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***Short Communication***

# Occurrence of *in vitro* flowering in coconut (*Cocos nucifera* L.)

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## ABSTRACT

**Immature inflorescence with outer spathe length of 5.5 cm size collected from West Coast Tall cultivar of coconut was used as the explant and rachillae bits were inoculated in Y3 media supplemented with 2, 4-D (1 mg $L^{-1}$). The cultures were incubated in dark for eight months and sub-cultured into the same media at monthly interval. The white shoot like outgrowths formed were sub cultured to ½ MS media fortified with 1 mg $L^{-1}$ each of NAA and BAP and subsequently transferred to light condition. After three months, the emerging shoot like structure was transferred to Y3 media fortified with NAA and BAP. Upon developing 3 - 4 leaves, the cultures were transferred to rooting media and root initiation was observed after two months. The transition of vegetative shoot to reproductive state was accompanied by some morphological changes including rapid emergence of long and thin leaves followed by emergence of pearly white inflorescence. Unlike normal inflorescence, the inflorescence emerged was terminal and was devoid of spathe. Prolonged subculture in the same media might have resulted in pH variation and subsequent reduction in organic and inorganic constituents of the media. The chemical stress experienced by the plantlet might have induced *in vitro* flowering.**

**Key words**: *Cocos nucifera,* immature inflorescence, hapaxanthic, prolonged subculture

The coconut palm (*Cocos nucifera* L.) is one of the most beautiful and useful trees in the world and all parts of this 'wonder palm' are useful in one way or other. Coconut, an out-breeding perennial tree, is seed propagated, exhibits great variation in morphological and agronomic characters. Vegetative multiplication of elite coconut palms is a promising possibility for producing uniform planting material with high yield and disease-resistance. Protocols for coconut micropropagation have been developed in various laboratories using different explant sources (Nguyen *et al*., 2015). Among various explants, the most extensively studied are the rachillae from inflorescence and plumule from zygotic embryos.

Flowering is a complex phenomena regulated by both internal and external factors and induction of *in vitro* flowering is very rare in most of the crops. Under natural conditions, flower formation normally commences when a plant attains maturity. Juvenile phase of a plant is genetically controlled and is species specific which means that a plant flowers only when genetic factors including photoperiodic response are congenial. However, these conditions can often be altered so that the plant can be induced to undergo an early reproductive phase. Such an attempt to induce flowering *in vitro* has been attempted in many plant systems. *In vitro* culture provides an ideal experimental system for studying the molecular mechanism of flowering. *In vitro* flowering studies has been conducted in many perennial crops e.g., bamboo (Joshi and Nadgauda, 1997), red hot pokers (Taylor *et al*., 2005), date palm (Allouche *et al*., 2010), oil palm (Nizam and Te-chato, 2012) etc. However, *in vitro* flowering in coconut has not yet been reported. Reducing duration of juvenile phase is an advantage especially in coconut with long pre-bearing period of 6-10 years. Here, in the process of establishing *in vitro* regeneration of coconut using immature inflorescence explants, strikingly, a few cases of *in vitro* flowering in coconut plantlets was observed. This paper aims

to present some observations connected with *in vitro* flowering of coconut palm and also tries to explain the possible factors involved.

The procedure followed by Shareefa *et al*. (2019) was used for immature inflorescence culture of coconut. Immature inflorescence explants with outer spathe length of 5.5 cm size were collected from 25 year old West Coast Tall variety and rachillae bits of 1 mm which were inoculated in Y3 media supplemented with 1 mg $L^{-1}$ 2,4-dichlorophenoxyacetic acid (2,4-D). The basal media also contained sucrose 40 g $L^{-1}$, charcoal 1 g $L^{-1}$ and agar 6 g $L^{-1}$. The cultures were incubated in dark condition at 27° ± 2°C and sub cultured in same media. After eight months, cultures were transferred to ½ Murashige and Skoog (MS) medium with 1mg $L^{-1}$ each of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). The cultures were initially kept in diffused light for one month followed by incubation in light condition for about 16 hours light (45-60 µmol $m^{-2}$ $s^{-1}$ PPFD) provided by white Light Emitting Diode (LED) tubes. After 4-6 months in light, the multiple shoots were separated from the parental clump and transferred for shoot regeneration to Y3 media with 1 mg $L^{-1}$ each NAA and BAP. After developing 3-4 leaves, the cultures were transferred to rooting media containing Y3 with NAA (2 mg $L^{-1}$) and BAP (2 mg $L^{-1}$) and Indole 3-acetic acid (2 mg $L^{-1}$) along with sucrose 30 g $L^{-1}$ for root initiation.

Within one month of dark incubation, the rachillae explants swelled and white outgrowths were observed in culture initiation media. The cultures when transferred to light conditions gradually turned green and developed multiple shoots which could be easily detached from parental clump. The separated shoots were transferred to shoot regeneration media for formation of well developed leaves. Root initiation was observed after two months in the rooting media.

*In vitro* flowering was observed in few plantlets cultured in the rooting media and such plantlets developed had four leaves and few root initials. In order to develop secondary roots, the plantlets were kept in the same media for a period of six months. The onset of *in vitro* flowering was accompanied by some morphological changes in the plantlets which include rapid emergence of long and thin leaves before the appearance of pearly white inflorescence. Unlike normal inflorescence, the emergence of inflorescence was terminal in the *in vitro* raised plantlets and the inflorescence was devoid of spathe (Figure.1).

The ability of explants to form flowers *in vitro* depends on numerous internal and external, physical and chemical factors and virtually all these factors interact in various complex ways (Compton and Vielleux, 1992). In the present study, induction of flowering was observed in plantlet cultured on Y3 media fortified with NAA (2 mg $L^{-1}$) and BAP (2 mg $L^{-1}$) and IAA (2 mg $L^{-1}$). The combined effect of auxin and cytokinin on *in vitro* flower induction has also described in a number of previous studies (Handro, 1983; Wang *et al*., 2002; Ammar *et al*., 1987; Jeyachandran and Bastin, 2013; Lin *et al.* 2005; Saritha and Naidu, 2007a; Sudhakaran and Sivasankari, 2002; Taylor *et al*. 2005; Thiruvengadam and Jayabalan, 2001). The role of cytokinins on *in vitro* flowering has been well documented (Wang et *al.,* 2001; Saritha and Naidu, 2007b). Cytokinins alone do not appear to be responsible for floral initiation. It is reported that cytokinins are known to interact with sucrose to cause the shift in the apical meristem from a vegetative phase to a reproductive one (Bernier *et al*. 2002; Bernier and Pe´rilleux, 2005). Sugars are primary sources known for reliable induction and development of flowers in many plant species such as rose (Vu *et al*. 2006), *Passiflora suberosa* (Scorza and Janick, 1980), *Vigna mungo* (Ignacimuthu *et al*., 1997) indicating that presence of carbon sources on the culture medium is necessary for floral stimulation.

There are many other physico-chemical factors which affected the *in vitro* flowering mechanism. Kolar and Senkova (2008) reported that reduced mineral nutrient availability accelerated *in vitro* flowering in *Arabidopsis thaliana*. The effect of Paclobutrazole, LEDs and sucrose on flowering of *Euphorbia milli* plantlets *in vitro* was studied by Dewir *et al.* (2007). In tobacco, important factors influencing in *vitro* flowering were light, growth regulators, carbohydrates and pH of the culture medium (Heylen and Vendrig, 1988).

The most essential part of plant tissue culture is the media which supplies hormones and necessary nutrients for growth and development. In the present investigation, maintaining cultures for six months in

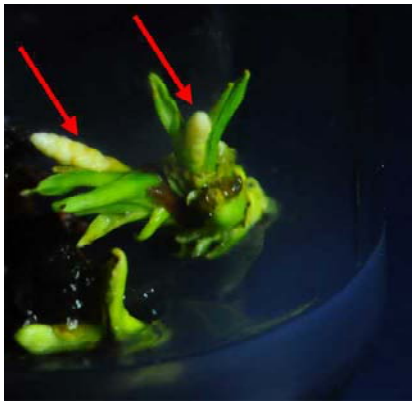

Fig.1a. Initial stage *of in vitro* flowering in coconut (arrow)


Fig. 1b. Fully emerged *in vitro* inflorescence

same media resulted in good root growth in plantlets, which also resulted in floral initiation. Prolonged culture of rooted shoots in media containing NAA and PBZ together with higher concentration of sucrose at 7% was reported to induce floral development in oil palm (Nizam and Te-chato, 2012). Delaying subculture may lead to hormone alternation and depletion of nutrients in the culture media. Therefore the altered chemical composition might have created a stress due to the increased passage time for subculturing.

It was interesting to note that *in vitro* flowering did not resemble flowering *ex vitro*, in that the inflorescences *in vitro* never matured and they subsequently senesced indicating that other factors, excluding cytokinins and a carbohydrate source, are required for continued normal development of the inflorescences. Cytokinins and sucrose therefore seem to act in the initial stages of floral initiation and development, however, full differentiation and maturation of the resulting flower bud requires involvement of other physiological factors.

The results of the current study revealed that contrary to natural flower formation, *in vitro* neoformed inflorescences were completely uncovered, *ie.*, lacking spathe. There are two types of developmental processes namely hapaxanthic and pleonanthic, in palms (Tomlinson, 1990). In hapaxanthic type, the growth of the axis of palm is determinate due to conversion of the vegetative shoot apical meristem (SAM) to the reproductive state, resulting in a short flowering phase and this phenomenon is observed only in less than 5% of palm species. The rest of the palm species are pleonanthic, with an indeterminate SAM, in which the vegetative growth continues while producing a reproductive meristem at each leaf axil. According to the Tomlinson model, under *in vivo* conditions, flowering in coconut is normally pleonanthic. However, in the present study, *in vitro* flowering was hapaxanthic as the inflorescence emergence was terminal resulting from the development of the apical bud which was devoid of any bract, which consequently gave rise to uncovered inflorescences. The flowers were malformed and never matured indicating that optimum interaction of light, temperature, plant growth regulators and nutrients are essential for flowering and normal maturation of flowers. Similarly, undersized and malformed flowers have been observed previously in other species (Ramanayake *et al.*, 2001). The malformation

occasionally observed in the flowers produced *in vitro* may have been partially due to competition and or nutritional deficiencies as reported in *Pentanema indicum* (Sivanesan and Jeong, 2007).

***Summary***

In the present study, prolonged subculture in the same media might have resulted in changes in the pH and reduction in concentration of organic and inorganic constituents of the media. The resulting chemical stress might have induced *in vitro* flowering in coconut. The interesting observation was that *in vitro* neoformed inflorescences were completely uncovered, lacking spathe and were terminal. The flowers were malformed and never matured indicating that optimum interaction of light, temperature, plant growth regulators and nutrients are essential for flowering and normal maturation of flowers. However, *in vitro* flowering can be efficiently used to understand the snapshots of physiological, hormonal and molecular regulation of flowering and such information gathered can be used to save time in future genetic improvement programs.

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