

Review

The Reproductive Biology of *Limonium sinuatum* and *L. perezii*—A Perspective for Future Breeding

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Abstract

This review examines the reproductive biology of *Limonium*, mainly *L. sinuatum* and *L. perezii*, and explores the breeding strategies employed for these species. *Limonium* is one of the 20 most cultivated genera in the ornamental plant industry worldwide, with novelty sought in traits such as flower color, stem quality and disease resistance. Approximately 85% of the species in this genus present pollen/stigma dimorphism and self-incompatibility, presenting both challenges and opportunities for breeding. Breeding strategies such as interspecific hybridization, ploidy manipulation and chromosomal mutation have been used to increase the diversity of material available to breeders. In this review, we highlight how insights into reproductive biology, combined with advanced breeding techniques, can accelerate the development of fertile interspecific hybrids and broaden the genetic base for future *Limonium* breeding programs.

Keywords: polyploidy induction; whole genome duplication; unreduced gametes; fertile interspecific hybrids



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1. Introduction

Limonium Mill., commonly known as “sea lavender” or statice, includes herbaceous perennial plants with value as ornamental crops. They are recognizable by their small, colorful, and elegant flowers that have a papery appearance when dry. The flowers commercialized worldwide as *Limonium* represent different species, e.g., *L. sinuatum*, *L. perezii*, *L. sinensis*, and *L. altaica* [1], and, as a cut flower, are mostly used as fillers for bouquets. *Limonium* hybrids have value in the international market for their novel combinations of morphological characteristics [2–5]. The ornamental plant industry continues to seek diversity and novelty in new cultivars with improved features, e.g., flower color, shape and size, and plant architecture, but also agronomic performance, e.g., stem yield, speed to flowering, and tolerance to biotic and abiotic stresses [6,7].

Different breeding strategies have been applied to *Limonium*, including intraspecific crosses [8], interspecific hybridization [3,9], ploidy manipulation [10,11] and chromosomal mutation [12–14]. Interspecific hybridization is limited by the genetic distance between species and cross-compatibility [6], with hybrids possibly displaying inviability, sterility or reduced vigor, commonly manifested as wilting and pale foliage [8,9] as reported in hybrids of *L. perezii* × *L. sinuatum* [3]. Interspecific hybridization remains an important

creator of germplasm diversity for breeding of new cultivars as it offers a rapid pathway for creating novel combination of characteristics [15]. Interspecific hybridization of *Limonium* has utilized the cross compatibility of *L. sinuatum* and *L. perezii* [3], *L. peregrinum* and *L. purpuratum* [9], and *L. latifolium* and *L. caspium* [2,16].

The aim of the current paper was to review available literature on the reproductive biology and breeding of *Limonium*, to support future breeding programs of this genus. In doing so, a particular focus was placed on *L. sinuatum* and *L. perezii* as the species with which the authors have most familiarity and which possess considerable economic importance as ornamental crops. These species are widely cultivated worldwide and valued for their colorful, long-lasting flowers, both as individual species and as parental lines of the interspecific hybrids developed by The New Zealand Institute for Bioeconomy Science Limited and marketed as the ‘siNZiiTM’ e.g., [4].

2. Reproductive Biology

Limonium species are apomictic or sexual [17–19], although a more recent report also demonstrates the occurrence of facultative apomixis [20]. Eighty five percent of *Limonium* species, including *L. sinuatum* and *L. perezii*, exhibit pollen/stigma dimorphism with the occurrence of two flower types [17]. The first flower type presents Type A pollen and a cob stigma while the other has Type B pollen and a papillate stigma (PAP) [17] (described in detail by [18,21,22]). Dimorphic species are self-incompatible, but cross-compatible, meaning flowers with a PAP stigma can be pollinated only by Type A pollen arising from a flower with a cob stigma [23]. Morphological distinctions between cob and PAP stigmata primarily arise from differences in the wall structure at the apical region of the papilla [21]. Specifically, in PAP stigmata, small papillae are apparent, whereas the aptly named cob stigma (resembling a corn cob) displays a smooth surface [21,24]. Additionally, the surface of Type B pollen has a coarsely reticulate pattern, while Type A pollen displays a less coarse reticulation [24]. For a more in-depth understanding of both pollen and stigma types, refer to [24].

A third stigma type known as capitate has been identified in some dimorphic species of *Limonium* and is accompanied by either A or B pollen type [18]. Other *Limonium* species are monomorphic, displaying different combinations of pollen and stigmata such as PAP stigma and Type A pollen (*L. mexicanum*), but other combinations can be found, such as capitate stigma and Type B pollen (*L. purpuratum*, *L. peregrinum*) or cob stigma and Type B pollen (*L. echioides*) [18,19]. The monomorphic species can be self-compatible (e.g., *L. peregrinum* [8,23]) or self-incompatible and reproduce by apomixis, e.g., *L. cosyrense* and *L. dodartii* [19].

3. Karyology

Limonium species have been reported as being primarily diploid, triploid, or tetraploid [25,26], although higher ploidy levels such as hexaploidy have been observed [25]. The chromosome numbers range from $x = 6$ to $x = 18$ per set [26–28], with the most common basic chromosome numbers being between $x = 6$ and $x = 9$ [17,27]. Triploid species in the genus are believed to have originated via hybridization between reduced and unreduced gametes from different species (allopolyploids) or from the same species (autopolyploids), resulting in various configurations in the numbers of chromosomes among triploid plants (e.g., *L. cosyrense* ($2n = 3x = 27$); [26]).

Both *L. perezii* and *L. sinuatum* are diploid species, but with different chromosome numbers [3]. *L. perezii* has $2n = 2x = 14$ chromosomes, while *L. sinuatum* has $2n = 2x = 16$ chromosomes. Hybrids between these two species have an odd number of chromosomes ($2n = 2x = 15$) and are sterile [3], which poses a challenge

for their continued breeding success. To restore fertility in such hybrid plants, chromosome duplication using techniques such as treatment with colchicine, oryzalin, or N_2O have been successfully applied to the whole plant, seeds, or gametes in various plant species [3,10–12,29,30]. Previous research on chromosome duplication in *Limonium* will be discussed in Section 13.2.

4. Apomixis

Apomixis refers to the asexual formation of seeds, i.e., without fertilization. The genetic composition of apomictic seeds is identical to that of the maternal parent (i.e., clones) [20,31]. The formation of seeds without fertilization can take place through different processes such as apomeiosis (meiotic reduction does not occur) or parthenogenesis, i.e., the egg develops an embryo without fertilization [31,32]. Apomixis results in no genetic variation among the progeny, but seed is produced and dispersed [32]. In 90% of apomictic species, the presence of functional pollen on the stigma is necessary for the formation of viable endosperm [31].

Facultative and obligate apomixis has been observed in *Limonium* species such as *L. binervosum*, *L. dodartii*, *L. multiflorum*, *L. ovalifolium*, *L. aucheri*, and *L. cyprium* [27,33–35]. This reproductive mode seems to be associated with reproductive biology [19,27], polyploidy [35] and geographical distribution [33]. For example, the role of ploidy in the occurrence of apomixis has been demonstrated in the tetraploid *L. multiflorum*, where meiotic abnormalities are frequent and apomictic reproduction results in stable populations [34,36]. Nevertheless, these results do not mean that polyploid species and genotypes always reproduce by apomixis. Tetraploid *L. sinuatum* genotypes obtained after whole genome duplication were found to reproduce sexually and, despite the occurrence of meiotic aberrations reducing their sexual reproductive capacity (e.g., reduced pollen germination), apomixis was not detected [37].

Seedlings developed via apomixis are identified using molecular markers or by flow cytometry [20,38]. When molecular markers are used, seedlings displaying the same DNA markers as the seed-bearing genotype are likely to be apomictic [20]. In the case of flow cytometry, apomictic seeds or non-hybrids could also be differentiated from interspecific hybrids based on their distinct DNA content, with apomictic seeds having the same DNA content as the species of the seed parent [38]. To ensure accurate differentiation, any potential intraspecific cross options that could confound the analysis should be ruled out and the DNA contents of the parents should be different, so the comparison of DNA content in 2C nuclei from the seedlings and the parents can be conclusive. For example, the nuclear DNA content of *L. sinuatum* is 6.42 picogram (pg), that of *L. perezii* is 8.69 pg, and their interspecific hybrids present an average nuclear DNA content of 7.59 pg [3]; in this example, seedlings from this cross with a DNA content close either to 6.42 pg or 8.69 pg (depending on which species is the female parent) could be considered apomictic, although this would need to be confirmed using molecular methods.

5. Floral Morphology

The floral structure of *L. sinuatum* and *L. perezii* is representative of the genus. Their florets have five sepals, five petals, five separate styles, five stigmas, five stamens and a unilocular ovary which contains a single ovule, thereby limiting fertilization to a single opportunity per floret ([19,23]; Figure 1). The florets are arranged in inflorescences composed of, in ascending order of complexity, spikelets, which constitute spikes, with these spikes organized in racemes, and this in turn forms the panicle, corymb or floral stem (Figure 2).

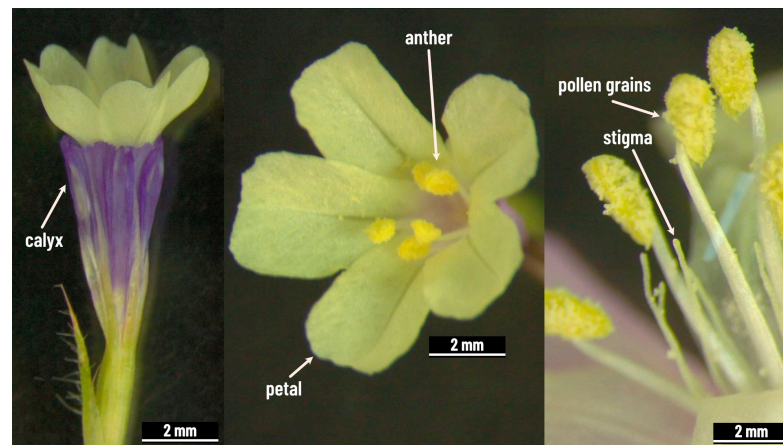


Figure 1. Mature, recently open floret of *Limonium sinuatum* with dehiscent anthers showing some of their constituent parts. Photos by Cordoba-Sanchez, J.

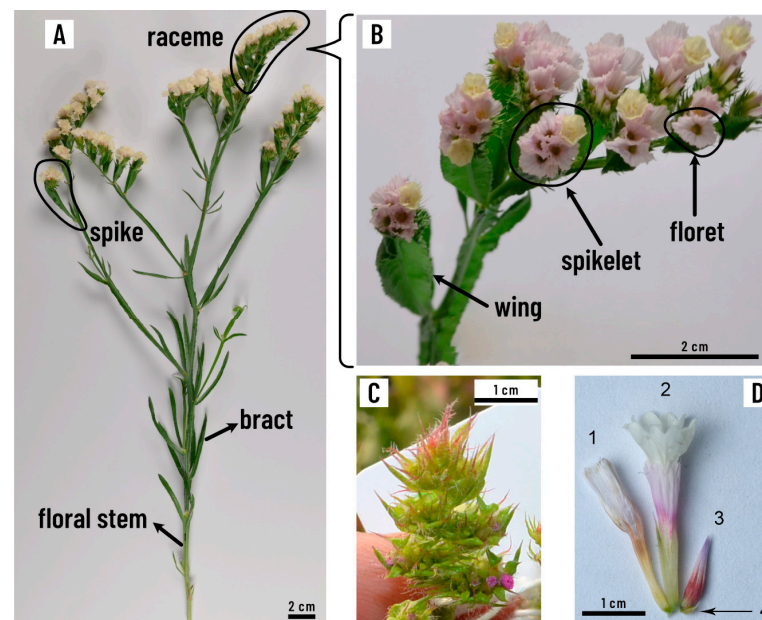


Figure 2. (A). *Limonium sinuatum* inflorescence showing the main constituent parts. (B). Detailed raceme. (C). Detailed spike with unopened florets. (D). Detailed spikelet composed of four florets numbered from 1 to 4. Photos by Cordoba-Sanchez, J. (A,B) and Siregar, S. (C,D).

A single *L. sinuatum* plant can produce more than ten floral stems at a time, with panicles or corymbs emerging from the plant's basal rosette. In contrast, a single *L. perezii* plant can produce between one and five floral stems per flush. In both species, each corymb can support more than 10 racemes (Figure 2A), and each raceme consists of five to nine spikes (Figure 2A). The number of spikelets within a spike varies but generally decreases toward the distal end of the inflorescence. In a spikelet there are up to four florets enclosed by a bract (Figure 2D).

6. Floral Development in *L. sinuatum* and *L. perezii*

Florets in a raceme do not open simultaneously. In *L. sinuatum* the first floret to open in a raceme is located at the proximal end of a spike, with subsequent florets opening acropetally toward the distal end [39]. In contrast, florets within a spikelet follow a centripetal maturation pattern, starting with the outermost floret and progressing inward toward the rachis [39]. Typically only one, or occasionally two, florets open at a time within

a spikelet, with the opening of each floret spaced one to two days apart for the first six florets. For later-maturing florets, this interval can extend to 3–14 days. Each floret is open for just one day.

The asynchrony in floret opening and the short duration of floret receptivity (one day) can have practical implications for breeders. For example, only a limited number of florets on an inflorescence are open and receptive to pollination on any given day, but this window can last for up to 14 days. Depending on the breeding goals, this pattern could either be advantageous or disadvantageous. One advantage is that the staggered flowering offers more opportunities for synchronization between species, as successful pollination often requires careful coordination between pollen donor and receptor plants, particularly when hybrids with different blooming schedules are involved. On the other hand, if the breeding goal is to target a specific stage of floret development the asynchrony in flowering may reduce the success of certain treatments, as will be discussed below in Section 7.

The characterization of floret opening patterns in *L. sinuatum* and *L. perezii* provides a basis for linking externally visible floral development with underlying cytological processes. This relationship is central to enhancing breeding efficiency by identifying developmental stages most suitable for biotechnologically assisted breeding. Section 7 further examines the association between floral development and meiosis and its relevance to this form of breeding of *Limonium*.

7. Association Between Floral Development and Meiosis in *L. sinuatum*

Understanding of the meiotic process in *Limonium* allows floret development to be correlated with meiotic progression; knowledge of this can be used to enhance breeding techniques such as polyploidization, as demonstrated in *Lilium* [40] and *L. sinuatum* [41]. This insight facilitates the use of specific biotechnological tools to restore fertility through, for example, chromosome doubling in pollen grains, as detailed in the following sections.

Each floret of *L. sinuatum* and *L. perezii* produces from about 100 to over 300 pollen grains across its five anthers, and only one ovule [22]. Under greenhouse conditions in Palmerston North, New Zealand (40.9006 °S, 174.8860 °E), with daily air temperatures set at 20 °C for heating and 24 °C for venting, and a natural photoperiod of 11 h in spring and 14.30 h in summer, the process of pollen development, from the onset of meiosis to anther dehiscence, spanned more than 14 days [37].

Meiosis can be synchronous between pollen mother cells (PMCs) and megaspore mother cells (MMCs) as reported in *Liriope spicata* [42]. In other species, however, it is asynchronous, and meiosis in the MMCs is delayed in comparison with meiosis in the PMCs [43–45]. Meiotic asynchrony has been established in *Limonium*, with various meiotic stages occurring concurrently within distinct PMCs [39]. However, the association between floret size and meiosis in the PMCs in *L. sinuatum* [39] and *L. perezii* has been established, providing a useful tool for breeding [37,41]. In *L. sinuatum*, c. 80% of the PMCs undergo meiosis in unopened florets with a diameter less than 0.8 mm and calyces protruding no more than 0.2 mm above the bract [39]. In a study done in *L. ovalifolium* and *L. multiflorum*, the relation between meiosis in the MMCs and floret size was determined, with meiosis occurring in unopened florets less than 2.5 mm in length [22]. However, no published studies have explored the correlation between meiosis in the MMCs and PMCs and floret size within the same species of *Limonium*.

8. Pollen Development, Morphology, and Viability

Meiosis in the PMCs results in tetrads containing four microspores, each enclosed by a callose wall that precedes the formation of the pollen wall (Figure 3; [46]). The post-meiotic development of the microspores begins after their individualization and release from the

tetrads (Figure 3; [47]). During the post-meiotic phase, the microspores grow, develop a large vacuole, and undergo an asymmetric pollen division known as pollen mitosis I (PMI), resulting in one larger vegetative cell and one smaller generative cell (Figure 3). At this stage, the fates of the two cells diverge, with only the generative cell proceeding to pollen mitosis II (PMII), which produces two sperm cells [48]. The timing and location of PMII varies among families and are associated with pollen type (i.e., binucleate or trinucleate; Figure 3; [46,47,49]). In the case of trinucleate pollen, such as that found in *Limonium*, a grain consists of a vegetative nucleus and two generative (sperm) nuclei at anthesis, indicating that pollen mitosis II (PMII) occurs in the anther before dehiscence (Figure 3; [46]). The presence of three nuclei before anther dehiscence allows for rapid germination but results in shorter viability compared with that of binucleate pollen [49,50]. This reduced viability limits the potential window for using trinucleate pollen in breeding programs, particularly when long-term pollen storage or extended breeding cycles are required. Additionally, the trinucleate pollen of *Limonium* has been difficult to germinate in vitro, with rates of only 40% reported [51] in comparison with trinucleate pollen from other plant species, e.g., *Arabidopsis* with 80% [52], or 87% in *Tanacetum vulgare* [50]. The low success rate of in vitro germination for trinucleate pollen of *Limonium* limits the effective assessment of its viability and functionality outside the plant, a useful step for selecting reliable pollen donors in hybridization programs.

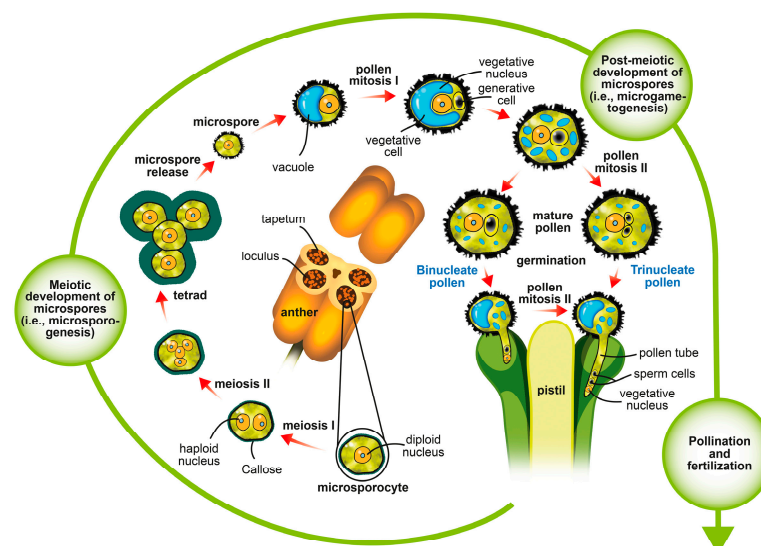


Figure 3. Schematic diagram illustrates key phases of pollen development, beginning with microsporocyte formation and culminating in pollination. Trinucleate pollen, depicted on the right side of the figure, consists of one vegetative nucleus and two sperm cells. On the left side, binucleate pollen is characterized by one vegetative nucleus and one sperm cell. Adapted with permission from [49], Global Science Books Japan, 2025.

In *Limonium*, mature pollen grains typically feature three apertures in the exine, which are specialized regions of the pollen wall that facilitate germination [39,46]. However, in some induced polyploids, the number of apertures can vary. In certain cases, no apertures were observed [39], while in other instances, following treatments with oryzalin or N_2O , polyploid plants produced pollen grains with four apertures [37,53].

The development and functionality of pollen grains are closely tied to changes in hydration status of the grain. For example, microspores increase their water content and become vacuolate during pollen wall development, leading to an increase in size. Subsequently, pollen dehydrates during maturation and prior to dispersal, followed by rehydration for germination and fertilization [54,55]. Trinucleate pollen, such as that of

Limonium, exhibits reduced dehydration before dispersal, which is associated with the shorter viability compared with that of binucleate pollen, as mentioned above [56]. The regulation of water content in pollen grains involves various physiological, molecular, and structural mechanisms, including the exine and vacuoles [56].

Pollen functionality and viability can be evaluated using several methods, including staining with modified Alexander’s stain [57], the tetrazolium test [58] or fluorescein-diacetate [58], in vitro germination of pollen [51], pollen size measurements [59], and seed set evaluations [60,61].

Pollen size may serve as an indirect indicator of pollen viability [59] and ploidy [62–64]. In *Limonium*, pollen size varies both between species [65] and within species, typically displaying a unimodal distribution [39]. In *L. sinuatum* and *L. perezii* [66], and as also proposed for other plant species, pollen with sizes half the average for that species are classified as micro-pollen [59,61], while pollen grains measuring 1.26 times the average diameter are considered unreduced (2n) [39,62–64]. Grains larger than 1.59 times the average diameter are categorized as tetraploid (4n) [63].

In summary, understanding pollen development and viability is essential for improving hybridization success and, consequently, for breeding. In *Limonium*, trinucleate pollen facilitates rapid germination but exhibits reduced viability, which limits its use in breeding programs. Assessing pollen size and aperture characteristics may provide valuable insights for breeding; however, additional challenges could still affect pollen functionality. In the following section, the review focuses on female gametophyte development or megagametogenesis. This developmental process is also critical in plant reproduction and affects the success of breeding strategies.

9. Megagametogenesis

Meiosis in the MMCs results in four haploid nuclei. However, depending on when cell plate formation occurs, the number of meiotic products is different from what is seen in the PMCs, where four microspores are always produced (discussed in Section 8 “Pollen development, morphology, and viability”; [46]). The meiotic product in the MMC can then be four uni-nucleate megaspores (i.e., monosporic), two bi-nucleate megaspores (i.e., bisporic), or one tetra-nucleate megaspore (i.e., tetrasporic). In each of these product outcomes, however, there is only one functional megaspore [67,68]. For example, in *Limonium ovalifolium*, a tetrasporic pattern known as the *Drusa*-type has been observed [41]. In this pattern, all four megaspore nuclei participate in the formation of the embryo sac (Figure 4).

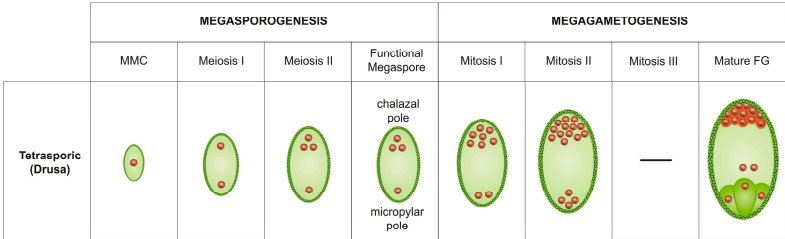


Figure 4. Diagram showing the main stages of megagametophyte development. First, during megasporogenesis, in the megaspore mother cell (MMC), meiosis occurs and a functional megaspore with tetrasporic pattern is produced. Then, during megagametogenesis, two rounds of mitosis give rise to an egg cell and two synergids at the micropylar pole, and a 6-nucleate embryo sac at the chalazal end. FG: female gametophyte. Adapted with permission from [67] Oxford University Press, 2025.

During megaspore development, multiple rounds of cellular division occur. These divisions are susceptible to disruption by anti-mitotic or meiotic agents, which can lead to chromosome duplication. While meiosis is the primary target for inducing unreduced gametes, in species like *Limonium*, which follow a tetrasporic pattern, the functional megaspore formed after meiosis undergoes two additional rounds of mitosis, followed by cell wall development [67]. In *Limonium*, the megasporocyte has a bipolar conformation with one nucleus at one pole (i.e., micropylar pole) and three nuclei at the other (i.e., chalazal pole) (Figure 4). The nucleus at the micropylar pole undergoes mitosis to give rise to the egg cell and the synergids. At the same time, the three nuclei at the chalazal pole fuse and then divide to produce a tetranucleate or 6-nucleate embryo sac, depending on the species (Figure 4; [22]). For example, in *L. ovalifolium*, the mature embryo sac is 6-nucleate and contains synergids, antipodals, egg cell and the central cell [22,69].

10. Fertilization

Double fertilization, first reported by [70] and further reviewed by several authors (e.g., [69,71]), occurs between one sperm cell and the egg cell to produce the diploid zygote, and between the other sperm cell and the diploid central cell (becomes diploid following fusion of the two polar nuclei) to produce the triploid endosperm [70].

Fertilization involves several steps apart from the fusion of sperm cells. The pollination process begins with the adherence of pollen to the receptive stigma, followed by pollen germination and then pollen tubes growing to reach and penetrate the ovule. Understanding the steps of pollination and fertilization allows breeders to identify the factors that influence successful fertilization and to devise strategies to overcome prezygotic barriers that impede fertilization in genetically distant crosses (i.e., interspecific, intertribal). For example, a study on interspecific crosses between six species of *Limonium* [23] examined crossability by analyzing the entire fertilization process—from pollination through pollen grain germination, pollen tube growth and ovule penetration, to fertilization of the egg cell. The researcher identified different crossability levels between species even in “compatible” crosses between several dimorphic species. In some crossing combinations between species, the pollen tube regularly penetrated the ovary while in others, pollen tube growth was restricted to the stigma. This knowledge allows breeders to consider using technologies such as cut-style pollination or mentor pollination where crossing barriers occur, as used in crosses between *Anemona coronaria* L. and *Ranunculus* L. [72] or in *Lilium* [73]. However, these techniques have not yet been implemented in *Limonium*.

11. Embryo Development

After pollination, and following fertilization of the egg cell, embryo development begins, presenting new opportunities for mitotic disruption and the potential induction of polyploidy, as discussed below in Section 13 “Breeding in *Limonium*”. Furthermore, a deeper understanding of embryo development enables breeders to intervene at the optimal moment, allowing the use of techniques such as embryo rescue, as described later.

Studies on pollination and fertilization in *L. perezii* [23] revealed that the pollen tube reached the embryo sac within as little as two hours after pollination [23]. The same research also studied embryo development, with globular embryos observed as early as three days after pollination (DAP). At 6 DAP, embryos were at either the globular or early heart stage, with most reaching the heart stage by 9 DAP. Torpedo-stage embryos appeared by 12 DAP, and from 15 to 30 DAP, the embryos continued to elongate. No further elongation was observed beyond 30 DAP. Embryo development was also studied in *L. perigrinum* [8] to investigate the reasons for poor seed set in self-pollinated flowers of this monomorphic member of the genus. Although pictures were not provided, a description of the stages

was given, wherein embryos undergoing normal development grew from 0.2 mm globular embryos at 9 DAP to a full size of 5.2 mm at 36 DAP. At 12 DAP the embryos were recorded to be at the early heart stage, with cotyledons beginning to grow. The embryos elongated, continued to elongate, and were at torpedo stage 21 DAP. Growth continued to 36 DAP but there was no further elongation beyond 36 DAP.

Studying the timing and stages of embryo development in *Limonium* provides a basis for applying techniques like embryo rescue (discussed in the next section) and polyploidy induction (refer to Section 13.2), both of which contribute to advancing breeding effort of the genus.

12. Embryo Rescue

Embryo rescue involves extracting the embryo from the ovule and cultivating it in a plant tissue culture medium [74]. The primary goal of this technique is to prevent embryo abortion [75,76]. Abortion of the embryo is more commonly observed after interspecific or interploidy crosses, often resulting from incompatibilities between the parental genomes or abnormalities during endosperm development [77].

In *Limonium*, embryo rescue may be preceded by ovule culture, which involves transferring immature, potentially abortive embryos within the ovule to in vitro culture [74,78]. For instance, to recover *L. perezii* × *L. sinuatum* hybrids, ovule culture has been successfully performed between 12 and 16 days after interspecific crossing, followed by the extraction of the embryo two weeks later for further growth and germination before subculturing [3,9]. Without embryo or ovule culture, no interspecific hybrid seedlings were successfully produced [3,9].

13. Breeding in *Limonium*

13.1. Interspecific Hybridization

In *Limonium*, interspecific hybridization has been successful in obtaining new genetic material and developing new commercial varieties. For example, hybrids between *L. latifolium* and *L. caspium* resulted in the variety ‘Ocean Blue’ [16] and other hybrids [2]. Interspecific crosses between *L. perigrinum* and *L. purpuratum* gave rise to the cultivar ‘Chorus Magenta’ [9], while hybridization between *L. perezii* and *L. sinuatum* gave rise to hybrids that form the basis of a series of cultivars known commercially as siNZii™ [3,79].

Interspecific hybridization can be limited by pre- and post-zygotic barriers operating to prevent hybrid formation or development [77]. The most common pre-zygotic barriers are geographic isolation, self-incompatibility [80], asynchronous flowering times [19], and failure of the pollen-pistil interaction [23,29]. In contrast, post-zygotic mechanisms to prevent the zygote’s development include embryo abortion, and if plants are produced, partial or complete infertility, leaf chlorosis (pale leaves) or albinism are examples of mechanisms reducing the fitness of the hybrid plants [15,81,82].

Limonium breeders have implemented strategies to overcome the post-pollination barriers. For instance, controlled crosses followed by ovule/embryo culture resulted in interspecific hybrids [3,9]. Despite this, however, the interspecific hybridization rate can remain low. For example, first attempts to hybridize *L. sinuatum* with *L. perezii* resulted in 12% of pollinated flowers containing fertilized ovules with less than 5% of these embryos developing into sterile interspecific hybrid plants [3]. In those cases, chromosome doubling was employed to restore fertility in the hybrid progeny.

13.2. Ploidy Manipulation

Ploidy is defined as the basic number of chromosome sets of a living organism [83], while manipulation refers to changing the number of chromosome sets of an organism, e.g., producing plants with ploidy levels higher than diploid (i.e., induction of polyploidy).

Polyloid induction in plants has been achieved through chemical treatment with mutagens, e.g., colchicine, oryzalin or nitrous oxide (N₂O) [12,84,85], or by crossing plants with different ploidy levels [85,86]. In some cases, breeders may rely on natural production of unreduced gametes, but in many genotypes the frequency of formation is too low for practical purposes.

In the case of polyploidy induction via chemical treatment, the chemical mutagens used (e.g., oryzalin, colchicine or N₂O) disrupt the mitotic/meiotic spindle by different mechanisms. Polyloid induction can be done at different stages of plant development, e.g., before pollination, after pollination, in seeds, or plantlets [12,53,62,87–89] depending on the aim of the treatment (Figure 5). For example, N₂O treatment can be used to block the first mitotic division of the zygote, resulting in polyploid seedlings, while application during meiosis results in unreduced gametes as detailed in the next sections.

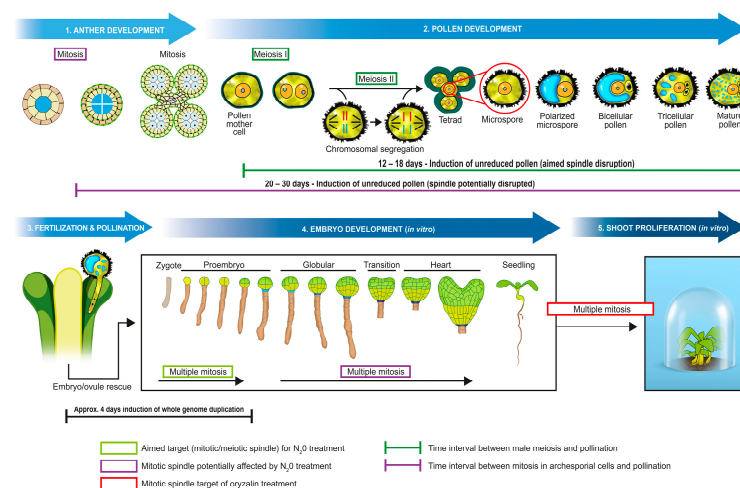


Figure 5. Diagram of the stages during plant development of *Limonium* when treatment with an agent such as oryzalin or nitrous oxide (N₂O) could be used to disrupt either the mitotic or meiotic spindle during microsporogenesis, microgametogenesis and embryo development. The time elapsed between either mitosis in the archesporial cells or meiosis in the pollen mother cell and pollination is shown, as well as the time elapsed between pollination/fertilization and the first developmental stages of the embryo (i.e., zygote and proembryo). Adapted with permission from [49], Global Science Books Japan, 2025; [54], John Wiley and Sons, 2025; [90], Springer Nature, 2025; [91], The Company of Biologists, 2025.

13.2.1. Ploidy Manipulation During Mitosis

Disrupting the mitotic spindle during division of somatic cells induces whole-genome duplication (WGD). The disruption of the mitotic spindle could take place in prometaphase, metaphase, or anaphase, causing chromosome mis-segregation (Figure 6). As a consequence, the sister chromatids will not separate between the daughter cells, and the two new cells will contain either double the number of chromosomes (e.g., tetraploid) or no chromosomes (i.e., nullisomic cell; [92]). The chromosome sets of the cell with the doubled number of chromosomes are genetically the same.

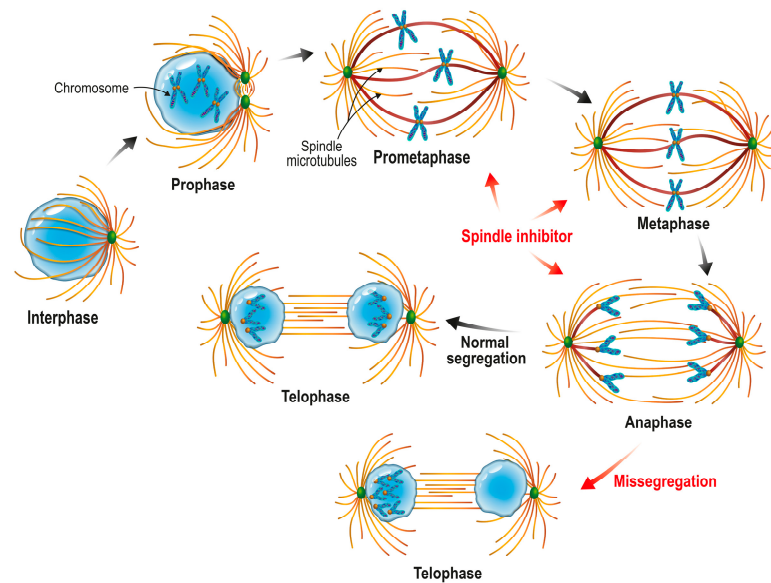


Figure 6. Phases of mitosis susceptible to spindle disruptors, e.g., nitrous oxide (N₂O) (red arrows). The most common consequence of the spindle disruption is chromosome mis-segregation, with one daughter cell having a doubled chromosome number while the other has no chromosomes (nullisomic cell). Adapted with permission from [92], Springer Nature, 2025.

The induction of WGD can occur from the first zygotic cell divisions through to plant multiplication in vitro (Figure 5). In *Limonium*, for example, WGD has been accomplished using oryzalin to treat in vitro shoots of *L. perezii* × *L. sinuatum* hybrids, which resulted in the production of fertile tetraploids from sterile diploid plants (i.e., 82% pollen viability compared with 1%), enabling its use in backcrosses [11]. Treating seeds of *L. bellidifolium* with colchicine resulted in 5% of the seedlings being tetraploids [12] and, more recently, N₂O was employed to treat embryos of *L. sinuatum* and *L. perezii*, in early development (between one and four days after pollination), resulting in production of between 16% and 35% polyploid seedling plants [10]. With a focus on the potential to save time within a breeding programme, the use of N₂O offers enables recovery of doubled plants directly from the single-celled zygote rather than needing to be somatically doubled following production of the diploid progeny arising from a cross not utilizing N₂O.

In this regard, while oryzalin treatments in vitro may last around five months, extending the total breeding cycle to as much as eight months, N₂O treatments are completed in just a few days, with the generation of a seedling with confirmed polyploid status taking up to six months [37]. As a consequence, N₂O becomes a significantly faster alternative.

13.2.2. Ploidy Manipulation During Meiosis

Ploidy manipulation before pollination aims to disrupt meiosis to produce unreduced gametes as mentioned above. The disruption could take place in meiosis I (i.e., First Division Restitution (FDR); [83]) or during meiosis II (i.e., Second Division Restitution (SDR); [64,83]). If the disruption of the meiotic spindle occurs in prophase I, it prevents the movement of the meiotic chromosomes to the opposite ends of the cells, with the chromosomes retained in the cell plate or scattered within the cell. Therefore, the homologous chromosomes are not equally distributed between the two nascent cells during telophase I ([64,83]; Figure 7 left). In contrast, spindle inhibition during meiosis II (SDR) allows for normal meiosis I. However, during telophase II, the sister chromatids remain together and cannot be separated to migrate to opposite poles of the cell. As a consequence, the daughter cell contains half the parental chromosomes, but each chromosome is formed by two identical sister chromatids (Figure 7 right; [61,83]).

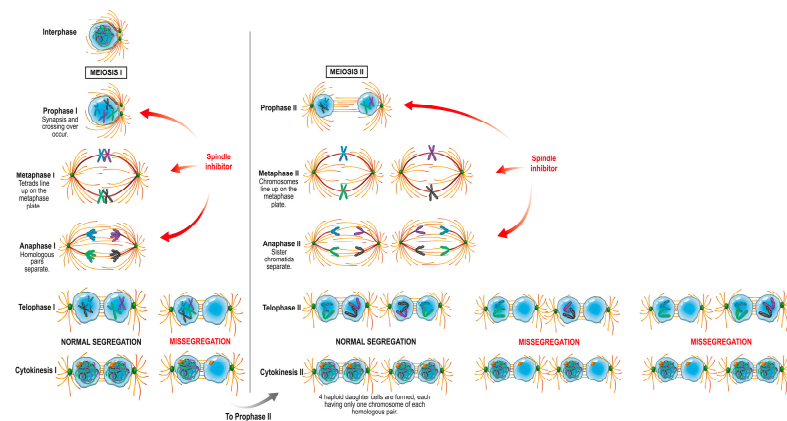


Figure 7. Phases of meiosis susceptible to treatment by a meiotic spindle disruptor (red arrows). If either prophase I, metaphase I or anaphase I is affected, First Division Restitution (FDR) occurs. However, if the meiotic spindle disruptor disturbs either prophase II, metaphase II or anaphase II, Second Division Restitution (SDR) occurs. In any case, the main consequence is mis-segregation of chromosomes.

It is also possible to generate unreduced gametes by inducing polyploidy in cells of the developing flowers, by treating pre-meiotic dividing cells that later give rise to the PMCs or MMCs [93]. Studies done in *Lilium* [64] confirmed the mitotic polyploidization of male archesporial cells, while in *Limonium*, unreduced gametes have arisen from either meiosis or from mitotic divisions within the somatic cells of the anther, with the latter being interpreted from the association between smaller pollen grains arising in *L. sinuatum* and meiotic disruption occurring 15 DAT [37,41].

Ploidy manipulation during meiosis affects not only the number of chromosome sets in the gametes but also the number of microspores or megaspores produced, which is evident in the type of polyads produced. For example, in interspecific wheat-rye hybrids, first division restitution (FDR) results in two unreduced cells (i.e., dyads) instead of the four expected haploid cells (i.e., tetrads; [94]; Figure 3); in *L. multiflorum*, second division restitution (SDR) could produce tetrads, triads or polyads [34]; and in *L. sinuatum*, the use of N_2O for induction of unreduced pollen resulted in pentads, dyads, hexads, and monads [41].

Ploidy manipulation before pollination that results in unreduced gametes may increase the interspecific hybridization rate, as reported in *L. perezii*, where an increase in progeny produced was noted even though the progeny were not polyploid [37]. In addition, the use of unreduced gametes in sexual hybridization facilitates the development of polyploid and fertile F1 interspecific hybrids [64,95,96].

The importance of polyploidy in breeding lies not only in its ability to restore fertility in hybrids. Other benefits include genome buffering, which reduces the impacts of deleterious genes, as well as increased heterosis, heterozygosity, shifts in reproductive modes, and enhanced phenotypic and genotypic diversity [83,97]. Additionally, polyploid plants may exhibit distinctive traits compared with their diploid counterparts. For example, in *L. sinuatum* and *L. perezii*, tetraploids have larger pollen grains, thicker leaves, larger floral stem diameters, and longer guard cells [10]. Specifically, in *L. sinuatum*, tetraploids also display increased leaf size, more prominent floral stem wings, longer flowers, and slower growth rates [10,53].

In summary, ploidy manipulation in *Limonium* has been demonstrated to restore hybrid fertility, enhance the rate of interspecific hybridization, and expand options for increasing genetic diversity, while also modifying commercially important morphological traits.

13.3. Radiation Driven Breeding

Gamma-rays, x-rays, or ion-beams have been used to induce chromosome mutations that might enhance desired traits, such as disease resistance, yield, or environmental adaptability [98,99]. In *Limonium*, x-rays were employed in the development of the commercial hybrid ‘Oceanic White’ [100]; gamma-rays produced six cultivars of the *Limonium* variety ‘Sea Pink’ and the cultivars ‘Tall Pink Emille’, ‘Misty Blue’, ‘Daifura Pink’ and ‘Super Lady’ [101]. In addition, C-ion beams were used to develop the color mutants ‘Kishu Fine Lavender’ and ‘Kishu Star’ [14].

The development of commercial cultivars following radiation treatments reveals its potential to increase genetic diversity. However, the efficiency of radiation for inducing novelty is highly variable. For example, the efficacy of the irradiation for inducing color mutations varies among genotypes, with 5 Gy of C-ion beams producing color mutation rates of 0% in ‘Kishu Fine Grape’, 2.4% in ‘Kishu Fine Lavender’, and 13% in ‘Kishu Star’ and resulting in three color mutations [14]. In another study, 20 Gy of gamma rays applied to shoots of *L. sinuatum* affected the number of leaves and the floral stem length, but not the flower color in the resulting plants [102]. In contrast, 1.0 Gy ion-beam irradiation on *L. sinuatum* shoots halted their development and multiplication, but no mutants were reported [103].

Despite its potential to introduce valuable genetic diversity, radiation-driven breeding presents challenges because of its inconsistent results across different genotypes and traits. These findings underscore the importance of optimizing radiation treatments and selecting suitable genotypes to maximize the breeding potential of radiation, especially for traits like color and other desired ornamental characteristics.

14. Future Breeding

The hybridization and chromosome doubling approaches discussed here constitute only part of the range of in vitro technologies applicable to *Limonium* breeding. Other promising techniques include mutagenesis, transformation, and gene editing, which offer further opportunities for generating genetic diversity and accelerating cultivar development.

Commercial *Limonium* production frequently relies on tissue culture propagation, a technique that can induce somaclonal variation, an important source of genetic diversity in several crops. However, reports of somaclonal variation in *Limonium* remain scarce, and its potential to enhance the genetic base of the genus has yet to be fully explored.

Somaclonal variation is most often associated with plant regeneration pathways involving a callus phase. For instance, regeneration from protoplasts of *L. perezii* [104] involved callus formation and resulted in minor morphological and cytological differences among 56 regenerated plants [105]. Some plants exhibited shorter stems and altered leaf length-to-width ratios compared with controls, but flower color remained unchanged. In contrast, analysis of a small number of plants of the *Limonium* hybrid ‘Misty Blue’ regenerated from adventitious shoots derived from root explants revealed genetic uniformity among the regenerated individuals, even though callus tissue was involved during shoot regeneration [106]. These findings suggest that the occurrence and extent of somaclonal variation in *Limonium* may depend on the species, genotype, and regeneration pathway, and thus remain an open and promising area for further study.

Beyond somaclonal variation, plant transformation and gene-editing technologies present significant opportunities for creating novel *Limonium* germplasm. The genus has shown broad responsiveness to in vitro culture, and successful *Agrobacterium tumefaciens*-mediated transformation has been reported in several species and hybrids, including *L. sinuatum* callus [107], transgenic shoots of an interspecific hybrid between *L. otolepis* × *L. latifolium* [108], and *L. gmelinii* [109]. More recently, Li et al. [110] developed a transforma-

tion and gene-editing protocol for *L. bicolor* for research purposes. Although gene editing has not yet been reported for the development of commercial *Limonium* cultivars, rapid advances in genome editing and transformation systems suggest that these technologies are likely to become integral tools in future *Limonium* breeding programs.

15. Conclusions

Insights into the breeding systems of *Limonium* have expanded opportunities for generating novel diversity and accelerating the breeding process. This understanding, combined with techniques such as interspecific hybridization and ploidy manipulation, has already led to the development of a wide range of interspecific hybrids. While hybridization often requires chromosome doubling to restore fertility, the phenotypic changes resulting from polyploidization alone may also offer new opportunities for developing cultivars with improved traits.

Although this review focused primarily on sexual hybridization and on two *Limonium* species, the knowledge, protocols, and principles described here are broadly applicable across other members of the genus and potentially to related ornamental crops. Beyond hybridization and chromosome doubling, other biotechnological tools such as mutagenesis, genetic transformation, and gene editing have also been applied to *Limonium*. The use of transformation techniques has likely been limited by regulatory constraints; however, the advent of targeted gene-editing systems, with an anticipated lower threshold for commercial use, presents new opportunities for breeders, research institutions, and the ornamental industry at large.

Future progress in *Limonium* breeding will benefit from deeper insights into the mechanisms underlying self-incompatibility and fertility control. Understanding these reproductive barriers at the molecular level could identify novel targets for manipulating male reproductive development and provide alternatives to cytoplasmic male sterility (CMS), thereby reducing the need for labor-intensive emasculation during hybridization not only in *Limonium* but also in other genera.

Ultimately, the integration of genomic technologies and conventional breeding can reveal the genetic basis of key ornamental and adaptive traits. Building comprehensive genomic resources and collaborative networks will be essential to translate these advances into practical outcomes.

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