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A general introduction of propagation and micropropagation techniques of Macadamia Nut (*Macadamia spp.*)

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ABSTRACT: Macadamia is categorized into a group of minor crop but can exhibit wide climatic adaptability. It has quick expanding industry demands for its high economic and nutritional values. Graftings, cuttings and micropropagations are the three major propagation methods of macadamia that can be utilized in a combination manner as seedlings with successful graftage can be encouraged to elongate and provide more scion woods for further multiplication such as supporting materials for cuttings. Cuttings can be sprouted to provide explant materials for micropropagation. Therefore, micropropagations are being used for commercial production of macadamia. Micropropagations are comparatively easy and convenient methods that can faster the introduction of any novel varieties into a new region, production of biotic stress tolerant plants, production of plants with greater uniformity and superior branching. Hence, this crop plant might hold an example for the other plants in the next few decades regarding much better application of advance propagation technologies and other genomic approaches.

KEYWORDS: Macadamia Nut, propagation, micropropagation.

INTRODUCTION

Four species of macadamia such as *M. ternifolia, M. tetraphylla*, *M. integrifolia and M. hildebrandi* belong to the tree family proteaceae are native to Australia and other ten species are assumed across the world [1],[2]. These trees favor warm region with high humidity and rainfall and growing in tropical climate regions but cannot tolerate any sort of chilling temperature. Despite, macadamia, an Australian originated plants but primarily it is commercialized in Hawaii and their germplasm enhancement contributes a major role for the chronological development of macadamia in several countries [1],[3–8]. In general, Hawaiian cultivars are more responsible for much production in the current world [1],[3],[9–11]. Commercial macadamia cultivars are recommended by the growing conditions so that it has been distributed in Hawaii [4],[8],[12], Australia [12], South Africa [12] and California [12]. Production of highly valued macadamia nut is massive in Australia [1] and day by day, it is emerging the international commercial food crop in Australian flora [4]. Most of the commercial farms of macadamia, propagation is usually used for multiplication and improvement of macadamia nuts. Grafting is one of the most important propagation method of macadamia because of the better quality seedling rootstocks and also ease of production particularly in Australia [9] and many other macadamia cultivated countries as well [1],[3],[5],[9]. Hidden Valley Plantations is a well-recognized Australian private macadamia breeding program which produces a series of broad genetic base macadamia cultivars (*M. integrifolia* × *M. tetraphylla*) comparing with Hawaiian cultivars. Ultimately, those are selected on the basis of yields, tree traits and ease of propagation by cuttings [2]. It has been noticed that macadamia genotypes mostly *M. tetraphylla* is uncomplicated to grow from cuttings rather than *M. integrifolia* [2],[9], but it has not been explicitly evaluated. Micropropagation is another means of propagation technique of macadamia that can be utilized to get rapid and vigor production of macadamia.

CYTOGENETICS

Survey reports of macadamia species have been accomplished and found that all macadamia species are diploid in nature having a total of 14 haploid chromosome number [12–14]. No disruptions are found in normal chromosome pairing or disjunction during *M. tetraphylla* and *M. integrifolia* hybridization are accomplished, and their F_1 progeny remains n ¼ 14 chromosome number [1],[13]. Five other genera of macadamia's subfamily Grevilleoideae of Proteaceae family have been surveyed and reported that they have relatively small chromosomes size [1],[15]. The evidence of the number of isozyme loci of macadamia has been sorted out by Peace *et al.*, [3] and Aradhya *et al.*, [3],[16] and they reveal that it is a diploid species rather than the other ancient tetraploid origins. Stace *et al.*, [15] also suggests that "if paleo-polyploidy has occurred in ancestral proteaceae then molecular genetics investigation of genera (macadamia) may reveal (extensive) gene silencing, which would have happened through the process of diploidization" [1].

GROWTH AND DEVELOPMENT OF MACADAMIA

It has been reported that four weeks to five or eight months are somewhere required for the germination of macadamia nuts after sowing of seeds. Seed dormancy actually causes the variation of seed germination among the different macadamia cultivars [1],[19]. A 15-year-old macadamia tree produces around 10,000 racemes during the flowering season [20],[21]**.** Pendulant type racemes bear a couple of hundred flowers (hermaphroditic) that look like perfect white to cream color at each whorl on the rachis. Each flower also consists an ovary having two ovules and a style covered with an extremely small stigmatic surface [12],[16],[20],[21]. In Hawaii, blooming of macadamia flowers usually occurs from November to May but in Australia it takes places from August to September which is also more numerous than in Hawaii [4],[5],[12]. It perhaps due to the fertilization of ovules in Hawaiian conditions starts in between 48 to 72 h after pollen germination [12],[20],[21]. It indicates the environmental factors involved in flower development. Macadamia is strongly mixed of out crossing, and cross-pollinated species that lead to increases in yield, and quality and size of kernels [20],[21]. Scions on rootstocks of most of the plant species have great qualitative and quantitative impact on crop yields controlling through nutrient accumulation and genetic variation among the seedlings [3], particularly in apple [17]. Macadamia also exhibits a small quantitative effects on strong rootstock [1],[4] but in case of yield, experimentally no significant difference has been found in five Hawaiian *M. integrifolia* cultivars using *M. tetraphylla* seedling rootstocks or cuttings of own-roots [1],[18].

PROPAGATION

Macadamia is usually propagated by graftings and infrequently using own rooted cuttings or clonal rootstocks [1],[3],[9]. The success of graftings of macadamia entirely depends upon the age of the scions. The scion of several years old branches typically performs more success rate than younger branches [22]. Clonal propagation of rootstock significantly guides for an uniform orchards because it can control the genetic variation on the selection of cultivars [1],[17]. Splice, side wedge and approach grafts are the most commonly exploited graft techniques for young trees. On the other hand, cleft and bark grafts are more practiced for top working of aged trees [12],[19],[23]. There is the evidence on the seedling rootstocks influencing in tree nutrition which eventually assists the variation of tree size, shape, vigor, nutrient contents, and productivity of the grafted orchards of macadamia [12]. Seedling rootstocks of macadamia habitually prefers their superior quality root systems and ease of production [2]. It has been observed that *M. tetraphylla* seedlings have a preference of more potential rootstocks to *M. integrifolia* orchards[12]. Cuttings and air layering are also another two propagating tools of macadamia, but in this manner trees become weak rooted that consequently vulnerable to strong wind-flow. Rooting of cuttings in macadamia is also influenced with various factors such as indolebutyric acid and bottom heat treatment [12],[24], season, cultivars, and carbohydrate reserves [16]**.** Alternatively**,** air layered trees reduce 2.5 to 3 years the juvenile period for the first fruiting of young trees [12]. Thus, air layering techniques have been successfully practiced in many other countries across the world.

Development of grafting of commercial macadamias (*M. integrifolia* and *M. tetraphylla*) are very time consuming process and also too expensive but in Australia they are commonly put up for sale as well [2]. Usually 12 to 18 months are required for the first flowering and at all around the cost is 14-29 AUD per tree from the beginning of the process until plantation [9]. Alternatively, cuttings are the most competent and reasonably cost-effective practices by which producing of tree plants through rapid clonal multiplication can be achieved [2]. Clonal macadamia trees have been accomplished by semi-hardwood cuttings under a mist system with a wide range of success rates[2],[9],[25]. It is thus the narrow genetic bases of Hawaiian *M.*

integrifolia cultivars are relatively complicated to utilize on Hawaiian industry [2],[4],[7],[9],[26].

Beaumont is an important hybrid of *M. integrifolia* × *M. tetraphylla* cultivars in South Africa. Potential yields and ease of propagation (cuttings) attributes of beaumont [2],[9] makes itself as the standard rootstocks in South African industry but Hawaiian cultivars perform very ordinary attitude [2]. Numerous advantages of cuttings over grafted stocks have been recognized by the most of the macadamia industries. They suggest that cuttings mediated tree plants usually provide inferior root systems rather than trees coming from seedlings or grafted stock [2],[9]. Various well adapted modified methods of cuttings are commonly being practiced in macadamia using around 3-5 mm in diameter and 15 to 20 cm long propagule tips [2],[12]. The tips are then dipped into rooting hormone before planting [2]. Cuttings are usually planted in the same collection day to prevent drying out [9]. No significant differences in tree quality can be found from cuttings when are practiced through good quality root systems and well cultural managements in the field intended for the first one and half year [2]. All cuttings are clones having several very important features such as no genetic variation, absence of rootstock suckers, lack of graft incompatibility between rootstock and scion but they are very common when plants are grown from seedling rootstocks [2],[9]. The macadamia industry didn't suggest yet any rootstock breeding program. On the other hand, well-established rootstock breeding programs are very common for the deciduous fruit trees. Screenings of potential candidate rootstocks are commenced into current breeding research for further development of breeding program in near future [2],[27]. Cuttings of *M. jansenii*, *M. tetraphylla*, and hybrids between *M. integrifolia* and *M. tetraphylla* are the good examples of superior rooting ability [2],[9]. The number of selection traits containing rooting ability, rootstock-scion compatibility, disease resistance, productivity and tree size are being investigated. Therefore, the breeders will have potential scopes to incorporate those selection attributes into broad genetic base of macadamia cultivars as well [2],[9].

MICROPROPAGATION

Tissue cultures are now widely used techniques for mass multiplication of various nut crops such as chestnut [16],[28], cashew nut [16],[28] walnuts [16],[29] and macadamia nut [30],[31]. Tissue culture techniques (Figure 1) for clonal propagation of macadamia have been improved since last decades [30–32] but there is lack of information regarding genetic variation of productive micropropagation [4]. It has been succeeded in regeneration of shoots from *Macadamia integrifolia* [33] and *M. tetraphylla* [30]. However, hybrids (*M. integrifolia* x *M. tetraphylla*) and different cultivars of macadamia are available but no efficient tissue culture techniques are established yet [25]. Generally, the degree of success of tissue culture systems and their commercial viability depend upon the characteristics of explants such as genotype, source or type of mother plants and history [30],[34–37].

Various genotypes of different explants alters their response in successful tissue culture systems through the balance of their endogenous hormones [16],[36],[38],[39]. Explants like nodal segments of *M. tetraphylla* are commonly being utilized in a successful tissue culture systems of crop plants [30],[31]. The readily available axillary buds in nodal segments may be required to trigger the bud break of leaf and cotyledonary tissue otherwise proliferation of adventitious buds [16],[40] and somatic embryos [41] would be achieved before any shoot regeneration [42]. The explants from *M. tetraphylla* response better performance comparing with *M. integrifolia* in *in vitro* regeneration which signify genotypic differences among them [16],[31]. It has been reported in *in vitro* regeneration systems that oxidation of polyphenols from explants exhibits high level of variations of some selected woody plants [30],[43–46]. Phenolic exudation of young explants other than the mature trees into the culture medium is a common problem in tissue culture of woody species [45],[46]. Phenolic exudation also accumulates in macadamia shoots varying with age. Shoot tip necrosis of macadamia has also been observed which can be influenced by the relative humidity in the culture vessels [30],[31],[42].

SUB-CULTURING AND ROOTING

Generally, physiological juvenility of the explants is one of the factors to the ability of vegetatively propagated woody plants [6],[47],[48]. A mature woody plant is often lack of juvenility so that it is the cause of difficulty in rooting of *in vitro* shoots [49]. *In vivo* rooting of macadamia cuttings also observes after six to nine months on moist sand without using rooting hormones because macadamia requires a long period *in vitro* condition to initiate rooting and shooting [33],[50]. Nodal segments are the most popular and suitable explants of shoot regeneration of macadamia [30]. Beside this, both grafted seedlings and matured field growing trees of macadamia also can be utilized as source of explant materials [42].

Shoot regeneration from cotyledonary explants is often possible because of their production of green embryogenic calli, roots and shoot primordia followed sub-culture on medium supplemented with different auxin and cytokinin group of plant hormones [42]. On the other hand, somatic embryos of *M. tetraphylla* has also capability to regenerate shoots [30] and this method also can be applied to explore the improvement of shoot regeneration from the hybrids of macadamia (*M. integrifolia* x *M. tetraphylla*) and their cultivars [42],[51]. In addition, approaches such as use of epicormic shoots sprouting from the tree trucks [52], serial graftings [30],[53] also suggest for tissue rejuvenation which ultimately overcome the rooting of *in vitro* shoots of woody plants [42]. Micropropagation has ability to multiply thousands of elite clonal material within a relatively shorter time which is very uncommon over other propagation methods. Development of any competent protocols for micropropagation of macadamia are directly associated with farm cost as propagules of macadamia should be provided to farmers at a more reasonable price [54]. *In vitro* storage [55] or cryopreservation [6],[43] can be followed to conserve elite macadamia varieties for future breeding through slow growth having cheaper cost compared to field and on-farm collections [4],[56]. Meristem culture has tendency to regenerate virus-free plants [57] while the endophytes of tissues in *in vitro* may also be induced resistance of the resultant macadamia trees to other disease phytotoxins [30],[58] along with somaclonal variation [59].

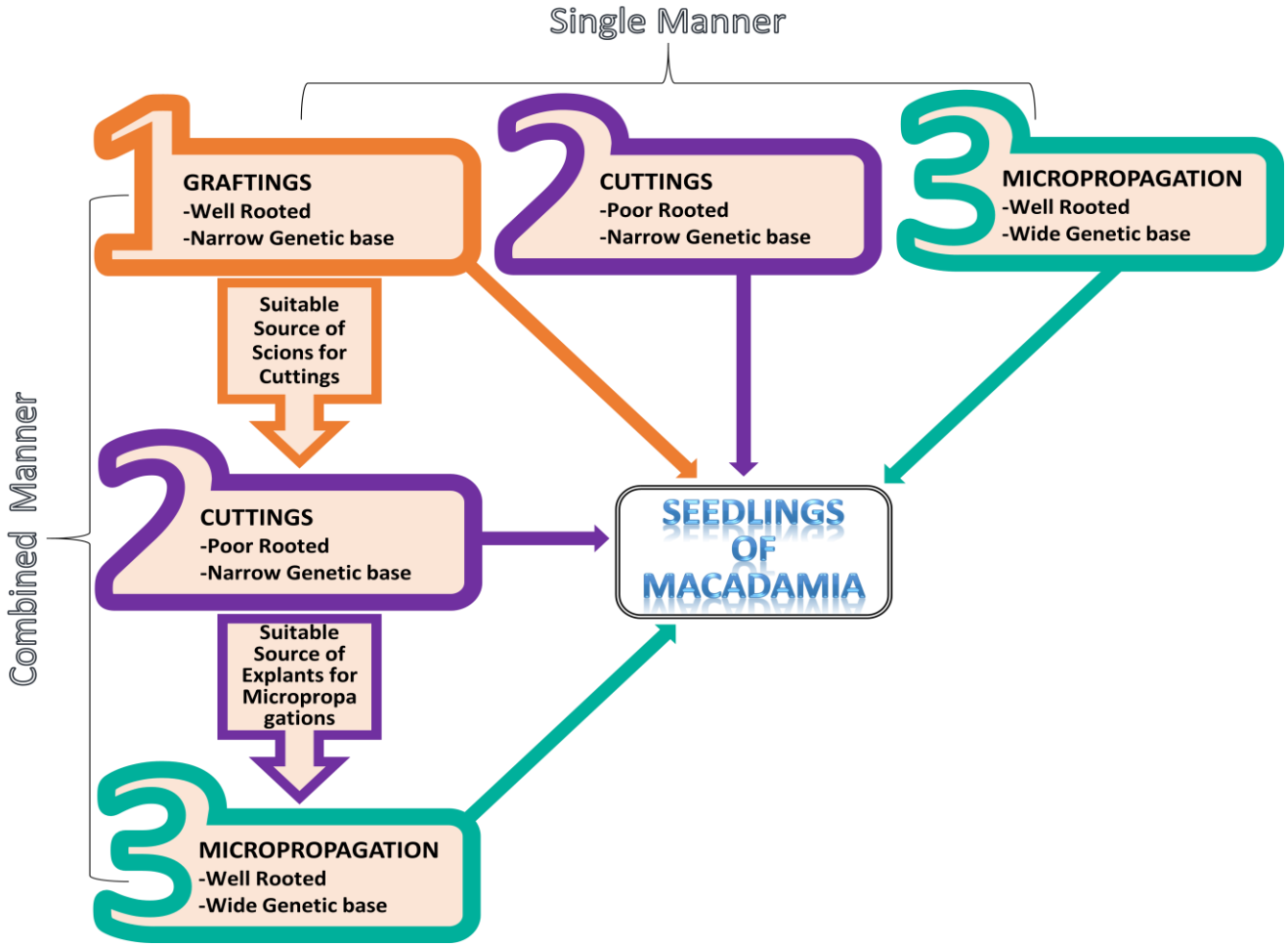

Figure 1. Schematic diagram of different propagation techniques of macadamia nut.

GENETIC VARIATIONS IN TISSUE CULTURE

Various genetic variation in germinability (degree of germination, rate of dormancy) [1],[19],[59] has been identified in several macadamia cultivars depending upon the thickness of the nut's shell [1],[3],[18],[26],[60]. Several studies reveal the significant differences among the macadamia cultivars on the subject of rooting and the success of cuttings [1]. Cultivars of rooting response and food storage (stem carbohydrate levels) of the mother plant are variable with each other, and there, no correlation has been found in both the germinability of seeds and the average strike success of cuttings from a cultivar [1]. Generally, it is

bit complicated to find out any support for the hypothesis of "Hawaiian-derived cultivars are relatively more difficult to root than Australian selections" [1],[9]. Genetic differences also promote the variation in nursery growth that has been explained in several studies [1]. Cuttings from more vigorous and robust cultivars in the nursery generally tend to be wide rate of success [1], on the other hand, less vigorous cuttings usually lead poor rooting systems which are apparently less satisfactory for the further steps of any breeding programs [1],[16],[61].

PROSPECTS AND FUTURE DIRECTIONS

Macadamia is classified into a group of highly valued nut tree crop and it is also prized for carrying their richly flavored nuts, sweet and soft flesh. From graftings and cuttings, the two major means of propagating materials of macadamia having narrow genetic base, this tree is preferably propagated by grafting because it requires only 12 to 18 months for the first flowering and producing commercial quantities of seeds. On the other hand, cuttings are also practiced for a while just because of its rapid clonal multiplication but not commercial purposes because it produces inferior rooting systems. Cuttings can be sprouted of hundreds of explant materials for micropropagation utilization. Micropropagation or *in vitro* regeneration is another prominent propagating tool of macadamia that can facilitate quicker multiplication of new rootstock and scion varieties with wider genetic base. The readily available auxiliary buds in nodal segments are commonly utilized as explants in a successful micropropagation of macadamia because it generates phenolic exudation on culture media which are very essential elements to make genetic variation of macadamia. Once this macadamia plant can be established from either of those propagating systems, the certain potentiality of this plant is able to continue its fruit bearing tendency over next 100 years. Hence, the three suitable propagating tools can be used in combination order to initiate several hundred plants from a desirable single mother plant. Beside this, the bright future research of many other relevant fruits and nut species such as almonds, chestnuts, hazelnuts, pecans, pistachios, walnuts etc. can be guided with this study to improve them in aspects of agronomic or commercial attributes.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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