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Polyembryony in Maize: A Complex, Elusive, and Potentially Agronomical Useful Trait

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Abstract

Polyembryony (PE) is a rare phenomenon in cultivated plant species. Since nineteenth century, several reports have been published on PE in maize. Reports of multiple seedlings developing at embryonic level in laboratory and studies under greenhouse and field conditions have demonstrated the presence of PE in cultivated maize (*Zea mays* L.). Nevertheless, there is a lack of knowledge about this phenomenon; diverse genetic mechanisms controlling PE in maize have been proposed: Mendelian inheritance of a single gene, interaction between two genes and multiple genes are some of the proposed mechanisms. On the other hand, the presence of two or more embryos per seed confers higher nutrimental quality because these grains have more crude fat and lysine than normal maize kernels. As mentioned above, there is a necessity for more studies about PE maize in order to establish the genetic mechanism responsible for this phenomenon; on the other hand, previous studies showed that PE has potential to generate specialized maize varieties with yield potential and grain quality.

Keywords: *Zea mays* L. polyembryony, genetic control, ploidy level, apomixis, xenia

1. Introduction

Polyembryony (PE) can be defined as the simultaneous emergence of two or more seedlings from one germinated seed [1]. The plant polyembryony phenomenon was discovered by Van

Leeuwenhoek in 1719 and reported in orange seeds and can be classified into two main types that are based on the cellular origin of embryogenesis either, gametophytic and sporophytic [2, 3].

This phenomenon occurs spontaneously in several plants species although at low frequencies. The term “polyembryony” also reports it as the division of one sexually produced embryo into many, and the resulting ones are genetically identical to each other, but distinct from their mother [4]. However, some PE versions have to feature of high potential with agronomical applications in maize [5]. This phenomenon is common in gymnosperms and less frequent in angiosperms [6]. Shukla in 2004 [7] reported about 59 families, 158 genera, and 239 vegetal species having this trait. Embryos in polyembryonic seeds may originate from embryo sac (ovule, zygote, synergids, and antipodes), nucellar tissue, or the integument [8, 9]. Therefore, may be monoploid (containing half (n) of the normal number of chromosomes), or diploid (with a normal number of chromosomes ($2n$)) [10–13].

Embryological studies in nineteenth and twentieth centuries demonstrated that the adventitious embryos present in a seed in addition to the sexual embryo can be formed based on different structures of ovule and embryo sac structures [14–18]. Maize PE has been studied for almost 100 years, judging from published reports [10, 19–29]. Although, this phenomenon has been studied by different authors, there are still many questions about the origin, causes, PE gene and its relationship with apomixis and pollen source, and the environmental effect on the expression of this feature [30]. This study provides a review of the present literature on this phenomenon, applications, and possible causes of PE and, particularly, discusses this phenomenon in maize.

2. PE in nature

PE has been reported in different plant species such as almond [31], citrus [32–34], mango [7, 35], peach [36], rice [37], soybean [38], strawberry [39], papaya [40], kiwi, apple [41], safflower [42], alfalfa [43, 44], lemon [45], grape [46], and olive cultivars [3]. Polyembryony was shown only in 8 of the 24 selected olive cultivars; this specificity of cultivar as in other fruit species agrees that polyembryony is also a genetically regulated character. The latter has two diploid ($2n$) embryos, one from zygote and the other from the nucellus [43]; potato and flax with two embryos, one diploid ($2n$) embryo from zygote and one haploid (n) from a synergid [47, 48]; wheat with two embryos in the same bag, an embryo of oosphere (n) and another from the fertilized ($2n$) synergid [49]; asparagus two diploid embryos from proembryo division [50]; citrus (*Citrus* spp.) with a normal embryo of sexual origin and others that develop from nucellus [31]; and papaya (*Carica papaya*) [40]. It has been assumed that the plants are of zygote origin, and there have been no genetic tests; occasionally, multiple embryos come from cultured ovules [46].

Most of the citrus cultivars are polyembryonic, for example, most lemon crops produce several embryos per seed, which is why it is necessary to rescue the zygotics, to reduce abortion and competition with nucleic embryos [45]. Polyembryony also has been reported among certain insects as parasitic wasps [51, 52] such as *Copidosoma floridanum* [53] and even mammals such as armadillo, which give birth to several offsprings, all twins [54]. Humans that originate in this way are the so-called identical twins, who are mostly genetically identical [55].

Polyembryonic, called embryo generation along with the zygotic embryo in a single seed, is widespread in angiosperms. The development of additional embryos may be induced by exogenous factors, such as pollen irradiation, higher temperatures, and herbicides, which are employed during and after flowering [56]. Polyembryony has been observed in sexual ferns and attributed to multiple fertilizations, and report this phenomenon in *Pteris tripartite* Sw. where they obtain from two to eight sporophytes, observed from a single gametophyte [57].

2.1. PE in maize

PE in maize is a phenomenon poorly studied. In addition, some research reports about this trait are contradictory. Sharman in 1942 [58] noted that a maize line had two embryos that emerged from a single caryopsis, whereby they were selected and dissected. The two embryos appeared to be completely separated except by the scutellum. This suggests that the twin characteristic showed up early and was probably caused by a longitudinal division or a constriction of the cell mass that was the stage of "pro-embryo." The above results suggested that both embryos were identical and produced typical plants with normal chromosome number $2n$.

Morgan and Rappleye in 1951 [24] induced PE in maize after exposing pollen to different X-ray doses and crossing females of the same line with that pollen; after sowing the obtained seeds, it was observed that the presence of PE was up to 18% of the seeds. Thus, concluded that treatment with X-rays causes a significant deviation from the normal reproduction process resulting in the formation of numerous embryos. They also reported that double embryo seeds produced plants with different heights, indicating that haploid plants may occur among the polyembryonic lines, resulting from plant crosses where pollen was exposed to X-rays. Earlier reports mentioned maize with multiple plumules and primary roots, but with a single scutellum, concluding that these plants did not come from two embryos, but from one abnormal embryo [21]. This feature was also mentioned by Kempton in 1913 [19] and Weatherwax in 1921 [20] and was called false polyembryony. In all cases, there occurred two stems and two primary roots. Besides, a case was found where three stems were attached to a single cotyledon. After two generations of a line with this trait, it was observed that this peculiarity was lost.

Pešev in 1976 [25] reported the derivation of several inbred lines from a population that formerly exhibited a few twin plants; the inbred lines showed the twin condition in frequencies that ranged from 2.1 to 25.3%. Pollacsek in 1984 [59] reported that in the Old French INRA F1254 line, it was found that 4.5% of the plants were with double stems and determined that the nature of this trait was an early fasciation that takes place during embryogenesis. This trait with incomplete penetrance had low probably due to oligogenic control.

In 1973, the Instituto Mexicano del Maíz at Universidad Autónoma Agraria Antonio Narro (IMM-UAAAN) located in Saltillo, Mexico, generated a maize population which presented polyembryonic seeds with a frequency of 1.5%. This material was improved with a process of recurrent selection for 5 years under the assumption that this may lead to a gradual increase of favorable alleles for PE, and at the same time, maintain high genetic variability [5, 26]. To avoid that selection carried to inbreeding, twin crosses were made with elite inbred lines from

a different origin [5]. This population in addition to genes for polyembryony had the brachytic two genes (*br2*). In 1991, 47% of the polyembryonic plants were observed in the population. Now, researchers decided to separate this population into two according to the phenotype in high or normal and brachytic (dwarf) plants. These have the brachytic2 (*br2*) gene [5]. The *br2* is a recessive gene that has an agronomic potential because it results in the shortening of the internodes of the lower stalk without an obvious reduction in other plant organs [60] that modulates polar auxin transport in the maize stalk. This gene encodes a protein similar to adenosine triphosphate (ATP)-binding cassette transporters of the multidrug resistant (MDR) class of P-glycoproteins (PGs) [61].

Four years later, the percentage of PE in both populations averaged 60%; the most common issue was found in seeds with double seedlings, but the number of seedlings per seed was as high as six (**Figure 1**). In 1996, each population (normal and brachytic plant height) was divided into two subpopulations, one with plants where PE frequency was high and one with plants where PE was low, having four different populations: the normal height plant and high polyembryony (NAP); normal height plant and low polyembryony (NBP); brachytic plant height and high polyembryony (BAP); and dwarf plant height with low polyembryony (BBP). In 1998, dwarf and normal populations reached 61 and 63% of PE, respectively [5]. The frequency of PE is currently 65 and 60% for the dwarf and normal populations, respectively;



Figure 1. Polyembryonic and nonpolyembryonic maize seedlings. (a) Left to right: Normal maize phenotype, twin maize (PE maize) seedling both normal and twin of 21 days old, triple, and quadruple maize seedling of 28 days old. (b) Sixfold seedling: multiple seedling almost independent, at least sharing scutellum. (c) Several ways in which twins seedlings are observed; there are also cases of two or more radicles per PE plants. Photographs provided by Jose Espinoza-Velazquez IMM-UAAAN.

the higher frequency of PE in these populations are twin plants (**Figure 1**), followed by triple and presenting uncommon seedlings—quadruple, quintuple, and sextuple [28]. Espinoza-Velazquez and Vega in 2000 [62] worked with subpopulations of IMM-UAAAN and reported that in the period 1995–2000 the selection for the PE has gained between 2 and 3% per cycle. They led the polyembryonic populations to levels above 60% PE, while the reverse selection (contrary to PE) groups rapidly leads to frequencies less than 6% PE.

3. Agronomic benefits from PE

Polyembryonic seed is an important feature due to commercial multiplication [63]. Citrus has a normal embryo of sexual origin and others that develop from ovule nucellus, so all these embryos from nucellus are identical to the parent plant so that they may be used as rootstocks by their rusticity and uniformity [64]. The PE is an extremely rare phenomenon in maize; however, this trait may confer great benefits since in this case, plants may have increased production and competitiveness because a seed may produce two to six normal plants favoring production because of the increase of number of plants and ears per surface unit [30].

Other benefits are lower production costs because with the same number of seeds, farmers can have more plants per unit area. So to plant a unit area will require less seed that will result in lower storage and transportation costs [5]. However, yield performance and population density experiments are needed to evaluate the improvement in grain yield because of polyembryonic maize varieties.

4. Nutritional benefits from PE

Pešev in 1976 [25] reported a significant increase in protein (4.5%), lysine g/100 g dry material (38–70.9%), lysine g/100 g protein (21.3–34.0%), and oil (3.5–13.6%) in polyembryonic maize grains compared to those with a single embryo. Other authors have reported a positive increase in polyembryonic maize dough, detecting a positive association between PE and oil content (22% higher than a native variety) with a high percentage of unsaturated oils and a better relationship between oleic and linoleic acids. The average of crude protein in polyembryonic maize is 10% and was 8% higher than a native variety. The crude fat content (FC) of grain in NAP and BAP populations showed an overall average of 6.2% [65]. This may be attributable to the positive correlation between PE and lipid concentration in the grain. FC quantitative superiority of maize PE may also be more qualitative because from 55 to 65% of the grains of an ear has two or more embryos [28]. This suggests that selection in favor of polyembryony increases indirectly grain content of nutriment as crude fat and lysine; a condition that could be exploited in the design of new varieties of PE maize, combining high yield and grain quality.

Gonzalez and collaborators [28] in a study on nutritional quality and quantity of PE grains derived from crosses between the IMM-UAAAN-BAP population (PE) and Tuxpeño Population

high oil content (HOC) of Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), to generate PE:HOC germplasm, direct and reciprocal crosses, as well as backcrossing to both parents, were performed. The authors obtained the following germplasm combinations (0:100, 12.5:87.5, 25:75, 37.5:62.5, 50:50, 62.5:37.5, 75:25, 87.5:12.5, and 100:0). These authors noted that crude fat content (CF) and lysine (Lys) may be raised increasing the doses of HOC and PE, respectively. The optimal combinations of germplasm PE:HOC for nutritional grain quality combinations were 50:50 (Lys = 2.7%; FC = 6.9%); these values were higher than those observed in common maize. The PE present in BAP population induced the highest value for lysine (4%). The PE in maize may be usable as an alternate route in the designing of varieties for special applications. In addition to the pattern for potential yield, the nutritional value of the grain, increasing quantity, and quality of protein and oil, which under the hypothesis that two or more embryos per seed, will increase the storage capacity of quality nutriment [66]. Cruz [67] studied the chemical, physical, and rheological properties of dough, tortilla, and grain of maize populations with high polyembryony. They concluded that the physical and chemical characteristics of polyembryonic maize are within those acceptable ranges for the production of food products, such as tortillas and flour.

5. Types of polyembryony

Analysis of different classifications of PE has shown that the main criterion for classification includes the origin of the initial cell, embryo formation pathways, and their genetic characteristics. The first classification system was proposed by Braun [68], who described four possible routes for the formation of adventitious embryos as a result of a merger of two or more eggs, developing several embryo sacs in the same ovule, or as result of a pro-embryonic division. According to Lakshmanan and Ambegaokar in 1984 [69], the PE is classified into “simple” or “multiple,” depending on the presence of one (single) or more (multiple) embryo sacs in the same ovule and events that can occur in both types. In angiosperms, after the first mitosis, the zygote is divided into two and then forms an embryo of each of the parts. It may also happen that the nucellus is divided into several parts from which originate many embryo sacs. Sometimes, only one of them is fully developed. In such a case, the seed embryo is formed from the union of gametes. This is a reproduction mode called apomixis that is a common event among flowering plants and is identified only by careful genetic study because the seeds look normal [70]. The PE may arise in angiosperms in four different ways: (1) PE for “cleavage” or division of the embryo to form more than one, (2) by the formation of embryos from different embryo sac cells to the egg cell, (3) by the development of more than one embryo sac within the same ovule derivative thereof from the megaspore mother cell or cells of the nucellus, and (4) by activation of a somatic cell or sporophytic ovule to form the embryo [71].

The PE per cleavage generates embryos from the zygote and sometimes from its suspensor within the embryo sac [69]. The synergids are the most common cells within the embryo sac that can form embryos, which can be fertilized by the egg cell and by the same pollen tube. However, in the absence of fertilization of the polar nuclei, the endosperm is not formed and the entire process collapses. Moreover, the process may also involve several pollen tubes to

fertilize the egg cell, polar nuclei, and synergids, achieving normal endosperm development [71]. Antipodal embryos are rare. Some authors question the possibility of forming embryos from antipodal cells [72]. Although, it has been observed that the number of seedlings per seed under greenhouse conditions of polyembryonic maize can be up to six (**Figure 1**), which approaches to the number of nuclei in the embryo sac. More studies are needed to elucidate the origin of PE in maize.

Embryos formed from the sporophytic cell ($2n$) are known as adventitious, and they are generated from the nucellus and integuments. In nucellar polyembryony, cells generally contain a starchy and dense cytoplasm, they actively divide and become embryonic masses directing their way into the embryo sac, and cell activation may be stimulated in an autonomous way or by pollen tube inserted into the sac or even by pollination. The angiosperms that are distinguished by nucellar polyembryony are *Citrus* and *Mangifera* [71]. Batygina and Vinogradova in 2007 [2] classified the PE into two main types: gametophytic and sporophytic. The first type is a PE related with the phenomenon associated with the formation of the adventitious embryo gametophytic cell: synergids and antipodal, also as an embryonic cell when the embryonic sac is developed further. While PE sporophyte is characterized by the development of adventitious embryos from sporophytic cells: mother (integumental and nucellar polyembryony) or daughter (polyembryony monozygotic twins).

In maize, it has been suggested that the PE is of suspensory type [8] as well as the zygotic type as described by Lakshmanan and Ambegaokar [69], where the embryos are arising spontaneously in suspensor cells from zygotic embryo. Erdelska [9] in a histological analysis (1996) suggests that PE is produced according to the origin of the embryos, their location in the grain (caryopsis), difference in structure (common tissues), and type of germination. From these concepts, the PE can originate in three ways: (1) two embryo sacs multi-embryonic commonly are located on opposite sides, or distance in the grain, which lack of common tissues and germinate independently, (2) cases of twins or triplets coming from individual egg cell, or embryo sac cells with multi-egg capabilities that are closely adhered, but separated by epidermal layers, with an endosperm in common and independent radicles and plumules, and (3) polyembryos arising from multiplication of egg cell cleavage spontaneously or after any induction, which share a common suspensors that are part of scutellum and radicle surface layers and due to this reason, embryos germinate with separated plumules but one root complex.

Moreover, in a study on morphology and anatomy of maize radicles as well as frequency of seedlings and multiple radicles per germinated seed, performed using two maize populations from the IMM-UAAAN polyembryonic germplasm as well as their direct and reciprocal crosses with Non-PE genotypes, it was found that the PE and multiple radicles trait occurred only in the progeny of the two polyembryonic populations and the hybrids between them. Some PE seedlings presented simultaneously multiple radicles, whereas other PE seedlings do not show these multiple radicles that were observed in variable number and conformation. In some cases from two to four roots, separated or merged with some degree of histological level, including the vascular cylinder. The average frequency of PE and multiple radicles was 62 and 14%, respectively [29]. This can be explained as a phenomenon of cleavage polyembryony by affecting cell division, making proembryonic form various embryonic axes that are attached by certain structures [73].

6. Possible causes of polyembryony

Despite the interest in the factor that may induce and affect the frequency of PE in different species, PE mechanisms and causes are not yet entirely clear. It is considered that the causes of PE are mainly genetic, although there is a strong environmental component in PE expression. One of the first suggested causes of PE was a hormonal imbalance [74], although recently PE has been attributed to genetic causes such as meiotic and/or mitotic chromosome irregularities and polyploidy hybridization. Polyembryony (polymeric embryos) can develop spontaneously in different plants with live flowers or can be induced in situ by various treatments, such as synthetic auxins, X-rays, or inhibitors of auxin polar transport [75]. Of these three, the most reported are irregularities during the meiotic and/or mitotic process, which are governed by the *ig* gene. It has been reported particularly in maize that the presence of multiple embryonic cells due to the mutant *ig* gene affects the number of mitotic divisions. However, there are not sufficient studies to ensure that mutation of this gene is associated with PE. On the other hand, it has been reported that PE can be increased by a selection or delayed pollination [8] suggesting a genetic component.

There are controversial reports on the genetic nature of PE. Shukla in 2004 [7] studied the genetic diversity of polyembryonic and monoembryonic mango and found that the two phenomena have a different genetic basis. Similar results were obtained by Andrade-Rodríguez [76] who used RAPD markers for identification of zygotic and nucellar seedlings in polyembryonic *Citrus reshni* and reported that it was possible to identify both types of seedlings. By contrast, Martínez-Gómez and Gradziel [31] analyzed the genetic structure of almond seedlings from mono- and polyembryonic seeds and found that the seedlings have a similar genetic composition in both types of embryos. It was also mentioned that variation of polyembryony may be affected by a type of pollinator, available pollen amount, plant nutrition, environment temperature, soil moisture and temperature, and air velocity. Therefore, factors affecting pollination or fertilization of seed development will also affect PE percentage and number of embryos per seed [77].

The occurrence of PE varies greatly and is influenced by environmental conditions. Plants from the same polyembryonic seed often are viable, although some of the plants may show a weak development of their leaves [31]. Andrade Rodríguez in 2005 [76] found that the environmental conditions during the growing season of *Citrus volkameriana* affected PE frequency; in addition, the fruit morphological characteristics do not indicate the PE frequency. These authors determined that the zygotic lines have a different RAPD pattern to nucellar lines and found that only 25.9% of the polyembryonic and 87.5% of the monoembryonic plants are of sexual origin and that in the polyembryonic seeds not all zygotic embryos were produced by the small embryos located in the micropyle.

There are reports where PE in maize was induced by treating the developing caryopses with 2,4-dichlorophenoxyacetic acid (2,4-D), on the second day after pollination finding that about 40% of the seeds were polyembryonic. The same authors also observed that polyembryonic caryopses were smaller than normal because of lower growth potential [8].

6.1. Polyembryony and apomixes

Webber [1] noted that many cases of adventitious cell formation in angiosperms are related with apomixes, and it is very likely that PE and apomixis can be interconnected. Apomixis in *Citrus* is known as polyembryony because multiple somatic embryos are developed simultaneously with the zygote embryo in the seed [78].

Genes that initiate and control apomixis will lead to the development of true reproductive hybrids for the genotype of a superior hybrids; apomixis can be divided into different categories: (i) adventitia or sporophytic type is where the embryos differ from the somatic cells in the eggs without the formation of megagametophyte; (ii) apospory, where the megagametophyte is to be developed from a somatic cell within the ovum; and (iii) diplospory with the development of megagametophytes of a nonreduced miaspore stem cell. Apomictic processes mimic many of the events of sexual reproduction to give rise to seeds without fertilization [79]. However, polyembryony has been characterized as the occurrence of more than one embryo in a seed, polyembryony in angiosperms may appear by excision of the proembryo, or formation of embryos by the cells of the embryonic sac [37].

Some varieties of citrus express a form of apomixis nucellar embryo in which adventive, the embryos are developed from the nucellus embryonic sac tissue. This feature appears in many seeds containing multiple embryos (polyembryony) [80]. Different species present several reproductive traits that appear to be interacting in the generation of PE. Gupta in 1996 [81] reported in guggul (*Commiphora wightii*) the occurrence of apomixis not pseudogamous (development of an embryo only from maternal chromosomes after activation of the egg by a sperm: sperm penetrates the egg, causes division, but there is no effective fertilization), nucellar PE and autonomous endosperm formation suggesting that plants have reproductive and survival strategies in the absence of male plants, but in the presence of males, sexual reproduction can occur. Moreover, in 2005, Mendes-Rodriguez [82] studied *Eriotheca pubescens*, which presents apomixis and adventitia polyembryony, found that in seeds, the zygote became a sexual embryo simultaneously with apomictic adventitious embryos that developed from nucellus cells. The adventitious embryo developed more rapidly than sexual ones, but they are morphologically similar so that 44 days after anthesis it was impossible to distinguish the sexual from the apomictic embryos.

Espinoza-Velazquez and De Leon in 2005 [83] asserted that maize populations might contain the ability to manifest asexual reproduction by seed, some form of apomixis. They were based on the history of polyploidy and polyembryony in the IMM-UAAAN populations and preliminary work on atypical reproductive behavior in maize. The introduction of apomixis in maize has been attempted through conventional backcrossing, using *Tripsacum* species as the source, from where can be generated viable seeds from intergeneric hybridization, which were produced in an apomictic way when they were pollinated using common maize [84]. This suggests that pollen source can influence apomictic embryo development. However, despite the effort to introgress apomixis into maize from its wild relative *Tripsacum dactyloides*, the attempts to generate apomictic maize have failed so far. As Leblanc [85] have concluded that "epigenetic information imposes constraints for apomictic seed development and seems pivotal for a transgenerational propagation of apomixis."

Several studies have discussed the evolution of apomixis and adventitious embryos on the subject of their similarity in regard to asexual propagation [86–89]. Given that there is a lack of clear distinction between PE, apomixis, and adventitious embryos, this is assumed because of all these phenomena have similarities in asexual reproduction. However, PE is distinguished from the other two processes on the basis of its requirement of sexual reproduction and genetic composition of their offspring. Since there is a clear distinction between PE, apomixis, and adventitious embryos, all of the above is assumed to have similarities in asexual reproduction.

7. Polyembryony and pollen source

In maize, various experiments have been conducted to show an effect of the origin and nature of pollen on grain development. This has been expressed as the difference in weight between the grains of selfing and those of cross-fertilization, where the grain weight of cross-fertilization increased 10.1% [21]. From the genetic point of view, the advantage of cross-fertilization can be interpreted in terms of complementarity among genes from male and female by some enzymatic systems in terms of heterosis [90]. The effect of pollen source has been reported affecting seed composition. In the case of QPM (high-quality protein maize), if normal maize pollen fertilizes QPM female plants, essential amino acid content in the grains is decreased; in the case of lysine, it is up to 30% by which the maize grains from QPM plants reach a protein quality similar to normal maize [91].

Villarreal in 2010 [30] conducted a study using 16 samples of maize grains, a product of crosses among four female and four different male lines (**Table 1**). He found a higher percentage of PE in the offspring of females with high PE levels crossed with a polyembryonic and genetically

	Line description	Coding
Female lines	1. Normal height and high polyembryony	NAP
	2. Brachytic line with high polyembryony	BAP
	3. Normal height and low polyembryony	NBP
	4. Brachytic line with low polyembryony	BBP
Male lines	1. Polyembryonic line and genetically related to female lines	PERE
	2. Polyembryonic line and genetically unrelated to female lines	PENORE
	3. Nonpolyembryonic line that is genetically related to female lines	NOPERE
	4. Nonpolyembryonic line that is genetically unrelated to female lines	NOPENORE

Table 1. Female and male polyembryonic and nonpolyembryonic maize lines.

unrelated male, compared to when the same female was crossed with a polyembryonic and genetically related male. These results suggest a possible genetic complementation conditioning maize PE and some possible maternal effects as well.

8. Genetic studies

Regarding genetic control, PE in maize has been reported as a trait of simple recessive inheritance [10, 13, 92], as well as a quantitative inheritance [5, 25, 26]. According to these authors, the manifestation of this character can arise from major effect genes (monogenic nature) or polygenes (quantitative nature). In the first type, one needs to emphasize the role of *ig* gene, which in a homozygote recessive condition generates in seeds with a monoploid embryo in 3% of the cases and in 6% PE [11], or by an unidentified recessive gene, as noted by Pilu [92]. However, Pešev [25], Rodriguez, and Castro [93] and Castro [26], cited by Espinoza [5], mentioned that inheritance of PE is quantitative, and the latter authors note that PE which they worked presented a heritability of 65%, calculated by the method of midparent-offspring regression method. There is evidence that maize PE has a heritable basis of a quantitative nature; however, inconsistent behavior, regarding fixing PE in genetic groups, suggests involvement of other genetic and reproductive phenomena such as nucleus-cytoplasm interaction and reductional type parthenogenesis. Microarrays and SSH have been used to identify the genes associated with polyembryony in *Citrus*. Studies have also been made to associate polyembryonic with heat stress [94].

As reported by Puri, polyembryony in rice is caused by insertion of mutagenesis, where they employ molecular tools for the cloning of the polyembryo gene (*Ospe*) in Basmati 370, and mention that for the F₃ population, the polyembryony was not segregated with the expected proportion, suggesting that there is variable penetrance and expressiveness for the mutant. Penetration is related when a phenotype is expressed for a particular genotype, which expressively refers to the degree to which a phenotype is expressed after penetrance, obtaining polyembryonic seeds of twins, triplets, and rare quadruplets that varied from 9.8 to 21.8% [95].

A study about the combination of PE germplasm with a nonpolyembryonic (Non-PE) source indicates a masking of PE trait in the F₁ generation in the crosses of the polyembryonic populations (NAP and BAP) with the Tuxpeño population that has high oil content and belongs to the CIMMYT collection [28]. Continuing with this experimental line, Espinoza Velazquez [96] reported on the probable genetic mechanisms involved in the PE expression. After analyzing the observed PE frequencies in the F₂ and RC₁ generation, they found that PE frequency did not agree on the expected in the case of a recessive gene but to the two interacting loci with epistasis of the kind of 15:1 double recessive for PE. A more recent study on PE reported by Musito Ramirez in 2008 [97] who worked with S₁ inbred lines derived from the NAP population (**Table 1**) found that inbreeding of S₁ lines did not increase PE frequency. Moreover, Espinoza-Velazquez in 2012 [29], after performing a histological study of 3-day-old radicles, belonging to genotypes derived from crossings among the NAP and BAP populations (**Table 1**) with the Tuxpeño HOC population, found that PE frequency and multiple radicles

(two or more roots per seed) were 60 and 14%, respectively; however, the traits were masked in the F_1 hybrids, manifesting that the PE as a recessive trait. Rebolloza in 2011 [27], who worked with BAP and NAP maize populations (**Table 1**), found that PE showed a Mendelian inheritance pattern by the action of two loci, with epistatic interaction of duplicate recessive type having a F_2 segregation of 15:1, with an incomplete penetrance of a range from 20 to 50%; thus, according to this source, the exotic germplasm with PE is being crossed. These findings corroborate the proposed inheritance mechanism suggested by Espinoza-Velazquez [96].

9. Future trends

Maize PE is a trait that has different practical applications. As demonstrated by several authors, polyembryonic maize contains higher grain nutritional quality which allows to develop PE varieties with high fat content (6.5%) and lysine (4%) [28, 30] also crosses between PE and non-PE genotypes produces hybrids that do not express the PE trait because of its recessive genetic condition, but fat and lysine in the grain remain high, which may help to generate hybrids with higher grain nutritional quality [28]. In the case of studies that attempt to explain the PE in maize, it is necessary to apply the advances in molecular biology for the identification of the genes that are involved in the control of this trait, and if it is possible to sequence these genes in order to provide greater information of this trait and increase its agricultural utility by inserting such genes in lines with high agronomic potential or for further molecular studies on PE and its relation to polyploidy, xenia, and apomixis.

10. Conclusion

Polyembryony in maize has been documented first by the presence of multiple plants simultaneously born from a seed and cytological studies that have confirmed this trait. The type of PE inheritance could be governed by major genes or genes of a quantitative nature. Besides, the presence of two or more embryos per seed gives an advantage to these genotypes for higher grain nutritional quality. However, more studies are required in order to fully understand the PE nature and control. On the other hand, reported studies showed that PE could be a useful trait in developing specialized varieties with yield potential and grain quality. However, there is not much molecular evidence that can help to fully understand the polyembryony trait.

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Appendices

CIMMYT	Centro International de Mejoramiento de Maíz y Trigo
FC	Fat content
HOC	High oil content
IMM-UAAAN	Instituto Mexicano del Maíz of the Universidad Autónoma Agraria Antonio Narro
Non-PE	Non-polyembryonic
PE	Polyembryony, polyembryonic
QPM	High quality protein maize
RAPD	Random amplified polymorphic DNA

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