | P5C10 | P5C11 | P5C12 |
| | P6C1 | P6C2 | P6C3 | P6C4 | P6C5 | P6C6 | P6C7 | P6C8 | P6C9 | P6C10 | P6C11 | P6C12 |
| | P7C1 | P7C2 | P7C3 | P7C4 | P7C5 | P7C6 | P7C7 | P7C8 | P7C9 | P7C10 | P7C11 | P7C12 |
| 1 | | | | | | | | | 1997 - C. | | | |

Allele 4

| e4 | P1C1 | P1C2 | P1C3 | P1C4 | P1C5 | P1C6 | P1C7 | P1C8 | P1C9 | P1C10 | P1C11 | P1C12 |
|----|------|------|------|------|------|------|------|------|------|-------|-------|-------|
| | P2C1 | P2C2 | P2C3 | P2C4 | P2C5 | P2C6 | P2C7 | P2C8 | P2C9 | P2C10 | P2C11 | P2C12 |
| 1 | P3C1 | P3C2 | P3C3 | P3C4 | P3C5 | P3C6 | P3C7 | P3C8 | P3C9 | P3C10 | P3C11 | P3C12 |
| | P4C1 | P4C2 | P4C3 | P4C4 | P4C5 | P4C6 | P4C7 | P4C8 | P4C9 | P4C10 | P4C11 | P4C12 |
|] | P5C1 | P5C2 | P5C3 | P5C4 | P5C5 | P5C6 | P5C7 | P5C8 | P5C9 | P5C10 | P5C11 | P5C12 |
| | P6C1 | P6C2 | P6C3 | P6C4 | P6C5 | P6C6 | P6C7 | P6C8 | P6C9 | P6C10 | P6C11 | P6C12 |
| | P7C1 | P7C2 | P7C3 | P7C4 | P7C5 | P7C6 | P7C7 | P7C8 | P7C9 | P7C10 | P7C11 | P7C12 |
| | | | | | | | | | | | | 5. |

Figure 3.13 Identification of the potential pool candidates for each allele with marker 44/45

Colored squares represent positive results. Saturated toned squares for allele 1 are the results with the highest confidence. For allele 2/3 and 4, all the results are supposedly reliable.

Step 2 – pools

12 super pools were chosen for allele 1 and allele 4, and we went back to the 7 pool plates. Each well in a pool plate corresponds to a BAC library plate.



Figure 3.14 Electrophoresis gel of the PCR products from the pool plates with the marker 44/45.

The three candidates that have been found are circled in yellow.

Three BAC plate candidates were then identified, 2 for allele 1, and 1 for allele 4, corresponding to plates 263, 663, 648.

| Address | allele | plate |
|---------|--------|-------|
| P3C11_F | 1 | 263 |
| P7C3_H | 1 | 663 |
| P7C12_F | 4 | 648 |

Table 3.4 Detail of the three candidates BAC plates identified for the marker 44/45.

A BAC colony with the 44/45 marker was already known for plate 648, so only plates 263 and 663 were further investigated.

Step 3 - Petri dishes

Plate 263 did not give any further amplification. However, for the plate 663, half row I-P was very clear. In plate 663, column 19, row I-P the band is likely to be a high molecular weight contamination.



Figure 3.15 Electrophoresis gel of the PCR products from the targeted half columns of the Petri dish with the marker 44/45.

Further investigation was made for both candidates. 663_19_I-P did not give any results. However, BAC 663_14_J was identified as carrying sequence for allele 4 at LOP.



Figure 3.16 Electrophoresis gel of the PCR products from the targeted half columns of the Petri dish with the marker 44/45.

In conclusion, two new BAC clones were identified by this work. One contained the Lsat46 marker in Allele 1 at the *LOP* locus and another contained the 44/45 marker in Allele 4 at the *LOP* locus.

Chapter 4 Mapping

4.1 Introduction

4.1.1 Deletion mapping

Several statements justify the choice of mutagenesis over classical genetic mapping techniques as a tool for mapping apomixis loci.

Genetic mapping relies on genetic polymorphism between individuals to map genes for phenotypic traits. However, apomictic loci are often found to be associated with highly repetitive chromosomal regions where recombination is suppressed (Grossniklaus et al., 2001; Ozias-Akins and Van Dijk, 2007; Hand et al., 2014). Genetic mapping is also not suitable for regions of low recombination rates ("recombination cold spots") because it wouldn't meet the adequate resolution needed for map-based cloning and the mapping of the present genes (Kumar and Jain, 2018). Indeed, "radiation hybrids usually have much more deletions (or breaks) per chromosome compared to the number of recombination events observed in genetic mapping populations" (Kumar et al., 2012 as cited in Kumar and Jain., 2018), which offers a better resolution.

Furthermore, the creation of a radiation-treated mapping population is in itself a quicker and cheaper process than a genetic mapping approach. For *P. praealta*, seeds of an apomictic plant were subjected to gamma irradiation using a ⁶⁰Co source. Regenerant plants that were found to be stable for a mutant phenotype were then tested for components of apomixis (Catanach et al. 2006, Bicknell et al., 2001). The most time-consuming process was the selection of the most informative lines.

The use of radiation can create a panel of deletion mutants with enough variability for mapping in a system without any prior genetic markers (Catanach et al., 2006). Moreover, "clonal reproduction makes apomicts very suitable to deletion mapping" (Van Dijk 2009), and apomictic traits have a dominant inheritance.

Previous studies showed that apomixis is controlled by two independent loci associated with apomeiosis and parthenogenesis in *P.praealta* (Catanach et al. 2006). The current study focused just on parthenogenesis and therefore the mapping of the *LOP* locus associated with parthenogenesis.

Pilosella apomicts are facultative apomicts. As sexual reproduction is not completely suppressed in *Pilosella*, a capacity to form a percentage of progeny via the sexual pathway remains.

"Off-types" coming from partial sex regularly occur in test populations and often show a different ploidy level than the apomict parents (Bicknell et al., 2003; Bicknell and Koltunow, 2004; Fehrer et al., 2007).

Facultative apomixis has also been revealed to exist in natural populations of the group of *Rosaceae* (Paule et al., 2011) and *Ranunculaceae* (Cosendai and Hörandl, 2010). There is a very tight link between mode of reproduction and ploidy (Mráz and Zdvořák, 2019). Checking the ploidy level of one plant is an easy way to get back to its reproduction mode.

4.1.2 Markers

Simple Sequence Repeats (SSR) and SCAR markers were chosen for this mapping studies. Simple-sequence repeats (SSRs) are short tandem repeated motifs for which the number of repeats at a given locus can vary (Tautz, 1989). Sequence Characterized Amplified Regions (SCARs) are markers designed from sequenced regions containing allelic polymorphisms.

SSR markers have many advantages over other molecular markers (Powell et al., 1996), they are:

- Genetically co-dominant (≠ presence/absence).
- Multi-allelic (discerning between the alleles at a locus)
- Abundant (there are a lot of repeated DNA sequences in apomixis-associated genomic regions)
- Widely dispersed across the genome
- High reproducible
- Easily and automatically scored

The main value of SCAR markers is that they are designed to analyze a specific location in the chromosome. They are therefore highly specific, but may not always be co-dominant.

4.2 Material and methods

4.2.1 Previous studies

Creation of the mutants collection (Catanach et al., 2006)

A collection of gamma-induced deletion mutants has been created as a mapping resource to identify markers associated with LOA and LOP loci for *Pilosella praealta*.

The experiment started with around 5,000 seeds coming from a single apomictic clone, treated by exposure to a ⁶⁰Co source. The seed was then sown, and any plants that lost their ability to reproduce by apomixis were selected. In the end, Catanach et al. developed a unique set of 79 viable *Pilosella praealta* apomixis mutants, including 24 with loss of apomeiosis, 30 with loss of parthenogenesis, and 25 that had lost both traits (Catanach., 2006). This provided an opportunity to establish the roles that these loci play in seed development and to evaluate interactions between apomixis and sexual reproduction.

The descriptive nomenclature for natural off-types has been used to characterize the mutants.



Figure 4.1 Possible pathways for the production of seed and its mutants forms.

(n+n): loa/lop: sexual reproduction (meiosis + fertilization), gives 2n = 4X
(2n+0): LOA/LOP: apomixis (avoidance of meiosis + avoidance of fertilization), gives 2n = 2X
(2n + n): loa/LOP mutants: fertilization of a 2n gamete with a reduced male gamete n, gives 2n = 6X
(n+0): LOA/lop mutants: formation of a seedling from a reduced, unfertilized (n) gamete, gives 2n = 2X

LOP mutants

In these mutants, the fertilization of an unreduced egg cell leads to an increase in ploidy (2n+n). As the mutant phenotype is 'Loss of parthenogenesis', the mutants were described as 'LOP mutants' and the locus as the '*P. praealta LOP* locus'. They still contain the LOA locus. In the current study, LOP mutants were used to detect mutation breakpoints near the LOP locus. All LOP mutants lack the LOP locus, so markers were used to test when the section of lost genomic DNA began.

LOA mutants

In these mutants, the parthenogenic development of a reduced egg cell leads to a reduction in ploidy (1n+0). As the mutant phenotype is 'Loss of apomeiosis', the mutants were described as 'LOA mutants' and the locus as the '*P. praealta LOA* locus'. They still contain the LOP locus.

Polyhaploids

Polyhaploids (see figure 17) are a useful resource to explore the LOP locus. As they are diploids, they contain only two copies of the LOP locus, which simplifies the mapping work. Unlike true apomixis that produces only clones, polyhaploids result from a meiotic process providing a valuable genomic diversity. Also, since fertilization doesn't take place in the development of polyhaploids, the meiosis only rearranges maternal alleles in these plants. The polyhaploids used were generated from an individual of *Pilosella piloselloides var praealta R35*, (commonly called R35) which was initially obtained from Dijon, France (Catanach et al., 2006). All polyhaploids have the LOP locus as it is necessary for their formation. Markers were used to detect and position recombination breakpoints flanking the LOP locus in R35.

AFLP markers

Amplified fragment length polymorphisms (AFLP) mapping was used in previous studies to distinguish the two loci implied in apomixis in *Pilosella praealta*. Comparisons of AFLP profiles identified clusters of markers linked to *LOA* and *LOP* loci that were present in the apomict but lost in the mutants (Catanach *et al.*, 2006).

4.2.2 SSR Marker design and PCR amplification

SSR markers were designed against the genomic reference sequence using Geneious to detect tandem repeats of di-nucleotides (AT, AG, CA, GT, CT, or CG). Primers were made to the conserved flanking sequence. The expectation was that each allele carries a different number of repeats, but flanked regions remained conserved enough to ensure the correct region was targeted. For each of the primers, the presence of polymorphism is assessed, as well as the position relative to the LOP locus (using the deletion mutants and polyhaploids).

The parameters for designing the primers were set as follows: primer length with ≈22 nucleotides, GC content of 40–60%, and optimum annealing temperature of at least 60°C. No self-dimerization. No off-target. amplification product size of:

1st set: 24 primers pairs producing fragments of 500-700bp

2nd set: 21 primers pair producing fragments of 250-400bp

3rd set: 10 primers pairs producing fragments of 200-400bp

4th set: 12 primers pairs producing fragments of 150 – 450 bp (not SSR)

SSR amplification was performed using 6 µL of Kapa 3g PCR mix, 0,9 µL of each primer (10 µM) (forward and reverse), 0,1 µL KAPA3G plant TAQ 0.25 unit, and 2 µL genomic DNA templates. PCR amplification was run using a T100 Thermal Cycler (BioRad, Hercules, California, USA) with the following program: 94°C for 3 min, followed by 45 cycles of 94°C for 30 s, then 30 s at the annealing temperature 60°C, 72°C for 1 min, and a final extension at 72°C for 30 min. PCR products were separated on 1% agarose gel and photographed under UV light. A fragment analyzer was then used to confirm SSR polymorphisms in amplified genomic DNA fragments and to precisely measure the size of the polymorphism.

1st set: 24 primers pairs producing fragments of 500-700bp

After a trial run of 24 pairs of SSR primers, 7 of them produced clear bands, stable amplification and potential polymorphisms for further analysis.

2nd set: 21 primers pair producing fragments of 250-400bp

It was realized that the target product's size was too big with the previous primers set to observe polymorphism in the fragment analyzer, so the size was reduced to 250-400 bp.

After a trial run, 11 primer pairs were selected.

3rd set: 10 primers pairs producing fragments of 200-400bp

Another approach has been used for this third set. All 10 of the primers were run through the fragment analyzer using a small testing set of templates. Only the ones showing clear differential pattern were selected for use with the extended template set.

4th set: 12 primers pairs producing fragments of 150 – 450 bp (not SSR)

As we approached the limit of the LOP locus, it became too difficult to design more SSR primers. The last primer set was developed using an automatic function of Geneious for designing primers within a constrained sequence range. A set of 12 of the primers was run through the fragment analyzer against a small testing set of templates. Only the ones showing a clear differential pattern were selected for use with the extended template set.

4.3 Results and discussion

Prior to this research, the region of the LOP locus was determined to be between the Lsat11 and 620T7 F1/R1 markers, an interval of 1.4 Mb. This estimate was based on marker positions against the SHASTA assembled Illumina sequence data. The positions of the markers are indicated in Table 4.1.

Table 4.1State of the mapping at LOP prior to this study. The markers are ordered according to their
position on the genome. The markers are ordered according to their position on the genome.



In green: expression of a marker for a particular individual at a precise position In red: absence of expression of a marker for a particular individual at a precise position As all polyploids arise from the action of LOP, they should all have a functional copy of this locus. The diagram only illustrates the polyhaploids found to have a recombinant breakpoint close to LOP. Over 2,000 polyhaploids were screened for recombination, so the seven listed polyhaploids all have breakpoints within 1cM of the locus. However, as recombination is suppressed in this region, a centiMorgan is still greater than 1Mbp at this locus.

One recombination breakpoint was determined to be between markers CDS23_32021 and 620T7 F1/R1 because one polyploid did not exhibit the 620T7 marker, but they all exhibited the CDS23_32021 marker. The CDS23_32021 and 620T7 F1/R1 markers are 95kb apart, indicating that the most proximal recombination breakpoint on this side of LOP lies in that interval. Consequently, most of the research effort was placed on determining the closest recombination breakpoint on the other side of LOP.

All of the deletion mutants had lost a large part of the LOP region, and they all exhibited a sexual-like phenotype. Some almost certainly had lost the entire locus for parthenogenesis, but LOP may also be a complex locus containing many genes, and some mutants may have just lost a critical part, but not all of it. g138, for example, has a breakpoint well inside of the interval found in all of the polyhaploids (Table 4.2), and it has a sexual phenotype. It is not clear if this means that the whole of LOP lies in the deleted region of g138 or that only a critical part of it does. As the deletion mutants have lost LOP, they were used as negative controls throughout the study.

The first set of primers tested gave two markers: 445_AG and 606_CT (see appendixes A.1 and A.2). 445_AG was clear enough but did not provide any more information than we already had from previous studies as it was very close to Lsat11. 606_CT was unclear, the PCR amplification failed for PH511 and there was a doubt for PH410.

The second set gave three markers: 754_AT; 907_CT; 1110_AG (see appendixes A.3, A.4 and A.5). 907_CT eliminated one of the polyploids (PH410) because it was only expressed in PH511 and PH544.

The third set gave four markers: 1117, 118R2, 1123, 1156 (see appendixes A.6; A.7; A.8 and A.9) and the final set gave two more markers: 1170 and 1267 (see appendixes A.10 and A.11).

After the data from the four primer sets was collated, it was found that the recombination breakpoint in the final polyhaploid (number 511) was positioned between markers 1267 and Lsat46 (Table 4.2). The interval between these markers is 70kb.

Table 4.2 Mapping of the LOP locus of Pilosella praealta

In green: presence of a marker for a particular individual at a precise position In red: absence of a marker for a particular individual at a precise position In yellow: markers developed in this study

| Markers-LOP | g179 | g138 | g115 | g136 | g116 | PH325 | PH191 | PH392 | PH410 | PH511 | PH544 | PH400 | PH 70 control | R35 wt |
|---------------------------------|----------|------|--------|------|-------------------|-------|-------|---------|-------|---------|-------|-------|------------------|--------|
| Phenotype | | | sexual | | | | | | a | pomicts | | | | |
| Lsat39 | + | + | + | + | 4 . 1 | + | | | | | | - | | + |
| Lsat01 | + | + | + | + | - | + | | + | | | | - | | + |
| marker 379 | + | + | + | + | - | + | + | + | - | - | | = | | + |
| F3 &R3 | + | + | + | + | - | + | + | + | - | - | - | - | | + |
| L sat10 | + | + | + | + | - | + | + | + | - | - | 10 | - | | + |
| I sat35 | + | + | + | + | | + | + | + ns | _ | - | | + | | + |
| Lsat04 | + | + | + | - | - | + | + | + | - | - | | + | | + |
| 445_AG | + | | + | 12 | | | | | 1.4 | - | - | | + | + |
| Lsat11 | + | + | + | - | | + | + | + | - | - | | + | | + |
| 606_CT | + | + | + | - | | | | | +? | F | _ | | + | + |
| 754_AT | + | + | + | | | | | | - | - | | | | + |
| 907_CT | + | + | + | | | + | | | + | - | | | | + |
| 1110_AG | - 1 | + | + | | | + | | | + | - | + | | | + |
| 1117 | | + | + | | | + | | | + | - | + | | + | + |
| 1118R2 | | + | + | - | | | | | + | - | + | | + | + |
| 1123 | - | + | + | | | + | | | + | - | + | | + | + |
| 1156 | | + | + | | | + | | | + | - | + | | + | + |
| 1170 | | | - | - | | + | | | + | - | + | | + | |
| 1267 | | | - | - | | + | | | + | - | + | | + | |
| Lsat46 F4R4 (position 1340) | | + | | - | - | + | + | + | + | + | + | + | | + |
| 1405_CT | - | | | - | - | + | + | + | + | + | + | + | | |
| 148_6FR | | + | | _ | - | + | | + | + | + | + | | | + |
| 44F & 45R | 1 | + | | | | + | + | + | + | + | + | | | + |
| 135 SP6 SSR1 F1R1 (BAC 135N06) | - | 19 C | - | | | + | + | + | | | | | | + |
| BAC135N06_Node11_F1R1 | - | 1 | - | - | - | + | + | + | | | | | | + |
| BAC335J15_Node12_F1R1 | <u>_</u> | - 92 | | | 14 | + | | + | 2 | | | | | + |
| CDS23 2332021 | 4 | | | | | + | + | + | + | + | | + | | + |
| 620 T7 F1/R1 | - | | - | - | | | + | + | | | | | + | + |
| 63_7FR | _ | - | | - | | _ | - | + | + | + | | | | + |
| Lsat43 | + | 24 | - 14 | 14 | - | 100 | | - | + | + | + | + | | + |
| F1R1 | + | | - | | - | - | - | - | + | + | | + | | + |
| gene B ssr1 F&R premix34 | + | 14 | - | 100 | 14 | 14 | - | - | + | + | | + | | + |

In conclusion, the mapping using recombination breakpoints in the polyhaploids set the estimated limits of the LOP locus between markers 620 T7 F1/R1 and 1267, an interval of 647kb. If the deletion breakpoint of g138 is taken into account, and assuming that LOP is a simple locus, this would imply that the locus lies between markers 620 T7 F1/R1 and 44F/45R, an interval of 527kb.



Figure 4.2 Detail of the markers and their position around the LOP locus

In brown: the deletion mutants In green: the polyploids

1.1.1.1 Cross between a P.praealta deletion mutant and P.aurantiaca

In a previous study (unpublished), a cross was made between a deletion mutant of *P.praealta* (g138) and an apomictic tetraploid of another species (*P.aurantiaca*). The purpose of this cross was to identify the dominant allele at *LOP* in *P.aurantiaca* as it was reasoned that all of the apomictic progeny should have this allele, and all the non-apomictic progeny should not have it. For this to work, a co-dominant marker was needed that could be used to identify the different alleles of *LOP* in this population. The SSR marker 1170, developed in this study, appears very valuable for this use because it is very clear, polymorphic, co-dominant, and it is close to the *LOP* locus (see Appendix A.10).



Figure 4.3 Detail of the fragment analyzer data for P.aurantiaca and the P.praealta deletion mutant g138 for the marker 1170 near the LOP locus.

The peak linked to the dominant allele at LOP (Allele 1) is circled in red. The allele numbering system was based on work in a previous study.

The peak representing the LOP allele for *P. aurantiaca* was found by process of elimination. This peak should be present in the data from all of the apomictic individuals and absent from the data from all of the nonapomictic individuals. Nine offspring from this cross were investigated (6 apomicts and 5 non-apomicts) using the fragment analyzer.



Figure 4.4 Fragment analyzer data for the apomictic offspring from a cross between the P. praealta deletion mutant g138 and P. aurantiaca, using the SSR marker 1170.

The dashed line represents the dominant allele at LOP (Allele 1). The allele numbering system was based on work in a previous study.



Figure 4.5 Fragment analyzer data for the non-apomictic offspring from a cross between the P. praealta deletion mutant g138 and P. aurantiaca, using the SSR marker 1170.

The dashed line represents the expected position of the dominant allele at LOP (Allele 1).

In conclusion, these results demonstrate that the 1170 marker is well suited for identifying the dominant allele at *LOP* in the species *P. aurantiaca*. It also confirms that the experimental phenotypic evaluations (apomict and non-apomict) were right as it brings genetic evidence to support the observed difference between the apomictic and non-apomictic plants in this population.

Chapter 5 Allelic diversity

5.1 Introduction

Apomixis can be seen as an aberrant way of reproduction in a world ruled by sexual reproduction. Apomixis is however known in over 400 taxa of angiosperms, many of which are very successful competitors in their habitats, so it is clear that apomixis must confer some ecological advantages for the trait to have persisted in so many taxa over evolution time. Sexual reproduction is particularly efficient in ensuring adaptability to rapidly changing environments thanks to the diversity created by recombination in each generation. Crow and Kimura (1965) noted that sexual reproduction would be beneficial over apomixis under certain conditions, such as a large population size subjected to a frequent occurrence of mutations. However, apomixis is a preferential mode of reproduction over sexual reproduction when the environment is stable (Tucker et al., 2009). Elite apomictic genotypes are cloned, and they multiply to settle down and often dominate in their environment. Where pollination is not required, this also provides a successful colonization strategy (Baker, 1959 ; Baker et Stebbins, 1965). This advantage also applies in environments where pollinators are scarce, such as mountains and high latitudes, and apomicts are particularly common in these places. The phenomenon of the unequal distribution of apomixis is called "geographical parthenogenesis" (Hörandl et al., 2008).

It has been described that asexual lineages accumulate deleterious mutations, and these can contribute to the ultimate extinction of a genotype (a phenomenon called 'Müller's ratchet') (Müller, 1932). However, residual sexuality typically persists in apomictic populations (Van Dijk, 2009) and this acts against the effects of Müller's ratchet. Sexuality occurs in the form of entirely sexual individuals within populations dominated by apomicts, and also as facultative apomixis where some of the seeds forming on a plant are sexually derived while others are produced by apomixis. Furthermore, most apomicts still produce reduced pollen even if fertilization is not required in the apomictic process. The result of all these mechanisms is that new genotypes do constantly arise in these populations to replace obsolete ones and some recombination does take place to ensure change over time.

According to the "Fisher-Muller" model, sexual reproduction is a paradox. It costs energy, and the beneficial traits it might create can be quickly lost through the generations due to a large amount of recombination. This disadvantage of sexual reproduction is usually referred to as the "cost of males" (Smith, 1971) or "the cost of meiosis" (Williams, 1975). Darlington (1939) proposed that apomixis would provide an escape from sterility, even though Darlington considered this would be "an escape into a blind alley of evolution".

It is now acknowledged that apomictic plants clearly have found a way to thrive probably because "apomixis preserves beneficial combinations of unlinked alleles in every generation for as long as apomictic genotypes persist in the population" (Sailer et al., 2020).

Allelic sequence divergence (ASD) is a phenomenon where alleles at a locus diverge in sequence over time. It occurs at most loci in plants that are open-pollinated because sexual reproduction homogenises allelic combinations within a population. It is believed to be repressed in apomictic plants as a result of repressed meiotic recombination (Corral et al., 2009). The existence of residual sexuality in apomicts, however, means this hypothesis is probably too simplistic because recombination is occasionally happening in these plants, as described above. That said, there are some parts of the genome of an apomict that are likely to be unaffected by residual sexuality, and they are the loci that control apomixis itself. These loci, which typically act in a dominant manner, must be intact for apomixis to occur. When they are inherited, the plant is an apomict and therefore reproduces clonally. If they are not inherited, or they are broken by recombination, then sexual reproduction (the recessive state) occurs. In apomictic populations, therefore, we predict that dominant alleles conferring apomixis will remain very similar (low levels of ASD) yet recessive alleles that confer sexuality will have higher levels of ASD. This hypothesis has never been tested before experimentally.

This chapter is about the sequencing of 34 individuals and different species of the *Asteraceae* family for three genes. One is part of the LOP locus, the three others being highly conserved genes placed near the LOP locus. The purpose of this study is to highlight the allelic diversity among the apomict and sexual species and discover how the alleles are distributed depending on their similarity from an evolutionary point of view. The technology used for the sequencing is the Nanopore MinION sequencer. The sequencing data were then used to build a tree.

5.1.1 Nanopore sequencing (MinION sequencer)



Figure 5.1 Nanopore MinION sequencer

Nanopore MinION is a pocket-size device commercialized by Oxford Nanopore Technologies. It is a long-read sequencing technology that allows a good contiguous assembly of a de novo genome. This technology can sequence long reads up to 100+ kilobase (Jain et al., 2018).

DNA molecules pass through a protein nanopore, leading to changes in the ionic current across a membrane (Bowden et al., 2019).

| Advantages | Disadvantages |
|---|--|
| Direct sequencing of nucleic acids allowing the detection of DNA modification Real-time data acquisition and analysis Ability to generate long reads | Requires quality DNA High cost Low throughput High insertion/deletion error rates associated with base-calling |
| $(T_{\rm rel})$ and $T_{\rm rel}$ | |

Table 5.1 Advantages and disadvantages of the use of Nanopore sequencer

(Tyler et al., 2018)

5.2 Material and methods

5.2.1 Plant material and DNA isolation

The plants used in the study are listed in Table 5.2. A total of 34 individuals were tested for each of the four genes. The ploidy of each plant was tested using flow cytometry to determine the number of alleles expected. Deletion mutants and segregants were used to determine which alleles represented the dominant allele associated with apomixis (designated allele 1). DNA was isolated using the method described in the General Materials and Methods section.

Table 5.2 List of the individuals sequenced with the abbreviation used in the rest of the thesis, their phenotype and ploidy.

| Name | Abreviation | phenotype | ploidy |
|--|-------------|-----------|--------|
| Pilosella piloselloides var praealta R35 | R35 | Apomictic | 4x |
| Pilosella aurantiaca | A-S | Apomictic | 4x |
| Pilosella onegensis | On | Sexual | 2x |
| Pilosella officinarum (Caen) | P-C | Sexual | 3x |
| Pilosella caespitosa (Tekapo) | C-T | Apomictic | 4x |
| Pilosella lactucella | Au | Apomictic | 5x |
| Hieracium policheae | Ро | Apomictic | 5x |
| Hieracium lepidulum | Le | Apomictic | 5x |
| Hieracium murorum | Mu | Apomictic | 5x |
| Pilosella cymosa | Су | Apomictic | 5x |
| Pilosella officinarum (Dijon) | P-D | Apomictic | 4x |
| Pilosella piloselloides D36 | D36 | Apomictic | 4x |
| Pilosella glacialis G45 | G45 | Apomictic | 5x |
| Pilosella LP | Pi-LP | Apomictic | 4x |
| Hieracium Lactucella | La | | 4x |
| Hieracium pelletarianum | L2 | Sexual | 2x |
| D2-10 | D2-10 | Apomictic | 2x |
| Taraxacum officinale | То | Apomictic | 3x |
| Hypochoeris radicata | Hr | Sexual | ? |
| <i>Pilosella glacialis</i> deletion mutant γ5 | G45-5 | Sexual | 5x |
| <i>Pilosella glacialis</i> deletion mutant γ57 | G45-57 | Sexual | 5x |
| <i>Pilosella glacialis</i> deletion mutant γ83 | G45-83 | Sexual | 5x |
| <i>Pilosella glacialis</i> deletion mutant γ110 | G45-110 | Sexual | 5x |
| Pilosella piloselloides var praealta | | | |
| deletion mutant | γ116 | Sexual | 4x |
| Hybrids (P.aurantiaca x P.piloselloides praealta γ138) | Аро 50 | Apomictic | 6x |
| Hybrid A-S x γ138 | Аро 72 | Apomictic | 6x |
| Hybrid A-S x γ138 | Аро 87 | Apomictic | 6x |
| Hybrid A-S x γ138 | Аро 67 | Apomictic | 6x |
| Hybrid A-S x γ138 | Аро 35 | Apomictic | 6x |
| Hybrid A-S x γ138 | Apo 81 | Apomictic | 6x |
| Hybrid A-S x γ138 | Sex 49 | Sexual | 6x |
| Hybrid A-S x γ138 | Sex 65 | Sexual | 6x |

The case of G45-110

G45-110 is a genetically transformed mutant that integrated a T-DNA containing elements of the Activator/Dissociator (Ac/Ds) transposons of maize. It had lost apomixis but continued to form seeds sexually. Indeed, aposporous initials were seen to begin differentiation but failed to divide. The meiotic apparatus in this plant continued to develop normally, and a compensatory increase in sexuality was observed (Ross Bicknell et al., 2001).

5.2.2 Candidate genes

Four genes were strategically chosen for amplicon sequence comparison according to their position compared to the LOP locus. One gene, *PpPAR*, can induce egg cell division without fertilization and was first described in Taraxacum (Underwood et al., 2020), which is also part of the Asteraceae family. It appears to be placed right in the middle of the LOP locus. It is believed to be involved in the expression of parthenogenesis. *Feronia* encodes a plasma-membrane-localized receptor kinase involved in growth and development. It maps to a locus approximately 4Mbp to one side of *PpPAR*. *Eif3e* encodes a highly conserved eukaryotic translation initiation factor, mapping to a locus approximately 4Mbp to the other side of *PpPAR*. The rRNA ITS sequence was used as a control for the taxonomic relationships between these plants. Ribosomal RNA genes are highly conserved and widely explored over species and the Asteraceae family. Lettuce is one of the rare Asteraceae that is also a crop, which makes it the member of this family that might be the most observed. The sequence used as a reference for this study was from *Lactuca seriola*, which is very close relative to *Lactuca sativa*; the synteny is high, especially for the ITS interval (around 100% similarities).

Although all four genes were amplified, barcoded and sequenced for all samples, only rRNA ITS and the PAR gene were analysed for ASD.

5.2.3 Primer selection

Several primers located around each target gene have been tested. The cheapest and quickest way to assess the validity of a primer in the first place is to run an electrophoresis gel. The first PCR was run according to protocol described in the next sub chapter with 40 cycles to ensure a clear amplification. The main criteria are:

- A clear and strong band without a smear to guarantee a clear result
- Consistency of the strength of the band throughout as many samples as possible

A first selection can then be made according to these criteria. The reduced set of primers is tested on a gel again with 25 cycles to verify that the PCR products are amplified. If the gel gives good results, the amplicons are then tested on a fragment analyzer to check their purity.

Eif3e

Primers for the *Eif3e* gene were developed in previous studies (McGee, 2013).

Feronia

Ten primers were tested for the *Feronia* gene with a test set of eight templates, including a positive control, negative control and six different species. Four primers were chosen for further investigation because they had clear bands, only one product or at least very distinct products. A new PCR was run with an extended test set of 24 DNA samples.

rRNA

Ribosomal primers have been widely studied, especially in *Arabidopsis* and are therefore recognized to be reliable primers. Five pairs of primers were chosen from the literature associated with their PCR protocol. They were directly run for an extended test set of 30 DNA samples, and all gave excellent results. The primer pair chosen was ITS5A/ITS4 (Downie and Katz-Downie, 1996; White et al., 1990)

PpPAR

Ten primers around the PpPAR gene generated using Geneious were tested with a test set of 5 DNA samples. Two primers were selected and tested with an extended set of 61 DNA templates. The ones that gave the strongest bands were chosen for the studies.

5.2.4 Amplicons barcoding

Sequencing is expensive, and it is common to limit the number of runs to limit costs. Barcodes, small specific sequences of around ten bases, are attached to each amplicon to facilitate multiplex sequencing of numerous samples and genes in the same run.

Barcode sequences are encoded at the termini of linear DNA through PCR protocols. The products are then pooled together for the sequencing. Each sample can then be de-multiplexed and sequences assigned to their original sample based on their barcode sequences.

For this study, 136 amplicons had to be tested (34 individuals/species tested for four genes). It was easy to identify each of the four genes because the primers used were different. However, it was necessary to be able to identify the 34 different plants used.

Instead of creating 136 barcodes for each gene + species pair, it was decided to develop 34 species barcodes and attach them to previously tagged primers (see figure 5.2). Three consecutive PCR reactions were set up to obtain amplicons with barcodes attached, resulting in a total of 408 reactions. The first PCR reaction amplified the selected genomic DNA region (not represented in the figure). The second PCR attach used tagged primers to add common tags to the amplicons. The last PCR attached the sample-specific barcodes using the tag sequence.



Figure 5.2 Detail of the PCR protocols to attach the customized barcodes to the tagged primers

PCR 1: Standard PCR to amplify a selected genomic region with the usual primers.

PCR 2: PCR with tagged primers. The primers were designed with an extra sequence, called a "tag" at their end that doesn't match the genomic DNA. As the PCR cycles progress, the amplicons formed are longer, and the Tag sequence is replicated in the complementary strand thanks to the free dNTPs. In the end, a new tagged DNA template is obtained to be used in the following PCR protocol. The same forward primer tags and reverse primer tags were used for all 4 reaction sets, but the gene-specific parts of the primers were different.

PCR 3: PCR with barcoded primers. The primers consist of the Tag + the species-specific barcode attached to it. The tag part of the barcoded primers matches the tag part of the tagged DNA's **complementary strand**. As the PCR cycles progress, the amplicons formed are longer, and the barcode sequence is replicated in the complementary strand thanks to the free dNTPs. In the end, a barcoded DNA sequence is obtained. All barcoded primers ended in a common 5bp sequence (GGTCA for forward primers and ACTGG for reverse primers). The common sequence allows for pore loading in Oxford Nanopore sequencing, which means the first 5bp are seldom correctly discerned.

Table 5.3 List of the customised barcoded primers

| | forward primer+tag | 5'-3 | reverse primer+tag | 3'-5' |
|---------|--------------------------|--|-------------------------|--|
| | ITS5A_F+tag | TTATTGCTTAAGAATACGCGTAGGGAAGGAGGAGGAGGAGGAGGAGGAGG | ITS4-R+tag | CGTATAGTTATTCGCCTCCTCCCTCACTTCTGTGCCTCGGTCT |
| | GeneX-2ndexon_F1+tag | TTATTGCTTAAGAATACGCGTAGACAGTATAACCTTCAGATGCTCAAT | GeneX-3rdexon_R1+tag | AACCAGGTCTTGTCTATCTTCGCCCTCACTTCTGTGCCTCGGTCT |
| | Feronia Consen F1+tag | TTATTGCTTAAGAATACGCGTAGCARCAMACSMTGGGTGATCRGTGG | Feronia L.s ExEx F4+tag | MSCSAGAACAGACTTACCCGCCCTCACTTCTGTGCCTCGGTCT |
| | ConsenHpPAR_F5+tag | TTATTGCTTAAGAATACGCGTAGGTTAAGATCCGGCACTTTAAACCG | ConsenHpPAR_R2+tag | CCAAAATTTGCAACCGTACTTTACCCCCTCACTTCTGTGCCTCGGTCT |
| | | | | |
| | | | | |
| species | species barcode | full forward primer 5'-3' | | full reverse primer 3'-5' |
| G45 | TTTGTTCGTTCGGCTCGCGTGA | GGTCATTTGTTCGTTCGGCTCGCGTGATTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTAGTGCGCTCGGCTTGCTT |
| G45-5 | TCTTTGGTTATCTAGCTGTATGA | GGTCATCTTTGGTTATCTAGCTGTATGATTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTAGTATGTCGATCTATTGGTTTCTACTGG |
| G45-32 | TGGCAGTGTATTGTTAGCTGGT | GGTCATGGCAGTGTATTGTTAGCTGGTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTGGTCGATTGTTATGTGACGGTACTGG |
| G45-45 | TCCATTACACTACCCTGCCTCT | GGTCATCCATTACACTACCCTGCCTCTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTCTCCGTCCCATCACATTACCTACTGG |
| G45-83 | AGAATTGTGGCTGGACATCTGT | GGTCAAGAATTGTGGCTGGACATCTGTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTGTCTACAGGTCGGTGTTAAGAACTGG |
| G45-110 | AGAATTGCGTTTGGACAATCAGT | GGTCAAGAATTGCGTTTGGACAATCAGTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTGACTAACAGGTTTGCGTTAAGAACTGG |
| R35 | CGCATCCCCTAGGGCATTGGTGT | GGTCACGCATCCCCTAGGGCATTGGTGTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTGTGGTTACGGGATCCCCTACGCACTGG |
| g116 | TCCCTGAGACCCTTTAACCTGTGA | GGTCATCCCTGAGACCCTTTAACCTGTGATTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTAGTGTCCAATTTCCCAGAGTCCCTACTGG |
| D36 | GCGACCCATACTTGGTTTCAG | GGTCAGCGACCCATACTTGGTTTCAGTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTGACTTTGGTTCATACCCAGCGACTGG |
| D-PH3 | TCAAGAGCAATAACGAAAAATGT | GGTCATCAAGAGCAATAACGAAAAATGTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTGTAAAAAGCAATAACGAGAACTACTGG |
| D2.10 | TGGCTCAGTTCAGCAGGAACAG | GGTCATGGCTCAGTTCAGCAGGAACAGTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTGACAAGGACGACTTGACTCGGTACTGG |
| C-T | CAGTGCAATGATATTGTCAAAGC | GGTCACAGTGCAATGATATTGTCAAAGCTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTCGAAACTGTTATAGTAACGTGACACTGG |
| A-S | CTAGACTGAAGCTCCTTGAGG | GGTCACTAGACTGAAGCTCCTTGAGGTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGAGTCTGGAGTTCCTCGAAGTCAGATCACTGG |
| Apo87 | TCGAGGAGCTCACAGTCT | GGTCATCGAGGAGCTCACAGTCTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTCTGACACTCGAGGAGCTACTGG |
| Apo81 | GCTGACTCCTAGTCCAGGGCTC | GGTCAGCTGACTCCTAGTCCAGGGCTCTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTCTCGGGACCTGATCCTCAGTCGACTGG |
| Apo35 | TAGCAGCACATAATGGTTTGTG | GGTCATAGCAGCACATAATGGTTTGTGTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTGTGTTTGGTAATACACGACGATACTGG |
| Apo67 | TCACAGTGAACCGGTCTCTTT | GGTCATCACAGTGAACCGGTCTCTTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTTTCTCTGGCCAAGTGACACTACTGG |
| Apo72 | CATTGCACTTGTCTCGGTCTGA | GGTCACATTGCACTTGTCTCGGTCTGATTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTAGTCTGGCTCTGTTCACGTTACACTGG |
| Apo50 | AACACACCTATTCAAGGATTCA | GGTCAAACACACCTATTCAAGGATTCATTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTACTTAGGAACTTATCCACACAAACTGG |
| Sex88 | CAGTGCAATAGTATTGTCAAAGC | GGTCACAGTGCAATAGTATTGTCAAAGCTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTCGAAACTGTTATGATAACGTGACACTGG |
| Sex65 | GCCCCTGGGCCTATCCTAGAA | GGTCAGCCCCTGGGCCTATCCTAGAATTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTAAGATCCTATCCGGGTCCCCGACTGG |
| Sex49 | TAGCAGCACGTAAATATTGGCG | GGTCATAGCAGCACGTAAATATTGGCGTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTGCGGTTATAAATGCACGACGATACTGG |
| Hr | TACGTCATCGTTGTCATCGTCA | GGTCATACGTCATCGTTGTCATCGTCATTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTACTGCTACTGTTGCTACTGCATACTGG |
| P-D | TCAGTGCATCACAGAACTTTGT | GGTCATCAGTGCATCACAGAACTTTGTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTGTTTCAAGACACTACGTGACTACTGG |
| P-C | TGAGGGGCAGAGAGCGAGACTTT | GGTCATGAGGGGCAGAGAGCGAGACTTTTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTTTTCAGAGCGAGAGACGGGGGAGTACTGG |
| Pi-LP | TGGCAGTGTCTTAGCTGGTTGT | GGTCATGGCAGTGTCTTAGCTGGTTGTTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTTGTTGGTCGATTCTGTGACGGTACTGG |
| Cy | CACCCGTAGAACCGACCTTGCG | GGTCACACCCGTAGAACCGACCTTGCGTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTGCGTTCCAGCCAAGATGCCCACACTGG |
| On | AGAGCTTAGCTGATTGGTGAAC | GGTCAAGAGCTTAGCTGATTGGTGAACTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTCAAGTGGTTAGTCGATTCGAGAACTGG |
| La | CTGGGAGAGGGTTGTTTACTCC | GGTCACTGGGAGAGGGTTGTTTACTCCTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTCCTCATTTGTTGGGAGAGGGGTCACTGG |
| L2 | CTGGGAGAAGGCTGTTTACTCT | GGTCACTGGGAGAAGGCTGTTTACTCTTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTTCTCATTTGTCGGAAGAGGGTCACTGG |
| Au | TGTAAACATCCTACACTCAGCT | GGTCATGTAAACATCCTACACTCAGCTTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTTCGACTCACATCCTACAAATGTACTGG |
| Mu | TCAGGCTCAGTCCCCTCCCGAT | GGTCATCAGGCTCAGTCCCCTCCCGATTTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTTAGCCCTCCCCTGACTCGGACTACTGG |
| Le | AGCAGCATTGTACAGGGCTATCA | GGTCAAGCAGCATTGTACAGGGCTATCATTATTGCTTAAGAATACGCGTAG | i | CCCTCACTTCTGTGCCTCGGTCTACTATCGGGACATGTTACGACGAACTGG |
| Po | AAGCCCTTACCCCAAAAAGTAT | GGTCAAAGCCCTTACCCCAAAAAGTATTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTATGAAAAACCCCCATTCCCGAAACTGG |
| То | TTATAAAGCAATGAGACTGATT | GGTCATTATAAAGCAATGAGACTGATTTTATTGCTTAAGAATACGCGTAG | | CCCTCACTTCTGTGCCTCGGTCTTTAGTCAGAGTAACGAAATATTACTGG |

PCR conditions for eif3e using KAPA2G Robust Hotstart Ready mix

The PCR started with 95°C denaturing for 3 minutes, followed by 25 cycles (reduced to 20 cycles for primer+Tag and primer+Barcodes) starting with 30 seconds at 95°C, 60°C for 30 seconds, followed by a 1 minute extension cycle at 72°C. The PCR ended with a 72°C for 3 minutes.

PCR conditions for rRNA using KAPA2G Robust Hotstart Ready mix

The PCR protocol was inspired by the protocol of Funk et al., (2012). It started with 95°C denaturing for 5 minutes, followed by a touchdown PCR starting with 45 seconds at 95°C, with a variable annealing temperature (60 - 55°C) for 1 minute, which decreased by 1 °C per cycle, followed by a 1 minute extension cycle at 72°C. The touchdown step was followed by 25 cycles (20 cycles for primer+Tag and primer+Barcodes) of 95°C for 45 seconds, 54°C for 1 minute, finishing with a 72°C extension cycle for 1 minute. The PCR ended with a 72°C for 7 minutes.

PCR conditions for Feronia using KAPA2G Robust Hotstart Ready mix

The PCR protocol started with 95°C denaturing for 5 minutes, followed by a touchdown PCR starting with 45 seconds at 95°C, with a variable annealing temperature (63 - 58°C) for 45 seconds, which decreased by 1 °C per cycle, followed by a 1 minute extension cycle at 72°C. The touchdown step was followed by 25 cycles (20 cycles for primer+Tag and primer+Barcodes) of 95°C for 45 seconds, 57°C for 45 seconds, finishing with a 72°C extension cycle for 1 minute. The PCR ended with a 72°C for 5 minutes.

PCR conditions for PpPAR

To improve the amplification of the long fragment associated with allele 1, the elongation time has been increased.

The PCRs for the first sequencing run were realized using KAPA2G Robust Hotstart Ready mix according to the following instructions:

The PCR protocol started with 95 °C denaturing for 5 minutes, followed by a touchdown PCR starting with 1 minute at 95 °C, with a variable annealing temperature (61 - 58 °C) for 1 minute, which decreased by 1 °C per cycle, followed by a 2 minutes extension cycle at 72 °C. The touchdown step was followed by 25 cycles (reduced to 20 cycles for primer+Tag and primer+Barcodes) of 95 °C for 1 minute, 57 °C for 30 seconds, finishing with a 72 °C extension cycle for 2 minutes. The PCR ended with a 72 °C for 10 minutes.

An additional step was added in the case of the *PpPAR* gene because of the presence of two fragments of very different sizes, representing different alleles.

The smallest fragment tended to be amplified more often than the longest one leading to a disequilibrium in the width of the bands on the electrophoresis gel. The long fragments were often lost over the PCRs.

To prevent that phenomenon, it was decided to band-stab the gel after the first step (technique according to Bjourson and Cooper., 1992) to isolate the amplicons of different lengths.

The PCRs for the second sequencing run were realized using a much more sensitive and efficient kit, RepliQa Hifi toughMix, according to the following instructions:

The PCR protocol started with 98°C denaturing for 5 minutes, followed by a touchdown PCR starting with 15 seconds at 95°C, with a variable annealing temperature (61 - 56°C) for 5 seconds, which decreased by 1 °C per cycle, followed by a 15 seconds extension cycle at 68°C. The touchdown step was followed by 25 cycles (20 cycles for primer+Tag and primer+Barcodes) of 98°C for 15 seconds, 55°C for 15 seconds, finishing with a 68°C extension cycle for 15 seconds. The PCR ended with a 68°C for 5 minutes.

5.2.5 Sequencing, sequence polishing and bioinformatics

The PCR products were quantified using an Invitrogen Qubit[™] 4 Fluorometer and adjusted to make one single tube with all the amplicons at the same concentration. They were then purified and concentrated to a final reaction volume of 50ul using Kapa beads.

After purification, 136 fmol samples of each library were sequenced on a MinION R9.4.1 flow cell according to the manufacturer's protocol.

The sequencer ran for 24h to reach the required quantity of data necessary to generate accurate *de novo* assemblies while at the same time maximizing the number of samples sequenced.

Data analysis

Following base-calling, the raw data was collated in the fastq format, a text document which, in addition to the fasta format, contains the sequence and reading quality of each nucleotide. All sequence reads were then categorized according to the species barcodes. The ordered dataset was then processed using a python script (called Adapter.py) within the software PORECHOP to remove the adapters and barcodes.

Further data analysis proceeded using Geneious. The cleaned data were ordered by species/variety, with four genes for each plant. Due to time constraints only two genes, *HpPAR* and rRNA, were analysed in this study.

Number of Sequences Sequence Length PAR Feronia eif3e PAR **r**RNA Long fragment

Sequence Lengths for 43,260 Nucleotide Sequences

Figure 5.3 Oxford Nanopore nucleotide sequences profile for P.cymosa showing the reads ordered by sequence length with the gene associated.

Isolation of PpPAR gene

For each variety, the reads were sorted by length to allow the selection of those with the correct fragment size. The dominant allele for PAR was expected to have an amplicon size of approximately 1890bp, and the recessive alleles were expected to have an amplicon size of approximately 620bp. Typically, more than 10,000 sequences were available for each plant/gene/allele size combination. The data were trimmed along the edges to focus on nucleotides sequences with the average expected size for each plant/gene/allele size combination.



Figure 5.4 PpPAR fragments for the dominant allele, trimmed to exclude sequences that were either very long or very short.



Figure 5.5 PpPAR fragments for the recessive alleles, trimmed to exclude sequences that were either very long or very short.

Mapping to reference

The reads were then aligned to the reference genome for the *Piloselloides praealta LOP* locus using the following instructions:

Sensitivity: Medium sensitivity/Fast

Fine tuning: Iterate up to five times (Recommended) Maps reads to the consensus from the previous iteration

This function only allows a few mismatches between the read and the reference. A mismatch is referred to in Geneious as a single nucleotide polymorphism (SNP). This detection of variations is taken as an advantage for the next step.

Finding Variations/SNPs:

SNPs are a valuable tool to assess allelic diversity. The genome from which the reads were sequenced differs from the reference genome to which the reads are compared.

SNPs are detected using the following instructions:

Minimum coverage: 60

<u>Minimum Variant Frequency</u>: 0.25 (A variation is not considered a consistent SNP if its frequency is under 25% of the total)

<u>Maximum Variant P-value</u>: 10^{-3} (0.1% to see variant by chance)



Figure 5.6 Example of the Geneious window for the detection of SNPs.

Each SNP was assigned a variant frequency which could be used to sort the data. The flow cytometry data previously indicated the ploidy for each species. If, for example, the species studied was a tetraploid, we expected to find four alleles. The valuable and believable SNPs then have frequencies of approximately 25%, 50% or 75%. SNPs with a low frequency are more likely to represent base-calling errors so were ignored. On the contrary, SNPs with a frequency of over 90% are likely to exist in all the alleles.

In the case of a tetraploid, the easiest way to proceed was first to target SNPs with a 50% frequency to separate the data in 2 sets and then the ones with a 50% frequency again for both groups. To do so, one SNP with the desired frequency was strategically chosen. 2 new files are created with each one of the two possibilities for this SNP (same as reference or variant), and the operation was repeated for each file.

A good way to be convinced of the sorting reliability was to choose a SNP that combined the best with other SNPs with the same frequency.

A few individuals were left out for the analysis, specifically the segregating population from the cross between a *P.praealta* deletion mutant and *P.aurantiaca*. This cross is of greatest value in assigning alleles associated with the dominant allele at LOP. For *PpPAR* this allele sequence was clear in the sequence data set due to the different amplicon sizes.

Generating consensus sequence :

Once the data was sorted into a number of files corresponding to the number of alleles of the species, a consensus sequence was generated for each allele in Geneious using the assembly function and the following instructions:

<u>Highest quality (60%)</u>: Bases matching at least 60% of total adjusted chromatogram quality (Recommended) <u>Assign Quality</u>: Total (Sum quality of contributing bases minus non-contributing bases Call Sanger heterozygotes >50%

5.2.6 Tree construction

Trimming of allele LOP

Before building the trees, the data from the *HpPAR* gene needed to be further adjusted. Since the amplified gene comprised of allelic fragments of different lengths, the dominant LOP allele sequence was cut to the same length as the recessive alleles.

To do so, the LOP allele of each species was aligned with one of the recessive alleles using the Pairwise Alignment (aligns a small number of similar length sequences such as genes) with the following instructions: <u>Global alignment type</u>: Global alignment with free end gaps

Cost Matrix: 65% similarity

This helped to find the right-sized motif in the paired file that could be found again in the original file. The allele LOP is then trimmed at each end of the motif.

Geneious Tree Builder

Three trees have been built for the studied genes, two trees for *HpPAR* and one for rRNA using the Tree Builder function of Geneious using the following instructions: <u>Alignment type</u>: Global alignment with free end gaps <u>Cost matrix</u>: 65% similarity <u>Genetic distance Model</u>: Tamura-Nei <u>Tree Buil Method</u>: Neighbor-joining <u>Outgroup</u>: No outgroup <u>Gap open penalty</u>: 12

Gap extension penalty: 3

Published sequences from additional species from the *Asteraceae* family were looked for to improve the tree. Indeed, the entire genome of some crops from the *Asteraceae* family has been sequenced without all the functions associated with their genome being known.

A BLASTN search was made against the LOP allele of the *PpPAR* gene to find other *Asteraceae* with the same pattern.

Two species partially matched the sequence: *Helianthus annuus* (for a putative gene coding E3 ubiquitinprotein ligase LIN-1) and *Lactuca sativa* (for an uncharacterized gene called LSAT_8X112340).

The FASTA files were downloaded and aligned against the genomic reference with 65% similarity. The region of interest was located and copied. The copied motif was found on the original file and chopped at the right size to be later integrated into the tree building with the following change:

Cost matrix: 51% similarity

At 65% similarity, it was impossible to compute the genetic distance for these sequences because at least one pair of sequences (Ongense_3 and *Lactuca sativa*) did not overlap (or had only ambiguities in common) in the alignment.

The three trees were presented with the shape that was considered to be the most indicative. For clarity, the LOP allele was called 1 in all cases and the recessive alleles were annotated from 2 to 6.

5.3 Results/discussion

5.3.1 PpPAR

Primer selection and amplification

The electrophoresis gel of the PpPAR gene revealed the presence of two sizes of amplicons of respectively 600 bp and 1.8kbp.



Figure 5.7 Electrophoresis gel of six samples for PpPAR gene shows two sizes of amplicons.

For y136 and P. onegensis, only one PCR product was amplified. For R35, G45 and Pilosella officinarum two sizes of PCR products were amplified.

The gel shows one band corresponding to a small fragment around 600bp for every species and a longer fragment of around 1.8kbp for only some of them.

It has been observed that the presence of this additional long fragment matches with the apomictic phenotype.

Indeed, sexual plants possess only copies of the short fragment, while apomictic plants possess at least one copy of the longer fragment. It is now known that a type II transposon is placed in front of the PAR gene in the case of the dominant allele at LOP and appears to have a role in parthenogenesis.



Figure 5.8 Illustration of the PpPAR gene in Pilosella piloselloides var praealta (4x).

It is present in 4 identical copies in the tetraploid sexual plants. In apomictic plants, one copy is present in a longer fragment corresponding to the gene preceded by a transposon.

Theory of epigenetic regulation

Transposons are DNA sequences that can "jump" from one place to another in the genome, and by doing so, they can modify the expression of a gene. For a long time, they were considered as "junk DNA", but their influence on the genome has probably played a significant role in the evolution (Muñoz-López and García-Pérez, 2010).

"In higher plants, the activation or movement of transposable elements (TEs) covers a significant proportion of the genome, influencing the evolution of the genome, alternation in gene expression, and frameshift mutations" (Fiaz et al., 2021). The role of retrotransposons in apomictic development has also been proposed. A retrotransposon in *Cenchrus ciliaris* was shown to have differential activity in apomictic and sexual plants (Rathore et al., 2020).

Characterization and role of PpPAR transposon

Inverted repeat sequences can be found in this transposon sequence, which would tend to confirm that this transposon belongs to the type II class of transposons as this class is characterized by a transposase gene flanked by Terminal Inverted Repeats. The repeat sequences at *PpPAR* indicate that this transposon forms part of the hAT transposon superfamily. This superfamily is widely distributed among eukaryotic kingdoms and is mainly found in genomic fragments. This is an ancient family of transposable elements that probably appeared at the early stages of plant-animal-fungi separation (Rubin et al., 2001).

Data analysis of the PpPAR gene

The sequencing of *PpPAR* gave between 20,000 and 40,000 reads per amplicon. The analysis of the sequencing data showed that there was indeed only one copy of the longer fragment, representing the dominant allele (Allele 1).

The analysis of a mutant form of G45 (G45-110) indicated only three allele could be determined yet the wild type apomict G45 had 4 alleles determined. The missing allele in G45-110 was the dominant Allele 1, which is consistent with this mutant lacking this sequence and therefore being sexual. The dominant allele was also missing in a deletion mutant of G45 that is also sexual (G45-5). One surprising result was that G45 was recorded as a pentaploid using flow cytometry but only four allele sequences were found. We suspect that one recessive allele exists in 2 identical copies in this plant.

The number of reads for G45-45 and G45-83 were too low (only 5 for G45-45 and 16 reads for G45-83) to reach any conclusions about these plants. A similar problem occurred with *Pilosella caespitosa* (C-T) where only 4 reads were recorded.

For the species *Hieracium lepidulum (Le), Hieracium policheae (Po), Hieracium murorum (Mu)* only four alleles at PAR were discovered yet they were all were recorded as being pentaploids by flow cytometry. Interestingly, although they are all apomicts no allele was found that had a transposon in the promoter of the PAR gene. These species all belong to the genus *Hieracium*, a sister genus to *Pilosella*. The conspicuous lack of sequence for one allele at *PAR* may mean that the primers used (which were designed to *Pilosella* sequence) may not have amplified the dominant allele in these plants. More work is needed to test this possibility.

Hieracium pelletarianum (L2) is a sexual diploid, but no distinct polymorphism could be observed, indicating that either only a single allele amplified or that both the alleles present were the same in sequence. *Taraxacum officinale* (To) and *Hypochoeris radicata* (Hr) did not give any consistent data for HpPAR. The hypothesis is that the PCR did not amplify well enough in these plants with the primers used.
Tree construction for the PpPAR gene



Figure 5.9 Cladistic tree of PpPAR in 16 species using a circular graphic.

The evolutionary tree showed five distinct clusters. The LOP allele (allele 1) of the different plants fall together in a single clade while the other alleles at PpPAR are mixed between the different clades. This supports the hypothesis that dominant alleles at LOP have a common ancestry, and they are less prone to ASD than the other alleles at the locus as they never exist in sexual biotypes. All known dominant copies of PAR are numbered '1'. All other copies are randomly assigned numbers greater than 1.



Here is another representation including two species of the same family, *Lactuca sativa* and *Helianthus annuus* which emphasizes how close some species are to each other compared to these two distant relatives.

Figure 5.10 Cladistic tree of PpPAR in 16 species using a straight-line graphic and rooted with two related species (*Lactuca sativa* and *Helianthus annuus*).

As expected the more distantly related species *Lactuca sativa* and *Helianthus annuus* fall apart from the other *Pilosella* and *Hieracium*. This representation shows that the species from the genus *Hieracium* (*Hieracium policheae* (Po), *Hieracium Lepidulum* (Le) and *Hieracium Murorum* (Mu)) cluster together (in green), separated from the plants in genus *Pilosella*. *Lactuca sativa* and *Helianthus annuus* fall apart the groups.

5.3.2 rRNA

Primer selection and amplification

The amplification for the ribosomal gene was clear for all of the species and most of the primer sets. The primers ITS5A and ITS4 were chosen for further use.



Figure 5.11 Electrophoresis gel for the rRNA gene with primers ITS5A/ITS4 x 30 DNA templates.

Only one band was expected, but this band is probably broadened due to a poor dye.

Data analysis of the rRNA gene

The sequencing of the rRNA ITS amplicons typically gave around 20,000 per amplicon. The analysis was quite easy and quick as there was one one allele for each species. This was expected as rRNA genes are typically very similar in species and it was confirmed by the data where SNPs gave mostly variant frequencies higher than 90%. The genome reference used in this study was that of *Lactuca serriola* in which rRNA ITS sequence is only present in one copy.

Tree construction for the rRNA gene

The tree for rRNA, which is a highly conserved gene, gives an indication of the natural branching of the different species.



Figure 5.12 Phylogenetic tree for rRNA x 17 species.

It was interesting to see that *Hieracium policheae* (Po), *Hieracium Lepidulum* (Le) and *Hieracium Murorum* (Mu) separated out. They are all close relatives so, unsurprisingly segregate together, but they also separated from Pilosella species, which confirms the clear observations made with the *PAR* gene.

The *Pilosella* sequences are separated into three clusters:

The plants *Pilosella officinarum*: *Pilosella officinarum (Caen)* (P-C), *Pilosella officinarum (Dijon)* (P-D) and *Pilosella Lewis Pass* (Pi-LP) were predictably in the same cluster because they are from the same species although they are geographically very different. However, the presence of *Hieracium pelletarianum* (L2) and *Hieracium lactucella* (La) in the same group is surprising.

This group also appeared in the *PAR* tree with only one difference: both *PpPAR* alleles of *Pilosella lactucella* (Au) fall in this group.

Pilosella onegensis (On) and *Pilosella lactucella* (Au) fall together but are actually not that far from the previously described cluster.

Pilosella piloselloides var praealta R35 (R35), *Pilosella piloselloides D36 (D36), D2-10* (D2-10), *Pilosella aurantiaca* (A-S), *Pilosella cymosa* (Cy) form a third group. In the PpPAR tree, they were broken down into two groups together with *Pilosella onegensis* (On).

Hypochoeris radicata (Hr) and *Taraxacum officinale* (To) are species apart as expected as they are only distantly related.

Note that since the rRNA genes keep consistent in plant species, ploidy level doesn't play any role in the positions of the different species in the tree. For example, *Pilosella onegensis* (diploid) and *Pilosella lactucella* (pentaploid) are closely clustered.

5.3.3 General comments

Additional experiments would be required to extend this phylogenetic tree model.

The entirety of the G45 mutants, *Hieracium lepidulum (Le), Hieracium policheae (Po)* and *Hieracium murorum (Mu)* need to be tested for PAR again, because we missed the LOP fragment for several of them. I would recommend using the Hifi enzyme for the PCR that is more precise and then trying to spot the band on the gel. If it is identifiable, a sample of amplicons could be extracted by band-stabbing and amplified by themselves by PCR. If the band is not identifiable, extraction of the appropriate part of the gel and the purification of the amplicons is worth considering.

Pilosella caespitosa (C-T), *Hieracium pelletarianum* (L2), *Taraxacum officinale* (To) and *Hypochoeris radicata* (Hr) need to be tested again for the four genes because the number of reads given by the sequencer was insufficient.

Finally further studies of the two other genes *Feronia* and *eif3e* should also bring more consistency to the tree model.

Chapter 6 General Discussion

The discovery of apomixis and perspectives

It has been a long time since John Smith first observed apomixis in 1841. However, the study of apomixis has lagged behind sexual reproduction because few major crops use it. Also, breeders have long shunned apomixis because the production of clones leads to a lack of genetic diversity, and that makes the job of plant improvement harder. However, the study of apomixis has increased in recent decades with the potential application of conditional apomixis to improve the plant breeding process. Apomixis brings promising perspectives in plant breeding and it carries the hope of food security in a context of global warming where it is more than ever necessary to accelerate the production of adapted varieties. The introgression of apomixis could make it possible to create high performance, true-breeding hybrid lines in a single generation. This would save considerable time. The absence of recombination would make it possible to fix the traits without losing the hybrid vigor, so apomixis would also limit the costs of maintaining parental lines and avoid the need for male sterility.

Evolutionary biology of apomixis

Understanding the molecular mechanisms of apomixis will assist our understanding of the evolutionary consequences of this trait. It has long been noted that, in plants and animals, sexual and asexual biotypes tend to have different ranges. This phenomenon is called "geographic parthenogenesis" (Vandel, 1928). Stebbins (1950) noted that autonomous reproductive systems seem to be preferentially present in unfavorable climates and/or where unreliable pollinator services are available. The tendency of *Pilosella* to use apomixis when the population is isolated or in a colonization mode was first reported by Gadella (1991). Similarly, it has been shown that apomictic species are more strongly represented in stable environments, for example, at high altitudes with slight environmental variation. Under these conditions, sexual reproduction represents an unnecessary energy cost and is often a slower process for producing seeds than cloning.

In the mid-1970s, evolutionary biologists began to study the paradox of sex (van Dijk and van Damme, 2000). Indeed, sexual reproduction, although being the most widespread mode of reproduction and considered the most efficient, contains disadvantages such as the cost of maintaining the males. From this point of view, apomixis might appear to be a much simpler process since it often involves only one parent. As genetic tools have improved, more and more new wild species have been identified that use apomixis. More than 400 apomictic species are now known, and this is clearly an underestimate of its distribution. It is now clear that apomixis has evolved independently in different clades of flowering plants (Carman 1997; Whitton et al. 2008). This represents a case of Convergent evolution, the appearance of a trait in different species that were not inherited from a common ancestor. Typically, convergent evolution results from similar, optimized adaptive responses in different taxa to a shared selection pressure. Again, this strongly suggests that apomixis offers advantages over sexual reproduction in some environments.

Origins and transmission of apomixis

Carman showed the existence of a strong correlation between gametophytic apomixis and polyploidy in more than 126 genera (Carman 1997). Polyploidy can lead to genome disruption and it is therefore a significant mechanism driving speciation among angiosperms. Odd ploidy levels (e.g., triploidy, pentaploidy) have been reported to be linked with the presence of apomixis, although tetraploidy is the most common ploidy level among apomicts (Asker and Jerling 1992). It is commonly agreed that apomixis derives from sexual reproduction by disrupting the developmental pathways of seed formation. However, apomixis can originate from mutations or from hybridization between divergent sexual species.

Carman (1997) put forward an hypothesis that climatic fluctuations of the Pleistocene could have modified the areas of distribution of plants and this brought together different ecotypes which then hybridized. Ecotypes with putatively-precocious embryo sac development would have had the opportunity to form polyploids with ecotypes with putatively-delayed embryo sac development. Asynchronous female development in these polyploids would then have resulted in an efficient apomixis system.

Another model of how apomixis might arise was postulated by Nogler (1994). He noted that a failure in meiosis would result in a difference in ploidy in the offspring through the action of unreduced gametes. A mutation implying a non-disjunction of the germline chromosomes in meiosis would create diploid gametes with 2n chromosomes (instead of n). After fusion between two such gametes, we will obtain a 4n zygote.

Another mutation would then be needed in the parthenogenesis locus because the avoidance of meiosis in itself wouldn't produce a viable product. Indeed, the individual would otherwise 'polyploidize itself out of existence' due to the exponential increase of ploidy level with the fertilization of each generation" (Stebbins, 1950).

Apomixis in P. piloselloides var. Praealta

P.piloselloides var. Praealta is a facultative apomict that uses apospory, one of the two gametophytic apomixis pathways. In gametophytic apomixis, the embryo is formed from an embryo sac-like structure that didn't undergo meiosis and is not fertilized. Two dominant loci control apomixis in *P.piloselloides var. Praealta*. The *LOSS OF APOMEIOSIS (LOA)* locus is involved in creating an embryo sac derived from a somatic cell of the ovule without meiosis. The *LOSS OF PARTHENOGENESIS (LOP)* locus is involved in the spontaneous formation of an embryo from the embryo sac without fertilization.

Before this study, previous work in Ross Bicknell's team (unpublished) estimated the size of the *LOP* locus in *P.piloselloides* to be 1.4Mb. This study made it possible to reduce the known sequence of the *LOP* locus in this plant to an interval of 647kb. No recombination event was observed within this interval in a sample of 2,000 recombinant plants, meaning the resolution of mapping was less than 0.2cM. This work, therefore, refines the search area for genes involved in parthenogenesis and it more precisely defines the exact location of the *LOP* locus in the genome. In addition, the isolation of BACs within the *LOP* locus provided a tool for checking the accuracy of the haplotype-phased genome assembly. It is indeed essential for future studies, to work on a sequence that is as close as possible to the real one.

PAR is a gene placed in the middle of the *LOP* locus. Discovered in *Taraxacum*, it turned out to be also present in *P.piloselloides*. As in *Taraxacum* the dominant allele is recognizable by an additional sequence upstream of the gene, corresponding to an ancient transposon. The transposon was present in all of the apomictic plants studied in the current study and absent from all sexual plants studied, without exception. This includes mutants of wild type apomicts that were converted to sexual phenotypes. Now that the sequence of PAR is known, it would be interesting to integrate this transposon upstream of a recessive gene in a sexual plant to check if apomixis is induced. This operation can be done by using *Agrobacterium tumefaciens* that has shown to be effective in *P.piloselloides*.

At least six other genes remain to be studied in the *LOP* locus of *P.piloselloides*. The genes can be deduced thanks to recognizable Open Reading Frames (ORF) patterns and from mapped transcript reads. A transcript database is available for this plant to progress this work (Bräuning et al. 2018). A panel of transgenic *P. piloselloides* could be created using *Agrobacterium*-mediated techniques, and screened using Green Fluorescent Protein (GFP) as a marker. By combining the GFP marker with promoter elements of the identified genes at *LOP*, the expression of those genes could be inferred by the patterns of GFP fluorescence.

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In particular, GFP monitoring provides knowledge of the tissue, timing and subcellular localization of protein products and therefore gives an indication of the function of a gene. *Agrobacterium tumefaciens* could also be used to knock out candidate genes at LOP using gene editing and the role of that gene on embryo development could then be assessed.

A recent study showed the efficiency of CRISPR/Cas9 gene editing in *P. piloselloides*, which would facilitate this work (Henderson et al., 2020).

Allelic variability at PpPAR

When part of a population separates to colonize another environment, it only includes a sample from the pool of alleles available in the mother population. This is called the founder effect. These populations contain less genetic diversity and are more sensitive to genetic drift. Apomicts are often good colonizers as they are less dependent on compatible partners and fertilizing insects, so the founder effect is expected to be particularly common amongst these plants. This is partly why some authors have described apomixis as an evolutionary dead-end, because successive founder events will lead to genetic impoverishment (Darlington 1939). The lack of recombination in apomictic plants also prevents them from getting rid of deleterious mutations that accumulate in a lineage, a mechanism known as Muller's ratchet (Muller, 1932). This is also expected to reduce the viability of apomictic lineages over time. However, apomixis, far from being an evolutionary dead-end, seems to have a persistence that defies the laws of nature. One reason for this may be that, although individual lineages (genotypes) may degrade due to accumulated deleterious mutations, apomicts typically exist in populations of many clones, so as one genotype becomes obsolete, new ones emerge to replace it. Another result of the suppression of recombination around apomixis loci is that these loci are often regions rich in repeat sequences (Kotani et al., 2014; Conner et al., 2015). This can then lead to hemizygosity and further compound the reproductive isolation of apomicts. The repeatrich nature of apomixis loci was confirmed in this study at LOP by the mapping work. It made it difficult at times to design primers that were specific to the sequence.

In *P. piloselloides* apomixis and sexual reproduction are not entirely independent pathways. Apomixis in aposporous *Pilosella* (formerly *Hieracium*) seems to be superimposed on the sexual path, where apomixis redirects the fate of cells with gametic potential. The continued existence of sexual reproduction in *Pilosella* populations means that genetic exchange is still occurring and that cross-over between alleles will influence the patterns of allelic variation in the population (Hand and Koltunow, 2014). This effect, however, is not likely to occur between dominant alleles for LOP as they lie in a region of non-recombination (Chapter 4).

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Consequently, we hypothesized that allelic variation would be greater in sexual alleles than in dominant alleles at *LOP*. In this study and for the first time in the literature, it was shown that dominant alleles of the *PAR* gene in eight species the genus *Pilosella* were almost identical, while the recessive alleles at the same gene were more variable amongst and within species. It was also confirmed that, despite the accumulation of mutations, the dominant *PAR* allele at *LOP* remained highly conserved, presumably because they are under strong selection.

In conclusion, the primary findings of this study were:

- Parthenogenesis *P.piloselloides var. Praealta* is controlled by the locus *LOP*, which is a region of suppressed recombination with a high level of repeat sequence.
- The LOP locus mapped to a 647kb interval in this species.
- A precise sequence for two alleles at the *LOP* locus was checked using BACs isolates
- The study of allelic diversity for the *PAR* gene within the *LOP* locus showed a high similarity of the dominant alleles among species for this gene and greater variability amongst recessive alleles.

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Appendix A

Fragment analyzer data of the markers identified in the mapping study

Viewed on the GeneMarker software



A.1 Marker 445_AG

The LOP allele and another recessive allele are on top of each other. When only two peaks are visible, the LOP allele is absent.

A.2 Marker 606_CT





A.5 Marker 1110_AG

A.6 Marker 1117

95

A.8 Marker 1123 LOP Allele 4 Allele 2/3 Allele MG_049_230920_1123_PH70_D04.fsa 390 398 400 404 406 386 388 392 394 396 402 PH70 10,000 Positive control 5.000 LOP + allele 2 or 3 0 394.9 397.3 398.3 MG_049_230920_1123_g179_C03.fsa 386 388 390 392 394 396 398 400 402 404 406 10,000 g179 5,000 Negative control 0 392.9 396.7 397.9 MG_049_230920_1123_g115_C04.fsa 394.5/2595 × 390 398 400 402 406 386 388 392 394 396 404 10,000 g115 5,000 0 393.0 395.0 397.3 MG_049_230920_1123_g138_D03.fsa 394.8/-2682 × 388 390 392 398 400 402 394 396 404 406 386 10,000 g138 5,000 0 392.9 394.8 396.6 398.0 MG_049_230920_1123_PH325_A03.fsa × 390 388 392 394 396 398 400 402 404 406 386 10.000 PH325 5,000 0 394.8 397.1 MG_049_230920_1123_PH410_B04.fsa 391.4/7483 × 386 388 390 392 394 396 398 400 402 404 406 10,000 PH410 5,000 0 393.0 395.0 397.0 MG_049_230920_1123_PH511_B03.fsa 388 390 392 394 396 398 400 402 404 406 386 10.000 PH511 5,000 392.7 397.3 MG_049_230920_1123_PH544_A04.fsa 390.6/10729 ×

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A.9 Marker 1156

A.10 Marker 1170

A.11 Marker 1267

Appendix B

Detail of the SNPs for each allele of the HpPAR gene for each species

Each decision step is presented, the final alleles are framed in a darker line.

| Hieracium lepidulum | 5 alleles | | | | | | | | | | |
|--------------------------------|----------------------------|------------------|--------------|--------------|----------------|----------------|-------------|---------|------------------|----------------|-----|
| DOMINANT ALLELE | missing (PCR didn't pic | ck it up) | | | | | | | | | |
| RECESSIVE ALLELES | 4 alleles | | | | | | | | | | |
| | | | | | | | | | | | |
| - 179 AG (55%) | 1 - 11-1- | | | | | | | | | | |
| - 507 - (43%) - 507 C (57%) | 1 allele | | | | | | - | | | | |
| - 507 G (57%) | Tailcie | | | Insertio | n of 9 nucleot | tides, | |) | | | |
| - 507 - (43%) | 1 allele | | | more ris | sk to have ran | dom errors | 1 2001 | | | | |
| - 507 G (57%) | 1 allele | | 6 | Which | explains the v | ariant frequen | icy of 70% | This to | as not taken ir | to | |
| | | | | (should | be around 10 | 0%) | | accou | nt in the rest o | f the explanat | ion |
| | | | | | | | | | | | |
| | | | | - | -> Part of t | he 9 nucleotid | les pattern | | | | |
| CNIDa | | | - | 4 | | | - | | | | |
| Name | AG | | AGGCGGAGT | GT | | | | | | | |
| Type | Polymorphism | | Polymorphism | Polymorphisr | Polymorphis | m | | | | | |
| Minimum | 179 | | 412 | 412 | 507 | 1 | | | | | |
| Maximum | 180 | | 411 | 411 | 507 | | | | | | |
| Change | GA -> AG | 1 | +AGGCGGAG1 | +GT | (G)3 -> (G)2 | 2 | | | | | |
| Polymorphism Type | Substitution | | Insertion | Insertion | Deletion | | | | | | |
| Variant P-Value (approximate) | 55.0% | | 70.1% | 25.9% | 41.8% | | | | | | |
| vanant P value (approximate) | 0 | | v | 0 | 2.46-200 | | | | | | |
| | | | | | | | | | | | |
| Le 179AG (55%) | | | | | | | | | | | |
| Name | AG | | Polymorphic | | | | | | | | |
| Minimum | 170 | | 507 | | | | | | | | |
| Maximum | 180 | | 507 | | | | | | | | |
| Change | GA -> AG | | (G)3 -> (G)2 | | | | | | | | |
| Polymorphism Type | Substitution | | Deletion | | | | | | | | |
| Variant Frequency | 97.8% | | 43.2% | | | | | | | | |
| Variant P-Value (approximate) | 0 | | 1.3E-128 | | | | | | | | |
| Le 179AG (55%) + 507- (43%) | Q | | | | | | | | | | |
| Name | AG | | anna - mar | | | | | | | | |
| Туре | Polymorphism | 1 | Polymorphism | | | | | | | | |
| Minimum | 179 | | 507 | | | | | | | | |
| Change | GA .> AG | - | (G)3 .> (G)2 | | | | | | | | |
| Polymorphism Type | Substitution | - | Deletion | | | | | | | | |
| Variant Frequency | 97.6% | | 100.0% | | | | | | | | |
| Variant P-Value (approximate) | 0 | | 0 | | | | | | | | |
| Le 179AG (55%) + 507G (57%) |) | | | | | | | | | | |
| Name | AG | | | | | | | | | | |
| Туре | Polymorphism | | | | | | | | | | |
| Minimum | 179 | | | | | | | | | | |
| Maximum | 180 | | | | | | | | | | |
| Change Polymorphism Type | GA -> AG | | | | | | | | | | |
| Variant Frequency | 98.0% | | | | | | | | | | |
| Variant P-Value (approximate) | 0 | | | | | | | | | | |
| | | | | | | | | | | | |
| Le 179GG (45%) | | | | | | | | | | | |
| Name | | G | | | | | | | | | |
| Type | | Polymorphism | Polymorphism | | | | | | | | |
| Maximum | | 180 | 507 | | | | | | | | |
| Change | | A -> G | (G)3 -> (G)2 | | | | | | | | |
| Polymorphism Type | | SNP (transition) | Deletion | | | | | | | | |
| Variant Frequency | | 97.2% | 41.0% | | | | | | | | |
| Variant P-Value (approximate) | | 0 | 8.5E-84 | | | | | | | | |
| 1 a 17000 (AEN) + FAT (1994) | | | | | | | | | | | |
| Le 1/9GG (45%) + 507- (43%) | | G | | | | | | | | | |
| Type | | Polymorphism | Polymorphism | | | | | | | | |
| Minimum | | 180 | 507 | | | | | | | | |
| Maximum | | 180 | 507 | | | | | | | | |
| Change | | A -> G | (G)3 -> (G)2 | | | | | | | | |
| Polymorphism Type | | SNP (transition) | Deletion | | | | | | | | |
| Variant P-Value (approximate) | | 0 | 0 | | | | | | | | |
| - (opp. constant) | | | - | | | | | | | | |
| | | | | | | | | | | | |
| Le 179GG (45%) + 507G (57%) |) | - | | | | | | | | | |
| Name | | G | | | | | | | | | |
| Minimum | | 180 | | | | | | | | | |
| Maximum | | 180 | | | | | | | | | |
| Change | | A -> G | | | | | | | | | |
| Polymorphism Type | | SNP (transition) | | | | | | | | | |
| Variant Frequency | | 97.2% | | | | | | | | | |
| variant P-value (approximate) | | 0 | | | | | | | | | |

| Hieracium murorum | 5 alleles | | | | | | |
|--|--------------------------|--------------------------|------------------|--------------------------|--------------------------|------------------------|-----------|
| EE1A (2004) | | | | | | | |
| - 507 - (42%) | 1 allele | | | | | | |
| - 507G (58%) - 551C (68%) | 1 allele | | | | | | |
| - 507 - (42%) - 610G (55%) | 1 allele | | | | | | |
| - 610C (45%) - 599 - (40%) | 1 allele } | Or 1 allele | CAUTION, only | 5 SNPs | | | |
| - 599 T (60%) | 1 allele | | other hypothesis | conly 4000 sequences : | so, maybe the 5 a | dieles are not well re | presented |
| | | | | | | | |
| | | | | | | | |
| SNPs | | | | | | | |
| Type | Polymorphism | A Polymorphism | | | C Polymorphism | | |
| Minimum | 507 | 551 | | | 610 | | |
| Change | 507 (G)3 -> (G)2 | 551 C->A | | | 610 G->C | | |
| Polymorphism Type | Deletion | (transversion) | | | (transversion) | | |
| Variant P-Value (approximate) | 42.5% 3.5E-126 | 32.3% | | | 27.7% | | |
| | | | | | | | |
| | | | | | | | |
| Mu 551A (32%) Name | | A | | | | | |
| Туре | Polymorphism | Polymorphism | | | | | |
| Minimum Maximum | 507 | 551 | | | | | |
| Change | (G)3 -> (G)2 | C-> A | | | | | |
| Polymorphism Type Variant Frequency | Deletion 43.8% | (transversion) | | | | | |
| Variant P-Value (approximate) | 3.6E-44 | 0 | | | | | |
| | | | | | | | |
| Mu 551A (32%) + 507- (42%) | | | | | | | |
| Type | Polymorphism | A Polymorphism | | | | | |
| Minimum | 507 | 551 | | | | | |
| Change | 507 (G)3 -> (G)2 | 551 C->A | | | | | |
| Polymorphism Type | Deletion | (transversion) | | | | | |
| Variant Frequency Variant P-Value (approximate) | 100.0% 4E-205 | 100.0% | | | | | |
| | | | | | | | |
| Mu 551A (32%) + 507G (58%) |) | | | | | | |
| Name | | A | | | | | |
| Type Minimum | | Polymorphism 551 | | | | | |
| Maximum | | 551 | | | | | |
| Polymorphism Type | | C -> A (transversion) | | | | | |
| Variant Frequency | | 100.0% | | | | | |
| Variant P-Value (approximate) |) | 0 | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Mu 551C (68%) | | | | | C | | |
| Туре | Polymorphism | | | | Polymorphism | | |
| Minimum | 507 | | | | 610 | | |
| Change | (G)3 -> (G)2 | | | | G -> C | | |
| Polymorphism Type | Deletion 42.0% | | | | (transversion) | | |
| Variant P-Value (approximate) | 4E-70 | | | | 0 | | |
| | | | | | | | |
| Mu 551C (68%) + 507 - (42%) | | - | - | | | | |
| Name | Polymorphism | - | | | C | | |
| Minimum | 507 | | | | 610 | | |
| Change | 507 (G)3 -> (G)2 | | | | 610 G-> C | | |
| Polymorphism Type | Deletion | | | | (transversion) | | |
| Variant Frequency Variant P-Value (approximate) | 100.0% | | | | 45.0% 1.5E-295 | | |
| (and (approximation) | | | | | | | |
| Mu 551C (68%) + 507- (42%) | + 610G (55%) | | | | | | |
| Name | Dot | | | | | | |
| Minimum | Polymorphism 507 | | | | | | |
| Maximum | 507 | | | | | | |
| Polymorphism Type | (G)3 -> (G)2 Deletion | | | | | | |
| Variant Frequency | 100.0% | | | | | | |
| vanan Prvane (approximate) | 1.0E-1/3 | | | | | 1 | |
| Mu 551C (89%) + FAT (49%) | + 610C (45%) | | | | | | |
| Name | 5100 (45%) | | т | | С | | |
| Туре | Polymorphism | | Polymorphism | Polymorphism | Polymorphism | | |
| Maximum | 507 | | 599 | 599 | 610 | | |
| Change | (G)3 -> (G)2 | | G->T | -G | G->C | | |
| Variant Frequency | 100.0% | | 61.0% | 37.3% | 100.0% | | |
| Variant P-Value (approximate) | 6.3E-166 | | 0 | 4.4E-60 | 0 | | |
| | | | | | | | |
| Mu 551C (68%) + 507- (42%) | + 610C (45%) + 599- (40 | %) | | | 6 | | |
| Туре | Polymorphism | - | | Polymorphism | Polymorphism | | |
| Minimum | 507 | | | 599 | 610 | | |
| Change | (G)3 -> (G)2 | | | - G | G->C | | |
| Polymorphism Type | Deletion | | | Deletion | (transversion) | | |
| Variant P-Value (approximate) | 100.0% 2.5E-62 | | | 100.0% 6.3E-124 | 100.0% 6.3E-168 | | |
| | | | | | | | |
| Mu 551C (68%) + 507- (42%) | + 610C (45%) + 599T (60 | 0%) | | | | | |
| Name | Datasa | | | T | C | | |
| Type Minimum | Polymorphism 507 | | | Potymorphism 599 | Polymorphism 610 | | |
| Maximum | 507 | - | | 599 | 610 | | |
| Change Polymorphism Type | (G)3 -> (G)2 Deletion | | | G -> T (transversion) | G -> C (transversion) | | |
| Variant Frequency | 100.0% | | | 100.0% | 100.0% | | |
| Variant P-Value (approximate) | 4E-87 | | | 0 | 2.5E-274 | 1 | |

| Hieracium policheae | 5 alleles | | | | | | |
|--|--------------|--------------|---------------------|---------------------------------------|--------------|-------------------|----------------|
| DOMINANT ALLELE | missing | | | | | | |
| RECESSIVE ALLELES | | | | | | | |
| 200 CATOCOTO (400() | | | | | | | |
| - 399 CATGCGGTC (43%) - 507- (44%) | 1 allele | | | | | | |
| - 507G (56%) | 1 allele | | | | | | |
| - 399 CATTCGGTC (41%) | | | | | | | |
| - 507- (44%) | 1 allele | | | | | | |
| - 507G (56%) | 1 allele | | | | | | |
| | | | | | | | |
| 51 SNPs variant frequency >8 | 0% | | | | | | |
| + SNPs | | | | | | | |
| Name | C | TTGG | GG | CATGCGGTC | CATTCGGTC | Datamarphiam | A |
| Minimum | 184 | 199 | 201 | 400 | 400 | 507 | 694 |
| Maximum | 184 | 202 | 202 | 399 | 399 | 507 | 694 |
| Change | T -> C | ATTT -> TTGG | TT -> GG | +CATGCGGTC | +CATTCGGTC | (G)3 -> (G)2 | T -> A |
| Polymorphism Type | (transition) | Substitution | Substitution | Insertion | Insertion | Deletion | (transversion) |
| Variant Frequency | 43.6% | 42.2% | 49.1% | 43.2% | 41.1% | 41.6% 8.5E-167 | 51.0% |
| valiant P-value (approximate) | | Ū | | 0 | | 0.52-107 | |
| | | | | | | | |
| Po 399T 41% | 6 | TTOO | | | CATTOCOTO | | |
| Type | Polymorphism | Polymorphism | · | · · · · · · · · · · · · · · · · · · · | Polymorphism | Polymorphism | |
| Minimum | 184 | 199 | | | 400 | 507 | |
| Maximum | 184 | 202 | | | 399 | 507 | |
| Change | T -> C | ATTT -> TTGG | j | | +CATTCGGTC | (G)3 -> (G)2 | |
| Variant Frequency | (transition) | Substitution | | | Insertion | A1 7% | ··· · |
| Variant P-Value (approximate) | 0 | 0 | | | 0 | 1.8E-70 | |
| | | | | | | | |
| Po 399T 41% + 507G 56% | | | | | | | |
| Name | С | TTGG | | | CATTCGGTC | | |
| Туре | Polymorphism | Polymorphism | | | Polymorphism | | |
| Minimum | 184 | 199 | | | 400 | | |
| Change | 184 T-> C | ATTT -> TTGG | | | +CATTCGGTC | | |
| Polymorphism Type | (transition) | Substitution | í | | Insertion | | |
| Variant Frequency | 85.2% | 89.2% | | | 89.9% | | |
| Variant P-Value (approximate) | 0 | 0 | | | 0 | | |
| | | | | | | | |
| Po 399T 41% + 507- 44% | | | | | | | |
| Name | С | TTGG | | | CATTCGGTC | | |
| Туре | Polymorphism | Polymorphism | | - | Polymorphism | Polymorphism | |
| Maximum | 184 | 202 | | | 399 | 507 | |
| Change | T -> C | ATTT -> TTGG | 5 | | +CATTCGGTC | (G)3 -> (G)2 | |
| Polymorphism Type | (transition) | Substitution | | | Insertion | Deletion | |
| Variant Frequency | 81.1% | 87.3% | | | 86.7% | 100.0% | |
| Variant P-Value (approximate) | 0 | .0 | | | 0 | 0 | |
| | | | | | | | |
| Po 399G 43% | | | | | | | |
| Name | | | GG | CATGCGGTC | | Dahmarahir | A |
| Type | | - | Polymorphism 201 | Polymorphism | | Polymorphism | Polymorphism |
| Maximum | | | 201 | 399 | | 507 | 694 |
| Change | | | TT -> GG | +CATGCGGTC | | (G)3 -> (G)2 | T -> A |
| Polymorphism Type | | | Substitution | Insertion | | Deletion | (transversion) |
| Variant Frequency Variant P-Value (approximate) | | | 98.5% 0 | 89.1% | | 43.9% 6.5E-89 | 88.3% 0 |
| | | | | | | | |
| Po 399G 43% + 507G 56% | | | | | | | |
| Name | | | GG | CATGCGGTC | | | A |
| Туре | | | Polymorphism | Polymorphism | | | Polymorphism |
| Maximum | | - | 201 | 399 | | | 694 |
| Change | | | TT -> GG | +CATGCGGTC | | | T -> A |
| Polymorphism Type | | | Substitution | Insertion | | | (transversion) |
| Variant Frequency | | | 98.8% | 88.2% | | | 88.8% |
| vanant P-value (approximate) | | | 0 | 0 | | | U |
| Do 2000 426 1 507 4 50 | | | | | | | |
| Po 399G 43% + 507- 44% | | | 66 | CATGCGGTC | | | A |
| Type | | | Polymorphism | Polymorphism | | Polymorphism | Polymorphism |
| Minimum | | | 201 | 400 | | 507 | 694 |
| Maximum | | | 202 | 399 | | 507 | 694 |
| Change Dolymorphicm Type | | | TT -> GG | +CATGCGGTC | | (G)3 -> (G)2 | T -> A |
| Variant Frequency | | | 98.2% | 90.1% | | 100.0% | 87.8% |
| Variant P-Value (approximate) | - S- | | 0 | 0 | | 0 | 0 |

| Pilosella aurantiaca | 4 alleles | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|--|---|--|---|---|---|---|--|--|---|---|--|---|---|--|--|---|--|---|--|---|---|--|---|---|---|--|--|---|---|---|---|---|--|--|--|-----------|---|--|--|--|---|--|---|
| DOMINANT ALLELE Name Yiph Meansur Meansur Change Polymospherin 7(er. Variant Prequency Variant Prequency Variant Preductors | False Polynorphan 215 215 (TGH-c(TGH Dector) 26.4% 436.322 | Polymorphism 300 305 (TJ) 4 (TJ) Deletion 90.2% | A A77 677 0 × A (ransisor) H 3% 0 | Poynostisun Sor Sor Sor Solars Solars Bases B | smorphism Poly 521 522 64-500 364-500 364-500 364-500 364-500 364-500 | norphan Pe 822 827 827 84800 9 | A yespitan Pol 736 5-> A tansfort 0 0 | ymurgehysti Pu 738 78 35 -> (T)4 0eksiss 28,7% 28,7% 26,170 | A Aymosphanist 853 852 496 + (A)8 Interfore 35.4% 2.56 ±2 | C ulymorphani 012 012 T→C (framitor) 96.7% 0 | 2046 2046 2046 (T)4 = (T)9 Detenso 25.0% 2.0% 201 | C L234 2214 T ~C (transition) HLPS S | openuphtan 1346 1346 (13-5) (59 Deletion 27.4% | 2003 2003 2003 (7)0 - 0(7)5 2003000 2003000 2003000 2003000 2003000 | lymospelsun Po 1884 1884 1884 1884 1884 0 872,0% 9 0 | Nymorganian Pro 1991, 1991, 1993, 1974 - (C)8 0 Alan 98,3% 0 | 8,mugAsan P 1902 1800 CJ7 → (C)6 Deletion 80.0% 0 | AC skymochan 2008 2007 +AC braction 29,0% 0 | | | | | | | | | | - - - - - - - - - - - | | - - - - - - - - - - - - - - - - - - - | | | | | | | | | | | - | | | |
| NECESSIVE ALLELES 4354+ (10%) 455- (70%) 35407 (50%) 3547C (83%) | 2 abors 1 abor 1 abor 1 abor | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A-5 Name Type Mismure Sausnare Change Polymostrain Type Variant Frequency Variant Propercy | C Polymorphan IB4 IB4 T > C (tanotion) SLON R | € 220 220 6 ↔ C 044544545 84.05 0 | A 254 224 © ⇒ A Massilice) 224% 0 | 0 | murphice Poly 305 205 205 20 ⊂ [T]3 0 0 0 0 | TC monthem Pol 154 255 7 → TC totilution 0 0 | T 364 364 6 4 T 804 904 904 904 904 904 904 904 904 904 9 | COC 307 360 17 → COC 0005000 39 95.1% 0 | C Springhour P 371 371 G > C Universion 25.4% 0 | ST3 375 40T Didesce 97.4% 0 | T Distriction 205 205 A + T Stateworkers 9 5555 0 | G A elemonykam AOG AOG (Samanon) BG Afte 0 | ettesces skywopten 419 413 413 413 413 413 5ectos 5ectos 10 10 10 10 10 10 10 10 10 10 10 10 10 | C 4/1 4/2 7 × C pacetor) 95.5% 0 | 6 426 426 A > 6 Sarutos 0 | A Aproxiphism Po Alli G o A Samelloni 27.2% 0 | C Autophan Ali Ali Ali Ali Ali Ali C aniversities Sa Pa | € 403 403 7 → C 7 a C | T djano og daan 402 402 C → T (Fundison) 30.0% 0 | TOA Rojmorpisce P 454 656 GGO > TGA Subclasses D 45 0 | G 308 308 A → G 3484000 34840000 34840000 0 | ColymanyHean P 521 525 525 525 525 525 525 525 525 6 10 42 | 0 530 530 7 -> 0 74 -> 0 74 -> 0 | S20 S20 S20 C(1)2 → (1)2 Dester 35.4% | A grouption P GIS SIS Q > A Turnston) St Ps Q | A See List Q + A Provident Q = A D are 0 0 | 0 Ski7 Ski7 Ski7 A > 0 (saceton) 0 | T 940 940 940 940 940 940 940 140 140 140 140 140 0 | egymaphanofe egy egy egy Dekter etge 0 | A dynophan P d29 d29 d > A Rissitoti Mike 0 | Synoplays P 945 40 Celebon 19.45 8 | T00 400 607 443 → T00 444 → T00 21.8% 0 | T STB STB A o T T B T B T STB C STB STB STB STB STB STB STB STB | A File File File File File File File File | 0117 Polymoption 738 740 AGC → GTT Subsettion 96.7% 0 | GTCAOTT Polymorpho 542 748 COSEC-9 GTR Satultasion 88.5% C | AGTT CAT | TOG G totplean Polymo 740 74 350 750 AT - studies Salad ALPS 98. 0 3 | G 1 Typhian Proym GP 2 GG AG British 15 15 15 15 15 15 15 15 15 15 | TT A tophon Put 752 753 153 153 154 155 155 155 155 155 155 155 155 155 | AAACGT freephan, Pr 750 750 853 AAACGT 4000400 853 N 9 | 0 760 762 252 C 3 G 253 St.95 9 9 | AT proceinary 0 755 755 755 755 85 85 85 85 85 85 85 85 85 85 85 85 8 | AAATG Symophism 768 772 ISA -> AAATI Isbester 13% -> 96.9% 4.75.206 |
| A-6 428 A+ (895) Name Type Microsoft Maximum Change Polymouthins Type Variant President Variant President Variant President | C Polymorphism (54 (54 (54) (54) (54) (54) (54) (54) (| C Crotymogilium 200 200 0 - C Jacosenium 86.8% 0 | | Pogmorphysic Po 275 275 Toja o (Toja () Cestion 27.3% 25.14 | pmorp94ym 305 305 44 ⇒ [7]3 5460ws 68,7% 98,7% 98,2% | Po | T Jonephani Pol 304 314 8 > T 90 > T 90 > S 90 S 90 S 90 S 90 S 90 S 90 S 90 S 90 | CGC progibien Per 367 358 IT -o CGC ANDARA 96.055 0 | 6 4/morphism P 201 201 9 → C 9 → C 9 → C 9 → C 9 → C | 0), morphism 373 375 -457 0, assign 99,2% 0 | T Still Stil | G A 400 400 400 400 400 400 400 400 400 40 | 611193C66 Intymopham 418 413 413 413 413 413 80 90 25 90 0 | C 401 401 Y - C (transitivi) 96.4% | 6 406 406 406 406 406 406 406 406 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 | AC 4/95 4/95 4/0 30 0 AC doubleton 0 0 0 0 0 0 0 | € 800 401 A → C 9000000000 802% 0 | | | - | € (9)-maiphane 500 500 A → 0 (100-500) 95.5% 0 | Sta Sta Sta Este (Sta Categor 6 SE - 30 | 6 0µthorphan P 530 130 ₹→6 144001440 0 | 630 530 530 (1)3 ~ (1)2 Detenso 25.55 | A tymophism Pr 505 505 610 A partologi 80.9% 0 | A Sis Das Das Daschil B29% 0 | 0 biymorghium 50 50 517 ★→5 (hanation) 81,3% 5 | | 422 452 (17)9 ~ (17)4 Doktor A2E-182 | A 0,000,000,000,000 509 629 629 629 629 629 629 629 62 | Rymorphism Pr 140 40 Constan 9 | TOS Animphan Pai Sel Sel Ani TOS Sel Sel S | T T T T T T T T T T T T T T | Α 712 712 712 712 712 712 712 712 712 712 | GTT Potenciption 700 700 ADG > GTT Statistics 92.29 0 | GTCAGT Poymoptia 782 240 CCGGTC > 6TC Subcision 882% 0 | HOT . | | Polym AG Salas BB | TT / nugblass Pos 787 787 100 TT PTTTS 6000000 10 6.7% 0 | AAACDT Grocption 786 390 CG - AAACD1 whattidion 81.8% 0 | 6 (monthem Por 760 760 C or G assurated 85.3% 9 9 | AT procession P 755 766 755 766 755 755 755 75 75 75 75 75 75 75 75 75 | AAAT Januphan 768 771 1020 – AAAT Satukase 105 – 98,2% 0 |
| A 8 KB - [299] Name Tyte Miserum Materian Change Polycosphon Type Variat Frequency Variat Frequency | C Puljenorptoen 186 186 T->C (tanalor) 61.3% | C Polytosyteme 220 220 0.0 + C Pressurement 0.055 | A Diskenseptisien 274 274 G + A Pransision) 85.0% 0 | 0 1 | program Prop 305 325 94 - (1)3 6 94 - 20 74 - 20 75 74 75 75 75 75 75 75 75 75 75 75 75 75 75 | TC monoplasm Pro 254 125 2 → TC bolistics 9 | T 264 205 Ω → T 0 9 46.3% 0 | CGC progRation 387 285 F ⇒ CGC pathone 81.59 0 | C 371 371 9 → C M 3% 0 | ulymophani 373 375 -AQT Datatos 96.99 0 | T 385 385 Å⇒T 940-00 94.4% | 0 405 405 A > 0 four-ulicor) 94.4% 5 | 0 | C 401 421 421 7 > C (function) 83.446 0 | Q Annuchtan Cli Cli A = 0 Karsten J J I IN Q | 0 | С Арториян Р 452 452 А + С анториян Р авру 1 0 | C 163 163 163 17 + C (mansion) 10 0 10 0 | 7 djatusjation 402 400 € > T (transfort) 10 20 9 | TGA Balansister 400 400 400 400 400 400 400 500 500 500 | 6 508 508 508 A > 0 framatori 96.4% 0 | Pulymorphism Pr 523 523 524 524 525 525 525 525 525 52 52 52 52 52 52 5 | 6 50 50 50 50 50 50 50 50 7 → 0 50 7 → 0 50 7 | vay-nurphick dr. 330 (17)3 -> (17)2 Deletion 28.3% | A 610-010000 Pr 525 530 6-0-A (taropico) 45.2% 0 | A Stat Stat G = A (Tyrother) At des 0 | G 547 547 647 A⇒G (ransiden) 0 | T opmorphase 7 365 365 0.5 T tanservices 0 | 622 522 1712 - 5718 Dekeisse 6 | A synophism 625 625 0.0 A (tunieto) 7538 0 | on-noushiae Pr 645 651 45 0 Exercise 82.4% | TDQ SAR SAR SAR SAR TDQ SAR TDQ TDQ | T nooptoon Pray STR 678 A->T Noortoon Ta Noortoon Ta Noortoon Ta | A 712 732 75 A 75 A 75 B 75 B 75 B | GTT Polymourset: 700 240 AGG > GTT Settlemen B6.5% 0 | GTCAGTT Pounceste 740 740 2006/TCCA > GTV 5006/CCA > GTV 5006/CCA > GTV 5006/CCA > GTV 5006/CCA > GTV 5006/CCA > GTV 5006/CCA > GTV 500/CCA > | ADITH CAT | 106 5 nuqueur Palyno 748 77 710 77 9 100 7 - 0 100 7 - 0 100 8 0 3 1 | a anglese Pulyes 10 Pulyes 10 Pulyes 10 Add ensering Salar 10 Add 10 Pulyes | TT A togbins Poi 752 1-5 TT FTTTC 66.4% 0 | AAACGT tymocation 780 780 CG -> AAACGT elesionten 9 82.4% | 6 Anno(Shan) Put 765 753 60 € 50 80.7% 0 | AT perceptions p 705 706 706 706 706 706 706 706 706 706 706 | AAATG Hymogesen 700 772 IGA -> AAATS Substance LON: -> 04.9% 2.167 175 |
| A-6 438 - (246) - 35407 (546) Mason Tojor Morenan Masimum Changie Pulymogratin Type Variant Frequency Context in Solite. (approximate) | C Prijerospisos 184 (184 T -> C (Terrolos) (Terrolos) 0 80.5% 0 | C Prdymos/Rece 220 0 + C (Patence) 80.75 0 | | Polymouphum Po 27% 27% Trian + (Tags () Colotion 27.3% 3.45° 12 | 205 205 205 205 205 205 205 205 205 205 | pe pe | Y 354 355 G = Y Y0 88,2% 0 | CDC proglam Pri 367 169 17 → CGC oxidullar _0 87.5% 0 | 6 371 371 6 3 6 80 26 80 28 0 | 0),maybloot 373 375 461 0,0050 62,0% 0 | 1 205 205 A → 1 205 BLAS 2 2 | 6 401 400 A > 6 francisco 90,7% 0 | TTGGCCGGA 410 410 410 505 TTGGCGGA 502050A 502050A 502050A 5110 74 | C 421 421 7 → C puestion 87.7% 0 | 6 838 825 8 = 0 = 0 8 = | AC cromptogram 440 140 140 140 140 140 140 140 | C Apresidion 402 452 A > C More 90 78.7% 0 | | | - | 6 500 500 A + 6 200 27,2% 0 | 104pmont/15000 FF 523 523 504 + (60)2 504 + 500 504 + 500 500 + 50000000000 | 0 500 500 7.46 0 | 520 520 520 (7)0 → (7)0 Deletion 28,295 1,75:24 | A Arresphere Pr Stati Stati Stati Stati Stati Stati Respired Respi | A Signa (1) (2) (2) (2) Sida Sida Gi (2) A (2) | 0 olymcophism 547 547 Å > 6 (hansidon) 77.9% | | edynaughten 0 622 622 (TT7 → (T)6 Dekster Min (Th Min (Th | A 625 625 6 + A Parelori Uras | Aprice priver 1- 165 -66 Contrast -7825 -0 | TGS Anno Denn Par 665 667 Anno TDS anno DDS anno DDS 0 | ¥ 475 475 A→T 1 9 | A Hoophism 712 712 712 713 713 713 712 713 713 712 713 712 713 712 713 712 712 712 712 712 712 712 712 712 712 | GTT Pogeouption 206 200 AGG -> GTT Subdition 36.7% 0 | 07CAGY Vityroogtig 262 CES60TC -> 670 Substation 98.7% 0 | TOK | | Prayer AG Subs M | TT / / Nothern Por No No TT ITTIC MARKIN SS 6406 BL2 9 | MARCST Americation Pr 755 750 CS - AMACOT absolution 0 75 - 251295 0 | 6 263 765 6 - 6 - 6 80.96 81.96 5 | AT second | AAAT openanghoue 700 771 ISOS > AAAT Salentescon ISNs > \$7,9% 0 |
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| Variant Frequency | 26.8% | 88.7% | 36.8% | 31.0% | 62.9% | 26.4% | 59.9% | 25.9% | 31.2% | 28.2% | 63,4% | 27.9% | 87.1% | 98.2% | 79.9% | |
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| Minimum | 275 | 305 | 507 | 521 | 622 | 758 | 852 | 1046 | 1274 | 1346 | 1744 | 1883 | 1884 | 1991 | 1992 | | | | | | | | | |
| Maximum | 276 | 305 | 507 | 521 | 622 | 758 | 852 | 1046 | 1274 | 1346 | 1744 | 1883 | 1884 | 1991 | 1992 | | | | | | | | | |
| Change | (TG)4 -> (TG)3 | (T)4 -> (T)3 | (G)3 -> (G)2 | (G)4 -> (G)3 | (T)7 -> (T)6 | (T)5 -> (T)4 | (A)8 -> (A)7 | (T)4 -> (T)3 | A -> T | (C)3 -> (C)2 | A -> G | (T)6 -> (T)5 | (T)6 -> (T)5 | (C)7 -> (C)6 | (C)7 -> (C)6 | | | | | | | | | |
| Polymorphism Type | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | (transversion) | Deletion | (transition) | Deletion | Deletion | Deletion | Deletion | | | | | | | | | |
| Variant Frequency | 25.8% | 90.4% | 38.0% | 31.6% | 61.5% | 26.7% | 60.5% | 25.5% | 31.1% | 28.4% | 63.3% | 27.7% | 87.1% | 97.9% | 78.9% | | | | | | | | | |
| riant P-Value (approximate | 2E-160 | 0 | 0 | 8.8E-53 | 0 | 2.6E-186 | 2.1E-107 | 2.5E-292 | 0 | 1.1E-100 | 0 | 4.9E-86 | 0 | 0 | 0 | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| ECESSIVE ALLELE | 1 allele | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | |
| Name | A | G | C | | | A | | AGTTGGCGG | | | C | G | | GTT | T | A | T | GGT | T | AAACG | T | GCATGAAAT | G | A |
| Type | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism |
| Minimum | 190 | 196 | 220 | 275 | 305 | 310 | 373 | 418 | 507 | 521 | 568 | 589 | 622 | 738 | 741 | 743 | 745 | 747 | 752 | 753 | 759 | 761 | 770 | 771 |
| Maximum | 190 | 196 | 220 | 276 | 305 | 310 | 375 | 417 | 507 | 521 | 568 | 589 | 622 | 738 | 741 | 743 | 745 | 749 | 752 | 757 | 759 | 769 | 770 | 771 |
| Change | T -> A | A -> G | G -> C | (TG)4 -> (TG)3 | (T)4 -> (T)3 | G -> A | -AGT | +AGTTGGCGG | (G)3 -> (G)2 | (G)4 -> (G)3 | T -> C | A -> G | (T)7 -> (T)6 | A -> GTT | G -> T | C -> A | G -> T | CCA -> GGT | A -> T | GTTTT -> AAACG | C -> T | GCCCCGGG -> GCATGAAA | C -> G | G -> A |
| Polymorphism Type | (transversion) | (transition) | (transversion) | Deletion | Deletion | (transition) | Deletion | Insertion | Deletion | Deletion | (transition) | (transition) | Deletion | Insertion | (transversion) | (transversion) | (transversion) | Substitution | (transversion) | Substitution | (transition) | Substitution | (transversion) | (transition) |
| Variant Frequency | 88.1% | 87.1% | 88.9% | 26.4% | 89.8% | 87.8% | 94.1% | 73.7% | 36.2% | 32.6% | 91.1% | 91.1% | 66.6% | 94.8% | 98.5% | 94.9% | 98.1% | 95.7% | 89.7% | 92.3% | 99.0% | 86.5% -> 87.8% | 77.0% | 55.4% |
| riant P-Value (approximate | 0 | 0 | 0 | 5.9E-109 | 0 | 0 | 0 | 0 | 1.9E-207 | 1.8E-43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.3E-26 |

| Pilosella piloselloide | s D36 | 4 alleles | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|---|--|--|---|--|--|---|--|--|---|--|---|--|--|--|-----------------------|-------------------------|-------------------|------------------|---------------------|--------------|-----------------------------|-----------------------------|--------------|--------------------------|---------------------------|-------------------------|---------------------|-------------------------|----------------------------|-------------------|
| DOMINANT ALLELE | 1 aleie | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Name Type Misimum Masimum Change Polymophism Type Variant Prequency Variant Prequency | Polymorphism 275 276 (TO)4 -> (TO)3 Deletion 25.9% 9.1E-232 | Polymorphism 305 305 (T)4 -> (T)3 Deletion 90.5% 0 | Polymorphism 507 507 (0)3 -> (0)2 Deletion 38.3% 0 | Polymorphism 521 521 (G)4 -> (G)3 Deletion 31.6% 4.1E-263 | Polymorphism 622 622 (1)7 -> (1)6 Detetion 83.4% 0 | Polymorphsm 758 758 (T)5 -> (T)4 Deletion 27.8% 1.5E-314 | Polymorphism 852 852 (A)8 -> (A)7 Deletion 60.2% 1.3E-143 | Pulymorphian 1046 1046 (1)4 -> (1)3 Deletion 26.4% 0 | T Polymorphism 1274 1274 A-> T (transvension) 31.1% 0 | Polymorphism 1346 1346 (C)3 -> (C)2 Deletion 28.0% 2.4E-130 | G Polymorphism 1744 1744 A → G (transition) 62.9% 0 | Polymorphian 1883 1883 (T)6 -> (T)5 Deletion 28.2% 3.4E-137 | Polymorphisi 1884 1884 (7)6 -> (7)5 Deletion 87.1% 0 | n Polymorphism 1991 1991 (C)7 -> (C)6 Deletion 96.1% 0 | Polymorphism 1992 1992 (C)7 -> (C)8 Deletion 79.0% 0 | | | | | | | | | | | | | | | | |
| RECESSIVE ALLELES | 1. | | | | | | | | | 1 | | .17 | | | | | | | | | | | | | | | | | 1 | | |
| - 629A (31%) - 629G (69%) - 528C (51%) - 528T (49%) | 1 aleie 1 aleie 1 aleie | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SNP | | | | | | | r | | | - | | | | | | | | | | | | - | 17 T | | | 1 | 414000 | | | | - |
| Type | Polymorphism | G Polymorphism | C Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | n | Polymorphism | C Polymorphism | G Polymorphism | n Polymorphism | A Polymorphism | Polymorphism | Polymorphism | Polymorphise | n Polymorphism | Polymorphism | Polymorphism | G Polymorphism | Polymorphism | Polymorphism | O Polymorphism | Polymorphism | Polymorphism | G Polymorphism |
| Maximum | 190 | 195 195 | 220 | 275 | 305 | 310 | 373 | 385 | 418 417 | 463 | 469 | 507 | - | 521 521 | 568 | 589 | 622 622 | 629 | 641 | 545 645 | 646 | 738 | 742 | 748 | 750 | 752 | 755 | 763 | 765 | 768 | 773 |
| Change Enlamonthism Tutue | T -> A | A -> G | G->C | (TG)4 -> (TG): | 3 (T)4->(T)3 | G->A | -AGT Deletion | A -> T | +AGTTGGCGG | T->C | G->T | (G)3 -> (G)2 | | (G)4 -> (G)3 | T-> C (transition) | A->G | (T)7 -> (T)6 | G->A | -CAGA | -Q | -TGGAA | AGG -> GTT Substitution | CCGGTC -> GTCAGT | CAT -> TGG | T->G | AG > TT Supplication | Substitution | C->G | CC->AT | GGCGA -> AAATG | A->G |
| Variant Frequency | 60.9% | 88.7% | 90.5% | 28.9% | 90.5% | 53.9% | 95.6% | 26.7% | 73.5% | 29.2% | 30.0% | 27.9% | | 32.0% | 58.9% | 29.9% | 66.2% | 31.1% | 28.9% | 73.9% | 25.8% | 94.8% | 88.8% | 95.9% | 99.3% | 98.2% | 90.9% | 96.2% | 93.5% -> 93.6% | 72.1% -> 94.3% | 48.2% |
| Variant P-Value (approximate | 0 0 | 0 | 0 | 1.56-119 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10:54 | | 9.18-37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | e | 0 | 0 | 0 | 0 | 0 | | | 7.16-37 |
| D36 629A 31% | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Name | A | G | C | - | | A | Distance in the | | AGTTGGCGG | | T | | | - | C | ĵ. | | A | - | | - | GTT | GTCAGTTG | - | [| TT | AAACG1 | 0 | AT | AAATG | |
| Minimum | 190 | 196 | 220 | 275 | 305 | 310 | 373 | | 418 | | 469 | 507 | | 521 | 568 | | 622 | 629 | 641 | | <u> </u> | 738 | Poynorphism 742 | 1 | | Poynorgnism 752 | roymorphom 755 | 763 | 765 | 768 | |
| Change | 190 T->A | 196 A->G | 220 G->C | 276 (TGM -> (TG)) | 305 3 (TH-> (T)3 | 310 G->A | 375 -AGT | | 417 +AGTTGGCG6 | | 469 G-2 T | 507 (Gl3 -> (Gl2 | - | 521 (G)4 -> (G)3 | 568 T-2 C | | 622 (T)7 -> (T)6 | 629 G->A | 650 CAGAGTOGA | A | | 740 AGG -> GTT 1 | 749 CCGGTCCA -> GTCAGTTC | 9 | | 753 AG -> TT | 760 TTTTCG -> AAACG1 | 763 | 766 CC-> AT | 772 GGCGA -> AAATG | |
| Polymorphism Type | (transversion) | (transition) | (tratsversion | Deletion | Deletion | (transition) | Deletion | | Insertion | 1 | (transversion) | Desetion | | Deletion | (transition) | | Deletion | (transition) | Deletion | | | Substitution | Substitution | 1 | | Substitution | Substitution | (transversion) | Substitution | Substitution | |
| Variant P-Value (approximate | 25.8% | 90.9% | 92.8% | 28.9% 1E-58 | 89.5% | 85.8% | 97.2% | | 79.3% | | 84.5% 0 | 35.3% 9.3E-41 | | 33.0% 3.3E-30 | 92.9% | | -0 | 0 | 80.8% 0 | | | 95.7% | 88.0% | | | 98.5% | 91,7% -> 91.8% C | 94.0% | 94.4% -> 94.8% | 73.6% -> 95.0% 1.2E-185 | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 000 6000 (600) | | į. | | | 1 | | , | | | 1 | | - | 2 | 5 - C | 1 | | 1 | | | x | | 1 | | | | | | | - | | |
| Name | A | 0 | c | in a second | | A | | T | AGTTGGCGG | c | | | 0 | | c | 0 | La serie | | | La sa como | Í. | GTT | GTCAGTTO | 1 | 00 | TT | AAACGT | 0 | AT | AAATG | |
| Type Ministern | Polymorphism 190 | Polymorphise 196 | Polymorphism 220 | Polymorphism 275 | Polymorphism 305 | Polymorphism 310 | Polymorphism 373 | Polymorphism 385 | Polymorphism 418 | Polymorphism 403 | | Polymorphism 507 | n Polymorphist 508 | n Polymorphism 521 | Polymorphise 568 | Polymorphise sag | n Polymorphism 672 | | | Polymorphism 845 | N. | Polymorphism 738 | Polymorphism 742 | | Polymorphism 749 | Polymorphism 752 | Polymorphism 755 | Polymorphism 763 | Polymorphism 765 | Polymorphism 768 | |
| Maximum | 190 | 195 | 220 | 276 | 305 | 310 | 375 | 385 | 417 | 453 | | 507 | 508 | 521 | 568 | 589 | 622 | | | 645 | | 740 | 749 | | 750 | 753 | 760 | 763 | 766 | 772 | |
| Change Polymorphism Type | T -> A (transversion) | A->G (transition) | G->C (transversion | (TG)4 -> (TG): Deletion | a: (T)4 -> (T)3 Deletion | G->A (fransition) | -AGT Deletion | A -> T (transversion) | +AGTTOOCOC Insertion | (transition) | | (G)3 -> (G)2 Deletion | A -> O (transition) | {G}4 -> (G)3 Deletion | T->C (transition) | A-> G (transition) | {T}7 → (T)6 Deletion | | | -G Deletion | | AGG-3-GTT I Substitution | Substitution | B | AT -> QQ Substitution | AG -> TT Subsibilition | Substitution | (transversion) | CC-> AT Substitution | Substitution | (|
| Variant Frequency | 78.1% | 88.3% | 89.7% | 28.2% | 90.3% | 53.1% | 94.6% | 38.9% | 74.2% | 42.0% | | 25.4% | 25.8% | 35.1% | 51.0% | 47.1% | 61.0% | (| | 58.4% | | 95.2% | 88.8% | | 97.9% | 98.1% | 92.3% | 96.4% | 94.3% -> 94.4% | 72.4% -> 94.6% | |
| Vielani P. Valle (approximate | | | 1 | 1.36.00 | | | M | | | | | 1.36/11 | | 1 1.00.09 | | - | | | | | 1 | - · · | | 1 | | - V | | | former Mercenned | | |
| D36 629G (69%) + 528C (51% | 0 | | | | | | | | | | | | | | | - | | | | | | | | | | | | | | | |
| Name | A | 0 | c | Party and a second second | Park or or white | A | Post on cost in a | | AGTTGGCGG | · | | Belanashira | | Post of the local division of the local divi | C | 6 | - Half on configure | | | Park on the second | | OTT | GTCAGTT | | G | 17 | AAACGT | G | AT | AAATO | |
| Minimum | 190 | 196 | 220 | 275 | 305 | 310 | 373 | | 418 | | | 507 | | 521 | 568 | 589 | 622 | | | 645 | - | 738 | 74Z | - · | 750 | 752 | 755 | 762 | 765 | 768 | |
| Maximum | 190 | 196 | 220 | 276 | 305 | 310 | 375 | | 417 | | | 507 | | 821 (014 -> (01/2 | 568 | 589 | 622 | | | 645 | | 740 | 748 | | 750 | 752 | 760 | 763 | 766 | 772 | |
| Polymorphism Type | (transversion) | (transition) | (transversion | Deletion | Deletion | (mansition) | Defetion | | Insertion | | | Deletion | | Deletion | (transition) | (transition) | Deletion | | | Deletion | | Substitution | Substitution | 1 | (transversion | Substitution | Substitution | (transversion) | Substitution | Substitution | |
| Variant Frequency Variant P-Value Japproximate | 80.3% | 90.8% | 92.3% | 28.6% | 90.7% | 85,8% | 97.5% | | 77.8% | - | | 38.1% | 8 | 35.2% 3.1E-16 | 100.0% | 89.4% | 68.2% | | | 27.9% 3.2E-28 | | 95.5% | 89.1% | | 99.2% | 98,3% | 91,5% C | 95.9% | 93.5% -> 93.7% | 74.4% -> 95.3% 2.1E-157 | |
| the second second | | | | | - | | | | | | | | | | | | | | | | | | | | | | | | | A.45. 444 | |
| D36 629G (69%) + 568T (49% | 1 | | } | | den. | 5 | | 1 | 1 | ý | | | | 2 | 1 | 1 | } | | | | } | 4 | | · | | | | 5 | e- | | |
| Name | A | G Polymorphice | C | Dolumontain | Polymontes | | Polymorphics | T | AGTTOGCOG | C | | | G | Polymorphise | | - | Potencenhiser | | | Polymorphice | | GTT Polymorphism | GTCAGTTG | | G | TT Polymorphism | AAACGT | G Potemorphism | AT | AAATG | |
| Minimum | 190 | 196 | 220 | 275 | 305 | | 373 | 305 | 418 | 463 | | | 508 | 521 | | | 622 | | | 645 | 1 | 738 | 742 | I | 750 | 752 | 755 | 763 | 765 | 765 | |
| Change | 190 T-2 A | 195 A-> 0 | 220 G-2 C | 276 (TGM-2 (TO)) | 305 | | 375 -AGT | 385 A-> T | 417 +AGTTGGCOK | 453 T -> C | | | 508 A-2 G | 521 (G)4 -> (G)3 | | | 622 (T)7 -> (T)6 | | | 645 | | 740 AGG -> GTT / | 749 CCOGTCCA -> GTCAGTTC | 6 | 750 T-2-0 | 753 AG -> TT | 760 | 763 | 796 CC-3 AT | 772 00C0A -> AAATO | |
| Polymorphism Type | (transversion) | (transition) | (transversion | Deletion | Deletion | | Defetion | (transversion) | Insertion | (transition) | | | (transition) | Deletion | 1 | | Deletion | | | Deletion | | Substitution | Substitution | 1 1 | dransversion | Substitution | Substitution | (transversion) | Substitution | Substitution | |
| Variant Prequency Variant P-Value (approximate | 75.8% | 85.7% | 87.0% | 27.9% 2E-27 | 89.9% | | 91.6% | 73.8% | 70.5% | 81.0% | | | 50.4% | 35.0% 4.1E-15 | R. | | 2.3E-166 | | | 90.2% | | 94.9% | 89.3% | | 99.3% | 97,9% | 93.2% | 97.0% | 95.0% -> 95.1% | 70.7% -> 94.0% 3.2E-175 | |

| Pilosella | glacialis | 5 alleles |
|-----------|-----------|-----------|
| | | |

DOMINANT ALLELE 1 since

| Nume | | | | | | | C. | | . C. | | | | |
|--------------------------------|---------------|-------------------------|-----------------------------|--------------|--------------|-------------------------|-------------------|-------------------------|------------------|---------------------------------|--------------|-------------------------|-------------------------|
| Type Pak | Apreciption 1 | Polymoighten. | Polymorphism | Polymorphism | Polymorphism | Polynophism | Fulymorphism | Palynophism | Polymorphism. | Polynophism | Polynophon | Polymorphism | Polymorphism |
| tuliniamum | 278 | 306 | 508 | 522 | 623 | 750 | 833 | 1347 | 3,767 | 1,864 | 3885 | 3,992 | 1995 |
| Masterum | 277 | 206 | 500 | 522 | 623 | 759 | 930 | 1347 | 1787 | 1984 | 3085 | 1990 | 1990 |
| Change (TO | 204 -> (TC32) | $(T)4 \rightarrow (T)3$ | $(0)(3 \rightarrow (6)(2))$ | (G)4 > (G)3 | (T)7 -> (T)6 | $(T)5 \rightarrow (T)4$ | $T \rightarrow C$ | $(C)0 \rightarrow (C)2$ | 0.00 | $(T)_{10} \rightarrow (T)_{10}$ | (T)8 -> (T)8 | $(C)7 \rightarrow (C)8$ | $(C)7 \rightarrow (C)6$ |
| Polymorphesm Type 1 | Deletion | Debtion | Deletion | Celetion | Deletion | Deletion | (nanotice) | Deletion | (Interpretation) | Deletion | Celetion | Deletion | Deletion |
| Variant Proceducy | 26.0% | 90.2% | 25.9% | 31.8% | 49.2% | 27.3% | 58.7% | 26.4% | 38.7% | 27.1% | 47.7% | 97,9% | 77.3% |
| Vielant P-Vielan (approximate) | 30-148 | | 1.00-150 | 9.32-59 | - 0 | 1.46-45 | 0 | 2.682-03 | - 0 | 1.225-04 | 0 | 0 | |

RECEISIVE ALLELES 3 alleles

- 3650 (45%) 1 aliele (pr 2 similar) - 3657 (45%) - 2517 (46%) 1 aliele - 2512 (56%) 1 aliele

This SNP down's follow any other SNP patron Or doesn't enter in any combination

| Reference Name Type Meamum Mastrum Change Popresiphics Type Valuer Propresery Valuer Proble (approximate | T Posymuthiam 173 173 € ↔ T (tunstion) 17 #% | C Aptroception 180 2 T -> C (transition) 0 0 | A 0 exploan 10. 297 26 387 29 A 4 ≥ 0 transie 0 0 0 | C Poignacphi 221 0 0 0 0 0 0 0 0 0 0 0 0 0 | nam Polymorphum 250 250 C -> T (transmorp) 36.4% 0 | A Polymorphium 275 6 ⇒ A (ranston) 25.Ps 0 | | Noteman (1) 300 200 (1)4 ⇒ (1)3 Deleter 82.7% 0 | T Notymeophian 365 0-3 T Prawnerskyp 0 0 | COC hyperaptican 300 370 TOT + COC Substitution 55.2% | C Mymorphian 312 322 6 + C hansurraion 56.45 0 | Slymorphium 37% 32% -ADT Dektson 85.4% 0 | T NAythanpitian 3880 3880 A ⇔ T Tannoersian) Matem 0 | 0 clymorphism 407 407 A = 6 manufact 18 2% 0 | AGTTEGEGG Polymorphism 428 438 ASTTEGEC80 Insertice 36 7% 0 | C 422 422 (Inscription) 80.8% 0 | | | AC Polymosphism 460 461 665 ÷ AC 54205hation 26.15 0 | C 452 453 A = C (itanevenion) 28.8% 0 | C Polymorphan 454 454 T-a-C (runcion) 10.5% 0 | Poly (P | 0 hoggilanti (r) 909 909 909 909 909 909 909 90 90 90 90 | G Aymosphian 500 510 AG = G Celetico 26.5% | olymiarghiam 522 522 534 ← (trij3 Deletion 26.4% | 0 Aproxybition 531 535 7 + 0 arover clorit 40 #5 0 | Putyers 5 6 (scan 28 | A Compliant Polymer MD 5 In A A Compliant Integration (International Integration (International Integration (International Integration (International Integration (International Integration (International Integration (International Integration (International Integration (International Integration (Integration (International Integration (International Integration (International Integration (International Integration (Integration (International Integration (International Integration (International Integrational Integration (International Integration (International Integration (International Integration (International Integration (Integration (International Integration (International) Integration l) Integrational Integrational) Integrational Integratio | 0 ophism 45 48 49 40 obcri 19 | | Polymorphism 623 623 (T)P -> (T)P Doletion 0 | A Polymorphian 630 630 6 - A (transition) 66 2% 0 | Pulymorphium 645 645 6 6 Defence 6 0 | 100 Polymorphism 600 600 GAA ⇒ 100 Sabasilistice N6.3% 0 | T EPB E75 A -0 T MARK 0 | 077 tolymorghism 759 745 656 + 677 64.5% 0 | OTCAGTTOG Polymorphan 761 751 507CCAT -> OTCA Subsiliation 82.3% 9 | 017104 01704 0 | rghoun Pro 0 4 TT TTTN Mator Si 16 | AAACOT dyneghiam 758 766 05 -> AAACOT labolisticn 0 | 0 764 764 6 + 0 (tanuerson) 300.0% 0 | AT lymosphiam 250 257 DD - AT desidation 86.3% 0 | AAATO Paymoptian 769 775 CGA = AAATD Substance 89% = 98.7% 0 | |
|--|--|--|---|---|--|--|---|--|--|---|---|--|---|---|--|--|--|----------------------------------|--|---|--|------------|--|--|---|---|----------------------------------|--|---|---|---|--|---|---|--|---|---|--|--|---|--|--|---|---------------------------------|
| G45 9950 495 Norme Type Maximum Maximum Owenp Polynorphism Type Variant Prequency Variant Prequency | | Diskon 2 7 7 7 7 83 | A 0 sophan 205 207 0 A 207 0 A 207 0 A 0 0 B6.0% 0 0 | C Poymocale 221 223 6 + C 1000 | 10 7 9 | | Patymorphism 8 219 217 (F694-6(F68) Celetion 25.4% 3.45-203 | olymosphium 300 300 (1)4 - (1)3 Defetion 88.3% 0 | | | 2 | Aymosphsan 374 315 -AGT Detecn 86.2% 0 | | | | C 422 422 Y -> C (Downlition) B4.JP5 0 | 007 Polymentium 425 425 party2 -> (6617)8 Insertion 9 0 | | | | C Palymorphism abid T-5-E (Suscition) BLETS 0 | 009 | 6 5000300 900 900 1-7-6 estilors 0.4% 0 | | olymurgitisum N22 Si22 Si34 → (6)3 Celetion 32.4% 7.50-49 | | | | C Polymon 634 10 10 10 10 10 10 10 10 10 10 10 10 10 | C phion Polymorph R28 R28 F-24 Parenter C R28 Parenter 0 | | A Pagencephean 630 63-9 A (transition) 91.3% 0 | | | | 0.71 Vajamospinan 779 Naj Mada - 6777 Salatistation SELEN: 0 | GTCASTTOD Postmorphan 745 TR SSTOCAT - GTCA Substitution 82.0% | 0 004/mic 755 555 56500 967764 967764 967764 967764 967 967 90 90 90 90 90 90 90 90 90 90 90 90 90 | operation of the second | AAACGT Aymuytson 0 756 761 05-3 AAACGT Monthloon 0 95.0% 0 | 0 /MJ 754 E + 6 (Intervention) 306.0% | AT Jan Jan JEP DE -> AT SEJ75 | AAATO Najanagkan 789 715 664 -> AAATO 5445katon AJPL -> 96,9% 3.06-300 | |
| G40-3657 55% Name Type Namun Maaiman | Polynosylliam Pr 173 173 | C Amorphism 100 130 | 0 Putymorp 157 347 | tisan C Patymorphi 221 223 | ian Popeogram | A Polymorphian 270 275 | | Oynexylian 1 325 228 | Y Addymorphism 305 305 | COC relymorphises 358 370 | C Oyeorjaliye 372 372 | alymorphism 1 374 376 | Y Nythophism 300 300 | 0 ntymospilaum 427 427 | ASTTERCOS Pelymustism 429 428 | C hdymoghian 452 452 | | Ö Polymorphises 437 437 | AC Polyneighten 640 641 | C Profymorphon 453 453 | | Poly | 0 maghlain Pr 500 100 | 0 dymorphism 509 510 | ttymoghism 1522 1622 | O Aprovation NIL SIL | 512 5 523 5 | A dophian Polymo HD D S HD D S | b nytřesm 48 | - | Polymorphise 623 625 | A Polymorphism 630 630 | Pulymorphian 640 640 | TGO Polymorphism / colo col | 7 ulymorphism 679 679 | GTTI Islymorghism 729 741 | GTCABTTSG Polymorphism 748 79s | Pulpino Pulpino 75 | gahisen Pu a | AAACOT dymostaan 756 145 | G Adynerytism 754 784 | AT penosphism 200 367 | AAAT Indymurgitean 789 772 | 0 togetuoptike 175 275 |

| Musimum Change Polymorphism Type Vasant Frequency Vasant P Volke: (approximate) | 177 1300 C-o-T T-o-C (namiliar) 0 0 | 307 A = 6 (tansitise) 48.7% | 221 6 + C (turoversion) 86.7% | 253 275 C → T 0 → A Yumstori) (transition) ML PN 42.5% 0 0 | 9 9 9 10 10 10 10 10 10 10 10 10 10 10 10 10 | 305 G-3 T (Tamoremont) 380.8% 0 | 3/0 TGT > CGC Substitution BLPS 0 | 312 6 + C Material 99.2% 0 | 275 -A07 Detetion 336.9% | 300 A -> Y A provention() (from 97.8% 9 0 | 67 4 0 6 446171 mitori) inter 13% Mitori | att 060096 rtcar 0 0 0 0 0 0 0 0 0 0 0 0 0 | 437 A ⇒ 6 (transition) at ans 0 0 0 | AGO A - C (harsverskor) Bil dra | 100 A 3 (Tartal 0 44.9 0 | 6 510 6 A5 + 6 Doktor 8 28.9% 0.36 125 | 1027 (6)4 ↔ (6)0 T Detetion (trans 38.3% T 2.36.517 | Sill Sill ⇒ 6 (7)3 ⇒ (7)3 (7)3 ⇒ (7)3 Chillion 2 2% 28.2% 0 9.66-120 | 2 0 0 A A 0 0 (Parsitor) 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 | ITP Do | 23 63 • (7)6 6 0 whoe (trans 19% 96.7 0 0 | A G bert Defetion 8 97.2% | GAA -> TGG Substitution \$2.2% 0 | 670 A> T (transversion 85.8% 0 | 741 AGG = GYT 20 Subsidiation 96.6% | 750 DIGSTOCAT = GTCASTTO Substitutor 82.5% 0 | 254 AG-OTT Constitution BE7% | Nos TTTCG -> AAACGT Substitution NA_2% C | (154 C = 0 (100000000) 300.0% | 367 C -> AT 660 millitors 54 87.8% 85.9 0 | 2712 10 - AAAT doubles 16. + 85.76 0 | 775 A-2-6 transitio 87.0% 0 |
|---|---|--|--|--|--|--|--|---|---|---|--|---|--|--|--|--|---|--|--|--------------------|---|--|---|---|--|---|---|---|---|---|---|---|
| G46 34657 55% + 2517 44% Rome Type Maxmun Maxmun Maxmun Maxmun Valant Programy Valant Programy Valant Programy | T C grouphism 173 185 173 185 C ⇔ T T ↔ C (transition) 54.1% 87.4% 0 0 | m 0 Polymouthau 237 237 4 + 0 (second of 0 98,2% 0 | C Polymorphiam 223 221 G ⇒ C Planeversion) 0 | T A sprinciplium 253 275 253 275 C + T G + A framino) 186.0% M.96 0 0 | 100 Polymouphian 300 300 (174 + (173 Dolman 300 300 300 300 300 300 300 300 300 30 | T Polymophism 265 0 → T (transversion) 1960 PN 0 | COC Polymorphilam 300 370 FOT > COC Substitution 6 | C dymarathane 372 372 6 + C caraversioni 98.4% 0 | 276 276 275 4607 266mon (82 216.0% 0 | T Jacopitani Polyn Jaco Jaco Jaco Jaco Jaco Jaco Jaco Jaco | 0 A011 0000000 Putyo 807 4 00 A011 00 B00 100 100 100 100 100 100 100 | 00C00 C optian Polymorphian 08 422 06C00 T + C (transition) 85 0 0 | AC Poyncoptian 480 481 06 ⇒ AC 5abitation 81.85 0 | | A Pulyreoption 558 69 A (rambol) 32 Ph 6 | 0 Polymorphium i 500 A5 ⇒ 6 Delateo 33.5% 1.90:152 | colymorphism 522 522 523 524 ↔ (50)3 7 Celetion 34.2% 4.30~30 | 0 00000000000000000000000000000000000 | 2 | Poge Cry Da | A coghian Piajano 23 63 23 63 c)(T)# G o (Dana (Dana 0 0 | daam Polymooph 640 640 640 640 640 640 640 640 640 640 | 000 000 000 000 000 000 000 000 000 00 | 7 Projencophice 875 8-> T (transversion 96.7% 0 | 0.77 Polymorphium 789 741 AGG + GTY 72 Substation 94575 0 | 01С401100 Рофторяан 783 791 сертсски → 61Скопто 545/86 9.2% | TT Osijimorphism 753 754 AG or TT Subcanakor 98,7% 0 | AAACOT Polymorphism 763 753 TTTCG -> AAACOT Substitution 83.7% 0 | Coperception 754 754 C = 0 Steamerstort) 5 Steamerstort) 5 | AT morphism Pos 360 787 C + AT antihabon Sul BL3% 0 | AAAT (morphism Pol) 769 772 05 - AAAT dolladion 9 0 2 | 0 773 773 8 2 4 0 8 2 |
| G45 3857 50% + 250C 54% Name Tyle Mermath Maanum Change Polymorphich Type Vaciant Pregaming | C Polymouthan 136 136 1-3-0 (transfor) 82.34 | | C Polymorphism 221 0 → C (tamorphon) 87.76 | | Polymorphom Polymorphom 2778 306 307 200 (1764 - (1764) (178 - 4719 Detektion 25.2% 88.8% | 7 205 305 6-5 T (100547567) 380.7% | COC Patymosphesh 200 300 TOT -> COC Substitution 84.Ph | C dymostern Po 372 372 6.→ C terroresorj M.Ps | amospituan Prof 271 375 -4607 Deletion (Br 96.0% | T Vercepteum Polym 200 A -> Y Aroversian) (the STAN S | G ADTS segnitum Polym BY G ADTS arG ADTS mitim) by LIS St | 50209 C optism Pstymostism 09 422 060000 F-s-C form (transform) 05 0 0 | 6 Polymorphism 423 A ~ 6 (prantizm) B A | C Poismanphon dS2 dS3 A > C (Transversion) B5.1% | | 0 Popmorphase 505 505 A > 6 (transion) 38.45 | Notymorphism Polyn S22 (5)4 -> (5)0 Deletion (nam 37 Arts 7 | 0 sophum 532 532 546 semicol 236 | A 6 Pulymorphise Polymorphise 545 548 6 -> A A > 6 (transition) (transition) 84.4% \$7.2% | Polym ITP De | orgatisaria 23 23 24 25 25 25 25 25 25 25 25 25 25 25 25 25 | Polymoph 640 641 Deleter 14.25 | TGG Polymouthon 608 GAA -> TGG Substitutor BL04 | Tolynophia 675 675 A - T (transector 86.16 | G71 Polymorphom 238 751 ASG > G17 25 Dobrinktor 98,8% | GTCASTTOG Dogmorphon 162 TS DEGTCEAT -> OTCASTTO Debiliston 89.2% | TT Polymorphism 753 754 AG + TT Sobritution 98.4% | AAACUIT Disyntospison 706 705 TTTEG - AAACUIT Substitution 94.4% | 6 Najmogitism 754 6 - 6 (ramorston) 346,2% | AT / / moption Pos 150 E + AT 66006 Initiation Sci 87.45 87.45 87.45 | AAATG ymorgfoat 709 772 SA -> AAATG dediadon No -> SC/N | |

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| Namo Tote Melenum Koamum Chalge Polyesophian Type Variad Prequency Variad Prequency Variad Prevale (approximate) | Polymorphism 325 535 (1534 -> (153 Deletion 25.0% 4.00-45 | Polymorphism 305 305 (1)4 ⇒ (1)3 Deletion 82.2% 0 | Polyanorpheae 507 507 (053 -> (052 Detence 38.5% 8.95-128 | Posymorpheni 821 521 (D)4 > (D)3 Delesse 233% 7.90-99 | Polymorphism 622 622 (1)7 > (1)8 Doletison 82,0% 0 | Polymorphism 758 758 (75 -> (754 Detension 28.0% 4.42-48 | A Polymorphism 853 852 (A)8 = (A)8 Insection 34.0% 0.00048 | C Polymorphise 932 932 T -> C (Interdisor) 96 215 9 | Polymorphism 1546 2546 (154 -> (15)3 Deletion 28.8% 2.96-135 | Polymorphase 1346 (C)3 = (C)2 Deletion 27.5% 3.16-32 | Polymorphism 3683 1583 (T)R = (T)S Detention 27.9% 26-35 | Polymorphyse 2004 2004 2004 (T)6 + (T)5 Defessor 86.0% 0 | Polymorphism 1991 1992 (C]7 -> (C)6 Detetion 92.3% 0 | Professional 1980 1980 (C37 -> (C36 Develop 72,046 1.46-200 | | | | | | | | | | | | | | | | | | | | | | | | | |
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| RECESSIVE ALLELES -2530 (53%) -2530 (45%) -664TDA (43%) -664TDA (43%) | 1 alere 1 alere 1 alere | | | | | | | | | | | | | | | | | | | | | | | | | | | | | antipati 2 codedene | | | | | | | | | |
| Name Type Meansan Kasimun Change Polynocoticer Type Variant Frequency Variant Programsy | C Polymorphise 184 184 T -> C (transition) 80.8% 0 | G Polymophia 196 A->G (ranshicr) M.JN 0 | G Putymosphile 208 A > G (transien) 62.6% 0 | TGA Polymorphism 220 222 GGC ⇒ TGA Substances 0 | A Polymorphism 222 223 C -> A (naroversion) 26.3% 0 | T Polymorphism 250 C -> T (nameteo) 42.1% 0 | 6 Polymorphism 253 C = 0 (hannemicor) 42.5% 0 | A Polymorphism 256 256 C -> A Manuscrater 4L4% 0 | 0 Pulymorphism 281 7 > 0 (harmerson) 42.8% 9 | Polymorphism 275 -0 Detesion 25.9% 6.36-50 | | Liter Liter | Polynooptian 305 305 (114 -> (110 Detetion 60.8% 0 | a tra unara | Polymorphism 373 375 440T Detetion 94.4% 0 | T Polymorphism 285 285 A → T (harmorphis) 28,4% 0 | | AUTTOOCOU Polymorphism 418 417 •AUTTOBCOO Imperian 0 | | TGA Polymorphism 404 409 600 > TGA Schultelice 0 | | A Polymorphism 450 0 -> A (transition) 25.4% 9 | 1997 - 1997 - 1 | G Solo 508 A > G (transloce) 29.0% U | Olymorphism P 521 521 1004 → (0)3 Deletion 100,76 | C Olymoophine P 565 6 -> C 41.8% 9 | 622 622 (1)7 -> (1)6 Deletion 67,3% 0 | Upmorphism P 645 645 -0 Deletice 81.4% 0 | T olymorphian P 065 095 € ⇒ T terminer) 92.9% 0 | G olymorphism 667 667 A > G (tranilics) 82,1% 0 | G S87 567 A = 0 (transition) 6 6 | GTT Polymorphism 738 740 AGG > GTT Substantion 95.PN 0 | GTCAOTT Polymogean 742 748 CCGGTCC > GTCA Subsidiation 85.5% 0 | 56 Polymorp 749 755 AT1 Subsitio 92,89 9 | 117 1000 Polymorphy 752 100 Ad | AAACOT Polymorphi 755 756 FTTTCG → A 5050588 91.7% → 91 0 | 0 bm Pulymoipt 763 563 6 × 6 0 (thansist 10% 96.2% 0 | AT Polymorphi 215 266 CC -> AT 545 545 545 545 545 545 545 54 | AAATG POyecophile 705 772 F GGC0A > AAATG Subulhilos 78,2% > \$5,2% 0 |
| La 2530 59% Name Type Maximum Maximum Change Polymorphism Type Variant Professory Variant Professory | C Polymorphan 184 T -> C (humitor) 86.5% 0 | 0 Polymosphar 130 230 A → G (transition) 25.2% 0 | 0 Polymorphism 208 A → 0 (hansitori) 82,5% 0 | 10A Polymorphism 230 222 GGC → TGA Substitution 82.4% 0 | | T Polymorphesm 250 250 C → T ((cersition)) 96.1% 0 | 0 Polymorphase 253 C = 6 (baseversion) 100.0% 0 | A Polymorphon 256 256 C → A stratoversion 93.2% 0 | G Projecospicer 281 285 7 → G (hanoversice) 81.5% § | | | | Projemorphism 205 205 (1)4 -> (1)0 Deletion 81,2% 0 | | Polymorphism 373 375 AQT Deletice 98.5% 0 | | 0 Potymosphar 405 A = 0 (hatsion) 15.8% 0 | AOTTOOCOO Polymosphan 418 417 •AGTTOSCOO Inserton 82.4% 0 | T Protymourpheae 4557 4557 G ↔ T (thansversion 36.5% 0 | TQA Popyroceptans 464 460 GGG = TQA Substitution \$2,2% 0 | | | | G tolymorpheue SOB SOB A ⇒ G (harstillon) 30.6% 0 | rolymosphism P 521 521 (0)4 ⇒ (0)3 Deletion 21.0% 2.56:33 | C SSD SSD 0.9 C Dataset SOI 48.3% 0 | 632 632 622 (T37 → (T)6 Debisor 6 3 0 | dymorphum P 645 645 6 G Deletor 8 8 19 9 | 7 04/morphics P 055 0-> T tsouverstor) 95.2% 0 | 0 05/morphism / 667 A = G (transition) 95.4% 0 | G Ndymorphism (887 A > G (Iterolice) ag ava 0 | 017 Polynorgfsam 728 740 AGG > GT7 Sebsebutor 95.3% 2 | OTCAOTT Polymophon 742 745 CCOOTCC > OTCA Sublision 85.9% 0 | 05 Polymon 249 250 41 - 40 Substat 540000 9 | 117 Hism Pidymospili 752 753 36 AG → 11 Ion Subsibilit 6 BEAN 9 | AAACOT Polymorph 758 760 1 FTTTCG + AA m Scatslag 92,4% 0 | 1 G Nm Polymorph 763 763 760 0 (Darever 98,79 0 | A7 Poj.mogile 785 16 100 CC → A7 5055866 54.2% 0 | AAATO Pojemoghism 718 727 1 00600A -> AAATO Dubijikation 74,8% -> 03,5% 9.8% -205 |
| La 253C (47%) Name Type Melesum Maenuus Cheege Podynorpitoin Type Vedant Frequency Vedant Prequency | C Polymosphar 184 184 7 + C (Tamsilon) 46,2% | C Polywoothise 136 136 A +> G (transition) 78,1% 0 | 0 Polymorphism 208 208 A ≫ G (Pantofor) 8 7% 6 | TGA Potymosphore 230 222 GGC + TGA Substitute 0 | A Dedycmorphism 222 222 C + A (Dataset store) 4L 45 0 | T Tolymorphism 250 250 G + T (Tarriston) 0 | | | Polymorphism 275 276 (1564 + (1569 Overlight 28.0% 4.36) 17 | Polymorphism 275 276 -G Ocietion 25.5% 2.68-27 | A Dotyrecepteers 207 G → A (Farston) 225% 5.55-261 | T Polymorphase 290 290 € ⇒ T (Paration) 41.PN 0 | Polymospinon 305 305 (T)4 ≠ (T)0 Deletion 86.4% 0 | A Progenospheser 310 210 G -> A (transition) 310 States 0 | Polymasphean 373 375 AG7 Debtico 90.6% 0 | T 205 305 6 ≥ T (tarserson) 86.2% 9 | | AGTTODCGO Polymorphane 438 417 *AGTTODCGG Insertion 333.95 0 | C Folyexophise 457 457 G + C (Tarona soon 30.0% 0 | AGA Dolymorphone 454 480 GGG = AGA Substitution 34.0% 0 | TGA Polymorphene dba db5 GGG > TGA Substation eLDN D | | 0 | G SCR SCR SCR A ≥ G (hansisce) 27.4% 0 | 104yrtophean 521 521 (614 ≥ (612 Deletion 23.45 2.45-43 | C 940 565 G ⇒ C 1419+150 M 05 0 | 622 8227 0 Celetics 0 Celetics 0 | olymorphism (* 540 645 G Oxfotos HUTS 0 | 7 605 605 665 6 > T 1atownsion 96.2% 6 | G 507 507 687 A ≠ G (Dansidon) 91.5% 0 | G 987 667 A→G (Tanistica) 47.5% 0 | GTT Polynorphism 738 740 AGG + GTT Galaxism 95.8% 0 | GTCAGITO Pogenetisse 742 745 GGGTCCA + GTCA Substance 88.1% 0 | 0 Polymorp 750 750 (Partice 19 19 19 19 19 19 19 19 19 19 19 19 19 | 177 Norm Pulymorphi 752 753 A G > 171 Substates 86.0% 9 | AAACOT Polencipa 755 7000 FTTTCG + A 90 Substitut 91,9% + 91 0 | G Set Poigeneepi Poigeneep | AT Polymorphi 205 206 500 50000000 50000000 50000000 50000000 | AAATO polymosphere 784 772 7 BOCCA + AAATO 19 Substation 19% 80.5% + 95.4% 7.42.230 |
| La 253C 47% + 484TGA 42% Name Tojon Malmoyn Change Polynophon Type Valuet Progency Valuet Progency | C Polymosphism 184 184 T-> C (transdist) 70.0% 0 | 0 Polymospisar 196 296 A -> 0 (transition) 94.8% 0 | G Polymorphian 208 208 A -> G (hwastor) 72.9% 0 | TGA Polymorphism 220 822 GGC > TGA Settomator 71.4% 0 | | T Polymorphan 250 250 C -> T (Sweated) 73.5% 0 | | | | | A Polynozybian 207 227 G > A (fransition) 0 | | Polymorphism 305 305 (TM -> (TJ3 Deletion 42.3% 4.35/252 | A Polymostikum 310 210 0-5 A (hexalice) 0-4.4% 0 | Polymorphism 373 275 -AGT Deletion 95.4% 0 | T Polymorphism 395 345 A → T (transversion) 85.4% 0 | | AGTTGGCGG Polynouthur 418 417 •AGTTGGCGG Itsertion 121.4% 0 | | | TGA Polymorphon 6% 6% GG0 > TGA Substitution 97.3% 0 | | | 0 500 500 500 500 500 500 (Inscided) 642% 0 | Adymosphan P 523 923 (G(4 -> (G)3 Detection 54.0% | C olymorphism 565 545 6-5 C barser serv 0 | Gymorphian P 622 622 (1)7 ~ (1)6 Deterion 68 2% 2.06 255 | Ulymurphian P 645 643 - G Deletion () 38.4% 2.76-127 | T 005 605 0 0 T 8 0 T 84.2% 0 | 0 9/moghtun 667 687 8-> 0 (transhor) 96,3% 9 | | G17 Polymorganum 738 740 AGG -> GTT C Tubothénan 96.4% 0 | GTCAGTTG Polymophism 142 749 CGGTCCA > GTCAH Dubsitution 83.2% 0 | 6 Polymorp 750 750 1710 1 - 44 (basser 98.49 0 | Asam Polymorphi 752 3 AG -> 17 scrt) Section 4 09.3% 0 | AAACOT Polymosphi 755 760 FTTTCG->AA Galasaya 91,9% 0 | G am Polycargo 763 763 763 763 763 00 00 00 00 00 00 00 00 00 00 00 00 00 | AT Folymorphic 766 CC -> AT CC -> AT AC 44.7% -> 94 0 | an AAAT Pogmoophism 700 711 700CG > AAAT 800094000 643% > 95.4% 0 |
| La 253C 47% + 48446A 38% Norm Type Mesmun Masimon Change Polymophon Type Variant Progressing | 5 400 7 | G Polymorphise 195 206 A > G (humilion) 82.3% | | | A Polymorphism 222 222 C → A (transversion) 94,8% | | | | | | | T 208 298 296 C = T (familion) 85.45 | Polymorphose 325 325 (1)4 ⇒ (1)3 Deletion 78.9% A 46-322 | | Polymorphism 373 375 40T Dention 90.9% | T Polymorphism 385 305 A⇒T (hansenson) 78.85 | in the second | ADTTODCOG Pojenostinen 438 437 •AGTTODCOG boertoo 78.4% | C Polymosphist 457 457 6 = C (barrownion \$1.0% | AGA Polymorphism 484 486 6660 -> AGA 5 Gutstituter 190.05 | | | 10/07/02/2007 507 507 507 507 507 507 507 507 507 | | Tolymorphism 521 521 (0)4 ⇒ (0)3 Dekton 327-14 | | Glymanphaen 7 622 622 (T)2 ⇒ (T)6 Dekrison 6.05.0% | esprecipitions P 645 645 65 Detellor 92.0% | T 005 005 G ⇒ T 10004000 105 G ⇒ T 100040100 0 | G 00/morphom 1 607 607 A > G (bienilion) 96.3% | 6 697 697 A = 0 (transition) 85.9% | STT Poyncothem 738 740 AGG > GTT (C Substitutor 95.8% | STCAGTTOD Polytocphism 742 750 SGTCCAT + GTCAI Sublishion 96.2% | 1 77 00 | TT Polymorphy 752 AG = TT Subsidiate 94.0% | AMACOT Polymorph 755 700 FFTFCG = A In Substan 81.3% 0 | 1 G nm Polymorpi 763 763 763 763 763 6450 763 763 763 763 763 763 763 763 763 763 | AT Polymorphi 785 266 CC > AT Substitute 82,9% | AAAT sm Polytoophore 705 F GGCG > AAAT Substitution 95.0% > 95.8% |

| Pilosella onegensis | s 2 alleles | | | | | | | | | | |
|------------------------------|----------------------|--|--|--|--|--|--|--|--|--|--|
| - 482C (45%) - 482T (55%) | 1 allele 1 allele | | | | | | | | | | |
| | | | | | | | | | | | |
| SNPs | | | | | | | | | | | |

| Name | c | c | A | | TC | T | COC | c | | T | 6 | | c | c | c | T | TGA | | 0 | | 6 | T | | A | | TOG | T | GTT | GTCAGT | TGG | | TT | AAACGT | 6 | AT | AAATG | 6 |
|--|--------------|----------------------------------|--------------|---------------------|--------------|-----------------|--------------|-----------------|--------------|-----------------|--------------|----------------------------------|--------------|----------------|---------------|--------------|---------------|--------------|--------------|--------------|-----------------|-----------------|--------------|----------------------------------|--------------|--------------|----------------------------------|--------------|---------------------|---------------|----------------|--------------|-----------------|----------------|----------------|----------------|------------|
| Type | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism (i | Polymorphism | Polymorphism | Polymorphism | | Polymorphism | Polymorphism | Polymorphism. | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphise | Polymorphism | Polymorphism | Polymorphism | Polymorphism. | Pr | olymorphism | Polymorphism F | Polymorphism | Polymorphism | Polymorphism | Polymorphi |
| Masimum | 184 | 220 | 274 | 305 | 354 | 364 | 367 | 371 | 373 | 385 | 406 | | 421 | 452 | 463 | 402 | 484 | 507 | 508 | 521 | 530 | 565 | 622 | 629 | 645 | 005 | 479 | 738 | 742 | 748 | | 752 | 755 | 783 | 765 | 768 | 773 |
| Maximum | 184 | 220 | 274 | 305 | 355 | 364 | 369 | 371 | 375 | 385 | 406 | | 421 | 452 | 463 | 402 | 485 | 507 | 508 | 521 | 530 | 545 | 622 | 629 | 645 | 667 | 678 | 740 | 747 | 750 | | 753 | 760 | 763 | 766 | 772 | 773 |
| Change | T->C | 6->C | 0->A | (T)4 -> (T)3 | OT -> TC | GoT | TOT-> COC | 0-00 | -AGT | A->T | A-> G | | T->C | A-> C | T->C | COT | 000 -> TOA | (013 -> (012 | A-> 0 | (0)4 -> (0)3 | T-> G | 0->T | (T)7 -> (T)6 | G->A | -0 | GAA -> TOO | T-A | AGG -> GTT | CCGGTC -> GTCAGT | CAT-> TOO | | AG->TT F | TTTCG -> AAACGT | 000 | CC -> AT | GOCGA -> AAATG | A-20 |
| Polymorphism Type | (transition) | (transversion) | (travsilion) | Deletion | Substitution | (transversion) | Substitution | (transversion) | Deletion | (transversion) | (transition) | T 1 | (transition) | (transversion) | (transition) | (transition) | Substitution | Deletion | (transition) | Deletion | (transversion) | (transversion) | Deletion | (transition) | Deletion | Gubstitution | (transversion | Substitution | Substitution | Substitution | 1 | Substitution | Substitution | (transversion) | Substitution | Subsiliution | Oransilion |
| Variant Frequency | 87.5% | 96.7% | 94.8% | 34.3% | 53.3% | 97.0% | 93.9% | 90.1% | 300.0% | 84.0% | 95.6% | | 96.2% | 43.1% | 54.3% | 55.2% | 53.2% | 39.5% | 34.3% | 37.2% | 71.7% | 53.2% | 69.2% | 55.2% | 99.8% | 90.4% | 98.9% | 95.2% | 88.5% | 95.8% | | 98.6% | 82.0% | 96.2% | 93.7% | 73.2% -> 94.8% | 30.0% |
| Variant P-Value (approximate | 0 0 | 0 | 0 | 0.000015 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 1.18-45 | 0 | 3.18-384 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | ő | 6 | 0 | 0 | 0 | 1.26-11 |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| On 482C (45%) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Name | c | C | A | | | т | CGC | C. | | T | 6 | ACTTOCCOG | c | с | | | | | | | 6 | | | | | TOG | T | GTT | GTCAGT | TGG | | TT | AAACGT | 6 | AT | AAAT | _ |
| Type | Polymorphism | Polymorphism | Polymorphism | Polymorphism | | Polymorphism | Polymorphism | Polymorphism I | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | | | | | | Polymorphism | Polymorphism | | Polymorphism | | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | 81 | olymorphism | Polymorphism / | Polymorphism | Polymorphism | Polymorphism | |
| Meterman | 184 | 220 | 274 | 305 | | 364 | 367 | 371 | 373 | 385 | 406 | 438 | 421 | 452 | | | | | | \$21 | 530 | | 622 | | 645 | 605 | 678 | 738 | 742 | 748 | | 752 | 755 | 763 | 765 | 768 | |
| Maximum | 184 | 220 | 274 | 305 | | 364 | 369 | 371 | 375 | 385 | 406 | 417 | 421 | 452 | | | | | | 521 | 530 | | 622 | | 645 | 667 | 678 | 740 | 747 | 750 | | 753 | 760 | 763 | 766 | 771 | |
| Change | T-> C | 6.20 | G-PA | (T)4 -> (T)3 | | GoT | TOT -> COC | 0.00 | AGT | A->T | A-> G | +AGTTOGCOG | T->C | A->C | | | | | | IG34 -> IG13 | T-2-G | | (TT2 -> (TD6 | | -0 | GAA -> TGG | A-PT | AGG -> GTT | CCGGTC -> GTCAGT | CAT -> TOG | | AG → TT T | TTTCO -> AAACOT | 0.00 | CC -> AT | GOCG -> AAAT | |
| Polymorphism Tupe | (transition) | (transversion) | (transition) | Dekrison | | (transversion) | Substitution | (transversion) | Deletion | (Interspersion) | (transidion) | Amonthian . | (bassilion) | (transversion) | | | | | | Detetion | (transversion) | | Defetion | | Deletion | Substitution | (transversion | Substitution | Substitution | Substitution | 1 | Substitution | Substitution | (transversion) | Galestination | Substitution | |
| Variant Frequency | 87.0% | \$7.0% | 94.5% | 38.9% | | 94.6% | 93.4% | 86.8% | 100.0% | 81.5% | 95.8% | 43.2% | 97.4% | 96.1% | | | | | | 40.3% | 88.8% | | 67.2% | | 99.9% | 90.6% | 98.4% | 96.2% | 88.9% | 95.25 | | 98.5% | 93.0% | 95.75 | 92.4% -> 92.4% | 93.6% -> 94.4% | |
| Variant D.Maker (approximate | 0 0 | 0 | 0 | 0.00000058 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | 4.15.40 | 0 | | 1.25.289 | - | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| Contract Contract Sectors and | | | | | | | | | | | | | | | | | - | | - | 110.10 | | | | | | | | | | | | | | | | | - |
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| On 482T (55%) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Name | c | c | A | | TC | T | COC | c | | T | 0 | | c | | c | T | TOA | | 0 | | 6 | T | | A . | | TOO | T | OTT | OTCAOTTO | | 0 | TT | AAACOT | 0 | AT | AAATO . | |
| Tube | Polymorphism | Polymorphism | Folymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism I | Polymorphism | Polymorphism | Polymorphism | 2 · · · · | Polymorphism | | Polymorphism | Polymorphism | Pohrnorphism | | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Pohmorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | Polymorphism | | Antonio P | okmonitism | Polymorphism / | Polymorphism | Polymorphism | Polymorphium | |
| Minimum | 184 | 220 | 274 | 305 | 354 | 364 | 367 | 371 | 373 | 345 | 406 | | 471 | | 463 | 480 | 494 | | 508 | \$21 | 520 | 545 | 822 | 629 | 645 | 665 | 478 | 738 | 742 | | 750 | 752 | 755 | 763 | 765 | 768 | |
| Maairman | 184 | 220 | 274 | 305 | 355 | 364 | 369 | 371 | 375 | 345 | 406 | | 421 | | 463 | 482 | 486 | | 508 | 521 | 530 | 545 | 622 | 629 | 645 | 667 | 478 | 740 | 7.49 | | 750 | 753 | 760 | 763 | 766 | 772 | |
| Change | T-2.C | 0.00 | 0.24 | (714-> (713 | OT o TO | O.o.T | TOT > COC | 0.00 | ACT | AnT | 4.20 | | TAC | | TAR | COT | 000 -> 704 | | 4.20 | 1014 -> 1013 | Tab | G.o.T | (117-2-(116) | 0.24 | -0 | G44-> 700 | A | 400 -> 0TT | COOTCCA -> OTCAOTTC | | T-2.0 | AD ATT T | TTTCO -> AAACOT | 6.0.0 | CC -2 AT | 00004-2 44470 | 4 |
| Dokenorshipp Tune | (Pranelline) | (transversion) | (manufactor) | Deletion | Substitution | (transumericae) | Schellingen | (transversion) | Deletion | Peacewareacol | (transition) | | (hospition) | | (transition) | (manufactor) | Substitution. | | (manuficer) | Deletion | (International) | (Instrumention) | Deletion | (transition) | Deletion | Substitution | (transmission | Submitteen | Future interest | 7 17 | (transportion) | Substitution | Substitution | (transportion) | Substitution | Futuritation. | 1 |
| Variant Frequency | 88.7% | \$7.6% | 66.7% | 34.6% | 83.0% | 98.1% | 94.7% | 82.3% | 100.0% | 88.0% | 95.9% | | 94.2% | | 88.5% | 100.0% | 98.0% | | 64.7% | 37.0% | 25.5% | 95.7% | 07.4% | 93.5% | 99.7% | 91.2% | 99.3% | 55.6% | | | 99.5% | 64.9% | 83.0% | 96.6% | 95.4% | 77.0% -2 95.9% | |
| Variant P-Value fapornale | | 0 | 0 | 1.16.21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | | 0 | 0 | 0 | | 0 | 2.15-70 | 0. | | | 0 | | | 0 | 0 | | | 0 | 0 | 0 | | 0 | 2.56-253 | |
| a second a construction of the second s | | | | · • • • • • • • • • | | | | - | | | | | | | - | | - 4 | | - 4 | - m.m 170 | | | | | | | · • | | | | | | | | | A | - |

| Pilosella officinarum | 3 alleles |
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| (Caen) | |
| - 647C (20%) | 1 alete |
| - 647 (80%)) | |
| - 232C (50%) | 1 akto |
| - 232T (SON) | 1 alete |

| Name Type Minersum Masimum Changa Programphian Type Variant P-Value (approxim | T Polymorphism 173 173 C -> T (harvikos) 6445 0 | C Polymorphism 185 185 T→C (familion) 200.0% 0 | T Polymopham P 190 290 C-9 T (barsitor) 40.0% | G 237 297 A -> G (transition) 320.0% 0 | G 209 208 A -> G (transitor) 380.4% 0 | TGA 221 223 GGC -> TGA Substitution 300.4% 0 | T Polymorphism 251 251 C→T (transition) 76.9% 0 | 0 Polymorphism 254 254 € → 6 (transvension) 15.95 0 | A Poymophan 257 257 C -> A (transvension 84.0% 0 | 0 Polymophism 262 263 T = 0 (barswenker) 78.5% 0 | | | | Polymorphism 305 305 (T)4 -> (T)3 Dekton 77.3% 0 | | | Polymorphon 274 375 467 Deletion 190.0% 0 | T Polymorphy 306 306 A → T Pransvenso 30.9% 0 | 0 Polymorpho 437 437 A⇒6 (hannikor 0 | ADTTODO Polymorph 413 413 +ASTTOGA Insertion 98.1% 0 | 100 tan 000 | TOA Polymorphis 485 487 0666 -> TO Subsidiation 206,0% 0 | A | 0 Polymosphio 500 A - 0 (transition) 38.2% 0 | Polymorphism 522 522 (6)4 -> (6)3 Dektion 23.4% 1.18-72 | | | C Polymorphism 565 565 566 6 -> C (transversion) 200.0% 0 | | 623 623 623 (TJP -> (T)6 Definition 64.4% 0 | .0 | T 055 055 6-3 T misersion) 536.8% 0 | 6 styrrotphon 555 60 A -> 6 (ramilion) 190.0% 0 | Poly Add Sat | 0117 728 741 8-> 017 545 0 | 6T 743 744 CC -> 6T Sabulitation 96,3% 0 | C Folymorphism 745 6 → C (tamiversion) 78.1% 0 | 6 | AOTTOO Polymorprison 745 751 TCCAT -> AGTTO Subsidiation 92.5% 0 | TT Polymorphism 753 754 4G -> TT Subsibilition 98,7% 0 | AAACGT Polymorpham 750 761 FTTTEG -> AAAC Subalikason 94.0% 0 | 0 Polynosphia 764 764 276 26-6 (temosmoo 96.0% 0 | AT Polymorphism 765 767 CC -> AT 5 Subsitiation 95.9% -> 96.9 0 | AAATG Polymorphi 759 773 660564-> A 5464540 87,8% > 91 0 | AAATO KAATO KARTA |
|--|---|--|--|--|---|---|---|--|---|--|--|--|---|--|---|---|--|--|---|--|--|---|--|--|---|--|---|---|---|---|---|---|--|---|---|--|--|--|---|---|---|---|--|---|----------------------------|
| P-C 647C (39%) Name Type Miserwan Klastnen Change Pagmoshion Type Varient Frequency Varient Frequency | T Polymorphism 173 173 €⇒T jtravelinn(92,7% 1020 0 | C Popmoqdiano 185 185 T → C (tarvellens) 298,0% | | 6 19jmugdism / 197 337 A - 6 (tansiton) 95,9% 0 | 6 208 208 308 A ≥ 0 (transitor) 180.05 0 | TGA Polymorphsen 221 223 GGC → TGA Sabrithation 100.9% 0 | | | | | A Polymorphism 275 G + A (Iteration) 80,1% 0 | A Polymorphism 256 256 G - A (transfor) 24,2% 0 | | Polymorphon 308 308 (T)4 -> (T)8 October 54.7% 2.25-49 | A Putymorphism 311 0.5 A (transition) 64,9% 0 | 6 Polymoruthian 2773 1773 T → 0 (transvertision) <u>66.495</u> 0 | Dolymorphise 274 278 407 Detetion 99,3% 0 | T Polymouths 386 A + T (baroversio 60.Ph 0 | 0 | AGTTOGO Philymouth 419 413 +AGTTOGO Insertion 82,9% 0 | 00 ten 000 | TOA Pulymorphia 485 487 000 → TO Subsidiar 98,8% 0 | 6 Polymorphie 490 490 T + 0 (Tarswende 77.2% | 6 500 500 A ≠ 0 (*ranston) 0 | Polymorphism 522 527 (0)4 + (0)3 Deterion H.1% 1.4(-14 | 0 Polymosptiam 531 535 T ⇒ 0 (hamsensket) 52 Ps 0 | A Polymorphism 520 G.+A (transfort) 75.6% 0 | C Polymorphism 585 585 6 + C (transversor) 306.0% 0 | TGT Polyimograym 805 904 TTO(2 → (TTO(2) Insertion 92 3% 0 | tiymouthun 9 623 623 (TT2 + (T)6 Deletion 73.8% 4.66: 170 | okmerythiam 646 646 -G Cektion 0 | T 966 966 966 965 969 969 100,0% | G Hymoiphish 980 980 (Familion) 180.0% 0 | Proj. | 017 738 741 5+017 5525 0 | 6T 741 744 744 00 + 0T Sobsibilitor 95,0% 0 | A Polymosphium 745 745 0 → A (transition) 65.Ps 0 | C Polymouthum 745 6 -> C (harownoice) 80 7% 4.66-208 | AGTTOG Potencrytism 740 751 TOCAT -> AGTTO Satisfactor 90.0% 0 | TT Polymorphan 751 754 A G + TT Sdetshullon 7 99,7% 0 | AAACGT Putymouphism 256 781 TTTTCQ -> AAAC Substitution 9 0 | G Polymorphis 764 764 764 764 764 97.45 97.45 0 97.45 | AT Polymorphon 767 767 767 60 -> AT 5 Subbliston 85.7% -> 85.4 0 | AAAT Polymorphi 750 772 GOCO -> A Soleman No 94,9% -> 9 | AAAT Ion N.2% |
| P-C. 647A (80%) Name Type Morean Masteria Change Polycontentor Type Variant P-requestory Variant P-value (upprovint | T Polymorphum 173 173 € ⇒ T Itransion 57.8% | e Polymorphem 185 115 T = C Jransford 380.0% d | F Polymospheren 220 C → T (transition) et ms 0 | 6 227 237 A ⇒ 6 (transition) 320.0% 0 | 6 209 200 A ≥ 0 (herestore) 350.0% 0 | TGA Polymorpham (221 222 GGC ⇒ TGA Substitutor 300.9% 0 | T Polymorphium 251 251 C => T (transition) 97.8% 0 | 6 Polymorphan 254 254 C = 6 (transversion) 89.4% 0 | A Polymorphism 257 C = A (framoversion 300.0% 1 | 6 Folymophan 262 210 T + 6 (hannerson) 81.5% 0 | | | | Polymorphism 205 205 (T)4 - (T)3 Deletion 82.7% 1 | | | Polymorphism 274 376 407 Deletion 196.0% 0 | Y Polymophie 306 A ⇔ T [Dansersio 27.2% 0 | 0 Polymorpha 427 437 A ⇒ 0 (harothor 23.7% 0 | AGTTODO | 266 Sun COG | 16A Polymophie 487 690 or 10 Substantion 200,9% 0 | | 6 Polymorphise 500 A > 0 (transition) 312N 0 | Projytocepsizen 522 522 00j4 e (6)3 00j45ten 32,9% 36,50 | | | C Polymorphism 188 6 € (framsenson) 198.0% 0 | | cymosphan 622 f017 ÷ f036 Deletion 6 C | 2 | T 668 669 0 → T 870-7500 200.75 0 | 6 5(morphun 688 688 688 680 (ramston) 180.0% 0 | Poly AGO Sub | 017 730 741 0-017 0 1455 0 | 6FC obmoshem 743 745 CO > 6TC Sebstution BLPh 0 | | 0 | AGTTOG Polymorphism 751 TCCAT - AGTTO Substitution 90.5% 0 | TY Dogmosphile 753 754 0 AQ->TY Substitution ML4% 0 | AAACGT Poijmophum 785 782 FTTTCO + AAAC Sukstaan 94,7% 0 | G Folymorphia 764 764 81 C + G (Famorena) 86.0% 0 | AT Disjenception 768 767 CC -> AT 5umstation 94.2% 0 | n Polymoph 700 000000 - A 00000 - A 00000 - A 00000 - A 00000 - A | G Horo Bior B7.2% |
| P-C_6674 (89%) + 232C (Name Type Mannan Masman Masman Change Polymoration Type Variat Programsy Variat Provider Opproven | 50%) · · · · · · · · · · · · · · · · · · · | C Polymosphane 185 T -> C (Introduction) 388.0% 0 | | G 227 A ⇒ G (transition) 280.0% 0 | 0 308 208 A → G (transition) 210.4% 0 | TOA 221 225 ODC > TOA Sabiahuton 200.9% 0 | T Polymorphism 251 251 C -> T (transferi) 84.4% 8 | 0 254 254 C > 0 (transversice) 00.05 0 | A Polymorphism 257 C > A (transversion 200.0% 0 | 0 Prájmogikism 282 7 0 0 (hansverske) 90.76 0 | | | Polyncophis 277 277 40 Deletion 25.5% 5.65-33 | Polymorphism 305 (T)4 -> (T)3 Dektour 52.4% 6 | | | Polymorphise 204 205 AGT Deletion 100.0% 0 | 1 | 0 Polymorphe 457 457 A > 0 (Pansilium 82.7% 0 | AOTTOOC Polymorph 413 413 413 413 413 413 413 413 413 413 | coo n | TQA Patymophis 405 407 GGC = TQ Substitutes 208.5% 0 | | 0 Volymorphing 500 500 A ≥ 0 (Passitor) 56.0% 0 | P-dynosphisa 522 532 (0)4 ⇒ (0)3 Deletion 31.8% 8.51-27 | | | C Polymorphism 566 6 in C (transversion) 100.0% 0 | | tdynoopteen 625 625 (T)3 ⇒ (T)6 Deletion 0 0 | | T 566 066 0 ⇒ T 200.P% 0 | 0 950 950 A ⇒ G (ranstor) 100.0% 0 | 1040 ADD 540 3 | 0TT norphism 720 741 5 - 0TT 1 1 1 1 1 1 1 1 1 1 1 1 1 | GTC Synophem 743 745 CG & GTC Subsitution 90.PN 0 | | 0 | AOTTOO Polymorphism 765 755 TCCAT -> AOTTO Substation 92.4% 0 | TT Polymospher 253 754 AG ⇒ TT Sutstitution BLTN 0 | AAACGT Pogenogitiser 750 753 753 753 753 753 754 754 754 754 754 754 754 754 754 754 | 0 1459/morphore 764 764 01 C >> 0 (Ransversor 98.0% 0 | AT Projenceptiest 785 CC -> AT Substatution 96.0% | AAATG Fotymosph 390 773 600CGA > A 5-85056 81.2% > 8 1.05-17 | AAATC Non 17.7% |
| P-C 667A (90%) - 232T () Name Type Mainten Mastham Change Polymorphism Type Variant Preguency Variant Preguency | 56% Polynorphism 173 173 173 173 273 273 173 173 173 173 173 173 173 1 | C Popriocation 155 155 155 155 7 → C (Barotion) 100.0% | T Potemophism P 190 190 2 -> T (transtore) 81.9% | 0 237 237 337 A > 0 (transition) 380.0% | 0 238 208 A ⇒ 0 (transition) 200% | TOA Polymorphism 221 223 GBC or TOA Sabothaton 350 Ph 0 | T Poprocristen 251 251 C → T (transition) 97.45 0 | Polymorphism 254 C -> G (transversion) 95 | A Poymorphism 250 250 C -> A (hanswerster) 300.0% | 6 Patymaphen 282 292 T > 0 (nancwosar) 56.76 | Polymorphese 276 277 (TG)4 -= (TG)1 Deterior 25.5% 8 16-59 | | | Polymorphism 305 308 (T)4 -> (T)3 Doktion 83.6% | | | Polymorphism 374 378 407 Deletion 100.0% | T Projemophy 306 A = T Pransversko 41.95 | 0 Popmorphi 417 437 A → G (transition (transition | ADTTOOC Polymorph 423 423 423 423 423 423 423 423 423 423 | 200 T esm Polymorp dig 400 0 a - 1 bitansuer 32 00 | 100 TOA 100 Polymophis 45 457 7 000 -> 70 5-8588.459 00 Polymophis 5-8588.459 00 Polymophis 5-859 5-859 5-95 | | Polymorphis 508 508 (6)3 ⇒ (6)3 Databan 28.0% | | | | C Polymorphism 568 9 -> C (fransversion) 100.0% | | 623 623 623 048604 048604 047% | | T 005 046 0-2 T antiversion) 120,05 | G Spenciphen Pot 555 560 A > Q (ranstor) 100 Ps | G 1000000000000000000000000000000000000 | 0TT 728 341 0 - GTT C balleton 1 8.5% | 0TC synophism 762 765 CG > 0TC Subsitution 8776 | | 0 | AGTTOG Polymutatism 746 751 TOCAT -> AGTTO Substation 92.76 | TT Polymosther 753 754 AG = TT Subseliation 98.75 | AAACOT Polymospham 756 751 TTTTCG > AAAC Substitution 92,2% | 0 Polymorphic 754 754 01 C > 0 (Ransverso) 96.2% | AT Polymorphen 785 787 CC -> AT 0 Jacobation 96.2% | AAAT Polymoph 369 772 GOCC -> A Sutoma 87,8% -> S | AAAT BOT BT 25 |

Pilosella officinarum 4 alleles (Dijon)

P-D DOMINANT ALLELE 1 alek

| P-D DOWNANT ALLELS | 1,0000 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|---|--|---|--|---|---|--|--|--|---|--|--|--|---|--|---|--|--|--|---|--|---|--|---|---|---|--|---|--|---|--|---|--|--|---|---|--|--|---|
| Name Type Misroum Maxmum Change Polymophism Type Variant Frequency Variant P-Value (approximate | T Pulymorphism 349 340 6 -> T (transversion) 48.5% 5.30-37 | Polymosphism 277 277 4 Dekreon 25.0% 1.12-115 | Polymorphism 306 306 (T)4 → (T)3 Deletion 85.6% 0 | Polymorphism Polym | Aymorphism Po 522 522 0)4 ⇒ (6)3 (1 2)4 to (6)3 1.00-71 | olymorphiseo 623 623 T)T → (T)6 Deletion 64.0% | 054mosphism 759 759 (1)5 -> (1)4 Deletion 28.2% 1.55-100 | C Polymorphism 933 933 T.⇒C (harsitor) 97.9% 0 | Polymorphism 1347 1347 (C13 -> (C12 Deletion 28.9% 5.22-60 | Polymosphism 1884 1886 (T)6 -> (T)6 Deketon 25.5% 0.76-20 | Polymorphern 1985 1885 (T)d -> (T)6 Deletion 86.2% 0 | Polymorphism 1992 1992 (CJ7 -> (C)6 Deletion 94.1% 0 | Pulymorphism 1993 2993 (CJ7 +) (C)8 Deletion 75.0% 4.45-259 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| P-0 RECESSIVE ALLELES - 2527 (45%) - 2620 (55%) 2110 (50%) 211A (50%) | 1 alete 1 alete 1 alete | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Name Type Merson Masmon Change Polymophism Type Variant Prequency Variant P-value Jappcomate | C Polymorphism 385 T -> C (transition) 51.4% | 6 Polymosphism 157 A = 0 (transition) 93.1% 0 | G Polymorphism 209 200 A⇒G (transition) \$2.9% 0 | T Polymorphism 217 217 A = T (transversion) 38.0% 0 | TGA Poi 221 223 GC ⇒ TGA Substitution 94.7% 0 | T dymorphism 251 251 C → T (transition) 55.2% 0 | 6 101/morphian 254 C => 6 banversion 55.4% 0 | A Polymorphism 257 257 C - 3 A (transversion) 55.2% 0 | 0 Polymorphism 252 252 T -> 0 (transvension) 55.4% 0 | A Polymorphism 275 0 -> A (transition) 35.1% 0 | | | A Polymorphism 296 0 = A (transition) 34.PN 0 | Polymorphism 306 (T)4 → (T)3 Dekton 52.5% 0 | A 211 211 6 → A (transition) 66.0% 0 | Polymorphism 2014 2016 40 T Deketon 96.5% 0 | T Polymorphism 386 8 = T (hansversion) 37.3% 0 | 6 Polymorphism 407 A > 0 (transition) 25.2% 0 | AGTTGGCGG Polymorphism 429 438 +AGTTGGCGG Intertion 98.7% 0 | T Polymorphism 458 6 → T (transversion) 47.2% 0 | | TGA Polymorphism 485 487 000 > TGA Substitution 95.7% 0 | | 6 tolymorphism P 509 A ⇒ 0 (transition) 25.7% 0 | *alymorphism P 522 522 (0)4 → (0)3 Deletion (31.1% 9.05-63 | C 500 500 0 → C (tansversion) 96.2% 0 | | Polymorphism | | T bolymorphism 666 6566 G → T (transversion) 97.5% 0 | G 668 668 A ⇒ G (transition) 96.8% 0 | GTT Polymorphism 229 741 AGG ⇒ GTT Substitution 94.375 0 | GTCAG Polymorphism 743 747 CCOOT -> GTCAO Substitution 85.2% 0 | TT06 Polymorphism 751 CCAT → TT00 Substitution 94.5% 0 | TT Polymorphom 753 754 AG = TT Subsitiation 97.3% 0 | AAACGT Polymorphism 756 751 TTTTCO ~ AAACO Substitution 92,7% 0 | G Polymorphism 764 764 (transversion) 97,2% 0 | AT Polymorphism 766 767 CC -> AT Substitution 95.4% 0 | AAATG Pojmoshom 709 773 IOCGA -> AAATG Substitution 82.3% -> 95.8% 0 |
| P-O. 252T (45%) Name Type Misrisan Maximum Change Polymophism Type Variant P-Value (approximate Variant P-Value (approximate | | G Polymosphere 197 197 ▲ → G (transition) 87.3% 0 | G Polymorphism 200 200 A ⇒ G (Parelifice) 88.0% 0 | 0 | TGA stymosphism 221 223 GC -> TGA Satisfution 88.6% 0 | | | | | A Polymorphism 275 225 G ⇔ A (hansidon) 76.4% 0 | | | A Polymorphism 298 0 -> A (hamilion) 78.45 0 | Polymorphism 306 306 (T)A -> (T)3 Deletion 38.256 6.7E-54 | A Polymorphism 1 211 G ⇒ A (transition) 85.8% 0 | 208ymooptissee 374 325 -440T Dektion 92.396 0 | T Dolymorphism 388 A ⇒ T (barrotice) 78.2% 0 | | AGTTGGCGG Polymonthism 419 418 +ADTTGGCGG Insertion 85.2% 0 | | | TGA Polymorphism 485 487 GGG > TGA Substitution 60.9% 0 | P | G 509 509 509 A ⇒ G (hanston) 70.2% | Nalymorphismi P 522 522 (G)4 -> (G)3 Deletion 10:45 1.11:47 | C S50 550 G ⇒ C (ransversion) 82.9% 0 | TGT Polymorphism 605 604 TTGJ2 → (TTGJ3 Inselfion 26.9% 0 | Polymorphism 1 623 623 (7)7 ⇒ (7)6 Doletice 65.9% 0 | olymorphian 646 646 646 0 0 0 0 | T 560 660 G ⇒ T (Tattoversion) 98.5% 0 | G 668 688 A ⇒ G (transition) 95.3% 0 | GTT Polymorphism 720 742 AOG > GTT Substitution B4.356 0 | GTCAGT Polymorphism 743 748 CCGOTC ~ GTCAG Substitution 88.4% 0 | 100 Polymorphism 749 751 CAT → TGG Substitution 94.2% 0 | TT Poymorphism 753 754 AG -> TT Substitution 87.7% 0 | AAACGT Poymorghism 756 761 TTTTCG -> AAACG Subsitiution 93.3% 0 | G Polymophsm 764 764 17 C ⇒ G (harmversion 87,4% 0 | AT Poymorphism 765 767 CC-3-AT Substitution 85.5% 0 | AAATG Poymorphism 759 773 IGCGA -> AAATG Substitution \$3,2% -> 95,9% 0 |
| P-0_2820 (19%) Name Type Morean Moanan Change Polymoshiom Type Variant Frequency Variant Frequency | C Polymorphism 185 180 T → C (transition) 84.0% 0 | G Potymospheses 197 A -> G (travestion) 97.8% 0 | G Potymouphism 200 200 A → G (transition) 96.3% 0 | P. | TGA dymorphism Po 221 223 GC → TGA labstitution 99.7% 0 | T olymorphism 251 251 € → T (transition) 97.8% 0 | 6 154 254 254 254 6 5 5 5 8 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 | A Polymotphasm 2557 C -> A (transversion) 96.2% 0 | G Polymorphism 262 252 7 → G (transversion) 300.0% 0 | | | Polymorphism 277 -0 Detetion 25.3% 1.18-100 | | Polymorphism 306 306 (T)4 → (T)3 Deletion 83.8% 0 | A Potymorphism 311 311 G → A (transition) 49.8% 0 | Polymospisum 334 325 -AGT Deletion 95.8% 0 | | G Polymorphism 407 A → G (transition) 39.1% 0 | AGTTGGCGG Polymosthism 410 +AGTTGGCGG Insention 8L4% 0 | T Polymorphism 458 6.⇒T (transversion) 73.8% 0 | A Potymotohism 476 6 → A (bassilion) 36.3% 0 | TGA Polymorphism 8 485 487 GGG → TGA Substitution 97.3% 0 | Polymorphism 508 508 (GJ3 → (GJ2 Deletion 34.3% 1.2E-05 | p | Polymorphism 522 522 (G)4 ↔ (G)3 Deletion 36.4% 7.48-53 | C 506 506 G ⇒ C (ranoversion) 96.2% 0 | | Polymorphism 623 623 (117 ⇒ (116 Deletion 60.0% 0 | 5 | T 866 666 G ⇔ T (ramute sion) 88,9% 0 | 0 olymorphiam 668 668 A → G (transilion) 98.8% 0 | OTT Polymorphism 720 741 AGG -> OTT Substitution 84.2% 0 | GTCAO Polymorphism 747 747 CCOGT -> GTCAG Subsitution 86.8% 0 | TTOG Polymosphan 748 751 CCAT → TTOC Sobstitution 94.3% 0 | TT Polymorphism 753 754 AG ⇒ TT Substitution 96,1% 0 | AAACOT Polymorphism 756 761 761 761 Subsistation 81.7% 0 | G Polymorphism 764 764 (transversion 96.5% 0 | AT Potymorphism 765 767 CC -> AT Substitution 84.5% 0 | AAATG Polymorphism 703 705 Subsidiation Subsidiation 80.5% -> 95.5% 0 |
| P-D 2020 59% + 3226 59% Name Type Meanurs Maximum Change Polymogramm Type Variant Frequency Variant Frequency | C Polymorphism 185 185 T -> C (Transition) 84.3% 0 | G Polymorphism 197 A -> 0 (sansition) \$7.8% 0 | 0 Polymorphism 200 209 A -> 0 (transition) 98.1% 0 | Pr 0 | TGA symorphism Po 223 GC → TGA satisfikition (96.8% 0 | T olymorphism P 251 251 € → T (transition) 97.9% 0 | G olymophium 254 254 € ⇒ G (tamoversion) 99.2% 0 | A Polymorphism 257 257 C -> A (transversion) 96.4% 0 | 0 Polymorphom 282 262 T -> G (transversion) 500.0% 0 | | Polymorphism 276 277 (TG)4 -> (TG)3 Deletion 25.5% 1.88:51 | | | Polymorphism 306 306 (T)4 -> (T)3 Detetion 82.6% 1,42-250 | | Polymorphism 374 276 -AGT Deletion 99.7% 0 | | | AGTTOGCDG Polymorphism 410 •AGTTOGCOG Insertion 78.9% 0 | T Polymorpheam 458 458 G -> T (transversion) 74.7% 0 | | TGA Polymorphism & 485 487 GGG -> TGA Substitution 96.6% 0 | Polymorpheam 508 508 (6)3 -> (6)2 Deletion 34.3% 1.7E-46 | | C colymorphism P 522 522 (G)4 -> (G)3 Deletion 20.8% 2.48-28 | olymorphism 566 566 € → C (transversion) 96.9% 0 | | Polymorphism 623 623 (T)? ~ (T)8 Deletion 87.5% 0 | | T 666 660 G -> T (ransversion) 98.0% 0 | G olymorphism 648 648 A > G (Itransition) 96.2% 0 | GTT Polymorphism 729 741 AGG > GTT Substitution 94.2% 0 | GTCAGT Polymophism 743 749 5000TC + GTCAG Substitution 84.8% 0 | TGO Polymorphism 740 751 CAT -> TGO Substitution 96.2% 0 | TT Polymorphism 753 754 AG > TT Substitution 96.3N 0 | AAACDT Polymorphen 756 761 TTTTCG > AAACD Subshibion 91.0% 0 | G Polymorphism 764 764 JT C > 6 (transversion 96.2% 0 | AT Polymorphism 785 787 CC -> AT Substitution 94,2% 0 | AAATG Pojwooptism 765 773 IOCOA > AAATG Substitution BLSN > 95.4% 1.56-283 |
| PLD 282G 5994 + 31114 59% Name Type Maximum Change Polymophism Type Warlant Frequency Warder Division forcements | C Polymorphizer 185 185 T → C (transition) 81.9% 0 | 0 Polymorphian 197 197 A → 0 (transition) 9 | G Pulymorphiant 200 200 A > G (transition) 90.6% | PC | TGA. Jysectshiam Po 223 GC → TGA latestitision (99.9% | T olymorphism P 251 251 C + T (transition) (97.8% 0 | 0 olyeroghism 254 254 C → G framversion) 90.05 | A Polymorphism 257 2537 C -> A (transversion) BL2N 0 | G Polyncephiam 282 382 T→G (transversion) 100.0% 0 | | | | | Polymosphiam 1 306 306 (T)4 → (T)3 Detetion 0 | A 211 311 G ⇒ A (transitor) 100.0% 0 | Adymorphism 274 275 -AGT Detelion 99% 0 | | G Potymorphism 407 407 A → G (transition) 73.4% 0 | AGTTOGCOO Polymotphism 410 +AGTTOGCOG Insertion 83.4% 0 | T Polymorphism 458 6 → T (transversion) 73.2% 0 | A Polymorphism 476 6.9 A (transition) 6.0% 0 | TQA Polymorphium # 485 487 GGG ⇒ TGA Substitution 95.2% 0 | Polymorphism 508 508 (G)3 → (G)2 Deletion 34.3% | | Polymorphism P 522 522 (G)4 → (G)3 Deletion 1.5F-23 | C obymosphism 566 566 G ⇒ C (transversion) 95.5% 0 | | Polymorphiam 623 623 (117 → (11)6 Deterion 0 | | T olymosphism F 666 666 G ⇒ T (tansversion) 91.0% | G olymosphiam 668 668 Å ⇒ G (transition) 97.25 | OTT Polymorphism 729 745 AGG > GTT Subsitution 842% | 0TCA Polymorphism 743 766 CCDS → GTCA Substitution 88.4% 0 | GTTOD Polymorphism 742 751 TOCAT -> GTTO Substitution 92.5% 0 | 17 Polymorphism 753 6 AG → TT Satistitution 92.21% 0 | AAACOT Polymorphism 750 751 TTTTCG -> AAACO Substitution 92.0% 0 | G Polymorphium 764 764 17 C → G (transversion) 91.0% | A7 Polymophism 766 767 CC → AT Subsitiation HJS 0 | AAATO Poymophiam 769 773 IGCGA → AAATG Sutsiliusion 78.2% → 95.6% 8.56.245 |

| iosel | la officinarum | -4 | alleles |
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| (Lewis Pass) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| DOMINANT ALLELE | 1 allele | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Name Type Mismum Maximum Change Polymophism Type Variant Prequency Variant Prequency | Polymorphism 275 276 (TG)4 -> (TG) Deletion 26.6% 9.52-194 | Polymorphese 305 325 (17)4 -> (17)3 Deletion 89.9% 0 | Polymorphism P 507 507 [6]3 -> (6)2 Orletion 36.4% 0 | Polymorphism Po 523 523 (0)4 -> (0)3 Deletion 32.2% 5.45-215 | lymorphism P 622 622 T)7 -> (T)6 Deletion 62.7% 0 | olymorphism 758 758 (T)5 -> (T)4 Detetion 28.9% 7.1E-240 | A Polymorphism #53 #52 (A)# ⇒ (A)9 insertion 33.4% 0.000034 | C Polymorphism 932 932 T -> C (transition) 58.0% 0 | Polymorphism 2046 2046 (T)4 -> (T)3 Deletion 26.7% 0 | Polymorphism 2346 2346 (C)3 -> (C)2 Deletion 28.0% 5.32-96 | Polymorphism 1803 1803 (7)6 -> (7)6 Deletion 27.1% 5.6E-76 | Polymorphism 1894 1884 (T)4 -> (T)5 Detetion 85.8% 0 | Polymorphism 1991 2991 (C)7 → (C)6 Deletion 93.7% 0 | Polymorphism 1992 1992 (C)7 -> (C)6 Deletion 74.1% 0 | | | | | | | | | | | | | | | | | | | | | | | | |
| RECESSIVE ALLELES | | | Very messy data | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| - 250C (50%) - 250T (44%) - 310G (47%) - 310A (53%) | 1 aleie 1 aleie 1 aleie | | unsure of the cho | nce for the 3 rd alle | nia | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SNPs Name Type Mixmum Change Polymophish Type Variant P-Value (approximate Variant P-Value (approximate | C Polymorphen 184 184 T -> C (transition) 41.9% 0 | 6 Polymopher 195 195 A → 0 (transition) 85.2% 0 | G 208 208 A → C (transition) 85.5% 0 | Po OS | TGA dymorphism P 220 222 GC → TGA katositution 84.3% 0 | T Yolymorphism 250 250 C → T (transition) 46.1% 0 | 6 Polymorphism 253 253 € → 6 (transversion) 43.0% 0 | A Polymorphism 258 259 C → A (transversion) 42,2% 0 | 0 Polymorphism 201 7 → 6 (Tarisversion) 43.0% 0 | A Polymosphaen 274 274 6 -> A (transkor) 38.4% 0 | A 297 297 G ⇒ A (transition) 25.4% 0 | Polymorphism 1 305 305 (T)4 -> (T)3 Deletion 45.4% 1.6E-291 | A Tolymoshism 310 310 G ⇒ A (transition) 65.2% 0 | Polymorphism 373 375 -AGT Deketion 93.2% 0 | T Polymorphism 385 A → T (transversion) 34.6% 0 | | AGTTOBCDG Polymorphism 458 417 •AGTTOCCCC Internos 74.2% 0 | T Polymorphear 457 637 637 (transversion 136.3% 0 | | TGA Polymorphism 484 486 GGG ⇒ TGA Substitution 89.0% © | | 0 Polymorphism 508 508 A -> 0 (transition) 27.1% 0 | Polymorphism 521 521 (6)4 ⇒ (6)3 Deletion 32.4% 56-125 | C Polymorphism 565 515 6 -> C (transversion) 89.5% 0 | Polymorphism 622 622 (7)7 → (7)8 Deletion 69.1% 0 | | T 605 605 605 605 (tensversion) 93.0% 0 | 0 667 667 A > 0 (transition) 94.5% 0 | 0117 Polymorphism 736 740 AOG -> 0117 Subsitiusion 95.3% 0 | GTCAGT Polymorphism 742 CCGGTC -> GTC/ Substitution 89.2% 0 | TGO Polymorp 748 750 GT CAT -> T Substan 95.5% 0 | G Nom Polymorphic 750 750 00 T → G 000 0 99.5% 0 | TT Polymorphise 752 753 AG > TT Substitution 98.4% 0 | AAACOT Polymorphism 755 TTTTC0 -> AAAC Subsitiation 91.5% 0 | 0 Polymorphism 763 763 07 C → 0 (transversion) 94.8% 0 | AT Polymorphism 765 CC -> AT Substitution 94.2% 0 | AAAT Polymoophism 785 773 GOCG > AAAT Substitution 94.5% > 94.5% 0 | G Potymorphism 772 772 A → C (transition) 82.9% 0 |
| Pi LP 250C 56% Name Type Mamum Mainum Change Polymophism Type Variant Prequency Variant Prequency | | 0 Polymorphism 196 296 A -> 0 (transition) 74.8% 0 | G Polymorphism P 208 208 A > G (transition) 74.4% 0 | T Tolymosphism Po 226 226 A -> T (transvenice) 36.5% 0 | T0A Aymorphism 220 222 GC → TGA abstitution 72.2% 0 | | | | | A Polymosphern 274 274 6 -> A (transition) 63.9% 0 | A Polymorphism 297 297 G -> A (transition) 64,2% 0 | Polymorphism 305 305 (T)4 -> (T)3 Deletion 38.4% 2.6E-63 | A 310 310 G > A (transition) 73.2% 0 | Polymorphism 373 325 -AGT Devision 83.9% 0 | T Polymorpheum 385 385 385 4.⇒ T (transversion) 60.5% 0 | | AGTTGGCGG Polymosphesm 418 417 •AGTTGGCGG Insertion 69.0% 0 | | | TOA Polymophen 484 660 -> TGA Substation 80374 0 | | G Polymorphism 908 938 A -> G (transition) 53.9% 0 | Polymorphism 523 523 (G)4 -> (G)3 Deletion 35.1% 5.4E-44 | C Polymorphism 565 565 6 -> C (transversion) 64.4% 0 | Polymorphism 622 622 (1)7 -> (1)6 Deletion 67.8% 0 | Polymorphism 645 645 -6 Deletion 33.0% 0 | T folymorphism 1 665 665 G → T (transversion) 92.0% 0 | G Polymorphism 667 657 A > 0 (transition) 92.5% 0 | OTT Polymorphism 738 740 AOG > OTT D Substitution 96.7% 0 | GTCAGT76 Polymorphism 742 749 COGTCCA +> GTC/ Substitution 85.7% 0 | 6176 | | TT Polymorphese 752 753 AG -> TT Substitution 98.0% 0 | AAACOT Polymorphism 755 760 TTTTCO-> AAAC Subsiduation 83.0% 0 | G Polymorphism 763 763 67 C → G (transversion) 97.8% 0 | AT Polymorphism 766 CC -> AT Substitution 95.2% 0 | AAATG Polymospikan 768 772 GGCCA → AAATG Subsitution 77.9% → 95.9% 7.7E-227 | |
| Pi LP 2507 44% Name Type Minman Maximum Change Polymophian Type Variant Prequency Variant P-Valee (approximate | C Polymorphism 184 184 T -> C (transition) 05.1% 0 | G Polymorphism 196 A → G (transition) 95.0% 0 | 0 Polymorphism 208 208 A ⇒ 0 (transition) 94.9% 0 | Pri G | TOA kymorphism P 220 222 GC → TGA kibistikion 83.7% 0 | T 250 250 C ⇒ T (transition) 290.0% 0 | G Polymorphism 253 253 C ⇒ G (transversion) 97,9% 0 | A Polymorphism 256 256 C → A (transversion) 96.7% 0 | G Polymorphism 281 291 7 → G (transversion) 93.4% 0 | | | Polymorphism 1 305 305 (T)4 -> (T)3 Deletion 62.4% 3.55-271 | A Polymorphism 310 G ⇒ A (transition) 53.2% 0 | Polymorphism 373 375 AGT Deletion 99.1% 0 | | D Polymorphism 406 406 A > G (transition) 41.4% 0 | AGTTGGCGG Polymorphism 428 417 •AGTTGGCGG Insertion 82.0% 0 | T Polymorphism 457 457 € ⇒ T (transvertison) 68.2% 0 | A Polymorphism 475 G > A (transition) 36.8% 0 | TGA Polymorphism 484 486 GOG ⇒ TGA Substitution 96.1% 0 | Polymorphism 507 507 (033 -> (032 Delation 35.5% 2.56-62 | | Polymorphism 521 521 (034 -> (033 Deletion 35.2% 7E-25 | C Polymorphism 565 565 G -> C (transversion) 541% 0 | Polymorphism 622 622 (1)7 → (1)8 Deletion 68.5% 0 | | T 005 065 0 ⇒ T (tansversion) 94.7% 0 | 0 967 667 A ≥ 0 (transition) 96.2% 0 | 01TT Polymorphism 738 740 ADG → 01TT D Subsidiation 96.0% 0 | OTCAOTTG Polymorphism 742 749 COGCCCA -> GTC/ Substitution 88.8% 0 | GTTG | G Polymorphic 750 ₹-> G (transversic 98.6% 0 | TT Polymorphon 752 753 AG > TT Substitution 98.2% 0 | AAACDT Polymorphism 785 760 TTTTCG -> AAAC Subsiliation \$2.2% 0 | G Polymorphism 763 763 67 C ⇒ G (Itansversion) 97.2% 0 | AT Polymorphism 765 766 CC → AT Substitution 95.1% → 95.2% 0 | A4A10 Polymorphism 788 772 GGCCA > AAAT0 Substitution 78/3% > 95.9% 1.36-257 | |
| Pi LP 250T 44% + 3100 47% Name Typio Maximum Maximum Change Polymorphom Type Variant P-Value (approximate Variant P-Value (approximate | C Polymorphism 184 154 T → C (transition) 85.4% 0 | G Polymorphian 196 A → G (transition) 95.2% 0 | G 208 208 A > G (transition) 94,2% 0 | 0 | TGA dymorphilim F 220 222 GC ⇒ TOA bubsitution 83.0% 0 | T 250 250 C ⇒ T (transition) 200.9% 0 | G Polymorphism 253 C = 0 (transversion) 97,4% 0 | A Polymorphism 256 256 C -> A (transversion) 96.2% 0 | G Polymorphism 281 T → G (transversion) 93.3% 0 | | | Polymorphism 305 (174 -> (173 Defesion 36.9% 3.96-111 | | Polymorphese 273 275 -AGT Deletion 98.7% 0 | | | AGTTGGCGG Polystorphism 428 427 •AOTTGGCGG Insertion 80.9% 0 | T Polymorphise 457 457 G ⇒ T (transversion 68.0% 0 | | TGA Polymorphism 456 GGG ⇒ TGA Substitution 94.1% 0 | Polymorphism 507 507 (6)3 ⇒ (6)2 Dektion 35.2% 18-28 | | Polymorphism 521 522 (0)4 + (0)3 Deletion 36.9% 26-15 | C Polymorphism 565 6 -> C (transversion) 93.3% 0 | Polymorphium 622 622 (1)7 -> (1)6 Deletion 88.0% 2.8E-292 | | T 605 605 0 ⇒ T (transversion) 95.2% 0 | G Potymorphism 667 A > 0 (transition) 96.4% G | 077 Polymorphism 738 740 740 740 800 + 077 Substitution 96.3% 0 | GTCAGTTG Polymorphism 742 749 COGTCCA -> GTC/ Substitution 82.7% 0 | отто | G Polymorphie 750 750 T→ G (transverse) 100.0% 0 | TT Potymorphism 752 753 AG → TT Subsiduation 98.1% 0 | AAACGT Polymorphism 755 780 TTTTCG > AAAC Substitution 91.9% 0 | G Polymorphism 763 763 07 C → G (transversion) 97.2% 0 | AT Polymorphism 765 765 CC → AT Substitution 94.9% 0 | AAAT Polymoghism 760 771 GGCG → AAAT Subsitution 94.0N → 95.7% 6 | |
| PI LP 2507 44% + 310A 53% Name Type Minimum Maximum Change Polymorphism Type Variant Programmery Variant Programmery | C Polymorphism 184 184 T-> C (transition) 84.7% | 0 Polymorphism 196 A -> G (transition) 94.9% 0 | 0 Polymorphism 208 208 A -> 0 (hanston) 95.6% | Pg Of S | TGA symophism F 220 222 GC > TGA substitution 9429 | T 250 250 C → T (transition) 100.0% | G Polymorphism 253 253 C -> G (transversion) 97.9% | A Polymorphism 256 256 C -> A (transversion) 97.1% | 0 Polymorphism 281 291 T→G (transversion) 93.5% | | | Polymorphism 1 305 305 (T)4 → (T)3 Deletion 4 96 194 | A Polymorphism 310 310 G -> A (transition) 109.0% | Polymorphism 373 375 -AGT Deletion 99-49 | | 0 Polymorphism 606 A⇒G (transition) 72.96 | AGTTOGCOO Polymorphism 428 417 •AGTTGGCGG Insertion 82.0 | T Polymorphism 457 457 G -> T (Dattovensort 00.00 0 | A Polymorphism 475 475 G -> A (transition) 43.9% | TOA Polymorphism 454 405 GGG -> TGA Substitution 97,9% | Polyanorphism 507 507 (G)3 -> (G)2 Defetion 28.6% | | Polymorphism 521 521 (G)4 -> (G)3 Deletion 34.1% | C Polymorphism 565 565 6 -> C (bansversool) 54.95 | Polymorphism 622 622 (T)7 → (T)6 Deletion 68.0% | | T fo5 665 645 G → T (transversion) 94.PN | 0 667 667 657 A > 0 (transition) 95.9% | OTT Polymorphism 738 740 ADG -> GTT D Substitution 95.7% | OTCAOTTO Polymorphism 749 COGTCCA -> GTC/ Substation 68.7% | атта | G Polymorphin 750 T-> G (transversio 96.4%) | TT Polymorphism 752 753 AG + TT 5 Substitution 96.49 | AAACOT Polymorphism 755 760 TTTTCG -> AAAC Substitution \$2.8% 0 | 0 Polymorphism 763 760 0T C -> G (blassversion) 97.2% 0 | AT Polymorphism 765 705 CC -> AT Substitution 95.7% -> 95.8% | AAATG Polymosphern 768 772 GGCGA -> AAATG Substitution 82.0% -> 96.2% | 2 |

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RIS DOMINANT ALLELE 1 alle

| Yantee Type Mermun Marmun Change Polyroophian Type Variant P-Value (approximate) | Polymosphan 300 (T)4 → (T)9 Deketion 78.7% 0 | Polymorphism Po 508 508 508 508 508 508 508 508 508 508 | olymsophiam Pr 522 522 034 -> (030 Extense 36.2% 0.000000 | Fymarphian Pol 623 623 7)P ← (7)9 12ektion 0 6027k 0 | (morphism Poly 250 36→ (7)4 (4) 264550 0 264550 0 264550 0 5.30-81 0 | morphan 853 853 8 ⇒ (A)T Seletion 64295 122-133 | ¥ I225 1225 A ⇒ T HIPS 0 1 | ymarghtan P 3347 3347 33 = (C)2 Oddaton 26.2% 3 65-28 | 6 vijmorphon 1745 1,245 A ≈ 0 (transhor) 0 0 | olymospham 1884 1884 (7)8 ⇒ (7)8 Dektika 25.4% 10000000214 | Polymorphism 3895 2895 (776 + (775 Datetion 88.7% 0 | Polymocalizari 2010 (C)7 -> (C)8 Deletion 98.0% 0 | Polymorphism 1990 1990 (CJ7 -> (C)8 Deletion 78.1% 1.42-126 | | | | | | dan e e e de e | | | | | | | | | | | | | | | | | | | | | | | | |
|--|---|---|---|--|--|---|--|--|--|--|--|---|---|--|---|--|---|---|----------------|---|---|---|--|--|---|---|---|--|---|---|---|--|--|--|--|--|---|--|---|--|--|--|--|
| R35. PECESSIVE ALLELES - 5364. (19%) - 5366. (19%) - 4536. (49%) - 4536. (52%) | 1 siele 1 siele 1 siele | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Name Type Missmum Mournum Change Polymorphoum Polymorphoum Variant Prequency Variant P-Value (approximate) | C Polymostism 185 180 T → C (fransition) 96.5% 0 | C Polynocythan 223 223 G -> C (tarsecsce) 97.P% 9 | T 253, 253 C ⇒ T (Tancilar) 25.0% 0 | A 273 273 275 G -> A Instables 0 4 | (rhoghian 276 277 (4 → (16)3 28,7% 6.4E-32 | mergibien Foly 305 4 ⇒ (1)3 17 3% 0 | T 305 305 0 → T 0 → T | 080 318 370 IT -> CGC dobusion 0 | C styrncoptions 177 177 G → C 177 177 G → C 177 177 0 → C 177 177 0 → C | algencephiaes 274 276 4637 Defetice 98.0% 0 | T 285 285 390 A ⇒ T (transversion) 87.7% 9 | 0 Polymorphism 407 407 A → 0 (ravestion) 87.8% 0 | AGTTODOOK Folynoufiae 433 433 •AGTTGGCD IDEFEN 30.4% 0 | C Pulyroophur 402 422 ¥ → C (hanalica) ML4% 0 | C Polymorphism 450 450 A > C (transversion) 60.000 0 | C Polymophise 484 494 Y or C (transition) 32.9% 0 | T Polymorphis at0 at0 c → T (humbler 32.4% 0 | TGA Polymorphi 485 487 000 > 10 Sabotuse 21.94 0 | ur) a | 0 Polyanarghi 309 A → 0 (transition 54.#5 0 | G Polymorph 500 510 AG -> G Doktor 27.0% 4.6E-7) | Polymorph 522 522 (6)# -> (5 Deleter 87.P% | 0 1500 Polymorph 500 500 17→0 0 17→0 10 1500 10 10 10 10 10 10 10 10 10 | kium 1 1 1 | A Polycorp 528 538 0 (+) (10000 52 89 0 | T T Polymouph 530 520 520 6 510 6 510 6 12,05 6 0 | A Harr Polymouth 545 545 0 - A (ranske 0 5129 0 | 0 894 Polymorph 548 548 548 6 0 9 9 9 9 9 9 | T Soli Soli Soli G - T (transvers 0 | um Polymorphic 103 103 (7)7 → (7) Contion 0 | A An Adymouphin 630 630 4 (Tatologi 842% 9 | en Polymorphis 648 648 648 648 648 648 648 648 648 648 | T00 Polymophan 500 CAA ⇒ TGD Subbaser 0 | T Poperophan 679 679 A → T (tarperson) 0 | GTT Polymorphism 739 743 AGG > GTT Subcatation S4.6% 0 | GTCAOT Polymorphem 745 CCENGTC -> GTCAO Subsitution 86.9% 0 | TT00 Polymorphism 740 750 TCCAT → TT0 Substantion BLP5 0 | 100 Polymorphan 740 751 00 CAT = 100 Substitution 95.9% 0 | TT Polymorphiam 753 754 AG ⇒ TT Substitution 98.3% 0 | AAACOT Polynosphan 755 TTTTCG > AAACO Subsensor 9LPs 9 | 0 Polymorphism 764 764 764 (c ⇒ 6 (c ⇒ 6 (c ⇒ 6) (c ⇒ | AT Polymorphan 760 757 CC > AT Substation 95.8% 0 | AAATG III Folymosphian 700 000008 -> AAAT Subalisace 78.4% -> 96.3% 4.56-295 |
| HIS SINA (DN) Name Tore Nemon Madeum Change Polymophan Type Variast Frequency Variast Frequency | 6 Polynosphan 185 1 - a C (tanstion) 97.9% 0 | C Polymorpham 223 223 6 ⇒ C (transvensor) 98.3% 9 | | | Poly (1) | morphism Poly 306 305 4 → (7)3 station gra 21:245 | Y ymorpham 305 305 0 + T 10 95.15 0 | 060 ymsybten 200 200 itt a COC duthatot 0 | € al/2 al/2 al/2 al/2 bio bio bio bio bio bio bio bio bio bio | 274 276 -907 Dektor 95.5% 0 | T Polymorphism 385 385 A + T (tansersion) 98,1% 0 | G Polymorphism 407 407 (translice) (translice) 80.1% 0 | AGTTGBCGK Polynophan 429 423 •AGTTGBCG Imarian 44.75 0 | € Polymorphon 422 423 T → C (hansilion) 96.3% 0 | E Polymorphan BO A = C (transervice) B5.9% 0 | | | | | 6 Potymerphi 509 509 A + 0 (transition 72,2% 0 | G Polymorph 509 510 AG = 0 Doktor 26,0000 | tom Polymorph S22 (0)4 + (0) Deleter 36.2% | G Sam Polymorph 531 533 7 -9 C (barroom 74.55 2 H | Rism Polymorph 521 533 (173 -> (1 Deletio 25.5% 1.56-2 | A Total | titon Polymoph 500 500 6 0-9 7 (tantuen 92.9% 0 | tian Polymoph 545 545 0 a 4 (ranski 95.2% 0 | G tem Polymorph 548 548 A == 0 (transitio 64.5% 0 | 40 | Pulymorphie 823 823 (1)17 o (11) Deletion 7 36-245 | A Potymorpho 630 630 630 630 640 640 81.0% 0 | en Polymorphic 648 648 648 648 648 648 648 648 648 648 | | | 677 Polymorphism 738 746 A06 → 07T Substitution M4,3% 0 | 611CA0 Polymorphism 147 CC060T → 617CA0 Substitution 81.2% 0 | | | TT Polymorphom 250 254 AG = TT Solitolikaico 92.4% 0 | AAAOG? Polymorphism 756 751 TTTTCG -> AAAO Substitution 91.5% 9 | G Polymorphism 764 764 C → 0 (hamsumison) 87.46 0 | AT Polymogilicat 358 357 CC + AT 51.9% + 96.09 0 | aAAAT Polymorphom 709 772 00000 → AAAT 5.20045600 0% 95.4% → 25.5% 8 |

| R35_536G (66%) | | | | | | |
|--|--|---|---|---|--|---|
| Name Type Meanum Maalmuth Change Profensophism Type Variant Prospency Variant Prospency | C φ C φ P φ P φ A 00 223 251 275 105 223 251 275 106 223 251 275 106 223 251 275 106 223 251 275 106 224 C ⊕ T 0 ⊕ A 108 224 C ⊕ T 0 ⊕ A 109 0 ⊕ A 0 ⊕ A 0 ⊕ A 0 0 0 0 0 | Polymorphic Dg/g train Cac/ project Cac/ project Cac/ project Display Display <thdisplay< th=""> <thdisplay< th=""> Dis</thdisplay<></thdisplay<> | С Г Но Реукрадни Роденсурнат Роденсурнат Роденсурнат Роденсурнат 422 453 464 463 465 422 450 464 463 465 427 450 464 463 465 427 450 454 463 467 9 450 7.4.5 7.4.5 463 465 9 8.9.9 20.4.6.5 7.4.5 20.4.6.5< | 0 0 | V A Figure 3 Figure 3< | Bit Cold T Protocol Technology TG0 Technol Tec |
| R35_536G (MA) + 4534 (484 | 50 | | | | | |
| Name Type Maximum Maximum Change Folymorphism Type Variant P-Value (approximate | C Q C Q T T Vidymouphism Polymouphism Polymouphism Polymouphism 148 221 251 251 T → C B → C C → T Polymouphism (maxiking) (B → C) C → T Polymouphism | Ροβοτογράτοι 2000 Ροβοτογράτοι 2000 Πρηγορίατοι 2000 Πρηγορίατοι 2000 <thπρηγορίατοι 2000 <thπρηγορίατοι 2000</thπρηγορίατοι </thπρηγορίατοι | C C C TotA 222 223 444 423 427 426 427 427 427 427 427 427 427 427 427 427 427 426 427 426 427 426 427 426 427 426 427 426 427 426 427 426 427 426 427 426 427 426 427 426 427 426 427 426 427 | O Operation Operation Operation Polymorphic Polymorphic Polymorphic Polymorphic 203 502 502 503 Polymorphic 204 502 502 704 Polymorphic | Y T A Non-spin to the spin | OFCOUNT TOTO TOO TUT AAACCT Op of Tut AT AAACT AT AAACT Total Participant Par |
| RIN. 538G (MPh) + 453C (52%) | | | | | | |
| Name Type Maximum Change Polymorphom Type Variant Freequency | C A Paymophian Cd morphism 160 223 170 275 170 Co-C 170 Co-C (manifor) (homomenum) 1975 Market | T COC T Odd (magnetistic) T Odd (magnetistic) T Odd (magnetistic) T T Mode Magnetistic Magnetistic <thmagnetistic< th=""> Magnetististic</thmagnetistic<> | Topocolo C Polynospina Poly | A 0 0 0 0 000 0.05 0.05 0.05 0.05 000 0.05 0.05 0.05 0.05 000 0.05 0.05 0.05 0.05 000 0.06 0.06 0.05 0.05 000 0.06 0.06 0.06 0.05 000 0.06 0.06 0.06 0.06 000 0.06 0.06 0.06 0.06 | Polymorphism TOG TOT OTAC Bilginophism | TOD TT Adv.0T 0 AT Adv.3T Dipungtion Pagenetism |