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Review Article

Status of Polyembryony in Tree Borne Oilseeds-a Review

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Abstract

Studies on mechanism in occurrence of polyembryony are vital for understanding the fundamental aspects of biology and also for practical purpose of genetic selection. Previously, polyembryony was reported in Mango and citrus; the commercial exploitation of it, in the field of Horticulture is well known. But, the reasons for occurrence is not clear, many authors have reported their observations and view about polyembryony. Similarly, the occurrence of polyembryony has been reported by plethora of workers in tree borne oil seeds at the nursery stage. The percentage occurrence of polyembryony in tree borne oil seeds ranges from 1 to 35% in *Calophyllum inophyllum, Amoora rohituka, Madhuca latifolia, Ponammia pinnata, Azadirachta indica, Garcinia indica, Garcinia mangostana* etc. But still, the topic is researchable and debatable due to lack of understanding in concept behind it. Generally, it is recommended to retain healthy shoots and cull out unhealthy ones for the growth and betterment of quality planting production. In this technology era, advance tools like markers can be used in identifying suitable reasons behind polyembryony and their potential in production of disease free seedling. Nowadays, the TBO's are in prime focus to minimize the gap between demand and supply of fossil fuels and their sustainable use. Therefore, this article is framed in order to focus the lights on concept of polyembryony and their mechanisms and also the possibilities in exploring the potential of pathways of seed development in tree borne oil seeds-a researchable issue.

1. Introduction

The major source of bio-diesel in India is non-edible oil seeds i.e., Tree borne oilseeds (TBOs). The requirement of TBOs in the country as per the National Oilseeds and Vegetable Oils Development Board, Gurgaon (India) is more than 5 mt. However, only 0.8-1.0 mt are being collected from different species such as Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*), Mahua (*Madhuca longifolia*), Jatropha (*Jatropha curcas*), Kusum (*Schleichera oleosa),* Pilu *(Salvadora oleoides*)*,* Bhikal (*Prinsepia utilis),* Undi (*Calophyllum inophyllum*), *Simarouba glauca,* Sal (*Shorea robusta*) and Jojoba (*Symondsia chinensis*). Some of the TBO species can be grown in wasteland under relative unfavourable environmental conditions and such species needs to be promoted. They meet dual objective of rehabilitating wastelands and energy substitute. The planting material was prepared by collection of seeds from forest and raising seedlings. Apart from this, superior germplasm collection, vegetative propagation methods such as cuttings, grafting sets has been developed in the recent years. As the demand for planting material and the energy demand is increasing day by day, production of quality seedlings in large quantity is essential for field plantation of TBOs. During the course of raising seedlings, some of the TBO'S produced abnormal seedlings such as polyembryony and albino in the nursery stage. Many workers have reported polyembryony in TBOs like *Calophylum innophyllum* (Gunaga et al., 2012), *Pongamia pinnata* (Reddy et al., 2006); *Madhuca latifolia* (Chavan et al., 2014b). Based on the existing reports and personal observations, it was noticed that polyembryony is a common phenomenon in TBOs. Besides reporting, the reason for its occurrence is not exactly pointed out. Some of the TBO'S are slow growing, late flowering and fruiting with long gestation period; in spite of nature of growth, if polyembryony is explored for commercial exploitation, some of the constraints can be sorted out, unlike in citrus and mango, which will add supplementary value in development of quality seedlings. Thus, this article will be focused in view of understanding the generalized concept of polyembryonic nature, phenomenon, mechanism and significance of polyembryony and research areas warranted for possible exploitation in production of quality planting material in Tree Borne oil seeds.

2. Evolution and Concept of Polyembryony

The term polyembryony can be put in different ways as defined by following authors:

• Polyembryony can be defined as production of two or more embryos with in an ovule (Webber, 1940).
• Presence of two or more embryos in the single seed (Naumova, 1993).
• Occurrence of more than one embryo in a seed (Maheswari, 1950).
• All cases in which there are clear indications of the potential or actual occurrence of two or several proembryos or embryos in a single seed, irrespective of the question whether they may ultimately give rise to viable seedlings (Bouman and Bosewinkel, 1969).

Polyembryony was discovered by Leeuwenhoek in 1719, when he found in orange seeds each containing two embryos. In 1878, Strasburger first demonstrated the nature of polyembryony in *Citrus, Coelebogyne, Funkia* and *Nothoscordum*. Braun (1860) reported polyembryony as known in twenty-one species, in thirteen genera, and in twelve families. Tisser et al. (1979) also mentioned, about 244 species from 140 genera belonging to 59 angiosperm families are existence. This suggests that polyembryony is more prevalent in Angiosperms.

Polyembryony has made an enigmatic approach for classification and further discussion by different scientists worked upon. Ernst (1918) has classified based on number of embryo sacs. i.e., True polyembryony [embryos derived from cells outside the sac (cells of nucellus/integument) and within the sac (embryos from synergids, antipodal cells, endosperm cells, suspensor)] and Pseudo/False polyembryony [formation of adventitive embryos in several embryo sacs originating from different nucelli in one or several ovules]. In future studies, Ernst classification paved way for lively discussion where the difference in opinion raised in false polyembryony. Webber (1940) agreed on classification but proposed to classify only on the formation of adventitive embryos upon their fusion or formation of more than one ovule in the nest of one seed-ovary whereas, Maheswari (1952) considered the formation of adventitive embryos from several embryo sacs in the same ovule. Johansen (1950) considered nature of embryos as important which tallies with the concept of polyembryony in animals. According to him, the category of true polyembryony is formed as a result of division of zygote or proembryo. In the new classifications, gametophytic (from gametophyte cells) and sporophytic polyembryony (from cells of mother and daughter sporophyte) has been framed based on genetic basis (Yakovlev, 1967; Bouman and Boesewinkel, 1969). In 1979, Camaron and Soost proposed classification of polyembryony based on frequency of occurrence. The occurrence of multiple embryo is less than 6% known as strictly monoembryony, from 6-10% known as nearly monoembryonic and the percent of multiple embryo is more than 10% known as polyembryony. In the later stage, Lakshmanan and Ambegaokar (1984) believed that presence or absence of sexual process is an essential factor in the formation of polyembryonic seeds and proposed to classify in to simple (embryos develop from zygote) and multiple polyembryony (embryos develop from unfertilized synergids or from cells of the nucellus and integument).

In plants, 78% of polyembryonic cases arise from maternal tissue (adventitious polyembryony), and 19% arise from cleavage of the fertilized embryo (cleavage polyembryony) (Ganeshaiah et al., 1991). Moreover, polyembryonic seedlings may be of same or different ploidy. In angiosperms, the frequency of polyploidy was ranges from 30% (Stebbins, 1971) to 70% (Masterson, 1994).

3. Mechanism of Polyembryony

Ganesiah et al. (1991) dealt evolution of polyembryony by taking examples from citrus and developed genetic and inclusive models to derive the conditions that permit evolution of polyembryony under maternal and offspring control. Expressions were derived for optimal allocation of resources between zygotic and nucellar embryos and concluded that polyembryony has evolved as a counter strategy by maternal parent to mitigate the consequences of sibling rivalry. Uma Shankar and Ganeshaiah (1996) argued on the topic of “Polyembryony in plants: a weapon in the war over offspring numbers?” and concluded sibling rivalry among seeds as the reason. The seed (offspring) in fruits compete for maternal resources and tries to reduce their sib population i.e. they may be selected to kill their neighbouring sibs in the fruit. The fight among offspring’s (seeds) leads to brood reduction i.e. seed abortion, causes fitness loss to the mother, and hence such brood reduction can be countered by maternal tissues through producing extra embryos in seeds.

In adventitious polyembryony, all embryos are genetically identical to each other and the maternal plant. For multiple embryos originating from maternal tissue such as the seed coat or endosperm, it has been proposed that polyembryony is a maternal strategy to counteract losses suffered due to sibling competition (Ganeshaiah et al., 1991). However, Willson and Burley (1983) suggested the possibility of facilitation, or at least reduced competition, among genetically identical offspring. Apart from this, in Gymnosperms i.e., *Pinus sylvestris* apoptosis/programmed cell death (PCD) (cells that are considered as threat to the organism are tightly regulated by cell suicide process is called apoptosis) is responsible for elimination of sub-ordinate embryos in a polyembyonic seed. Once dominant embryo is selected, subsequently the entire female gametophyte is affected by PCD; the cells of sub-ordinate embryos initiate self-destruction. The PCD follows rapid basic-apical pattern, first killing most basally situated cells and then proceeding towards apical region until all cells in embryonal mass is doomed (Filonova et al., 2002).

Under the growth circumstances on polyembryony seedlings, the competition for light is generally thought to result in size asymmetry between the competing individuals, with earlier germinating or inherently taller plants prevents light and having a proportionally detrimental effect on smaller plants (Schwinning and Weiner, 1998). The below-ground completion is size-symmetric, with roots acquiring resources proportional to their size (Schwinning and Weiner, 1998; Cahill and Casper, 2000; Schenk, 2006). There exists above-ground competition but less than below-ground. At the same time, if the resources are pre-empted by the competitor, none of the embryo individuals from polyembryonic seeds would be able to compete, regardless of differences in embryo size. Therefore, conflicting reports of symmetric and asymmetric competition still exists (Prati et al., 1997).

4. Causes of Polyembryonyw

Polyembryony is caused by a number of factors including the type of pollinator, the amount and viability of available pollen, plant nutrition, and environmental factors such as air temperature, soil humidity and wind speed (Yildiz et al., 2013). Several theories have been put forth to explain the causes by various workers but still the causes of polyembryony are not well understood and is a debatable issue. The views are:

- Polyembryony may occur due to hormonal imbalance (Leoroy, 1947).
- Cytological, disturbance in chromosome balance between the embryo, endosperm and mother tissue may lead to degeneration of the embryo (Rhoedes, 1961).
- Polyembryony also occurs due to genetic causes (Ganeshaiash et al., 1991; Maheshwari and Sachar, 1963; Castle and Meinke, 1993). In mango, it is controlled by single dominant gene (Aron et al., 1998).
- Adventive embryonic in mango was probably due to the effect of one or more recessive genes (Leroy, 1947).
- Hybridization may be an important factor that leads to polyembryony (Ernst, 1918).
- Production of polyembryonic seed may be hereditary (Atabekova, 1957).
- Number of embryos in citrus seeds was influenced by various factors such as age of the tree, nutritional status and orientation of branch of the tree (Furusato et al., 1957).

5. Polyembryonic Occurrence in TBO'S

In Tree Borne Oilseed trees, the occurrence of polyembryony i.e. twin, triplet or multiple seedlings are considered as abnormality or malformation and such seedlings are culled out from nursery because of unclear understanding about polyembryony in trees (Rai, 2014; Chavan et al., 2014b; Rane et al., 2012; Gunaga and Vasudeva, 2008a). Although the number of seedlings from a single seed is usually only two or three, it may exceed to as many as thirteen seedlings from a single seed (Cook, 1907). Seedling evaluation for vigour and health is an important aspect in any tree or crops. As per the International Rules for Seed Testing (ISTA), abnormal seedlings are those seedlings that do not show the potential to develop in to a normal plant when grown in good quality soil and under favourable conditions of moisture, temperature and light (ISTA, 1993). Such abnormalities may occur due to cleavage of embryo, proliferation of the zygote or development of adventitive embryos from independent sources or genetic factors or mutation (Swamy and Krishnamurthy, 1980). The morphological abnormalities like polyembryony, twin and triplet seedlings, albino, double embryo have been widely reported for different tropical oil trees in Table 1 and Figure 1. The nature of polyembryony and percentage of occurrence varies with the species. According to Cameron and Soost (1979), if occurrence of polyembryony is more than 10 per cent then the species is considered as polyembryonic in nature or else as monoembryonic. Therefore *Madhuca latifolia* (11%), *Amoora rohituka* (35%), *Azadirachta indica* (12.25%) and *Garcinia mangostana* (11%) can be considered as polyembryonic in nature. The field studies in twin or multiple seedlings are very few. Some of the studies by Verma and Bhatt (2006); Rai (2014) reported that the growth of twin seedlings of *Diploknema butyracea and Amoora rohituka* was stunted in all respects as compared to the normal ones, respectively.Thus, morphologically polyembryony has been included as abnormal seedlings, where different forms of growth are recorded during germination. They are:

- The radical and root system may be normal. But the first/second set of leaflets emergence may differ from original botanical nature of leaf shape (for example, in neem, the first set of leaflet initiated from the base of plumule, the second set of leaflet were strap shaped instead of being serrate) (Pandey et al., 2006).
- Instead of two leaflet emergence at the base of plumule,

Table 1: Reported polyembryonic cases in TBOS

Species name	Nature of Polyembryony	Percentage of occurrence (%)	Reference
Calophyllum inophyllum	Twin and triplet	5.13	Gunaga et al. (2012); Wanage et al. (2010)
Mammeaga suriga	Twin	-	Gunaga and Vasudeva (2008a)
Amoora rohituka	Twin, triplet and polyembryo	35.00	Rai (2014)
Madhuca latifolia	Twin, triplet	11.00	Chavan et al. (2014)
Mangifera indica	Twin	1.00	Kannur et al. (2005)
Pongamia pinnata	Twin	-	Reddy et al. (2006)
Azadirachta indica	Twin	12.25	Singh et al. (2001)
Garcinia indica	Twin, triplet	-	Gunaga and Vasudeva (2008b)
Garcinia mangostana	Twin	11.00	Abdullah and Isamil (2010)


Calophyllum inophyllum


Garcinia indica


Pongamia pinnata


Madhuca latifolia

Figure 1: Synoptic view of polyembryony in tree borne oil seeds

sometimes there may be one.

- The radical and root system were stunted. Two nature of system from cotyledons were observed.
- One of the cotyledons slightly pushed up with the

plumule.

- One of the cotyledons born roots.
- The seedlings may be of unequal size-One seedling taller and another shorter

The growth of twin seedlings was similar to that of normal seedlings. In neem, after 10 days the twin seedlings was separated from each other with emerging shoot apex and behaved as normal seedlings. They persisted up to 28 days (Pushpakar and Babley, 1995).

6. Identification of Polyembryony in Trees

Occurrence of polyembryony in TBOs is common but its identification in seed or seedling is difficult and complex task. Phenological or morphological identification of polyembryonyic seeds are very complex job and it requires special indices. Against this drawback, there are some techniques like biochemical and molecular approaches which are effective and expeditious tools to identify zygotic and nucellar seedling at an early stage. Gill et al. (2002) reported that the nucellar seedlings could be distinguished from the zygotic seedling on the basis of banding pattern of different isozyme markers. Molecular techniques are very effective, advance and reliable tool to study gene expressions by RAPD, RFLP, SSR and QTL markers. Das et al. (2007) identified the zygotic seedling through RAPD markers and reported that zygotic seedling (twin or triplet) usually had one or more extra band than nucellar seedling. RAPD differentiates zygotic from nucellar seedlings in early stage, as reported by Ochloa et al. (2012); Cordeiro et al. (2006) for mango and Rao et al. (2008) for citrus. The use of SSR markers is an effective method to identify nucellar seedlings as being genetically identical to their maternal parent and may significantly contribute to breeding programs by decreasing the space and time needed to develop new cultivars (Yildiz et al., 2013).

7. Exploitation of Polyembryony in TBO's

In recent research areas, polyembryonic occurrence has become a common phenomenon; its occurrence has been reported by various workers. The percentage occurrences of polyembryony in TBO's are ranges from 1% (Kannur, 2005) to 35% (Rai, 2014) in different species are given in Table 1. Despite documentation, the mechanism involved in it and the commercial exploitation is still a researchable and debatable issue. It is also noticed that the survival ability of polyembryonic seedling is very low. Although the causes for occurrence in polyembryony in other species are studied, in TBO's most of the authors have reported due to developmental errors/genetic factors. The exact reason behind this is still infancy. Some researchers have recommended keeping vigour shoot and culling out remaining shoots/roots in order to avoid competition and also to ensure better performance under field condition. The behavioural pattern of polyembryonic seedling has not been studied in detail.

Unlike the economic exploitation in Mango and citrus, identification and characterization from initial cells of sexual and adventive embryos is essential in order to know the problem of polyembryony. The genesis is needed to be studied in detail, since the origin and development depends on specific features of developmental biology. Pathways of seedling development may suggest a high polymorphism of sexual embryos related to ecological conditions (Batygina and Vinogradova, 2007). Inspite of this, Polyembryony is also closely linked with genetic heterogeneity in seeds but is usually undistinguished phenotypically. Moreover, ecological conditions also favour polyembryony (Eg: Mango seeds may be monoembryonic in one habitat and polembryonic in another, Juliano (1937). It is indirectly notifies chances of huge variability in seeds under natural conditions that may be altered by various biotic and abiotic factors. This factor is taken in to account in case of tree borne oil seeds; as the research on it was initiated in the recent past years and the most of the seed collections were from wild. So the variability not only exists in populations but also in developmental biology of seedling formation and development. This area is left emptied and there is lot of scope for research on economic exploitation of polyembryony in TBO's.

8. Significance of Polyembryony

Polyembryony has been found as a considerable value to plant breeders and horticulturist. The significance of polyembryony has been proved in horticulture i.e., the seedlings developed through nucellar polyembryony performed more superior than the clones multiplied through vegetative propagation. In Citrus and Mango, Polyembryony helps in large-scale propagation of selected genotypes. Nursery managers can use the nucellar embryos to propagate disease-free clone rootstocks or rejuvenate old clones that have lost their vigour through constant propagation.Adventive embryos can be raised by budding from the nucellus (clones of the mother tree) or from differentiated embryonal cells (clones of new generation). In adventitious polyembryony, all embryos are genetically identical to each other and the maternal plant (Blanchard et al., 2010). This genetic nature existing in polyembryony can be exploited. Moreover, twin seedlings provide excellent source of haploids, which can be used in heterosis breeding (Verma et al., 2009). These haploids are used to produce polyploids, aneuploids and translocations, for overcoming incompatibility in crosses with wild related species possessing economically important features (Tyrnov, 2000).

If embryos from a polyembryonic seed are genetically similar, "seed individuals" can serve as the larger proxy for individual fitness. Genotype fitness for a plant originating from polyembryonic seeds is additive (i.e., each embryo's reproductive output should be summed for each seed individual) compared with a plant arising from a single-embryo seed that has only this single source for reproductive output. Under natural conditions, if polyembryonic seeds increase the probability of a given genotype surviving, as well as increasing the probability of adding individuals to the next generation, then this reproductive strategy should be favoured over one resulting in single-embryo seeds (Blanchard et al., 2010).

9. Conclusion

TBOs are important renewable energy source for biodiesel production, but due to lack of quality planting materials many plantations have failed. So, here comes, the role of elite planting material right from variability in seed material to other biometric traits. Therefore, the reporting of polyembryony in TBO's will help us in understanding the nature and pathway of embryo and seedling development. Studies on the applied aspects of polyembryony may pave an opportunity for disease free planting material in TBO'S.

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