



UNIVERSITAT
POLITÈCNICA
DE VALÈNCIA

Departamento de Biotecnología

ivia
Institut Valencià
d'Investigacions Agràries

 **cirad**
LA RECHERCHE AGRONOMIQUE
POUR LE DÉVELOPPEMENT

Assessment of citrus reproductive biology for seedless mandarin production and its interaction with temperature

PhD THESIS PRESENTED BY
Rafael Montalt Resurrección

SUPERVISORS

Dr. Pablo Aleza Gil

Dr. Patrick Ollitrault

TUTOR

Dr. Jaime Cebolla Cornejo

2023

Abstract

Since the origin of citrus, ancestral natural hybridizations, thousands of years of cultivation and admixture phenomenon have resulted in the complexity of the reproductive biology of today's cultivated citrus, which often involves female and male sterility, self-incompatibility and apomixis in different degrees. The global objective of this PhD was to increase our knowledge on different reproductive aspects that are crucial for citrus breeding and propagation and their interaction with environmental conditions.

Temperature in the progamic phase is critical for the success of plant sexual reproduction. Pollen grain germination, stigmatic receptivity and pollen tube growth are the main components of this phase. The analysis of temperature effect on the progamic phase of citrus was our first objective. We developed an innovative method based on the microscopic observation of transversal slices from pollinated pistils collected daily, allowing a more comprehensive analysis of the pollen tube growth kinetics and dynamics along the pistil. Using three compatible crosses within the *Citrus* genus, we evaluated, with this method, the effect of four temperature regimes in every process during the progamic phase. Warm temperatures reduced the time needed by pollen tubes to reach the ovules and also accelerate pistil degeneration while cold temperatures produced the opposite effects. However, pollen tubes were able to reach the ovules in all crosses studied. Interestingly, we observed for the first time in Citrus both pollen germination and pollen tube growth at 10°C.

Mandarins account for 24% of total citrus production and seedlessness is a crucial quality criterion for the fresh fruit mandarin market. Parthenocarpic ability (PA) is the key for seedless fruit production when it is coupled with self-incompatibility (SI) or sterility. The second objective of this PhD dissertation was to evaluate PA and SI for mandarin varieties with relevant characteristics as parents for seedless mandarin breeding. For this purpose, we developed an efficient protocol based on emasculation, hand self-pollination and hand cross-pollination. Pollen performance was analysed by histological observations, together with fruit set and seed production. Six different behavior was observed among the nine varieties analysed. 'Clemenules' clementine and 'Moncada' mandarins were strictly self-incompatible with facultative and vegetative parthenocarpy; 'Imperial' mandarin and 'Ellendale' tangor displayed no strict self-incompatibility associated with facultative and vegetative parthenocarpy; 'Fortune' mandarin was self-incompatible with facultative and stimulative parthenocarpy; 'Campeona' and 'Salteña' mandarins were self-compatible with vegetative parthenocarpy; 'Serafines' satsuma was associated with male sterility together with facultative and vegetative parthenocarpy; 'Monreal' clementine was self-compatible and non-parthenocarpic.

Beyond the critical importance of SI for seedless fruit production, SI is an obstacle for breeding programs based on hybridization as it reduces crossing possibilities. The third objective of this PhD dissertation, was to compare the efficiency of the SI reaction breakdown by three factors previously identified in other plant species: temperature stress, bud pollination and polyploidization. The SI phenotype was characterized for two self-incompatible varieties 'Fortune' and 'Clemenules' by a histological study of pollen tube growth and ovule fertilization. A molecular marker analysis with SSRs and SNPs allowed us to demonstrate that all the obtained plants were zygotic from selfing. The

ABSTRACT

three methods were successful in recovering selfed plants, and bud pollination was the most efficient approach. Chromosome doubling was also efficient, but involved developing tetraploid plants. Cold temperature stress allowed us to obtain a few diploid selfed plants. However, this method proved much more complex to apply than bud pollination in specific breeding programs.

Marker-assisted selection (MAS) allows the selection of target genotypes at the seedling stage, making it a powerful tool, especially in tree species with long juvenile period, such as citrus. The 4th objective of this PhD dissertation focuses on the development of SNPs markers associated with polyembryony and male sterility. Polyembryony (a form of apomixis) is a desired trait for rootstock production, while monoembryony is researched for parents of sexual breeding projects. Male sterility is useful coupled with parthenocarpy for seedless fruit production and satsuma-derived nucleo-cytoplasmic male sterility (CMS) is the most prevalent in mandarins. In Chapter 4, a segregating offspring derived from 'Kiyomi' (a monoembryonic and CMS variety) x 'Murcott' (a polyembryonic and male fertile variety) was genotyped by GBS. It was also phenotyped for polyembryony and male sterility by the evaluation of the number of pollen grains per anther (NPGA) and apparent pollen fertility (APF). Thorough genetic association studies, we identified a genomic region in linkage group 1 associated with polyembryony and a genomic region on linkage group 8 associated with NPG. SNP markers closely linked with implied genes were developed for the two genomic regions.

Overall, this PhD dissertation provides new insights into citrus reproductive biology and the influence of temperature on it. The knowledge generated will allow to implement breeding programs more efficiently, particularly those aimed at obtaining seedless varieties. This knowledge can eventually be used to respond to some of the most pressing challenges posed by the current global warming scenario.