Trends in Developmental Biology
Vol. 16, 2023

Review

Polyembryony and its molecular mechanisms in angiosperms

Honglei Wang and Renze Heidstra*
Cluster of Plant Developmental Biology, Laboratory of Cell and Developmental Biology,
Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

ABSTRACT

Sexually reproducing land plants in general strictly obey the one seed-one embryo rule, whereby a single mature embryo originates from a single fertilized egg cell. Polyembryony in seeds plant is the development of more than one embryo per seed, and can be classified into so-called multiple and simple forms of polyembryony depending on the origin of the additional embryo. Although polyembryony represents a characteristic byproduct of apomixis, various forms of sexual (zygotic) polyembryony have been observed. However, the knowledge on these forms of polyembryony remains scarce, particularly in terms of identifying causal genes and elucidating molecular mechanisms. Here we provide a comprehensive overview of current knowledge on polyembryony, encompassing classification, mutants, genes, and we address the underlying mechanisms from the research performed in angiosperms.

KEYWORDS: polyembryony, angiosperm, apomixis, cleavage polyembryony, suspensor polyembryony, synergid polyembryony.

1. Introduction

Multiple births in humans involve the production of more than one offspring from a single pregnancy. They can occur as either monozygotic ('identical'), arising from a single zygote that undergoes division to form multiple embryos, or dizygotic ('non-identical' or 'fraternal'), where each offspring originates from a separate egg, each fertilized by its own sperm cell [1]. Similarly, plants also exhibit the phenomenon of multiple births, referred to as polyembryony. However, the concept of polyembryony in seed plants displays greater flexibility compared to the multiple birth in mammals. This is primarily due to the (acquired) totipotency of particular plant cells, which allows embryos to develop not only from zygotes but also from other plant tissues [2, 3]. It was initially proposed that a truly polyembryonate seed is one in which the embryos are produced solely from the zygotic proembryo, or from a mass of embryogenic cells [4]. This view on polyembryony is consistent with the multiple birth phenomenon in mammals. A later definition of polyembryony also included embryos developing from the synergids, antipodal cells, and sporophytic nucellar or integument cells [5]. Finally, a broad classification proposed to categorize polyembryony as either 'simple' or 'multiple', based on the presence of one or more embryo sacs within the same ovule [2].

Historically, research on polyembryony has primarily focused on morphological descriptions. Advances in molecular genetics have since identified multiple mutants and genes associated with polyembryony. Here we will discuss how the characterization of these mutants and genes enhanced our current understanding of molecular mechanisms underlying polyembryony. Extrapolating from this knowledge, we have refined the classification proposed by Lakshmanan and Ambegaoker, while keeping the overall framework.

*Corresponding author: renze.heidstra@wur.nl

2. Classification of polyembryony in Angiosperms

Polyembryony occurs when a single seed gives rise to multiple embryos. Although polyembryony is uncommon in angiosperms, several species have adopted it in their reproductive strategy. Polyembryos can arise by fusing of ovules, either before or after integument differentiation, or by formation of multiple embryo sacs from a common integument. For instance, in the herb *Trithuria konkanensis*, double embryo sacs have been frequently observed within same integument [6]. Such occurrences are referred to as multiple polyembryony (Figure 1) [2]. Also the natural zygotic polyembryony that is common in gymnosperms, and which occurs by the fertilization of eggs in more than one archegonium, represents a form of multiple polyembryony [7, 8]. In contrast, when polyembryos form in a single embryo sac, this is classified as simple polyembryony [2], which is the focus of this review.

2.1. Simple polyembryony

We can divide the simple polyembryony into three types depending on whether embryos develop upon a fertilization event (sexual), without fertilization (asexual), or both (Figure 1).

Purely asexual polyembryo development falls under the category of obligate (or gametophytic) apomixis (Figure 1). It entails polyembryos that are produced directly from the unreduced embryo sac within the ovule, or from the unfertilized egg (haploid) and synergid cells (haploid). However, this is rare in nature [2, 9-11]. The resulting seeds are genetically identical to the maternal parent, leading to clonal offspring.

In most apomictic plants both sexual and asexual reproduction occurs simultaneously in the same ovule, a phenomenon termed facultative apomixis (Figure 1) [12, 13]. As such, polyembryony is often a characteristic feature of apomictic plants. Natural polyembryony through apomixis in citrus is a well-known example and was first documented by Van Leeuwenhoek in 1719, when he observed two plants growing out from one seed [2]. In citrus, nucellar cells can differentiate into supernumerary embryos and coexist with the zygotic embryo, thereby producing multiple embryos in one ovule (Figure 2K) [12, 14]. In the Turk's cap lily (*Lilium martagon*), it was reported that a diploid and a haploid embryo can form within one ovule and the position of the haploid embryo located at the synergid cell position suggested it originated from a synergid cell (Figure 2 D,I,J) [15]. Similarly, screening for

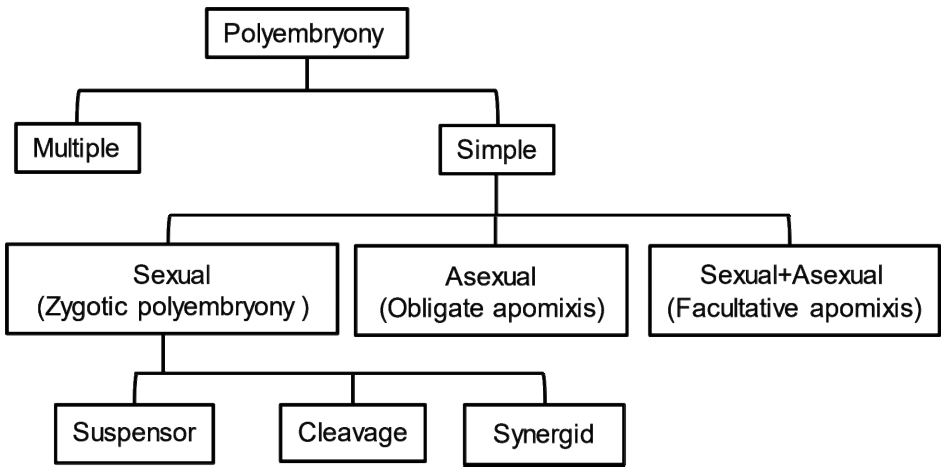

Figure 1. Classification of polyembryony in angiosperms. Polyembryos in angiosperms derive from one (simple) or more embryo sacs (multiple). The simple polyembryony is then divided into polyembryos developing from the zygote (sexual) or by apomixis (asexual) or a combination of both. Zygotic polyembryos can develop from the suspensor after reprogramming, develop after cleavage of the embryo proper, or by fertilization of a reprogrammed synergid cell.

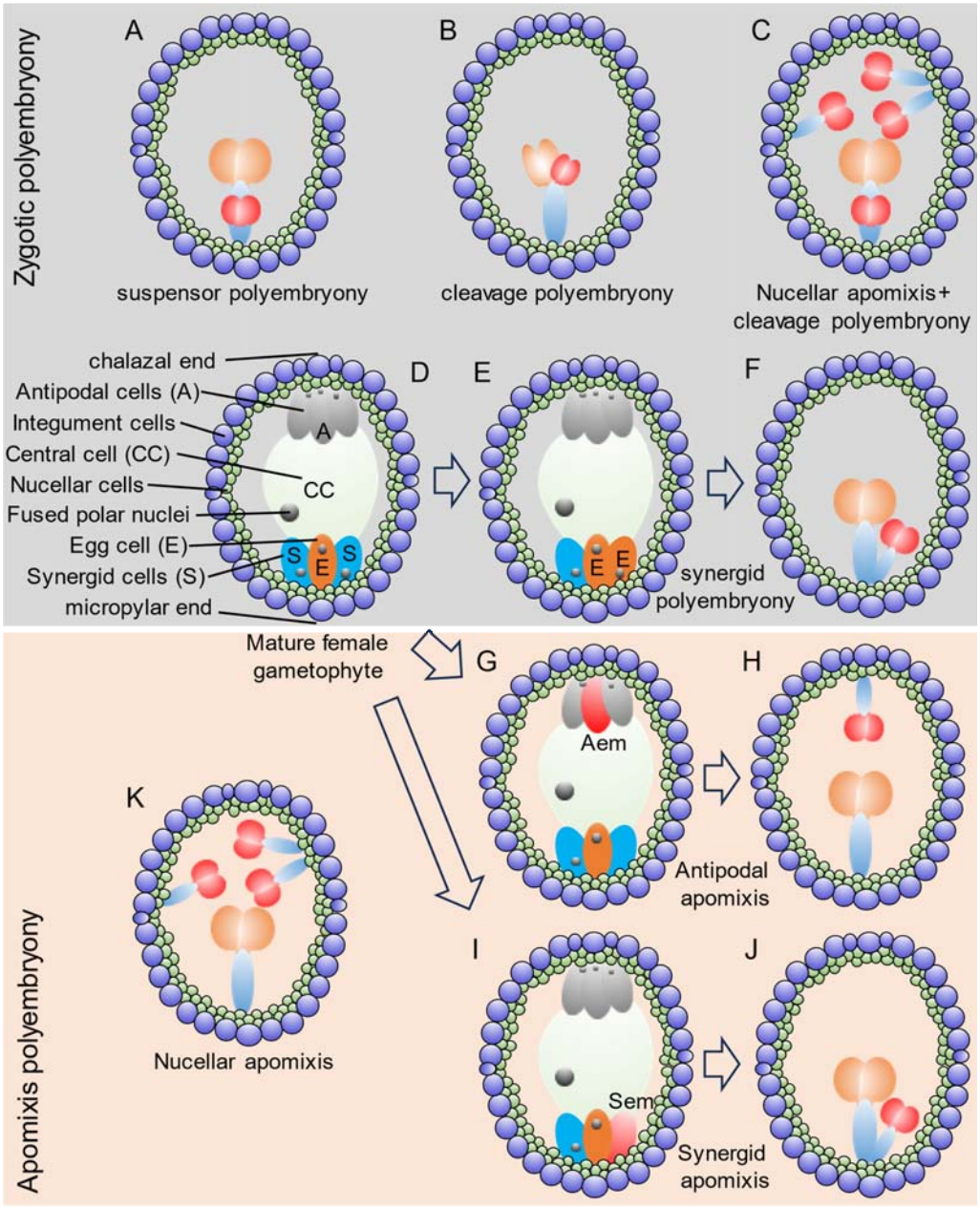

Figure 2. Simple polyembryony in angiosperms. Various forms of simple polyembryony roughly divided into zygotic polyembryogeny (A,B,E,F, grey background) and apomixis polyembryony (G,H,I,J,K, pink background). **A**. Suspensor reprograms embryonic fate and develops into an embryo. **B**. Embryo proper cell aberrantly divides (cleavage) to generate additional embryo. **C**. Combination of zygotic cleavage and apomixis nucellar polyembryony. **D-F**. One of the synergids reprograms into egg cell and is fertilized to generate secondary embryo. **D,G,H**. Secondary embryo develops from antipodal cell (Aem). **D,I,J**. Secondary embryo develops from synergid cell (Sem). **K**. Secondary embryos develop from nucellar cells by apomixis.
Arrows indicate order of events in synergid, antipodal apomixis and syndergid apomixis polyembryony. The mature female gametophyte in D consists of seven cells: a central cell (CC) with a large vacuole and a bigger nucleus (fused from two polar nuclei), an egg cell (E) positioned towards the micropylar end of the embryo sac and flanked by two synergid cells (S), and three antipodal cells (A) positioned at the chalazal end of the embryo sac. Surrounding the embryo sac are nucellar and integument cell layers.

supernumerary embryos in the rice germplasm identified four strains (API, APII, APIII, and APIV) that produce polyembryonate seeds, with rates ranging from 5 to 32% [16]. Among these strains, APIII demonstrated a notably higher incidence of polyembryony, and formed twins and triplets. The emergence of haploid extra embryos from the synergid cell position adjacent to the zygotic embryo suggests that polyembryony in APIII originated from synergid apomixis (Figure 2 D,I,J) [17, 18].

Polyembryony occurring through sexual reproduction may arguably be called zygotic polyembryony depending on the mechanism involved, and which we classify into three distinct forms: suspensor, cleavage and synergid polyembryony (Figure 1).

Suspensor polyembryony is observed in cases where additional embryos develop from cells of a uniseriate suspensor (Figure 2A). Early studies noted the occurrence of a large suspensor with multiple embryos in garden tulip (*Tulipa gesneriana*), where typically only one embryo persists. This indicated that the mechanism of polyembryony is due to the rapid growth of the suspensor [19, 20]. Similar observations were done in sawah lettuce (*Limnocharis emarginata*), where the suspensor cells may divide extensively, giving rise to multiple embryos [21]. Insights into the possible role of the embryo proper in the suspensor-derived embryos came from studies in the buttercup species *Eranthis*. There it was found that cells of the embryo proper were highly susceptible to acidic solutions, often resulting in their degeneration. This frequently led to renewed growth of the suspensor and the formation of a new embryo from the enlarged suspensor. These and other studies indicated that suspensors in various angiosperms possess embryonic potential, which becomes evident only when embryo proper is disturbed or removed [22-26]. These observations led to a model for intercellular communication proposing an inhibitory effect of the embryo proper to maintain suspensor identity and development [22, 27]. Subsequent molecular studies into the genes related to the proposed embryo-suspensor communication have mainly been obtained using the model species *Arabidopsis thaliana*.

Cleavage polyembryony is common in certain gymnosperm genera such as Pinus, wherein immature embryos split to eventually form multiple pro-embryos. Nevertheless, only one of these embryos progresses to maturity, whereas the remaining embryos are chosen through unknown mechanisms to undergo degradation through programmed cell death [8, 28, 29]. In angiosperms cleavage polyembryony is notably less prevalent, but it can result in the actual formation of multiple embryos. It is widely observed among orchids and also species such as the garden tulip (*Tulipa gesneriana*) and maize have been reported to produce cleavage embryos from the apical embryonic cells (Figure 2B) [20, 30-32]. Further research in maize showed that artificially inducing such a cleavage was possible by treating developing caryopses with 2,4-Dichlorophenoxyacetic acid (2,4-D) on the second day after pollination, resulting in about 40% polyembryony [33]. Interestingly, non-apomictic citrus genotypes have also been reported to display cleavage polyembryony. Here, supernumerary zygotic citrus embryos were formed specifically upon pollination of a diploid female parent with a tetraploid male (Figure 2C). However, reversing the parental roles in the cross eliminated the occurrence of cleavage embryo production. These results showed that cleavage polyembryony in citrus is dependent on ploidy of the male parent [34].

The final and distinctive form of zygotic polyembryony is known as synergid polyembryony. Normally, synergid cells flank the egg cell and their role is to attract the pollen tube and facilitate fertilization of the egg cell [35]. However, an additional haploid embryo can develop from a synergid cell by apomixis, or a diploid embryo can form that originates from a fertilization event of a synergid cell (Figure 2 D,E,F) [36, 37]. We categorize the latter case as zygotic polyembryony, wherein both the synergid and egg cell are fertilized and develop into embryos. An example of synergid polyembryony is presented in the grass *Pennisetum squamulatum Fresen*. Ovules in this species have been reported to show entry of more than one pollen tube into the same embryo sac, thereby allowing fertilization of a synergid in addition to the egg and central cell. The synergid origin of the second embryo along with a diploid chromosome count indicated that the supernumerary embryo originated from a fertilized synergid cell [36].

3. Mechanisms underlying angiosperm zygotic polyembryony

Across most angiosperm species, the 'one seed, one embryo' paradigm is rigorously executed. Nevertheless, mutants have been obtained that display the formation of additional embryos in a single embryo sac, providing insights into the underlying molecular mechanisms governing zygotic polyembryony originating from cleavage or by development from suspensor or synergid cells.

3.1. Cleavage polyembryony

Cleavage embryos can arise from splitting of the apical embryonic cells thereby creating additional developing embryos. One of the few genes reported to be involved in cleavage polyembryony is the *OsPE* gene from rice. The *pe* mutant was generated by T-DNA/Ds insertion mutagenesis in the indica rice cultivar Basmati 370. The polyembryony in *pe* mutant displayed variable penetrance ranging from 10 to 22%. Twin, triplet, and quadruplet embryos that were observed in the *pe* mutant originated from cleavage zygotes, and these could develop further into fertile and healthy plants. Identification of the T-DNA revealed its insertion in the promoter region of *Os03g0241300,* a gene that was expressed in shoot tissue. Unfortunately, the *OsPE* gene has been annotated as encoding a hypothetical protein in *Oryza sativa*, without functional homologs in other species, leaving the molecular mechanism to be resolved [38, 39].

Experiments involving dehydroascorbate reductase (DHAR) have provided some insight on the regulatory mechanisms underlying cleavage polyembryony. DHAR is involved in the recycling of Ascorbic acid and thereby regulates its concentration. Interestingly, overexpression of DHAR in tobacco could increase the rate of polyembryony from 0.034% in WT to 3.36% in transgenics. Morphological observations indicated that the first zygotic division generated embryos of equal size through transverse cell division instead of the normal asymmetric longitudinal division observed in WT. Interestingly, injection of ascorbic acid, or compounds involved in its biosynthesis or recycling, into ovaries during first two days after pollination also increased the rate of polyembryony [40].

3.2. Suspensor polyembryony

Although the function of the suspensor in supporting growth of the embryo proper has been examined in some detail, the molecular mechanism underlying developmental competence of the suspensor and the role of the embryo proper therein has recently seen renewed attention.

Development of the suspensor during zygotic embryogenesis in the dicotyledonous model Arabidopsis starts with an asymmetric division producing a smaller apical cell and a larger basal cell. Subsequent divisions of the apical cell contribute the majority embryo tissues, while the basal cell undergoes limited division, ultimately differentiating into a suspensor (Figure 3). The suspensor plays a pivotal role in embryonic development by pushing the embryo proper into the endosperm cavity and establishing connections with maternal and endosperm tissues, thereby facilitating the transfer of essential nutrients and growth regulators [41, 42]. As the embryo matures, the majority of suspensor cells undergo programmed cell death with the exception of the top-most suspensor cell, which serves as a precursor to the quiescent center and columella stem cells within the embryonic root (Figure 3) [43, 44].

The recessive *twn1* mutant has been obtained following T-DNA transformation, but the mutant phenotype did not segregate with the T-DNA in the progeny [26, 27], suggesting a complex or independent mutation event. Although *twn1* mutant seeds displayed normal germination, around 9% of these produced more than one seedling. These supernumerary seedlings originated from the suspensor but this aberrant development was accompanied by the apparently normal development of the embryo proper [26, 27]. Similarly obtained as a T-DNA insertion mutant, the *twn2* mutant was found by visually screening Arabidopsis seed morphology from F3 families of which the majority of seeds did not germinate. Of those that did germinate, around 40% produced suspensor-derived partial or full twin embryos [25]. Detailed analysis showed that the *twn2* phenotype was characterized by early proembryo cell division arrest accompanied by the production of twin embryos from the suspensor. The causal mutation was a T-DNA insertion in the 5' flanking region

of a valyl-tRNA synthetase (valRS) gene that resulted in an altered pattern of expression. The phenotypes of *twn* mutants together with additional suspensor-related mutants suggested a model in which an inhibitory signaling is imposed on the suspensor by the embryo proper [26, 27]. Identifying and characterizing the responsible genes should aid in unravelling the molecular nature of the signaling process.

To investigate the role of the auxin in the suspensor, auxin response was blocked by the expression of the non-degradable AUX/IAA bodenlos (bdl) protein using both ubiquitous and suspensor drivers. This induced loss of suspensor quiescence resulting in abnormal suspensor divisions, and marker analysis suggested at least partial loss of suspensor identity. As a side effect the *RPS5A* promoter-driven *bdl* occasionally induced development of a twin embryo from the suspensor. However, these germinated as rootless after seedlings, which was attributed to the importance of proper auxin response for root development [45]. Polyembryos derived from the suspensor could also be induced by ectopically expressing somatic embryogenesis-related genes, *RWP-RK DOMAIN CONTAINING 1* (*RKD1*), *RKD4*, and *WUSCHEL* (*WUS*), under the control of a suspensor-specific driver. In these transgenic plants, the periclinal division of the suspensor that was followed by expression of the *DRN* marker in polyembryonate ovules suggested that the secondary embryo development proceeds *via* a classical somatic embryogenesis process [46].

Recently, the mutant *meerling-1* (*mrl-1*) was identified and characterized, which exhibited a high penetrance of suspensor-derived polyembryony [47]. In this mutant, both embryo proper and suspensor-derived embryos were capable of developing into healthy plants, very similar to the phenotype displayed by *twn1*. In addition, *mrl-1* and *twn1* form a distinct clade of mutants where suspensor-derived embryo formation is not dependent on embryo proper damage. However, the F1 of a cross between *mrl-1* and *twn1* revealed that they represent mutations in different genes.

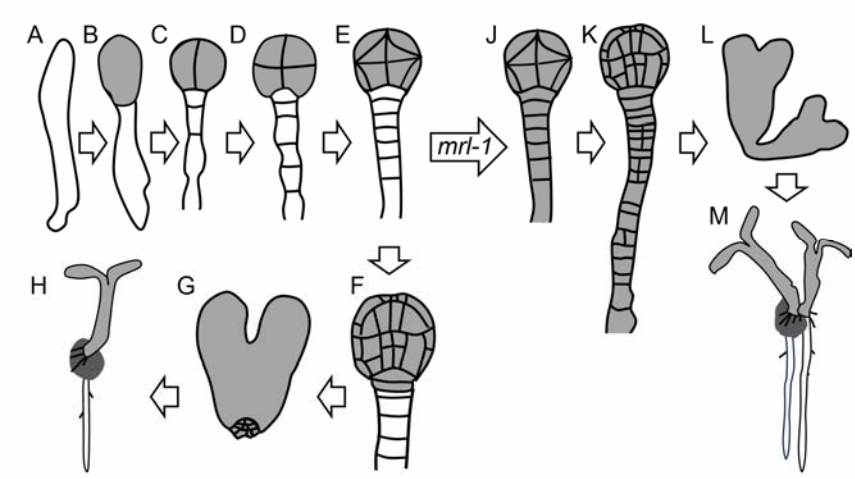

Figure 3. Arabidopsis embryogenesis and suspensor-derived polyembryony in *mrl-1*. **A-G**, Embryogenesis in WT. The elongated zygote (**A**) divides assymetrically to generate an apical precursor of the embryo proper (grey) and a basal cell that originates the suspensor (white) (**B**). Subsequent divisions generate the depicted two to four cell stage embryo (**C**), followed by the octant stage (**D**), dermatogen stage (**E**), globular stage (**F**) and heart stage (**G**) embryo. **H**, Single seedling germinating from a single seed. **J-L**, Suspensor-derived polyembryony in the mrl-1 mutant. The suspensor is reprogrammed to adopt embryo identity (grey) at around the globular stage (**J**). Subsequently aberrant periclinal division appears in the reprogrammed suspensor (**K**) preluding to the development of an additional suspensor-derived embryo (**L**). **M**, multiple seedlings germinate from one seed. Grey represents embryo identity.

The high penetrance of the *mrl-1* polyembryo phenotype allowed pinpointing the timing of secondary embryo formation at around octant stage [47]. In addition, this allowed efficient analysis of tissue specific markers DORNROSCHEN (DRN) [48] and ATPase [46] for embryo proper and suspensor fate, respectively. The DRN marker was initially correctly setup in the embryo proper in *mrl-1* and did not localize to the mutant suspensor cells until globular stage, after which transverse dividing suspensor cells were observed. Together with the initial normal suspensor morphology and the ATPase marker expression, this indicated that suspensor fate was partially compromised from octant stage onwards [47]. These results indicated that suspensor-derived polyembryony involves a reprograming process. This is in agreement with ablation studies where damage to the apical cell that was formed after the asymmetric division of zygote, resulted in basal cell reprogramming [49, 50]. Compared to the ectopic suspensor development upon interfering with auxin response or ectopic transcription factor expression, the suspensor reprogramming followed by ectopic embryo fate establishment observed in the *mrl-1* mutant and in ablation studies appears to represent a different mechanism.

The *mrl-1* mutant was obtained from a transformation experiment using *Agrobacterium tumefaciens*. Bulk segregant analysis revealed a genomic rearrangement resulting in an approximately 2Mb part of chromosome 1 being reverse duplicated in the 5'-UTR region of the *U3 SMALL NUCLEOLAR RNA-ASSOCIATED PROTEIN 18 (UTP18)* gene. The *UTP18* gene encodes a nucleolar-localized WD40-repeat protein involved in processing 18S pre-ribosomal RNA. The genomic rearrangement caused the promoter from an *ARMDILLO REPEAT* gene to drive *UTP18* expression, leading to reduced protein abundance and delayed early embryo proper development. This delay would trigger suspensor reprogramming towards embryo fate, leading to the development of supernumerary embryos [47]. In addition, *mrl-1* mutant complementation specifically in the embryo proper fully restored the polyembryony phenotype. Taken together this study supports the proposed model involving active signaling between embryo proper and suspensor for progression of normal embryo development.

3.3. Synergid (zygotic) polyembryony

The mature female gametophyte of Arabidopsis comprises a mature egg cell flanked by two synergid cells that attract the pollen tube. Interestingly, mutants have been obtained where synergids express an egg cell-specific marker, suggesting reprogramming into egg cell-like cells [51-53] [37]. One of these was defective in the *ALTERED MERISTEM PROGRAM 1 (AMP1)* gene. Previously the *amp1* mutant had been reported to display pleiotropic phenotypes, that included altered shoot apical meristems, overproliferation of suspensor cells, polycotyly, early flowering and increased cytokinin levels [54-56]. Although initially the *amp1* mutant was found to display suspensor-derived polyembryony, a later study observed reprogramming of synergid cells towards fully functional supernumerary egg cells, that gave rise to secondary embryos [37, 56]. During fertilization of *amp1* mutant, the two sperm cells that were delivered by a single pollen tube fertilized both the egg cell and the synergid-derived egg cell. As a consequence, the unfertilized central cell failed to form endosperm, resulting in developmental arrest of the embryos. However, pollination with pollen from a polyspermic mutant *tetraspore* (*tes*), that was capable of delivering more than two sperm cells, enabled the embryo sac to produce viable embryos [57].

The *AMP1* gene encodes an endoplasmic reticulum-associated protein and was reported to share the organization of motifs and domains with glutamate carboxypeptidases in plants and animals [37, 58]. Its carboxypeptidase function was proposed to modulate the levels of signalling molecules thereby impacting several aspects of plant development [59]. In addition, AMP1 was shown to be involved in inhibiting the translation of mRNAs targeted by miRNAs [58]. In the ovule, a genomic *AMP1:3xGFP* fusion, able to fully rescue the *amp1* phenotypes, showed strong expression in sporophytic tissue and synergids. Interestingly, not only synergid expression but also sporophytic or central cell expression of *AMP1* prevented supernumerary egg cells and formation of twin embryos, suggestive of an AMP1-dependent mobile signal to maintain synergid cell identity. It was concluded that one synergid is sufficient for pollen tube attraction after which the other

synergid needs to be eliminated in favor of central cell fertilization [37]. However, comparing synergid polyembryony in *amp1* with the reported polyembryony occurrence upon multiple pollen grain entry in the ovule in *Pennisetum squamulatum Fresen*, raises the question whether fertilization of the synergid cell requires its reprogramming to an egg cell-like fate. Whether AMP1 protein or RNA itself acts as a mobile signal or the AMP1 enzymatic activity generates specific signals remains unclear. In addition, the RNAi- related function and the pleiotropic mutant phenotypes suggest AMP1 is a multifaceted protein with different roles in various developmental processes.

4. Mechanisms behind apomixis in natural plant populations

Apomixis represents a mode of asexual reproduction in flowering plants that results in seed formation in the absence of meiosis or fertilization of the egg cell [9, 13]. Apomixis produces clonal offspring genetically identical to the maternal plant, which has attracted much attention due to its potential to preserve hybrid vigor in highly productive crop plant genotypes. Apomixis in nature is commonly observed in a small group of angiosperms, and polyembryony can occur as a byproduct in apomictic species.

Parthenogenesis is a form of apomixis in which an unfertilized egg develops into an embryo without fertilization. In dandelion (Taraxacum), parthenogenesis is controlled by the *PARTHENOGENESIS* (*PAR*) gene, which encodes a K2-2 zinc finger protein containing a transcriptional repressive EAR domain [60]. A miniature inverted repeat transposable element (MITE) insertion in the promoter region of this gene enabled egg cell expression, thereby facilitating the transition to embryogenesis. Interestingly, in apomictic forms of hawkweed (Hieracium), a similar MITE was found present in the promoter of a *PAR* homolog, presenting another case of parallel evolution. Although the parthenogenesis not necessarily produced polyembryos, ectopic expression of *PAR* in the lettuce egg cell occasionally induced the formation of multiple embryos within a single embryo sac. The polyembryo structures aborted at later stages, likely due to abnormal endosperm initiation, thereby failing to develop viable seeds [60].

Besides the synergid polyembryony that was observed in *Pennisetum squamulatum Fresen* [36], *Pennisetum squamulatum* species have also been investigated as an obligate apomict. In the latter case the embryo was directly formed from the nucellar cells within the embryo sac, in a process called apospory [61]. Apospory is governed by the apospory-specific genomic region (ASGR), a distinctive chromosomal segment characterized by its large physical size, hemizygosity, and lack of recombination. Within the ASGR, multiple copies of the *PsASGR-BABYBOOM-like* (*PsASGR-BBML*) gene are located [61]. *BBM* was originally characterized in Arabidopsis and Brassica, and its constitutive overexpression induced somatic embryogenesis whereas egg cell-specific overexpression of *BBM* was able to induce parthenogenesis [62, 63]. Similarly, the *PsASGR-BBML* promoter conferred egg cell expression in a sexual tetraploid pearl millet and driving the *PsASGR-BBML* gene from this promoter could induce parthenogenesis and the production of haploid offspring [64]. Interestingly, the Arabidopsis synergid-specific promoter *pAtDD45* driving *OsBBM1*-induced synergid cells to autonomously develop into embryos accompanied by normal fertilization of egg cells, resulting in the formation of twin seedlings in rice seeds [65].

Citrus and mango are two economically important crops that display facultative apomixis, producing a zygotic embryo but also multiple clonal nucellar embryos. The genetic basis of nucellar polyembryony in both species is due to specific insertion events in the promoter of the *RWP* gene, presenting a case of parallel evolution. The promoter insertions result in *RWP* overexpression in ovules, thereby causing polyembryony [14, 66]. The *RWP* gene is the homolog of *AtRKD4* (*GROUNDED)*, that functions in the maintenance of egg-cell identity in Arabidopsis. Transient overexpression of *AtRKD4* promoted the formation of ectopic embryos from somatic tissues [67]. The high expression of sexual embryogenesis–associated genes during apomixis in citrus indicated their close developmental relationship [14, 66]. Taken together, the involvement of somatic embryogenesis-linked genes in the process of apomixis suggests possible shared mechanisms.

5. Perspectives on polyembryony in fundamental research

From a broader perspective, the occurrence of polyembryony in angiosperms is attributed to the totipotency of plant cells. For example, the proembryonic cell can cleave into additional embryonic cells, suspensor cells can reprogram into embryonic cells, synergid cells can transdifferentiate into functional egg cells, and female gametophytes and nucellar cells can proliferate into embryos. Much research has focused on plant regeneration, e.g. from vegetative tissues where differentiated cells are induced to reprogram under the influence of hormonal regimes with or without simultaneous expression of developmental regulators and gain totipotency to subsequently develop somatic embryos or de novo shoots [3, 68, 69]. The molecular mechanisms underlying polyembryony in angiosperms remain largely elusive, but linking genes such as *BBM* and *RWP* suggests at least some similarities with processes governing apomixis and somatic embryogenesis.

Signaling appears to be another important factor in steering correct embryo development. Mutants like *twn1* and *mrl-1* appear to lack synchronization between embryo proper and suspensor development whereby, at least in the case of *mrl-1* it was shown that, the suspensor reacts on the delayed embryo proper development by reprogramming towards embryonic fate. The general molecular function of the *UTP18* gene that is mutated in *mrl-1* suggests that any factor able to delay embryo proper development prior to the globular stage will result in secondary embryo development from the suspensor. Indeed, examples are the *twn2* mutant with its altered valyl-tRNA synthetase expression and the apical cell ablation studies, that both resulted in basal cell reprogramming [25]. In addition, from the *amp1* mutant it follows that a mobile signal is required to maintain synergid cell identity [37]. Together, these scenarios support active signaling between the various cell types in the ovule to sustain the development of a single dominant embryo.

Studying polyembryony in angiosperm species that display low frequencies is impractical. Instead, research on the molecular genetic mechanisms may benefit by focussing on high-frequency polyembryony in species such as citrus or in mutants of model species such as Arabidopsis. So far, polyembryony research in these species has been mostly limited to cloning the causal genes, while the regulatory mechanisms remain largely unknown. For instance, as seen in Arabidopsis, the potential of the suspensor to develop into an embryo is suppressed by normal embryo proper development, indicative of signaling between the embryo and suspensor. In case of citrus, the induction of cleavage polyembryony specifically by tetraploid male pollination suggests that male-female crosstalk provides patterning information.

In the future, exploring the gene regulatory networks and signalling pathways during the initiation of polyembryony should enhance our understanding of the intricate communication networks underlying fate specification and reprogramming in plants.

ACKNOWLEDGMENTS

We thank Jana Wittmer and Tristan Wijsman for critical reading. Funding for this work was provided by a China Scholarship Council grant (H.W., file No. 202006990030).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Cummings, M. R. 2010, Human heredity: principles and issues, Brooks/Cole, Cengage Learning, Pacific Grove, Calif.
2. Lakshmanan, K. K. and Ambegaokar., K. B. 1984, “Polyembryony” in Embryology of Angiosperms, 445–474.
3. Su, Y. H., Tang, L. P., Zhao, X. Y. and Zhang, X. S. 2021, J. Integr. Plant Biol., 63, 228-243.
4. Johansen, D. A. 1950, Plant embryology: embryogeny of the spermatophyta, Chronica Botanica Co., Waltham, Mass.
5. Maheshwari, P. 1963, Recent advances in the embryology of angiosperms, International Society of Plant Morphologists, University of Delhi, Delhi.

6. Rudall, P. J., Remizowa, M. V., Beer, A. S., Bradshaw, E., Stevenson, D. W., Macfarlane, T. D., Tuckett, R. E., Yadav, S. R. and Sokoloff, D. D. 2008, Ann. Bot., 101, 941-956.
7. Raghavan, V. and Sharma, K. K. 1995, "Zygotic Embryogenesis in Gymnosperms and Angiosperms" in In Vitro Embryogenesis in Plants, 20, 73-115.
8. von Arnold, S., Clapham, D. and Abrahamsson, M. 2019, "Embryology in conifers" in Molecular Physiology and Biotechnology of Trees, 157-184.
9. Bicknell, R. A. and Koltunow, A. M. 2004, Plant Cell, 16, S228-S245.
10. Xu, Y., Jia, H., Tan, C., Wu, X., Deng, X. and Xu, Q. 2022, Horticulture Research, 9, uhac150
11. Asker, S. E. and Jerling, L. 1992, Apomixis in plants, CRC Press, Boca Raton.
12. Koltunow, A. M. 1993, The Plant Cell, 5, 1425-1437.
13. Koltunow, A. M. and Grossniklaus, U. 2003, Annu Rev Plant Biol, 54, 547-574.
14. Wang, X., Xu, Y., Zhang, S., Cao, L., Huang, Y., Cheng, J., Wu, G., Tian, S., Chen, C., Liu, Y., Yu, H., Yang, X., Lan, H., Wang, N., Wang, L., Xu, J., Jiang, X., Xie, Z., Tan, M., Larkin, R. M., Chen, L. L., Ma, B. G., Ruan, Y., Deng, X. and Xu, Q. 2017, Nat Genet, 49, 765-772.
15. Cooper, D. C. 1943, American Journal of Botany, 30, 408-413.
16. LI, Y. and Yuan, L. 1990, Acta Agronomica Sinica, 16
17. Mu, X., Jin, B. and Teng, N. 2010, Flora - Morphology, Distribution, Functional Ecology of Plants, 205, 404-410.
18. Mu, X., Zhu, Z., Cai, X., Sun, D. and Lin, J. 2002, Acta Botanica Sinica, 44, 1387-1395.
19. Coulter, J. M. and Chamberlan, C. J. 1903, Mophology of Angiosperms,
20. Ernst, A. 1901, Ueber Pseudo-Hermaphroditismus und andere Missbildungen der Oogonien bei Nitella syncarpa (Thuill.) Kützing und Beiträge zur Kenntniss der Entwicklung des Embryosackes und des Embryo (Polyembryonie) bei Tulipa Gesneriana L., München: Val. Höfling.
21. Hall, J. G. 1902, Botanical Gazette, 33, 214-219.
22. Haccius, B. 1963, Phytomorphology, 13, 107-115.
23. Yeung, E. C. and Meinke, D. W. 1993, The Plant Cell, 5, 1371-1381.
24. Marsden, M. P. F. and Meinke, D. W. 1985, Am. J. Bot., 72, 1801-1812.
25. Zhang, J. Z. and Somerville, C. R. 1997, Proc. Natl. Acad. Sci. USA, 94, 7349-7355.
26. Vernon, D. M. and Meinke, D. W. 1994, Developmental Biology, 165, 566-573.
27. Schwartz, B. W., Vernon, D. M. and Meinke, D. W. 1997, "Development of the Suspensor: Differentiation, Communication, and Programmed Cell Death During Plant Embryogenesis" in Cellular and Molecular Biology of Plant Seed Development, 53-72, B. A. Larkins, I. K. Vasil, Eds. Springer Netherlands, Dordrecht.
28. Cairney, J. and Pullman, G. S. 2007, New Phytol, 176, 511-536.
29. Bozhkov, P. V., Filonova, L. H. and Suarez, M. F. 2005, Curr. Top. Dev. Biol., 67, 135-179.
30. Maheshwari, P. 1952, Journal of Palaeosciences, 1, 319-329.
31. Batygina, T., Bragina, E. and Vasilyeva, V. 2003, Acta Biologica Cracoviensia Series Botanica 45, 21-34.
32. Sharman, B. C. 2006, New Phytologist, 41, 125-129.
33. Erdelskfi, O. and Vidovencovfi, Z. 1992, Sexual Plant Reproduction, 224-226.
34. Aleza, P., Juarez, J., Ollitrault, P. and Navarro, L. 2010, Ann Bot, 106, 533-545.
35. Higashiyama, T., Yabe, S., Sasaki, N., Nishimura, Y., Miyagishima, S.-y., Kuroiwa, H. and Kuroiwa, T. 2001, Science, 293, 1480-1483.
36. Sindhe, A. N. R., Swamy, B. G. L. and Arekal, G. D. 1980, Current Science, 49, 914-915.
37. Kong, J., Lau, S. and Jürgens, G. 2015, Curr. Biol., 25, 225-230.
38. Puri, A., Basha, P. O., Kumar, M., Rajpurohit, D., Randhawa, G. S., Kianian, S. F., Rishi, A. and Dhaliwal, H. S. 2010, Funct Integr Genomics, 10, 359-366.

39. Paul, P., Awasthi, A., Kumar, S., Verma, S. K., Prasad, R. and Dhaliwal, H. S. 2012, Plant Cell Rep., 31, 1779-1787.
40. Chen, Z. and Gallie, D. R. 2012, PLoS One, 7, e39147.
41. Kawashima, T. and Goldberg, R. B. 2010, Trends Plant Sci., 15, 23-30.
42. Peng, X. and Sun, M. X. 2018, Plant Reprod, 31, 59-65.
43. ten Hove, C. A., Lu, K.-J. and Weijers, D. 2015, Development, 142, 420-430.
44. Scheres, B., Wolkenfelt, H., Willemsen, V., Terlouw, M., Lawson, E., Dean, C. and Weisbeek, P. 1994, Development, 120, 2475-2487.
45. Rademacher, E. H., Lokerse, A. S., Schlereth, A., Llavata-Peris, C. I., Bayer, M., Kientz, M., Freire Rios, A., Borst, J. W., Lukowitz, W., Jurgens, G. and Weijers, D. 2012, Dev. Cell, 22, 211-222.
46. Radoeva, T., Albrecht, C., Piepers, M., de Vries, S. and Weijers, D. 2020, Development, 147
47. Wang, H., Santuari, L., Wijsman, T., Wachsman, G., Haase, H., Nodine, M., Scheres, B. and Heidstra, R. 2024, Plant Cell, 10.1093/plcell/koae087
48. Cole, M., Chandler, J., Weijers, D., Jacobs, B., Comelli, P. and Werr, W. 2009, Development, 136, 1643-1651.
49. Gooh, K., Ueda, M., Aruga, K., Park, J., Arata, H., Higashiyama, T. and Kurihara, D. 2015, Dev Cell, 34, 242-251.
50. Liu, Y., Li, X., Zhao, J., Tang, X., Tian, S., Chen, J., Shi, C., Wang, W., Zhang, L., Feng, X. and Sun, M. X. 2015, Proc. Natl. Acad. Sci. USA, 112, 12432-12437.
51. Groß-Hardt, R., Kägi, C., Baumann, N., Moore, J. M., Baskar, R., Gagliano, W. B., Jürgens, G. and Grossniklaus, U. 2007, PLOS Biology, 5, e47.
52. Moll, C., von Lyncker, L., Zimmermann, S., Kägi, C., Baumann, N., Twell, D., Grossniklaus, U. and Gross-Hardt, R. 2008, Plant J., 56, 913-921.
53. Kirioukhova, O., Johnston, A. J., Kleen, D., Kägi, C., Baskar, R., Moore, J. M., Bäumlein, H., Gross-Hardt, R. and Grossniklaus, U. 2011, Development, 138, 3409-3420.
54. Chaudhury, A. M., Letham, S., Craig, S. and Dennis, E. S. 2002, The Plant Journal, 4, 907-916.
55. Chin-Atkins, A. N., Craig, S., Hocart, C. H., Dennis, E. S. and Chaudhury, A. M. 1996, Planta, 198, 549-556.
56. Vidaurre, D. P., Ploense, S., Krogan, N. T. and Berleth, T. 2007, Development, 134, 2561-2567.
57. Spielman, M., Preuss, D., Li, F. L., Browne, W. E., Scott, R. J. and Dickinson, H. G. 1997, Development, 124, 2645-2657.
58. Li, S., Liu, L., Zhuang, X., Yu, Y., Liu, X., Cui, X., Ji, L., Pan, Z., Cao, X., Mo, B., Zhang, F., Raikhel, N., Jiang, L. and Chen, X. 2013, Cell, 153, 562-574.
59. Helliwell, C. A., Chin-Atkins, A. N., Wilson, I. W., Chapple, R., Dennis, E. S. and Chaudhury, A. 2001, Plant Cell, 13, 2115-2125.
60. Underwood, C. J., Vijverberg, K., Rigola, D., Okamoto, S., Oplaat, C., Camp, R., Radoeva, T., Schauer, S. E., Fierens, J., Jansen, K., Mansveld, S., Busscher, M., Xiong, W., Datema, E., Nijbroek, K., Blom, E. J., Bicknell, R., Catanach, A., Erasmuson, S., Winefield, C., van Tunen, A. J., Prins, M., Schranz, M. E. and van Dijk, P. J. 2022, Nat Genet, 54, 84-93.
61. Ozias-Akins, P., Roche, D. and Hanna, W. W. 1998, Proc. Natl. Acad. Sci. USA, 95, 5127-5132.
62. Boutilier, K., Offringa, R., Sharma, V. K., Kieft, H., Ouellet, T., Zhang, L., Hattori, J., Liu, C. M., van Lammeren, A. A., Miki, B. L., Custers, J. B. and van Lookeren Campagne, M. M. 2002, Plant Cell, 14, 1737-1749.
63. Chen, B., Maas, L., Figueiredo, D., Zhong, Y., Reis, R., Li, M., Horstman, A., Riksen, T., Weemen, M., Liu, H., Siemons, C., Chen, S., Angenent, G. C. and Boutilier, K. 2022, Proc. Natl. Acad. Sci. USA, 119, e2201761119.
64. Conner, J. A., Mookkan, M., Huo, H., Chae, K. and Ozias-Akins, P. 2015, Proc. Natl. Acad. Sci. USA, 112, 11205-11210.
65. Dan, J., Xia, Y., Wang, Y., Zhan, Y., Tian, J., Tang, N., Deng, H. and Cao, M. 2024, Plant Cell Rep, 43, 79.

66. Yadav, C. B., Rozen, A., Eshed, R., Ish-Shalom, M., Faigenboim, A., Dillon, N., Bally, I., Webb, M., Kuhn, D., Ophir, R., Cohen, Y. and Sherman, A. 2023, Horticulture Research, 10
67. Waki, T., Hiki, T., Watanabe, R., Hashimoto, T. and Nakajima, K. 2011, Current Biology, 21, 1277-1281.
68. Ikeuchi, M., Favero, D. S., Sakamoto, Y., Iwase, A., Coleman, D., Rymen, B. and Sugimoto, K. 2019, Annu. Rev. Plant Biol., 70.
69. Morinaka, H., Sakamoto, Y., Iwase, A. and Sugimoto, K. 2023, Curr. Opin. Plant Biol., 74, 102377.