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SHORT COMMUNICATION

Scope of novel and rare bulbiferous coconut palms (*Cocos nucifera* L.)

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Abstract A naturally occurring, rare bulbiferous coconut palm was identified at the Central Plantation Crops Research Institute, Regional Station, Vittal in Karnataka State, India, among West Coast Tall population. The palm produces only bulbil shoots in leaf axils in place of normal inflorescence. The identified palm happened to be twins in which one member was more vigorous than the other, but both of them produced only bulbil shoots instead of floral parts. Morphological and molecular studies on these palms revealed the main palm and their bulbil progenies are genetically uniform. The genetic uniformity of the twin mother palms and their bulbil progenies was confirmed through microsatellite analysis using 10 polymorphic SSR primer pairs specific to coconut and has been well demonstrated to differentiate coconut cultivars. The primary and secondary bulbil shoots were found to be capable of growing into independent plants making it possible to use them as propagules to develop a homogeneous clonal population hitherto unavailable in coconut. The bulbils showed axillary growth in 6–12th leaf axil which further again develop as secondary bulbils indicating the complete vegetative state of the palm. Comparison of shoot apices of a normal seedling with bulbil shoot revealed variation in cell growth pattern. Conservation of bulbiferous palms as a unique genetic resource needs to be taken up to utilize these rare sources for future breeding programmes, provided their seed-fertility can be restored.

Keywords Bulbil shoots · Coconut · *Cocos nucifera* · Palms

Palms are monocotyledonous plants belonging to the Family Arecaceae, that includes about 3,000 species under 190 genera (Tomlinson 1990). They possess many biologically distinctive peculiarities in Plant Kingdom, which make them important while studying the evolutionary history of plants. The palm species possess different growth habits under different habitats like their massive crown size, lignified wood, fibrous parts, juicy fruits to dry copra yielding edible oil, starch storing stems, and ability to grow in varied environments. Among these, a number of palm species are being grown on commercial scale around the world for innumerable uses of which, the coconut palm (*Cocos nucifera* L.) is widely cultivated in tropical areas including islands and coastal sands, mainly for its edible kernel oil and delicious tender-nut water. Although many high yielding selections and hybrids

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have been released for commercial cultivation in all the coconut growing countries, the rapid spread of improved varieties is limited due to the absence of viable vegetative propagation protocols for large-scale production. The need for clonal propagation in palms like coconut is very strongly felt, considering the scope of developing uniform populations for future genetic and breeding investigations.

Coconut seedlings, after planting, pass through a clear vegetative growth phase ranging from 18 to 36 months in the Dwarf, and 60–90 months in the Tall cultivars, after which the first flowering occurs in every leaf axil. This initial flowering is followed by regular production of inflorescence in the subsequent leaf axils. Coconut inflorescence is a highly branched spadix enclosed in a woody spathe, with branches called spikes, which further produce rachillae, with few female flowers (buttons) on the lower part of spikes and numerous male flowers on the upper part of spikes. In rare exceptional cases, these spadices, spikes, female flowers and male flowers get transformed into vegetative shoots either in part or fully due to genetic and environmental factors. These are known as bulbil-shoots on account of their similarity to bulbils seen in certain members of Agavaceae and Liliaceae families. Since bulbil formation is a rare phenomenon in nature, very few studies have been conducted. It has previously been described as a form of 'pseudo-vivipary', where the flowering process is aborted and the flower buds get changed into vegetative bulbils (Tooke et al. 2005).

In the present report, a twin coconut palm, producing a number of bulbil shoots in the leaf axils of both the vigorously growing main palm and its weaker twin, has been identified among a West Coast Tall coconut population at our Institute's Regional Station in Vittal, Karnataka State. The palm resembled normal coconut palms that were planted in the vicinity along with these palms, in its morphological appearance, except that they produced only bulbil shoots in place of normal inflorescence and hence do not produce nuts. Morphological and anatomical observations and molecular studies were carried out to reveal the potential of the identified palm. The morphological observations revealed an average retention of 32 fronds on the main crown with over 200 primary and secondary bulbil shoots on its leaf-axils exhibiting large number of smaller fronds (Fig. 1A). The bulbils were seen hanging from the stem even after the subtending leaf had withered and fallen off. The bulbil shoots on examination, revealed the production of secondary bulbils too in their leaf axils indicating the completely vegetative state of the palm and their bulbil progenies.

Few reports of bulbil-producing palms have appeared earlier (Davis 1948, 1967, 1978; Sudasrip et al. 1978), which reported spadices growing into simple bulbil shoots, compound bulbils, spikes developing into bulbils, partial foliation of spadices, young fruits, male and female flowers growing into bulbils and secondary bulbils. Among these varied transformations, proliferation of whole inflorescences or any floral parts into vegetative shoots in such exceptional palms is understandable as these flowers are themselves modified vegetative shoots ontogenetically. Coconut palms produce a compound much-branched inflorescence (spadix) in every leaf axil which originally commenced as a vegetative meristem that later gets converted into floral primordia. The transition of vegetative meristem to the flowering state has been considered to be controlled by genetic factors combined with environmental factors like temperature, daylight and the developmental state of the plant. In most flowering plants, there is a clear differentiation of vegetative and reproductive phases, whereas in palms like coconut, these phases are continued even after the first flowering, throughout its life time, wherein a single terminal bud continues to produce leaves where the axillary buds regularly get converted into inflorescence, which subsequently produced flowers and fruits. In case of bulbil-producing palms too, the terminal bud continues to produce leaves; however, the axillary buds grew into entire bulbils, or first grow as flowers and then get converted into bulbil shoots making the palms completely vegetative (Davis 1967).

In the present report, there was no floral structure noticed and the whole axillary growth forms the bulbil shoot with secondary bulbils arising from 6 to 12th leaf axil of the primary bulbil. This observation indicates that the bulbil production in this palm may not be merely due to environmental factors but through complex genetic factors resulting from either a mutation or expression of rare genes that existed in the population.

The earliest report of a bulbiferous coconut palm from Malabar, is by Iyengar (1922), followed by Davis (1948, 1967, 1978), and their potential use in clonal propagation was also demonstrated (Davis 1969;

Fig. 1 **A** Twin Coconut crowns with numerous bulbils, **B** Meristem of normal seedling, LS, **C** Meristem of bulbil shoot, LS, **D** Spathes in the bulbil shoot transforming into leaves, **E** Stem of the bulbil resembling peduncle of an inflorescence, **F** Shoot growing in vitro showing secondary bulbil, **G** Monomorphic bands detected for the twin mother palms and their bulbil shoots using the primer CAC 8. M Main Mother palm, TP Twin of the main mother palm, T1 to T11 Bulbils from weaker twin, M1-M7 Bulbils from stronger twin

Sudasrip et al. 1978; Davis et al. 1981), those palms yielded only bulbiferous clonal progenies, which were not utilized in furthering palm improvement programmes. The bulbiferous coconut palms reported earlier could not be utilized to their full potential either for development of a clonally propagated uniform (nut yielding or bulbil yielding) population or for crop improvement.

Since the bulbil shoots can possibly be used as propagules, the growth pattern of vegetative meristem from a normal seedling *vis-a-vis* the bulbil meristem was compared through histological studies. The shoot apices excised from bulbils and normal seedlings were fixed in Carnoy's fluid for 24 h and dehydrated in ethanol-butanol series and embedded in paraffin wax. Serial sections were taken using Leica microtome (RM 2145) at a thickness of 10 µm and stained with Periodic acid- Schiff's reagent. Photographs were captured on a Leica application suite. The growing apical bud appears to be conical in shape in normal seedling, whereas the bulbil shoot apex indicated a dome-shaped growing point (Fig. 1B, C). The finding is in agreement with an earlier report (Mohandas et al. 1976) in which thick fleshy globular tissues were observed while studying the anatomy of coconut bulbils. The growing cells in the normal seedling apical meristem appeared to be dense and active, whereas they were sparse in the bulbil shoot apex, indicating the variation in growth rate of cells.

A young bulbil shoot (equivalent placement on the crown to an unopened fully mature inflorescence in a normal coconut palm i.e. 8th leaf axil from the spindle) was carefully removed from the weaker twin palm and dissected to examine the growth and developmental pattern. At the time of emergence from the leaf axil, the shoot structure just resembled a normal inflorescence with a green spathe covering the growth. In a normal inflorescence, the spathe is vertically split open to expose the spike and spikelets with numerous male and fewer female flowers during inflorescence opening. Whereas in the bulbil shoots, the emerging spathe was observed to split vertically to expose spathes, one after another, and up to 7–9 whorls of such spathes could be seen with gradual increase in leafy ramifications on the top (Fig. 1D). The series of spathes arranged in whorls split one after another in the course of growth. After producing 7–9 spathes with increasing length of leafy tip, the subsequent spathe turned out completely to be a normal coconut frond with the base of the spathe being converted into a normal petiole with leaf sheath and the top as leaflets. This process has some similarity with a normal seedling derived from a seed nut in coconut. In seedlings too, depending on the cultivar, few scaly leaves (with more of petiole and less or no lamina) are initially produced (resembling spathes) and later normal leaves are produced with leaf sheath.

The bulbil-producing palm in the present report was observed to be highly vigorous, producing leaves simultaneously on the main bud and on the numerous primary and secondary bulbils. The secondary bulbils were initiated starting from 6 to 12th leaf axils of primary bulbils and then continuing with the other leaf axils subsequently. They first appeared as a small spathe and then subsequently grew as a branch of the bulbil shoot with leaves unfurling similar to the growth of primary bulbils. This indicates that if the bulbils are rooted to establish as new plants, they will also be bulbiferous. Similar observations have been made earlier in coconut in which the bulbil-derived palms produced only bulbils (Sudasrip et al. 1978; Davis et al. 1981). In the present case, this was confirmed through observation of direct vegetative shoot production from young secondary bulbil growths cultured in vitro using Y3 media. In vitro culture of these bulbils also was successful (Fig. 1F) indicating the possibility of culturing primary and/or secondary bulbils in vitro through which a uniform coconut population can be produced.

The bulbil shoots were seen with number of leaves ranging from 6 to 15 depending on the maturity and position of the bulbils. Due to the continuous production of bulbil shoots with many leaves in the main leaf axils and secondary bulbils arising from those primary bulbils, the mother palm crown appeared overcrowded resulting in drying and falling off of lower bulbils. Partially grown and some dried roots were also observed in few hanging bulbil shoots on the crown indicating their capability to grow as independent plants if detached and planted. The attachment of the bulbil stem to the main trunk is similar to the attachment of an inflorescence to the trunk in a normal palm and the base of the bulbil stem resembled the peduncle of an inflorescence (Fig. 1E). Observations on the bulbils that were extracted from the main and twin mother palm and planted in pots showed initiation of roots from the bulbil stem indicating the possibility of multiplying the bulbil-producing palms.

If the bulbil progenies are manipulated to produce normal inflorescences, then that population will have tremendous utilization in coconut breeding as they will be genetically uniform and such population is hither to unavailable in coconut.

In case of *Agave*, there was high level of genetic variability observed (Infante et al. 2003; Abraham-Juarez et al. 2009) among individual bulbils and also among bulbils and their mother palm, indicating their de novo origin from flowers followed by sexual reproduction resulting in the segregation. In coconut too, if the bulbils are produced from normal floral primordia, there may be chance for segregation. In the case of simple bulbil shoots, where the whole spadices get transformed into shoots, there would not be any genetic differences expected. Therefore, to confirm the genetic uniformity of the twin mother palms and their bulbil progenies, microsatellite analysis was carried out using ten polymorphic SSR primer pairs specific to coconut which has been well demonstrated to differentiate coconut cultivars. Genomic DNA was extracted from both the main and twin palm and their respective bulbils. The PCR products were analyzed using the MCE-202 MultiNa Microchip Electrophoresis System for DNA/RNA analysis (Shimadzu, Japan) using the DNA-500 kit. Monomorphic bands (Fig. 1G) were detected in the main palm, its twin and in all the bulbils from both the palms, indicating their genetic uniformity.

The initiation of inflorescence primordia takes about 36 months before anthesis in coconut (Perera et al. 2010). The earliest inflorescence primordium could be detected about 4 months after differentiation of the leaf primordium. While observing the requirements for transition stage of the vegetative meristem into reproductive meristem, it was suggested that intrinsic conditions such as the age of the palm or extrinsic conditions such as an appropriate day length or temperature regime, mediated through endogenous signals may be responsible (Huala and Sussex 1993). In bulbiferous palms, this transformation is arrested either due to the intrinsic factors (as extrinsic factors are the same for the surrounding normal palms too), or due to a combination of both intrinsic and extrinsic factors (Perera et al. 2010). Bulbil formation was observed after physical manipulation of the shoot apical meristem in *Titanotrichum oldhamii* (Hemsl.) Solereder (Wang and Cronk 2003; Wang et al. 2004), suggesting that in addition to environmental factors, hormonal regulation may also be involved. It is associated with rearrangements in tissue structure and accelerated cell multiplication in *Agave tequilana* Weber (Abraham Juarez et al. 2010). They have shown that *Agave KNOX* genes are functionally similar to Class I *KNOX* genes and suggested that spatial and temporal control of their expression is essential during bulbil formation in *A. tequilana*. Hence, bulbil formation may be based on a common genetic mechanism in different species and attributed to environmental conditions and varied intrinsic factors. However, the nature of the DNA signal that causes an axillary meristem to turn into a reproductive meristem in palms is still unknown. The possibility of manipulating the vegetative and reproductive phases has been reported through external treatments such as simple temperature and light treatment causing fruiting (reproductive) phase to get transformed to vegetative bulbil condition in *Poa alpina* L., whereas leaf pruning and terminal bud-pruning in *Arenga pinnata* (Wurmb.) Merr. palm stimulated vegetative shoots in the leaf axils (Sudasrip 1980). Hence, the possibility of inducing bulbils in selected superior normal coconut palms exists with further efforts, through which development of clonally multiplied, genetically uniform, superior coconut populations may be possible. Although no efforts have been reported in coconut for these manipulations, it is worthy to attempt the conversion of axillary vegetative buds of bulbils into reproductive buds and vice versa, considering the importance of evolving uniform populations and earliness in maturity.

Another novel opportunity of utilizing the bulbiferous coconut palms is, growing them for commercial production of palm 'cabbage', known as "Millionaire's salad". The edible palm 'cabbage' that is extracted out of the growing terminal bud, usually by sacrificing the palm, is considered a rich source of minerals and nutrients. In some coconut growing regions, senile palms meant for removal or 3–4 years old coconut seedlings are used for extraction of 'cabbage' for edible purposes, and in both the cases the palms are lost. Thus, the bulbiferous coconut palms could be utilized for 'cabbage' production, as the cabbage of bulbils were observed to be more numerous than from a normal seedling and the palm is not lost while extracting the bulbil shoot for 'salad cabbage' purpose. The possibility of utilizing these bulbiferous irreversible mutant clonal palms for sweet neera tapping (used for coconut sugar, honey, fermented drink etc.) also need to be explored, so that the normal nut-yielding palms are saved from being over-exploited for this product. Hence, the bulbil producing population could be used for product diversification owing to the perpetuation of bulbil production in the progenies.

Detailed studies on the transcriptional changes and genes controlling the morphological changes occurring during bulbil formation in coconut are needed. Hence, further investigations on the reported bulbiferous coconut palm will throw light on the possible manipulation of flowering through identification of genes that control the shoot development process in palms that could help in addressing concerns in development of viable vegetative propagation protocols, leading to evolving homogeneous populations. Elucidating information on control of bulbil formation at molecular level and the role of uncharacterized bulbil-specific genes in the process are the future challenges. As a first step, conservation of bulbiferous palms as a unique coconut genetic resource needs to be taken up to utilize these sources for future breeding programmes not only in coconut but also in other important palm palm species.

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