Synthetic Seeds: An Alternative Approach for Clonal Propagation to Avoiding the Heterozygosity Problem of Natural Botanical Seeds

Biswajit Ghosh and Sk Moquammel Haque

Abstract The seed is a functional element of sexual reproduction of higher plant. In nature, the humble beginning of the independent life of higher plants starts along with seed germination. Seeds are the "mysterious genetic capsules" which store the genetic information and carry forward to next progeny. The zygotic embryo present inside the botanical seed serves as propagule to produce offspring, and these embryos are always heterozygous because of the recombination during meiotic crossing over in the course of gamete formation as well as for mix-up of the genome of two different parents through cross-pollination. In seed-propagated crops, the agricultural yield is highly unstable due to heterozygosity among seed-derived plants. The answer of this problem is synthetic seeds—the functional mimic of botanical seeds.

Synthetic seed is one of the most promising tools of plant biotechnology, which could be tailor-made for horti- and agricultural improvement at present as well as upcoming days. As all the propagules used for synthetic seed preparation are produced through in vitro clonal propagation, which means they did not encounter two fundamental events of sexual reproduction, the meiotic recombination (during crossing over) and gametic fusion of two different parental genome (cross-pollination), both of these events can create new types of heterozygosity in zygotic seeds. Therefore synthetic seed-derived offspring are always true to type to their source plant. Although, unlike zygotic seed, new types of heterozygosity are never generated in synthetic seeds, the heterozygosity already existed in the mother plant is always transmitted in all synthetic seed-derived offspring.

However, the heterozygosity problem will be totally avoidable, and production of homozygous synthetic seeds is also possible only by using double haploid source plant, because double haploid plants are always truly homozygous. Otherwise synthetic seed technology can only aid to restrict the formation of new types of heterozygosity in offspring, which are abundant in botanical seeds.

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1 Introduction

In nature, the humble beginning of the independent life of higher plants (i.e. spermatophytes) starts from seeds. Spermatophyte is the most latest evolutionary embellishment of the plant kingdom and includes gymnosperm and angiosperm. The key character of spermatophyte is that they are seed bearing, unlike Pteridophyta and other cryptogams. It is really tough to overstate the significance of seeds for the evolutionary prosperity of the spermatophytes and the development of human civilization, improvement of human cultures, and their existence (Knapp 2015; Sabelli and Larkins 2015). Plants produce seeds for their most important purpose of life—reproduction. Although gymnosperm and angiosperm both are seed-bearing plants, gymnosperms are more primitive as they have uncovered seeds; in contrast the angiospermic seeds are enclosed within fruits. Plants store enough nutrients within the seed for utilization of their zygotic embryos (Ali and Elozeiri 2017). Seeds can be divided into two groups on the basis of their nutrient storage tissue—albuminous seeds (endosperm tissues serve for storage) and exalbuminous seeds (cotyledons serve for storage). Due to the presence of these nutrient storage tissues, seeds perform as an important element of the world's diet (Bewley 1997).

In higher plants the life cycle is divided into two phases—sporophyte and gametophyte (Haque and Ghosh 2016a). The dominant phase is diploid sporophytic stage where the main plant body occupies maximum span of the life cycle, whereas haploid gametophytic phase (pollen and ovule) is too much reduced and occupies very little span of the life cycle. The gametophytic generation starts from microspore (male gametophyte) or from megaspore (female gametophyte) and ultimately produces sperm and egg cell, respectively (Yadegari and Drews 2004). These haploid sperm and egg cells fertilize together to form a single diploid cell, i.e. zygote—the first cell of the sporophytic generation. Fertilization activates a complex cellular programme that converts two highly specialized haploid germ cells, the sperm and the oocyte, into a totipotent diploid zygote (Clift and Schuh 2013). The first part of the sporophytic development starting from zygote formation up to embryo maturation takes place within the ovule, and ultimately the ovule gives rise to a seed. The initial step in seed development is a double fertilization, where first fertilization occurs between sperm nucleus and egg nucleus to form the diploid zygote, whereas second fertilization occurs among one sperm nucleus and two central cell nuclei, resulting in the development of triploid nutritive endosperm (Sabelli and Larkins 2015). The components of mature seeds—embryo (propagule), endosperm (storage food), and seed coat (protective jacket)—are derived from the fertilized egg cell ($2n$), fertilized central cell ($3n$), and ovule integuments (diploid mother tissue), respectively (Drews and Koltunow 2011). Contemporary genetic studies point out that the

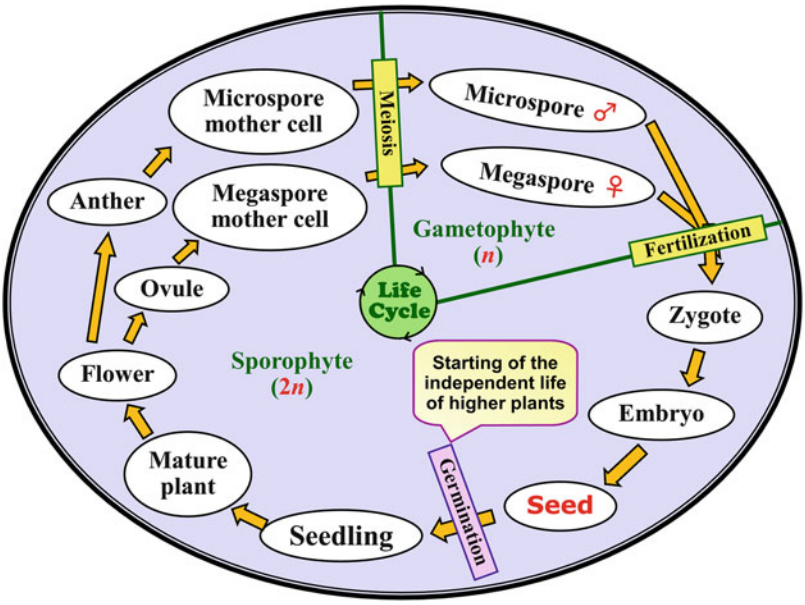

Fig. 1 Diagrammatic representation of the life cycle of seed-bearing plants

female gametophyte influences the events of seed development through maternal-effect genes as well as by regulating maternal contributions (Yadegari and Drews 2004; Drews and Koltunow 2011). The development of the seeds takes place in the mother plant; as a result zygote to embryo formation and their maturation are fully dependent on mother plants (Maheshwari 1950). Therefore, the independent life of higher plants eventually starts from seed germination, and the ultimate aim of their life is to produce seeds for the next generation (Fig. 1). Starting from zygote, the sporophytic plant bodies develop via embryo development, different phases of embryo maturation, embryo germination, and the vegetative growth (roots, stems, leaves) of the main plant body. After prolonged vegetative developmental phase, they attain certain maturity, and then transition from vegetative to flowering stage occurs (Haque et al. 2018). Female and male reproductive organs develop within the flowers, and ultimately haploid spores (mega- and microspores) are produced through reductive (i.e. meiosis) cell division (Haque and Ghosh 2017a).

"Evolution is the process of heritable change in populations of organisms over multiple generations" (https://www.nature.com/subjects/evolution). Evolution in living organisms is a very essential process which creates a gradual modification of all forms of life over generations. Without evolution, all life gets threatened and ultimately goes to extinction in ongoing changing environments of the earth (Burger and Lynch 1995; Parmesan 2006). The four major factors which lead to the evolution process are mutation, gene flow, genetic drift, and natural selection (Allendorf 2017). Evolution occurs in populations because of the modifications in allele frequency over time. Modification of allele frequency increases the heterozygosity

in individuals as well as in populations. Hence, more heterozygosity means more probabilities of speciation, i.e. formation of new species. This marvel of heterozygosity is utilized by the plant breeder as a weapon for creating new hybrid varieties (Acquaah 2012). At this point, heterozygosity serves as blessing for the progress of agriculture. But whenever a hybrid line was created, maintenance of genetic stability of this hybrid line is very essential, which is only possible through clonal propagation of this hybrid plant (Wang et al. 2019). Clonal propagation is none other than a vegetative mode of propagation, where all the offspring truly maintain the genetic make-up of their parent plant by avoiding the genetic recombination, i.e. meiotic crossing over. Some of the organisms have ostensibly evolved without sexual reproduction for several centuries (Schön and Martens 2003). Due to human-exercised selective pressures, the clonally multiplied food crops incorporate an enormous range of ecological, morphological, and phylogenetic diversity (McKey et al. 2010). Somatic mutations are the cause of genetic variation among clonally propagated domesticated crop plants which supports the adaptive evolution (Whitham and Slobodchikoff 1981).

Seeds are the functional component of plant reproduction, from where the independent life of higher plants starts. In general, the botanical seeds comprise three basic criteria—(1) first and foremost it contains a propagule in the form of zygotic embryo, (2) the zygotic embryo is covered by a hard jacket, i.e. seed coat for mechanical protection, and (3) it contains storage food for zygotic embryo in the form of nutritive tissue, i.e. endosperm or cotyledon (DuPont 2012). Apart from these three basic criteria, though not all, most of the seeds have another important features—seed dormancy (Yildiz et al. 2017). During dormancy period the seeds cannot germinate even in the presence of favourable environmental conditions (temperature, humidity, oxygen, and light) required for germination. Seed dormancy is a trait acquired by the spermatophytes during evolution to subsist in adverse environments such as low or high temperature, salinity, and drought (Yildiz et al. 2017). Dormancy can compare with “sleep” and dormancy-break with “wake-up”. After dormancy-breaking the seed germinates and give rise to a seedling. However, dormancy-break is very unpredictable because the threshold stimulus needed to encourage germination differs extensively among individual seeds; therefore all seeds among the same population do not germinate synchronously (Bewley 1997). Seed germination frequency is considered as a determining factor for plant productivity (Ali and Elozeiri 2017). Although seed propagation is the leading mode of reproduction of higher plants, there are some drawbacks like all seedlings are genetically not true to type, production of seed is not possible throughout the year but restricted to a particular season only, and few species aren’t able to produce seeds throughout their life. Hence, an alternative of botanical seed is strongly desiderated to address the above-mentioned drawbacks.

Synthetic seeds are functionally alternatives of botanical seeds and are tailor-made and developed in laboratories. Synthetic seed is one of the most promising plant biotechnological tools which could be expedient for agricultural improvement at present as well as upcoming days (Haque and Ghosh 2014). This technology has been established to utilize somatic embryos or some other micropropagules like

shoot tips, nodes, etc. as seed analogues effectively in the greenhouse or field and their commercial planting (Ara et al. 2000). Nowadays, the synthetic seed is an ardent topic of research, and importance of this technology can be predicted by the huge number of scientific works continuously done on it. A casual perusal of the scientific search engines (https://www.sciencedirect.com/) reveals that over 4450 publications related to "synthetic seed or artificial seed" have been published in the last 3 years (2017–2019, accessed on February 4, 2019). In crop plants, the maintenance of genetic stability of the high-yielding variety and retain of the high-yielding features in next generation is very essential, which is possible only through clonal propagation (Bhojwani and Razdan 1996). Since all zygotic seeds are heterozygous (except when parents are inbred line), therefore seed-derived plants are genetically not true to type to their parents; henceforth, all the desired characters of the parents may not be expressed in offspring. Heterozygosity is a realistic problem for those crop species, whose planting material is zygotic or botanical seeds. Over botanical seeds, the synthetic seeds have some advantages like synthetic seed-derived seedlings are true clones of their source plant, and it can be produced in huge quantity throughout the years (Bapat and Mhatre 2005).

2 Propagation Through Seeds

Seed propagation is the method of plant reproduction through seeds. Maximum plant species naturally reproduces through seed propagation. As well, farmers also take advantage of seed propagation for cultivation of agricultural and horticultural crops. Seeds are an essential element of the life cycle of higher plants, as they store the hereditary information essential for the next progeny to disperse, inaugurate, grow, and finally reproduce to perpetuate the species (Nambara and Nonogaki 2012). Seed formation in higher plants begins along with the developmental decision to switchover from a vegetative to a reproductive phase of development (Simpson et al. 1999). The seeds contain a lot of secrets that have yet to be discovered, that's why Nambara and Nonogaki (2012) mentioned seed as "mysterious genetic capsules". In this type of plant propagation, seeds can be germinated, post-germination development occurs, and ultimately a seedling was developed. In recent years, an understanding of the seed biology especially seed dormancy and germination has been greatly progressed (Nonogaki 2017). In nature, germination postponement due to dormancy keeps certain seedlings safe from possible damage of detrimental weather or from seasonally migrating herbivores (DuPont 2012). Seed germination is a very crucial event in agricultural aspects, and yield may directly depend on the percentage of seed germination. There are four environmental factors which affect germination—water, oxygen, light, and temperature; and germination is rapid and uniform at optimal temperature and moisture (DuPont 2012). Seed vigour is a most important agronomic trait determined by longevity during storage, germination capability, and growth of the seedling in field condition (Daniel 2017). Two types of seeds are found in nature, albuminous seeds and exalbuminous seeds (Fig. 2).

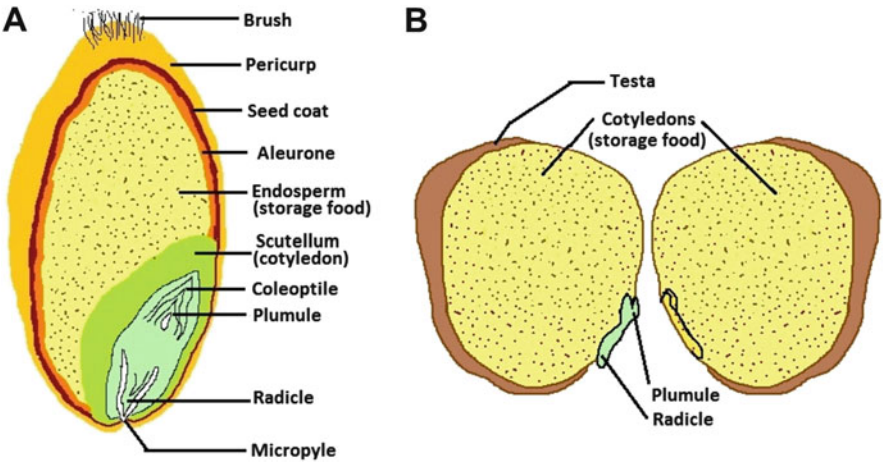

Fig. 2 Diagrammatic representation of (**a**) albuminous seed (wheat) and (**b**) exalbuminous seeds (chickpea)

Albuminous seeds have endosperm, a special nutritious tissue for food storage, which remains persistent even at maturity (e.g. rice, wheat, castor seed, etc.). Here cotyledons merely performed as nutrient-sucking organs. In contrast, the exalbuminous seeds are those seeds where endosperm is used up by the developing embryo and cotyledons turn thick and fleshy and serve as food storage tissue (e.g. *Alisma plantago* seed, chickpea, jackfruit seed, etc.). In dicotyledonous albuminous seeds, the endosperm is solely made with uniform living reserve cells, whereas, in monocotyledonous albuminous seeds, the starchy endosperm mostly consists of nonliving storage tissue, enclosed by the living aleurone layer (Joët et al. 2009).

3 Propagation Through Isolated Zygotic Embryo Culture

Zygote is the progenitor of subsequent generation, which forms an embryo through sequential developmental stages (Bhojwani and Dantu 2013a). Zygotic embryo culture refers to an aseptic excision of the zygotic embryo (generally in immature conditions but sometimes at mature stage) from seeds and their in vitro culture in artificial nutritive medium (in absence of endosperm tissue) with aim to obtaining complete plants. Excision of embryos from seeds and their in vitro culture were first time initiated by Hannig in more than 115 years ago (Hannig 1904). Three main utilities of zygotic embryo culture are:

1. A rare hybrid can be obtained through immature zygotic embryo rescue and their culture, because in reciprocal cross among two distantly related species (during

interspecific and intergeneric hybridization), the fertilization occurs but endosperm tissue is not developed or degenerated; as a result further maturation of embryo hampers due to lack of nutrition, and ultimately embryo is aborted if not rescued (Sahijram et al. 2013). Nowadays it is potential to rescue hybrid zygotic embryos which are mostly aborted even at the early stage of development, i.e. globular stage, and the method of hybrid zygotic embryo culture has come to be an important part of the plant breeding methodology (Bhojwani and Dantu 2013a; Wang et al. 2019). In vitro zygotic embryo culture is now very popular and being regularly used to produce rare hybrids which may be possibly not produced by the conventional breeding method because of sexual incompatibility between the male and female parents at postfertilization stages (Rajamony et al. 2006; Eeckhaut et al. 2007; Sahijram et al. 2013; Gupta et al. 2019).

2. The zygotic embryo culture is also exploited for overcoming the dormancy of recalcitrant seeds (Raghavan 2003). In case of coat-enhanced seed dormancy, the embryos excised from these seeds remain not dormant (Bewley 1997). Therefore, the excised embryo of coat-enhanced dormant seed when cultured in vitro on nutrient medium can grow by breaking the dormancy.
3. In addition, zygotic embryo-derived callus possesses a high regenerative capacity as compared to mature organ (leaf, stem, root)-derived callus; hence zygotic embryo is a good source of explant for developing callus-mediated indirect organogenesis or embryogenesis and plant regeneration. There are so many micropropagation protocols that had been established in gymnosperms and angiosperms where zygotic embryos are used as initial explants (Fitch and Manshardt 1990; Bodhipadma and Leung 2002; Chaturvedi et al. 2004; Zhang et al. 2006; Yang et al. 2008; Konieczny et al. 2010).

4 Heterozygosity in Seeds

Zygosity is the degree of resemblance among the genetic alleles for a particular trait in an organism. While some traits exhibit the occurrence of just a single allele, lots of others show the existence of two or more alleles for a particular locus within a population. In diploid plants, one allele is inherited typically from the female parent and another from the male parent. On the basis of similarity or dissimilarity of DNA sequence among these homologous alleles, the genetic trait is considered to be homozygous or heterozygous, respectively (Fernandez 2013) (Fig. 3). Hence, heterozygosity is the form of having two dissimilar alleles at a locus, and it is fundamental for studying genetic variation within populations. Mutation, natural selection, genetic drift, and migration play critical roles on maintaining heterozygosity in populations (Allendorf 2017). During meiosis, crossing over among two non-sister chromatids of homologous pair resulted reciprocal exchange of genetic materials, which ultimately mixed up the hereditary factors of male and female parents and transgresses in offspring (Clift and Schuh 2013). Hence, sexual reproduction especially crossing over plays a crucial role on creating as well as

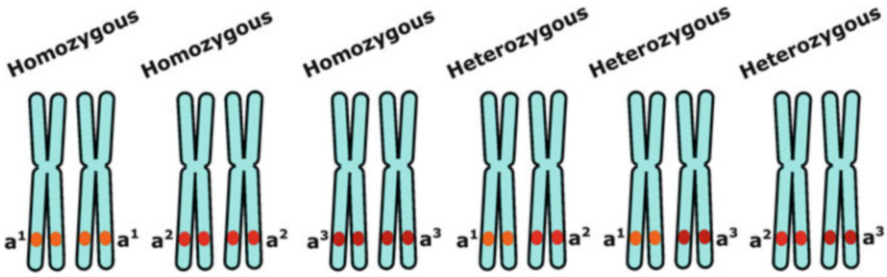

Fig. 3 Graphical representation of homologous chromosome showing heterozygosity and homozygosity concept. If three different alleles (a^1, a^2, a^3) of the same gene are present in a population, then three types of homozygous (a^1a^1, a^2a^2, a^3a^3) and three types of heterozygous (a^1a^2, a^1a^3, a^2a^3) individuals may present within this population

maintaining the heterozygosity. Therefore, all the botanical seeds containing zygotic embryo, which are produced by the random fusion of two meiotic products, i.e. sperm and egg cell, are always heterozygous in nature.

4.1 Heterozygosity and Plant Breeding

During Mendel's time, people have no idea regarding the genetics, but farmers realized that plants may perhaps be changed vividly through cautious selective breeding. The resilient, strong, disease-resistant wild relatives of crop plants were crucial for cross-breeding programme (Acquaah 2012). Since then, simultaneously with natural cross-breeding, the human being was also trialled with various types of cross-breeding to obtain high-yielding, disease- and drought-resistant hybrid plants which are better for cultivation. Mendel's famous experiment on *Pisum* revealed segregation of traits, established the function of gametes as the carriers of genetic factors, and established the mutual significances of segregation and recombination (Pupilli and Barcaccia 2012). Heterozygosity, genetic diversity, natural selection, and mutation, all of these may lead speciation and hence evolution. For example, an only ancestor species of weedy coastal mustard in due course of evolution gave rise to over half dozen of accustomed European vegetables (Hanson 2013). Hence, heterozygosity is one of the main causes of genetic diversity and speciation (Avise 1977; Allendorf 2017).

Plant breeding is a mechanism for the improvement of plants by hybridization or selective mating for the benefit of human beings. Traditionally it serves as tool for the production of new plant varieties for upliftment of agriculture and horticulture (https://www.nature.com/subjects/plant-breeding, accessed on February 4, 2019). In plant breeding the "inbred" line is those plants where every single locus is homozygous. Generally inbred lines are produced by repeated self-pollination followed by selection for minimum 6–10 consecutive generations to attain the almost homozygous condition (Prigge et al. 2012). Nowadays, apart from the conventional breeding

method, another popular and time-saving in vitro biotechnological method is available for truly homozygous line production—i.e. double haploid plant production (Ren et al. 2017; Kleter et al. 2019). These truly homozygous or inbred lines are very essential in a plant breeding programme for hybrid production (Dong et al. 2019). Plant breeding has given rise to many new varieties of seed crop with high levels of carbohydrates, proteins, fats, or certain combination of those three in their seeds (Krebbers et al. 1997).

4.2 Demerits of Heterozygosity

In today's agriculture, hybrid crops are very important for their high-yielding potentiality, but required lucrative phenotypes are lost in the progeny of subsequent generations due to genetic segregation (Wang et al. 2019). Once the hybrid line is produced, most of the time we want exact true-to-type offspring, which permits the continuation of the desired phenotypic characteristics of the hybrid cultivar and helps to maintain the stable high yield. When the requirement is true-to-type plant production, then the above-mentioned (see Sect. 4.1) merits of heterozygosity are considered as demerits. Zygotic embryo present inside the botanical seed serves as a natural propagule to produce offspring, and these propagules are always heterozygous because of the crossing over during meiosis as well as cross-pollination among two different parents. Phenotypic expression from heterozygotes failed to maintain same agronomic quality compare to that of source plant (Küpper et al. 2010). Now, when the target is to produce true-to-type parental plants, sexual reproduction creates problems and does not provide desired characters at that time; alternatively clonal propagation is appropriate to serve the purpose (Wang et al. 2019).

4.2.1 Heterozygosity Formation Due to Crossing Over in Meiosis-I

Meiosis, the exclusive and essential event of the life cycle of the entire range of sexually reproducing organisms (Wijnker and Schnittger 2013), is the procedure through which a diploid sporophytic cell gives rise to haploid spore cells which grow further to develop the gametophyte and ultimately the gametes (Schwarzacher 2003). The first meiotic division (i.e. meiosis-I) is very crucial for sexually propagated plant species because of the two major events. First is the reduction of the chromosome number to half of their somatic number to produce haploid gamete; therefore the meiotic division is alternatively known as reduction division. During fertilization the gametic fusion of microspore and megaspore (i.e. ♂ and ♀ gametes) gives a diploid zygote. Thus reduction division is the only way to maintaining the chromosome number characteristic of the sexually reproduced species. Second is recombination in crossing over that takes place during the pachytene stage of first meiotic prophase, where the hereditary factors from male and female parents get mixed due to the reciprocal exchange among non-sister chromatids (Schwarzacher

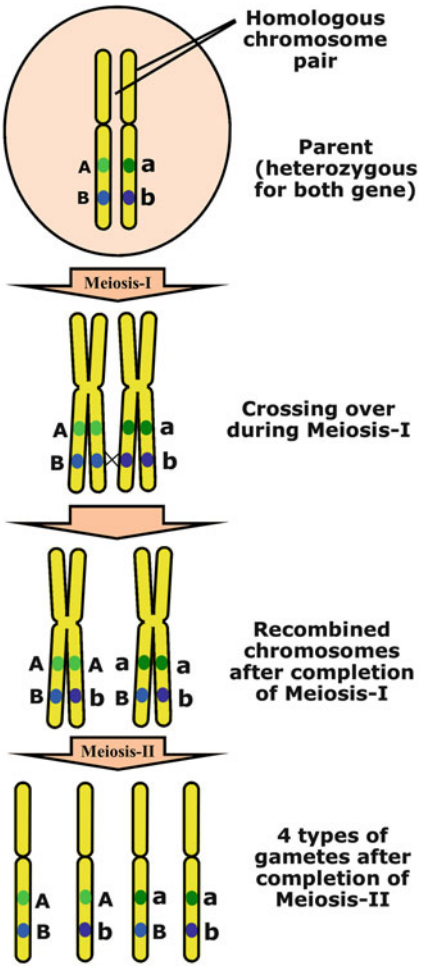

Fig. 4 Diagrammatic presentation about how meiotic crossing over creates new recombinant types in gametes. Present example considers only one pair of homologous chromosomes and only two genes in two loci, and both genes have only two alleles, but in nature, several pairs of homologous chromosomes are present in each individual, and few thousands of genes reside in different chromosomes as well as multiple alleles of each gene are present in population which remain more complex and create diverse types of heterozygous gametes

2003). This type of mix-up of genetic materials causes rearrangement of alleles and enhances the probability of heterozygosity, and segregation may arise among the progeny (Zhang et al. 2019). For example, if we consider only two genes and both have only two alleles (A, a, and B, b) and crossing over takes place in one locus and other genes have not participated in crossing over, then after meiosis four different types of gametes (AB, Ab, aB, ab) will be produced (Fig. 4). If crossing over does not happen, then only two types of gametes (AB, ab) will be produced. Hence, crossing over increases the recombinant types in gamete and therefore increases the chances of heterozygosity in offspring. However, the present example (Fig. 4) considers only one pair of homologous chromosomes, only two genes in two loci, and both genes having only two alleles, but in nature, more chromosomes are present in every individual, and a huge number of genes (few thousands) reside in different chromosomes, and, most of the time, multiple alleles of each gene are present in

population which obviously creates more complexity and generates several new types of heterozygous gametes.

4.2.2 Heterozygosity Formation Due to Random Cross-Pollination

In the previous section (see Sect. 4.2.1), it was already discussed about the role of meiotic crossing over on creation of new types of heterozygosity. Now, even if we don't consider the crossing over phenomenon (i.e. let's assume that the crossing over did not happen), all offspring are not always true to type because of the random cross-pollination. Specifically, when pollen and ovule are from different parents, their genetic make-up is also different in nature (Acquaah 2012). Therefore, all zygotes produced in a particular plant may not be genetically identical because though the female recipient germ cell was fixed, the male donor (i.e. source of pollen grains) is different. For example, consider only two genes and both have four alleles (A^1, A^2, A^3, A^4 and B^1, B^2, B^3, B^4), male parent with $A^1A^2B^1B^2$ and female with $A^3A^4B^3B^4$ (Fig. 5). Let's assume that the crossing over did not happen and then after meiosis two dissimilar types of gametes (A^1B^1, A^2B^2) are produced in male parent and two additional types of gametes (A^3B^3, A^4B^4) are produced in female parent. As a result of random cross-pollination, four totally new types of heterozygosity are produced among offspring ($A^1A^3B^1B^3$, $A^1A^4B^1B^4$, $A^2A^3B^2B^3$, $A^2A^4B^2B^4$), and none of them are similar with any parent ($A^1A^2B^1B^2$, and $A^3A^4B^3B^4$). However, in the present example (Fig. 5), it considered only one pair of homologous chromosome, two genes in two loci, and both genes having four alleles (two alleles in each parent), but in nature, several pairs of homologous chromosomes are present in each individual, and few thousands of genes reside in different chromosomes as well as multiple alleles of each gene are present in population which remain more complex and create diverse types of heterozygosity. The next-generation sequencing technique provides the facility to screen 10–100 of thousands of loci all over the genome for detecting heterozygosity, which has reformed our understanding of heterozygosity in natural populations (Allendorf 2017). Hence random cross-pollination also causes mix-up of different alleles present in the population and also enhances the probability of new form of heterozygosity in offspring (Acquaah 2012).

5 Clonal Propagation and Its Importance

Multiplication by means of non-sexual mode of propagation when all the multiplied copies are genetically identical to their parent is called clonal propagation. Plant population produced from a particular individual plant through non-sexual mode of propagation creates a clone. All clones are genetically true to type to their source plant. In natural condition, clonal plant propagation occurs by vegetative propagation or by apomixis (Park et al. 2016). Several plant species that propagate clonally (non-sexually) are also capable of sexual reproduction (Bailey 2018). Clonal

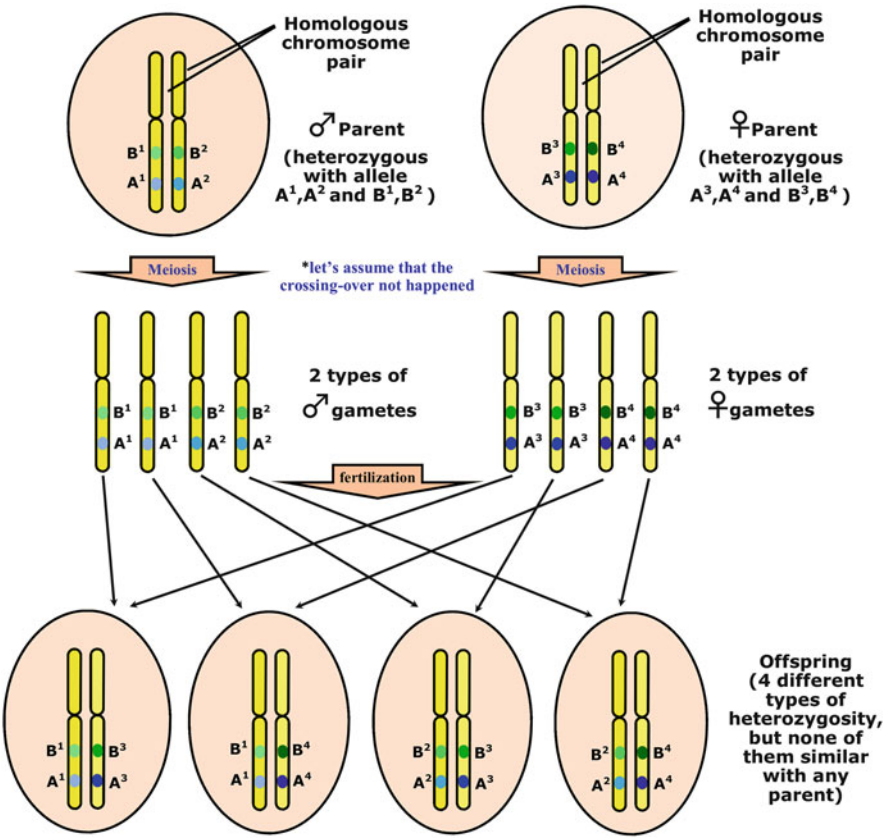

Fig. 5 Diagrammatic presentation about how cross-pollination enhances the probability of heterozygosity in offspring. Four totally new types of heterozygosity are produced among offspring ($A^1A^3B^1B^3$, $A^1A^4B^1B^4$, $A^2A^3B^2B^3$, $A^2A^4B^2B^4$), and none of them are similar with any parent ($A^1A^2B^1B^2$, and '$A^3A^4B^3B^4$). Present example considers only one pair of homologous chromosome and two genes in two loci, and both genes have four alleles (two alleles in each parent), but in nature, several pairs of homologous chromosomes are present in each individual, and few thousands of genes reside in different chromosomes as well as multiple alleles of each gene are present in a population which remains more complex and creates diverse types of heterozygosity

propagation has some advantages over sexual mode of reproduction—fixation of valuable agronomical traits, control of gene flow from wild-to-crop plant, and easiest way of multiplication (Bhojwani and Razdan 1996). As well, the clonal propagation also has some drawbacks like restriction on genetic diversity, deleterious mutations, retain of pathogenic entities and transfer to subsequent progeny (McKey et al. 2010).

Variability arising between seed-derived plants can be omitted by avoiding sexual reproduction and following vegetative mode of propagation. Unwanted gene flow from wild-to-crop plant can be controlled through clonal propagation (McKey et al.

2010). Clonal propagation is very useful when exact true-to-type plants are required. In this type of propagation, all offspring are genetically identical to their parent, so all the important characters of parental plant are truly unchanged among all progeny. Clonal propagation is the easiest way of multiplication and highly practical in the case of tree propagation, particularly in fruit cultivation; because a seed-derived tree needs several years to reach maturity, whereas a vegetatively propagated (through grafting or gootee) tree requires comparatively much less time to reach the fruiting stage (Bonga 1982; Park et al. 2016). Clones are very useful in the field of agriculture on the way of maintaining the steady production (Bhojwani and Razdan 1996).

6 Different Modes of Clonal Propagation

In higher plants, mainly two types of clonal propagations are observed in nature, viz., vegetative propagation and apomixes (Fig. 6). Besides the natural mode of propagation, human beings too have established a number of methods for artificial vegetative propagation of numerous valuable plant species (Megersa 2017). Again, natural propagation always happens in in vivo condition, whereas man-made artificial methods of propagation through vegetative mode are either in vivo or in vitro condition.

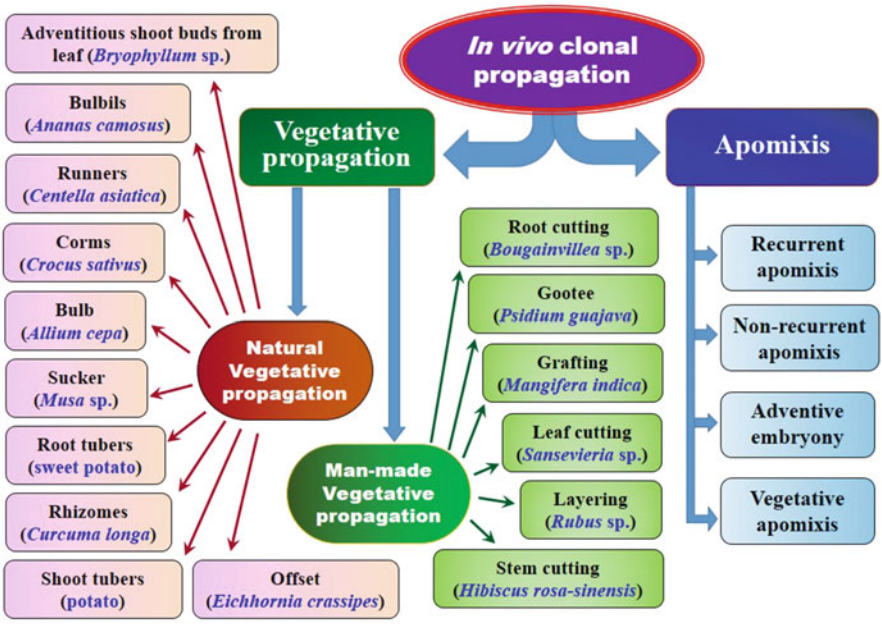

Fig. 6 Schematic representation of different paths of in vivo clonal propagations

6.1 Vegetative Propagation

The vegetative mode of propagation is very common and adopted by numerous plant species, even though many of them are also capable of sexual reproduction (Bailey 2018). Different plant organs like root, stem, and leaves are modified in a different way for vegetative propagation (Megersa 2017). Few of the very common structures which are modified for vegetative reproductions are root tubers (sweet potato), adventitious shoot buds from root (*Albizia lebbeck*, *Aegle marmelos*), bulbs (onion, *Ledebouria revoluta*, *Drimiopsis botryoides*), rhizomes (ginger, *Curcuma longa*, *Alpinia calcarata*, *Kaempferia angustifolia*), tubers (potato, *Oxalis tuberosa*), runners (strawberry, *Centella asiatica*), offset (*Eichhornia crassipes*), corms (*Crocus sativus*, *Gladiolus*), bulbils (*Ananas comosus*), sucker (*Musa* spp.), adventitious shoot buds from leaf (*Bryophyllum* spp.), etc.

In higher plants, where natural way of vegetative reproduction is absent, few special methods had been developed by humans for clonal propagation. These methods are mainly applied for the propagation of horticultural as well as fruit plants (Bailey 2018). Some of these man-made methods are as follows—Cutting is a method where a plant part, usually a stem or root or leaf, is separated from mother plants and sometimes treated with plant hormones and then planted in moist soil. Adventitious organs are developed from the cuttings, and ultimately a new plant was produced. Rose, *Hibiscus rosa-sinensis*, and *Chrysanthemums* spp. are some common examples of stem cutting; similarly *Bougainvillea* spp. are propagated through root cutting and *Sansevieria* sp. through leaf cutting. Grafting is another method where a shoot tip (or scion) was collected of the desired source plant and grafted on the stem of another healthy seed-derived plant (or stock). In due course, the tissue of stock and scion become attached together to form a complete plant (e.g. Mango, *Adenium*). Few other popular methods are layering (*Rubus* sp.), gootee (*Psidium guajava*, *Citrus*), etc. (Bailey 2018).

6.2 Apomixis and Clonal Seed Propagation

Apomixis refers to a process of non-sexual propagation through seeds, in absence of the meiotic cell division as well as gametic fusion, producing clonal offspring of maternal origin (Spillane et al. 2004). In 1908 Winkler for the first time coined the term "apomixis" to mean "substitution of sexual reproduction by an asexual multiplication process without nucleus and cell fusion" (Winkler 1908). Since this is a fertilization-independent, spontaneous natural development of the embryo from somatic cell ($2n$) without any gametic fusion, so perhaps it can be said in other words as "natural somatic embryo". In agriculture the apomixis is employed as a reproductive tactic for clonal plant production by seeds (Spillane et al. 2001; Bicknell and Koltunow 2004; Pupilli and Barcaccia 2012). The plants produced through apomixis are known as apomictic plant, which is not very uncommon

among higher plants, and more than 400 species of about 40 families are apomictic (Bicknell and Koltunow 2004). In higher plants apomixis is defined as the asexual development of a seed from the female parental tissues ($2n$) of the ovule, bypassing two utmost fundamental events of sexual reproduction—meiosis and fertilization, leading to the development of an embryo. The momentary definition of apomixis defines an end product, but the developmental procedures that lead to this end result can differ broadly (Ozias-Akins 2006). Primarily four kinds of apomixis are found in nature:

1. Recurrent apomixis: here an embryo sac develops from the megaspore mother cell where meiosis has not happened or from some adjoining cell; therefore, the egg cell is diploid. An embryo develops directly from the diploid egg cell ($2n$) escaping fertilization. Some examples of recurrent apomixis are somatic apospory, diploid apogamy, and diploid parthenogenesis.
2. Nonrecurrent apomixis: here an embryo develops directly from a typical haploid egg cell (n) without fertilization; as a result the embryos will also be haploid. Nonrecurrent types of apomixis are rarely found in nature. Particular examples of such types of apomixis are haploid parthenogenesis, haploid apogamy, and androgamy.
3. Adventive embryony: here embryos were developed from cells of nucellus or integuments, outside the embryo sac. In addition to such adventive embryos, the regular zygotic embryo may also develop concurrently within the embryo sac, thus generating polyembryony situation, frequently found in *Citrus* spp.
4. Vegetative apomixis: here instead of flowers, some vegetative buds or bulbils are produced in the axil of inflorescence, and they can be regenerated without any struggle. Such types of apomixis are observed in *Agave*, *Poa bulbosa*, and some grass species.

Apomixis is a beautiful attribute for the improvement of crop cultivation because it facilitates the formation of huge genetically identical populations and maintains hybrid vigour through continual seed production (Spillane et al. 2001; Hand and Koltunow 2014). In plant breeding the apomixis prospered several advantages. During sexual reproduction, cross- and self-fertilization followed by segregation have a tendency to modify the genetic configuration of offspring. Inbreeding and abandoned outbreeding as well have a tendency to interrupt heterozygote superiority in such offspring. In contrast, apomicts have a tendency to protect the genetic configuration as such (Spillane et al. 2001). Apomicts are also proficient of conserving benefits of heterozygote generation after generation. Thus, apomixis offers remarkable advantage in plant breeding where genetic consistency maintained over several generations for both heterozygosity (in hybrids of both outbreeders and selfbreeders) and homozygosity (in selfbreeders) is the remarkable motive (Hand and Koltunow 2014). Furthermore, apomixis may also offer an effective utilization of maternally inherited factors, if present, reflecting in the subsequent offspring.

It is mostly believed that zygotic embryogenesis (sexual reproduction) and apomictic embryogenesis (asexual reproduction) both follow alike developmental

pathways in the course of embryo and seed development (Pupilli and Barcaccia 2012). But the offspring of an apomictic plant are always genetically uniform to their mother plant.

6.3 *In Vitro Clonal Propagation*

Nowadays, in vitro plant cell and tissue culture method is considered as one of the basic components of modern plant biotechnology (Neumann et al. 2009). In vitro methods of plant propagation always follow the vegetative mode of regeneration. Plant growth and development occur in in vitro aseptic and controlled environmental condition in the presence of artificial nutrient medium and plant growth regulators (Jha and Ghosh 2016). The prodigious advantages of in vitro aseptic technique of clonal propagation (i.e. micropropagation) are that an enormous number of disease-free true to type plantlets can be produced within little space and short time span plus season-independent round-the-year production (Bhojwani and Razdan 1996; Altman and Loberant 1998; Anis and Ahmad 2016). Theoretically all living plant cells are "totipotent" and have the capability to produce a whole plant from any single cell. In vitro culture technique is obviously the best platform to utilize the cellular totipotency of the plant cell for clonal propagation (Bhojwani and Dantu 2013b). The most significant and unique capacity of in vitro culture system is—irrespective of the nature of the explant source (root, leaf, shoot tip, node, internode, flower parts, pollen, ovule, zygotic embryo, endosperm, etc.)—a complete plant can be produced via axillary or via adventitious regeneration through organogenesis or embryogenesis (Fig. 7). In axillary regeneration methods, shoot tips and nodes are used as explant, and plant growth regulators (especially cytokinins) are used for inducing axillary branching by breaking the dormancy of shoot buds which are already present in their axil. Profuse branching is induced through this process, and complete plantlets are produced from these multiplied shoots followed by root organogenesis. The adventitious regeneration refers induction of plant organs or embryos from unnatural position, i.e. from where they are not grown in in vivo natural conditions. Plants can adventitiously propagate through two different primary morphogenic pathways, i.e. either through organogenesis (unipolar organs are formed) or through somatic embryogenesis (bipolar embryos are formed). Again, both organogenesis and embryogenesis may go through either direct morphogenic pathway without any callus phase or through indirect morphogenic pathway via callus phase (Fig. 8). The callus-mediated path has been accompanying with an augmented risk of genetic instability and henceforth increasing somaclonal variations among regenerated plants (Hervé et al. 2016). Therefore, the plants produced through direct morphogenic pathway are more reliable when target is clonal propagation, because comparatively more somaclonal variants are induced in callus-mediated regeneration (Bhojwani and Dantu 2013c).

The in vitro somatic embryogenesis process has been routinely used as large-scale micropropagation method (Ghosh and Sen 1989, 1991, 1996; Haque and Ghosh 2016b; El-Esawi 2016). Somatic embryogenesis is a typical example of

Fig. 7 In vitro clonal propagations of different plants through different paths. (**a**–**c**) Multiple shoot inductions from node culture of *Bacopa chamaedryoides*, *Hemidesmus indicus*, and *Physalis minima*; (**d**) multiple shoot inductions from shoot tip culture of *Kaempferia angustifolia*; (**e**, **g**, **h**) adventitious shoot induction from leaf explant via direct shoot organogenesis in *Solanum americanum*, *Bacopa monnieri*, and *Tylophora indica*; (**f**) direct shoot organogenesis from internode explant in *Bacopa chamaedryoides*; (**i**) direct somatic embryogenesis from leaf explant in *Ledebouria revoluta*; (**j**, **k**) indirect somatic embryogenesis via callus phase in *Ledebouria revoluta* and *Drimiopsis botryoides*; and (**i**) indirect shoot organogenesis via callus phase in *Tylophora indica*

cellular totipotency concept which is expressed in a huge number of plant species (Verdeil et al. 2007; Loyola-Vargas and Ochoa-Alejo 2016). All living plant cells cannot be deliberated as totipotent per se, but few of them can reclaim totipotency in

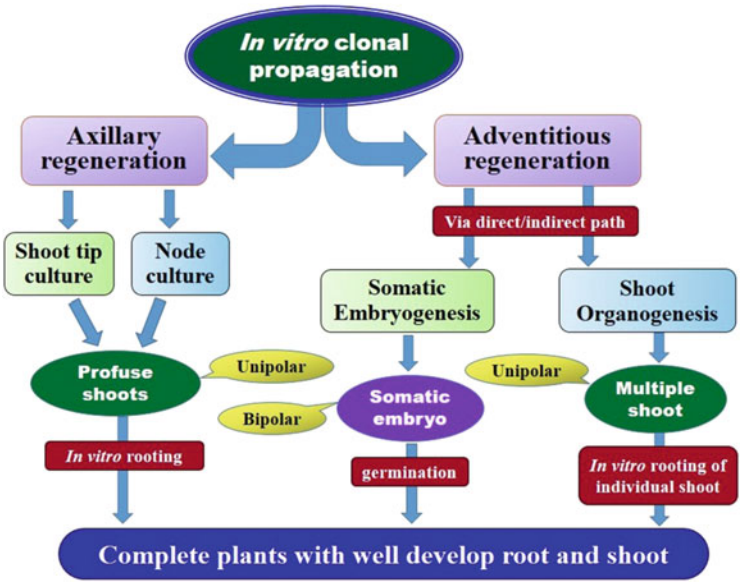

Fig. 8 Schematic representation of different paths of in vitro clonal propagations

suitable environments (Fehér et al. 2016). Somatic embryogenesis is considered to be developmental reprogramming of somatic cells or non-sexual cells towards the embryonic pathway followed by development through typical morphological stages (Yang and Zhang 2010) that are similar to the zygotic embryo development (Leljak-Levanic' et al. 2015). In somatic embryogenesis process, the somatic cell is distracted from their usual fate and reprogrammed an entire ontogenic developmental process to form embryos without any gametic fusion or zygote formation. Somatic and zygotic embryogenesis represent similar developmental events in which single cells obtain embryogenic cell fate and redifferentiate into mature embryos (Harada et al. 2010). For evidence, the developmental study between zygotic and somatic embryos of oak (*Quercus robur*) exhibited nearly four to seven identical developmental stages among them (Palada-Nicolau and Hausman 2001). During fertilization, two haploid (n) gametes fuse together to form a diploid ($2n$) zygote. The zygote is truly a totipotent single cell, from where an embryo is formed by way of gradual differentiation process. The embryo produced from a single zygotic cell is known as zygotic embryo, which is the propagule present inside the botanical seeds. Hence, fertilization is a must-needed process on the way of zygotic embryo production. The zygotic embryo is a bipolar structure having an embryonic axis and cotyledons. Monocotyledonous embryos have single cotyledon, while dicotyledonous embryos have double. The embryonic axis contains radicle (root initial) and plumule (shoot initial) at their two ends. In the course of somatic embryogenesis, the fertilization did not happen; instead, the embryo is developed directly from diploid ($2n$) somatic cell without fusion of two haploid (n) gametes. Therefore, the somatic embryogenesis is

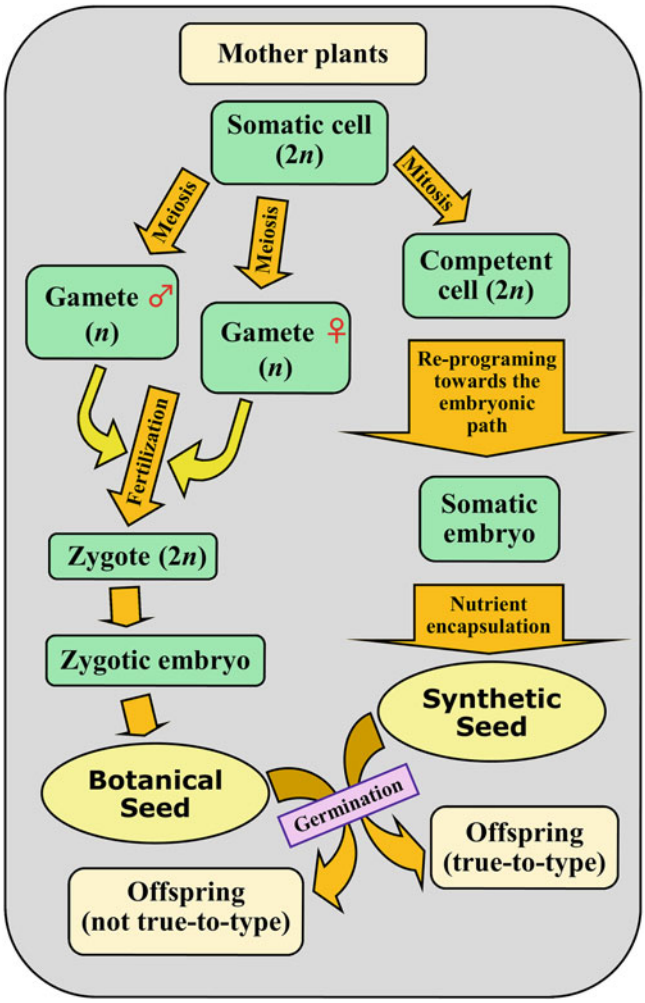

Fig. 9 Schematic representation of the comparative development of zygotic and somatic embryos as well as botanical and synthetic seeds

alternatively known as non-zygotic embryogenesis (Yang and Zhang 2010; El-Esawi 2016). The development of both zygotic and somatic embryos is schematically presented in Fig. 9.

De novo shoot organogenesis is also another good example of in vitro clonal propagation. Dissimilar to somatic embryogenesis, only unipolar (shoot pole) structures are developed during de novo shoot organogenesis (Yumbla-Orbes et al. 2017). The complete plantlets are produced from these de novo shoots pursued by root organogenesis.

7 Clonal Propagation of Genetically Modified (GM) Crops

Transgenic or genetically modified (GM) crops are those plants that have been improved genetically by means of recombinant DNA technology (Key et al. 2008). The population of human beings is growing faster than expected and predicted to reach almost ten billion people by the year 2050, therefore making food security is the vital social issue for the next three decades (Herrera-Estrella 2000). The food availability for everyone, particularly in developing countries, is possible through the cultivation of GM crops (Herrera-Estrella and Alvarez-Morales 2001). In the last two decades, the agricultural lands planted with GM crops have increased more than 100-fold, which clearly indicates that crop biotechnology is today's fast-growing promising area (Mall et al. 2018). America is the chief manufacturer of GM crops, including cotton, maize, soybean, and canola, representing 80% of the entire production of GM crops worldwide (Mall et al. 2018). Bt cotton, Bt brinjal, and GM papaya are well grown in Asian countries (James 2010). Regeneration of plants from the genetically transformed cells is indispensable to the success of genetic engineering and is only possible using in vitro tissue culture techniques (Darbani et al. 2008). The in vitro plant tissue culture system was recently described as "a battle horse in the genome editing" through a novel CRISPR/Cas9 technology (Loyola-Vargas and Avilez-Montalvo 2018). Ones the GM crops introduced through recombinant DNA technology (RDT), cross pollination and subsequent seed formation fail to retain the GM trait. Hence once again, the clonal micropropagation is only a viable option for large-scale production to address the huge requirements of GM crops. Clonal micropropagation of different transgenic plants species like *Betula platyphylla*, *Tylophora indica*, *Lactuca sativa*, and many others is well studied in the last decade (Pua and Davey 2007; Darbani et al. 2008; Zeng et al. 2010; Roychowdhury et al. 2013; Pniewski et al. 2017; Mall et al. 2018).

8 Synthetic Seed: A Modern Approach for Clonal Seed Propagation

Synthetic seeds are nothing but a functional mimic of botanical seeds, which was manufactured in laboratories and therefore alternatively known as manufactured seed or artificial seed (Sharma et al. 2013). Synthetic seed is one of the most promising modern plant biotechnological tools which could be useful for agricultural improvement at present as well as upcoming days. Application of synthetic seed technology is the perfect approach for micropropagation and conservation of important plant species, owing to their several advantages, including genetically true to type nature, comfort in handling and transportation, round-the-year production, and effectiveness in relation to space, time, labour, and cost (Niazian 2019). Recent advancement of in vitro clonal propagation systems opens a door for use of high-

quality high-vigour clonal plants in agri-horticultural field (Anis and Ahmad 2016). However, for commercial application of micropropagation, several steps like large-scale multiplication, in vitro root induction, their acclimatization, and planting are needed, which is more laborious and expensive. In this context, synthetic seed can provide a better option for cost-effective delivery system of in vitro-propagated clonal plants (Sharma et al. 2013) and may prove to be an effective alternative of the botanical seeds in future. Synthetic seeds offer a little-cost, high-volume clonal propagation system (Roy 2013). The advantages of synthetic seeds over other tissue culture-based propagation methods are easy to handle and potential for long-term storages (Rai et al. 2009).

8.1 Concept of Synthetic Seed

The idea of artificial seed or synthetic seed was the brainchild of Japanese botanist Toshio Murashige; he coined the word "artificial seed" for the very first time in 1977 (Murashige 1977). The definition of an artificial seed was first time given by Murashige (1978), as "an encapsulated single somatic embryo, i.e., a clonal product that could be handled and used as a real seed for transport, storage and sowing, and that, therefore, would eventually grow, either in vivo or ex vitro, into a plantlet". Therefore, synthetic seed production was previously restricted to only those plants in which somatic embryogenesis had been successfully standardized. Later, Bapat et al. (1987) proposed to expand the synthetic seed technology to the encapsulation of various in vitro-derived propagules other than somatic embryos, and they used axillary buds of *Morus indica* as a first example of this new application. Up-to-date perusal revealed that more than 20 scientific review papers were already published on the topic "synthetic seed", which clearly reflects the exact importance of this technology in modern days (Table 1). On the basis of research and review papers existent so far, synthetic seeds can be differentiated into two types—(1) encapsulated desiccated synthetic seed and (2) encapsulated hydrated synthetic seed. In botanical seed, after maturity the zygotic embryo enters in dormancy period when all cells of the embryo enter into quiescent (i.e. G_0 phase of the cell cycle) resting phase (Bewley 1997). For the first type, encapsulated desiccated synthetic seed preparation needs desiccation of propagules, which helps to improve the storage capability (or dormancy period) of the synthetic seed by aiding to enter the propagules in quiescent resting phase. However, this type of synthetic seed is less popular because of their low rate of germination as compared to encapsulated hydrated synthetic seed. For the second type, encapsulated hydrated synthetic seeds had to be developed by hydrogel encapsulation of propagule. This method was first time used by Redenbaugh et al. (1984) and was patented by them in 1988 (Patent # 4,780,987). In the present day, hydrogel encapsulation method is the most effective and broadly accepted technique of synthetic seed production (Sharma et al. 2013). Aiming for better understandings on how to prepare synthetic seed more successfully, Rihan et al. (2017b) studied the accumulation of dehydrin proteins during the maturation of

Table 1 Important scientific review papers related to the topic "synthetic seed" or "artificial seed"

Year of publish	Title of the review paper	References
2018	Synthetic seed—future prospects in crop improvement	Chandra et al. (2018). https://ijair.org/administrator/components/com_jresearch/files/publications/IJAIR_2688_FINAL.pdf
2018	Manufactured seeds of woody plants	Hartle (2018). https://doi.org/10.1007/978-3-319-89483-6_8
2017	Artificial seeds (principle, aspects, and applications)	Rihan et al. (2017a). https://doi.org/10.3390/agronomy7040071
2017	The usage of cryopreservation and synthetic seeds on preservation for plant genetic resources	İzgü and Mendi (2017). https://juniperpublishers.com/ijcsmb/pdf/IJCSMB.MS.ID.555583.pdf
2017	Synthetic seed technology in vegetables—A review	Khatoon et al. (2017). http://www.envirobiotechjournals.com/article_abstract.php?aid=7536&iid=224&jid=3
2017	Synthetic seed technology	Magray et al. (2017). https://doi.org/10.20546/ijcmas.2017.611.079
2017	Synthetic seed technology and its applications: A review	Tripathi (2017). http://biotech.journalspub.info/?journal=IJPB&page=article&op=view&path%5B%5D=157
2016	Development of synthetic seed technology in plants and its applications: A review	Nongdam (2016). http://www.currentsciencejournal.info/issuespdf/Nongdam.pdf
2015	Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent, and matrix	Gantait et al. (2015). https://doi.org/10.1007/s11738-015-1847-2
2015	Artificial seed: A practical innovation	Panwar (2015). http://www.rroij.com/open-access/artificial-seed-a-practical-innovation.pdf
2014	Synthetic seeds: A boon for conservation and exchange of germplasm	Kumara et al. (2014). http://advancejournals.org/bmr-biotechnology/article/synthetic-seeds-a-boon-for-conservation-and-exchange-of-germplasm/
2013	Synseed technology—a complete synthesis	Sharma et al. (2013). https://doi.org/10.1016/j.biotechadv.2012.09.007
2013	Synthetic seed production; its relevance and future panorama	Siddique et al. (2013). https://doi.org/10.21276/ajptr
2012	Production and applications of artificial seeds: A review	Ravi and Anand (2012). http://www.isca.in/IJBS/Archive/v1/i5/13.ISCA-JBS-2012-106.php
2012	Synthetic seeds: A review in agriculture and forestry	Reddy et al. (2012). https://academicjournals.org/journal/AJB/article-full-text-pdf/FEF310B30197
2011	Alginate-encapsulated shoot tips and nodal segments in micropropagation of medicinal plants. A review	Kikowska and Thiem (2011)

(continued)

Table 1 (continued)

Year of publish	Title of the review paper	References
2011	The green revolution via synthetic (artificial) seeds: A review	Helal (2011). http://www.aensiweb.net/AENSIWEB/rjabs/rjabs/2011/464-477.pdf
2009	The encapsulation technology in fruit plants—A review	Rai et al. (2009). https://doi.org/10.1016/j.biotechadv.2009.04.025
2001	Artificial seeds and their applications	Saiprasad (2001). https://www.ias.ac.in/article/fulltext/reso/006/05/0039-0047
2000	Synthetic seeds: a novel concept in seed biotechnology	Bapat (2000). http://www.barc.gov.in/publications/nl/2000/200009-02.pdf
2000	Synthetic seed: prospects and limitations	Ara et al. (2000). https://www.jstor.org/stable/24104316
1998	Recent perspectives on synthetic seed technology using non-embryogenic in vitro-derived explants	Standardi and Piccioni (1998) https://www.jstor.org/stable/10.1086/314087
1993	Embryogeny of gymnosperms: advances in synthetic seed technology of conifers	Attree and Fowke (1993). https://doi.org/10.1007/BF00043936
1992	Artificial seeds	Senaratna (1992). https://doi.org/10.1016/0734-9750(92)90301-O
1991	Somatic embryogenesis and development of synthetic seed technology	Gray et al. (1991). https://doi.org/10.1080/07352689109382306

botanical seeds of cauliflower and their significant role in the drought tolerance of seeds, and these findings could help on quality improvement of artificial seeds.

8.2 *Considerable Criteria in the Designing of Synthetic Seed*

At the time of synthetic seed preparation, the following three basic properties of botanical seeds have to be fulfilled—(1) primarily it must contain a propagule which later grows up as a plantlet (like zygotic embryo in botanical seed), (2) it should contain a nutrient medium which serves as storage food for plant propagule (like endosperm or cotyledons in botanical seed), and (3) the plant propagule should be covered by a hard protective layer for mechanical protection (like seed coat in botanical seed) (Fig. 10).

8.3 *Preparation of Synthetic Seed*

Somatic embryo is the ideal propagule for encapsulation to produce synthetic seed because of its bipolar nature (Gray et al. 1991; Ghosh and Sen 1991, 1994), but

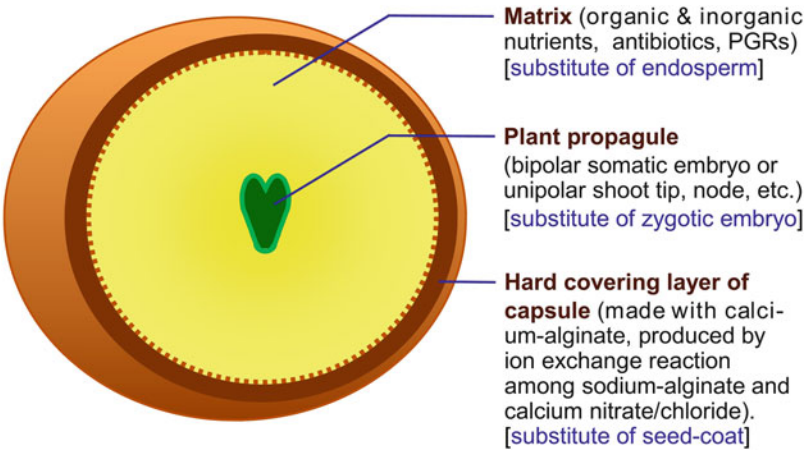

Fig. 10 Diagrammatic representation of the concept of synthetic seed and their different parts

successful somatic embryogenesis protocol is not established in many important plant species. Alternatively, any parts of plant which have the ability to grow can be used as non-embryogenic propagule (Fig. 11). In this context, recently different parts of plant organs like shoot tip and node (containing apical and axillary shoot buds), protocorm-like bodies (PLBs), corm, rhizome, micro-bulblet, micro-tuber, etc. are very popularly used for synthetic seed preparation (Fig. 12 and Table 2).

Nutrients present in encapsulation matrix are used by encapsulated plant propagule for their nutritional requirement. Different formulations of basic nutrient media which are used for in vitro plant culture are also used in encapsulation matrix with slight modification (Gantait et al. 2015). Although all the basic nutrients are same, calcium salts are not added. For example, if MS nutrients (Murashige and Skoog 1962) are used in encapsulation matrix, then calcium chloride is replaced with sodium chloride, which is devoid of calcium ions but fulfils the requirement of chloride ions. Apart from inorganic nutrients and sodium alginate, carbohydrates in the form of sucrose or glucose are also needed. In addition to inorganic and organic nutrients, plant growth regulators, antibiotics are also used in encapsulation medium (Sharma et al. 2013).

Another important requirement of encapsulation matrix is hydrogel. More than a few encapsulating agents such as agarose, potassium alginate, sodium alginate, gelrite, sodium pectate, sodium alginate with carboxymethyl cellulose, guar gum, carrageenan, tragacanth gum, gelatin, etc. have been experimented as hydrogels (Ara et al. 2000; Rai et al. 2009; Sharma et al. 2013). Among all of these gelling agents, sodium alginate achieved maximum popularity due to its adequate viscosity, rapid gelation, low cost, as well as non-toxicity for plants (Gantait et al. 2015).

For the preparation of encapsulated hydrated synthetic seeds, the encapsulation medium (or matrix of synthetic seed) should be prepared at first. Encapsulation medium contains all inorganic and organic nutrients (without calcium ion) of any

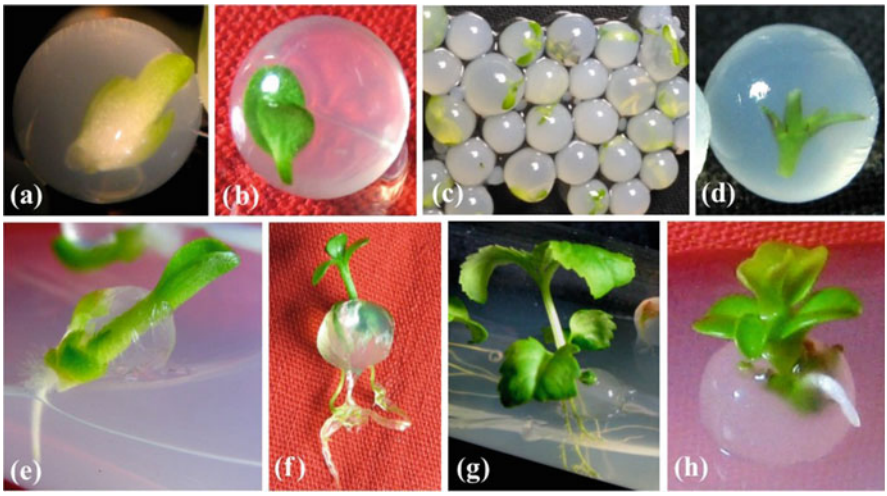

Fig. 11 Synthetic seeds prepared from embryonic and non-embryonic explants. (**a**–**d**) Freshly prepared seeds produced by encapsulating the somatic embryo, shoot-tips, and nodal segment, respectively. (**e**–**h**) germinated seeds, (**a**, **e**) synthetic seed of *Ledebouria revoluta* before and after germination, (**b**, **f**) synthetic seed of *Bacopa monnieri* before and after germination, (**c**, **g**) synthetic seed of *Bacopa chamaedryoides* before and after germination, and (**d**, **h**) synthetic seed of *Tylophora indica* before and after germination

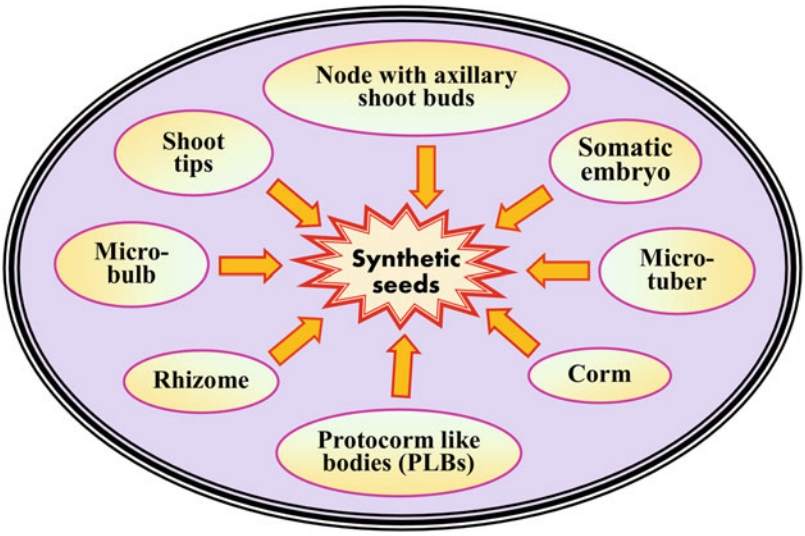

Fig. 12 Schematic representation of different types of explant used for synthetic seed preparation

Table 2 Important publications (in 2016 and onwards) on synthetic seed informing about the recent trends on use of different explants for encapsulation

Year of publish	Plant species	Encapsulated explant type	References
2016	*Solanum tuberosum*	Axillary buds	Ghanbarali et al. (2016). https://doi.org/10.1007/s11240-016-1013-6
2018	Sugarcane	Bud chip	da Silva et al. (2018). https://doi.org/10.5539/jas.v10n4p104
2018	Sugarcane	Micro-shoots	Badr-Elden (2018). https://ejbo.journals.ekb.eg/article_5168_2f3913c2d70d6a019aa587b3a90fd465.pdf
2018	*Althaea officinalis*	Nodal segments	Naz et al. (2018). https://doi.org/10.1080/11263504.2018.1436610
2018	*Capparis decidua*	Nodal segments	Siddique and Bukhari (2018). https://doi.org/10.1007/s10457-017-0120-7
2018	*Ceropegia barnesii*	Nodal segments	Ananthan et al. (2018). https://doi.org/10.1007/s11627-018-9934-x
2017	*Erythrina variegata*	Nodal segments	Javed et al. (2017). https://doi.org/10.1016/j.indcrop.2017.04.053
2016	*Manihot esculenta*	Nodal segments	Hegde et al. (2016). http://isrc.in/ojs/index.php/jrc/article/view/407/290
2018	*Salix tetrasperma*	Nodal segments	Khan et al. (2018). https://doi.org/10.1016/j.bcab.2018.07.002
2018	*Sphagneticola calendulacea*	Nodal segments	Kundu et al. (2018). https://doi.org/10.1007/s11738-018-2633-8
2017	*Tylophora indica*	Nodal segments	Gantait et al. (2017b). https://doi.org/10.1016/j.hpj.2017.06.004
2017	*Vitex trifolia*	Nodal segments	Alatar et al. (2017). https://doi.org/10.1080/14620316.2016.1234949
2017	*Spathoglottis plicata*	PLBs	Haque and Ghosh (2017b). https://doi.org/10.1016/j.hpj.2017.10.002
2018	*Ansellia africana* (Leopard orchid)	Protocorm-like bodies (PLBs)	Bhattacharyya et al. (2018). https://doi.org/10.1007/s11240-018-1382-0
2018	*Plumbago rosea*	Shoot tips	Prakash et al. (2018). https://doi.org/10.1007/s12298-018-0559-7
2017	*Rauvolfia serpentina*	Shoot tips	Gantait et al. (2017a). https://doi.org/10.1016/j.jarmap.2017.01.005
2017	*Rauvolfia serpentina*	Shoot tips	Gantait and Kundu (2017). https://doi.org/10.1007/s12210-017-0637-8
2018	*Taraxacum pieninicum*	Shoot tips	Kamińska et al. (2018). https://doi.org/10.1007/s11240-017-1343-z
2018	*Urginea altissima*	Shoot tips	Baskaran et al. (2018). https://doi.org/10.1007/s13205-017-1028-7
2018	*Nerium oleander*	Shoot tips and first nodal segments	Hatzilazarou et al. (2018). https://doi.org/10.1080/14620316.2018.1542283
2017	*Withania coagulans*	Shoot tips and Nodal segments	Rathore and Kheni (2017). https://doi.org/10.1007/s40011-015-0577-y

(continued)

Table 2 (continued)

Year of publish	Plant species	Encapsulated explant type	References
2016	*Zingiber officinale*	Shoot tips and Somatic embryos	Babu et al. (2016). https://doi.org/10.1007/978-1-4939-3332-7_28
2016	Mountain garlic	Shoot tips or Micro-bulbs	Mahajan (2016). https://doi.org/10.1007/978-1-4939-3332-7_23
2016	*Citrus* spp.	Somatic embryo	Micheli and Standardi (2016). https://doi.org/10.1007/978-1-4939-3061-6_30
2016	*Bacopa monnieri*	Somatic embryos	Khilwani et al. (2016). https://doi.org/10.1007/s11240-016-1067-5
2016	*Curcuma amada*	Somatic embryos	Raju et al. (2016). https://doi.org/10.1007/s13580-016-0096-7
2017	Date palm	Somatic embryos	Bekheet (2017). https://doi.org/10.1007/978-1-4939-7159-6_7
2016	*Ledebouria revoluta*	Somatic embryos	Haque and Ghosh (2016b). https://doi.org/10.1007/s11240-016-1030-5

suitable formulation of basic medium (e.g. MS medium), sucrose, sodium alginate, plant growth regulators, and antibiotics. Suitable plant propagules are mixed with encapsulation medium, and then sodium alginate-containing medium dropped into a solution of calcium salt (calcium chloride or calcium nitrate). Each drop (bead) containing a single propagule should be maintained in calcium solution for 10–20 min with gentle shaking. Ion exchange reaction takes place on the outer surface of the beads where Na^{+} ion of the sodium alginate is replaced with Ca^{+2} to form a hard layer of calcium alginate (Jha and Ghosh 2016). Polymerization of calcium alginate resulted in the construction of hydrogel capsules with single propagule inside—i.e. synthetic seed. The capsules containing gel matrix actually perform as a repository of nutrient which assists in the survival, as well as growth and development of propagules (Gantait et al. 2015).

9 Applications of Synthetic Seed to Avoiding Heterozygosity

Applications of synthetic seed are not only restricted to avoiding the heterozygosity problem but have a wide-ranging list of utility (Fig. 13). In general, synthetic seed technology is utilized for the following purposes—(1) for micropropagation; (2) for short-, medium-, or long-term conservation; (3) for clonal or true to type seed production; (4) for large-scale seed production aimed at commercial use; (5) for season-independent round-the-year seed production; (6) for propagation of non-seed-bearing plants; (7) for easy handling and transportation; and (8) for exchange of germplasm between different countries by lowering plant quarantine requirements as for the germ-free condition of the plant propagules (Ara et al. 2000; Rai et al. 2009; Sharma et al. 2013; Hartle 2018; Chandra et al. 2018).

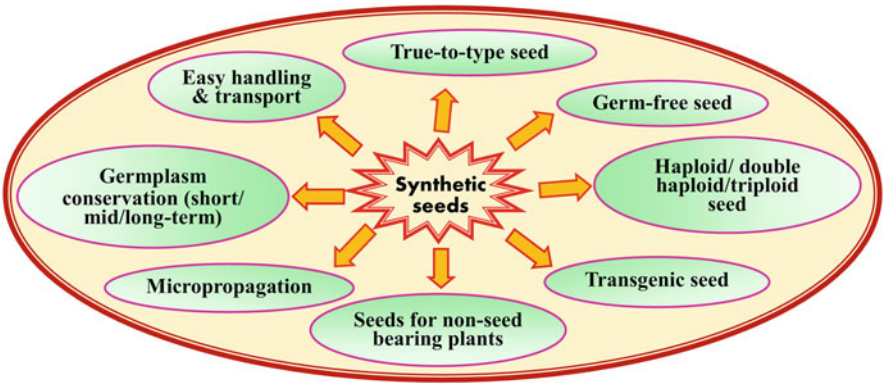

Fig. 13 Schematic representation of different applications of synthetic seeds

Now keeping aside the general benefits, let us come back to our main focus, i.e. avoiding heterozygosity through synthetic seeds. Two phenomena, namely, meiotic crossing over and gametic fusion, are unavoidable events of sexual reproduction. In diploid plants, one chromosome of each homologous pair originates from male parent and another from female parent. During crossing over, the genetic materials of two different chromosomes of the homologous pairs get mixed and recombined due to the reciprocal exchange among non-sister chromatids (Schwarzacher 2003). As a result, new types of heterozygosity are generated in haploid gametic cells. Now another event is gametic fusion where a lot of new forms of heterozygosity are generated. During fertilization, male and female gametes may perhaps from two different parents participate; as a result two totally different sets of alleles come together in zygotic cell (Acquaah 2012). Hence, in comparison with any one of the parental plants, the newly formed zygote contains many new combinations of alleles, i.e. so many new forms of heterozygosity are created in zygote.

Since all the propagules used for synthetic seed preparation are propagated vegetatively, which means they escape meiotic recombination (during crossing over) as well as gametic fusion of two different parental genomes (Clift and Schuh 2013), they ceased the chances of the formation of new types of heterozygosity in seed. Although the heterozygous conditions which are already present in source plant (from where clonal propagations initiated) can't be eliminated, they can be shifted to all offspring.

10 Summary and Future Prospects

Synthetic seed is an up-to-date tool of plant biotechnology which manufactured in laboratories and serve as efficient alternative of botanical seeds. Since vegetatively propagated propagules are used for synthetic seed preparation, all the manufactured

seeds are genetically true to type of their parent. Nevertheless, the heterozygosity that already existed in a mother plant cannot be eliminated but can also be transmitted in all synthetic seed-derived offspring.

However, if the production of homozygous synthetic seeds is wanted in realistic form, the only method is double haploid source plant selection, because double haploid plants are always truly homozygous (Prigge et al. 2012; Kleter et al. 2019). The clonal propagation of double haploid plant gives rise to a huge number of homozygous clones. If those clonal propagules are used for synthetic seed preparation, then the heterozygosity problem will totally be avoidable. Otherwise synthetic seed technology can only help to restrict the construction of new forms of heterozygosity in successive regeneration cycle, which are abundant in zygotic or botanical seeds.

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