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Short Communication

High frequency occurrence of soft endosperm mutant *Macapuno* coconuts in Andaman Islands and their embryo culture

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Abstract

A relatively high frequency occurrence of mutant soft endosperm coconuts called "Dahi nariyel" in the Andaman Ordinary Tall variety is reported. These coconut types are similar to the popular "macapuno" coconuts. Embryo culture is the only method to propagate such novel mutants. We generated few plantlets in Y3 media through embryo rescue. Germination of embryo commenced in a month's time and the seedlings got developed into a three leaf stage plantlet in about 9 months. The scope of these coconut types for the islands and local prosperity is presented.

Key words: Coconut, macapuno, dahi nariyal, Andaman and Nicobar Islands

Coconut is the principal crop of the Andaman and Nicobar Islands and occupies around 50% of the cultivable area [1]. It was introduced during the 18th century in the Andaman group of Islands from the Nicobar Islands [2]. The Andaman Ordinary Tall is the widely cultivated coconut cultivar in these Islands. In Andaman, a rare mutant coconut types with soft endosperm called as "Dahi nariyel" from certain coconut palms are known to local people but reported only recently [3, 4]. Here we report the presence of such coconut palm type in high frequency in the South Andaman Islands and its similarity to the "Macapuno" or "fully filled coconuts" which have been developed into a delicacy in the South East Asian countries such as Philippines, Thailand and Indonesia. A rare coconut endosperm mutation that is manifested as soft extra thick kernel filling partially or completely the nut water cavity and containing some amount of thick, mucilagenous oily substance was first reported from the Philippines [5]. This rare mutant is widely occurring and is called Dua Sap in Vietnam, Maphrao Kathy in Thailand, Kelapa Kopjor in Indonesia, Dong Kathy in Cambodia, Thairu Thengai in India and Dikiri Pol in Sri Lanka.

The occurrence of such soft endosperm mutants in the mainland India is very rare. The Macapuno nuts are less frequent and indistinguishable from the normal coconuts by appearance. Only certain palms holding this genetic mutation bear nuts of this type and less than 15% (1-3 nuts in a bunch of 25-30 nuts) of the nuts in a bunch are of this new type. The macapuno nuts can be identified by the absence of the nut water splashing sound while shaking the nut. The chief fibre in macapuno is pectin while that of normal coconuts is hemicellulose. The presence of pectin in macapuno coconuts makes it highly suitable for jam making and confectioneries. The kernel of nine month old macapuno coconut is semisolid with the nut water cavity intact and filled with viscous fluid. As it progresses in age, the semi solid endosperm fills almost 50% of the nut water cavity in ten month old coconut and completely fills the cavity in eleven month old coconuts. Depending on the nature and extent of the semi solid endosperm filling the nuts are classified as A, B and C type respectively [6]. It was reported that the macapuno character is produced by a recessive gene [7]. In macapuno trees, the recessive pollen self fertilizes with recessive ovary resulting in a homozygous recessive genotype that yields

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macapuno coconut. This genetic rarity causes the formation of fewer number of macapuno coconuts in a single bunch and therefore in a tree. If embryo cultured recessive macapuno genotypes are planted nearby the recessive pollen from the neighbouring macapuno, palm will fertilize the palms thus maximizing the percentage of macapuno nuts in a palm. These indications prompted us to know the approximate occurrence of this novel coconut in Andaman islands and to explore its propagation potential through embryo culture.

A search for dahi nariel palms was initiated through key informants such as coconut climbers and progressive farmers in coconut plantation in South Andaman district and 15 dahi nariyel palms were identified and confirmed by harvesting and splitting the nuts. In addition, two palms were also identified in the Havelock Islands located about 30 miles of Port Blair. These palms were marked for the future source of dahi nariyal nuts for research purposes. The occurrence of other dahi nariyel palms was judged by conducting a survey of the number of such nuts obtained in the 10 copra making units situated around Port Blair. The period of survey was from April 2012 to March 2013. Each copra unit processes around 10,000 to 20,000 nuts/week. The nuts of same size and shape obtained in a single day from a particular copra unit were grouped together and assumed to be sourced from a single tree. If the dahi nariyel coconuts obtained at different seasons are sourced from a single garden then it is considered to be sourced from the same tree. Therefore, a total of 130 soft endosperm coconuts were found during the year (Table 1).

In the past, various researchers have tried different protocols for the *in vitro* culture of Macapuno coconuts with slight modifications [8, 9]. Taking into account the literature, we standardized the embryo culture protocol with some modifications. About 128 nuts were collected over a period of 1 year and the collected nuts were immediately brought to the laboratory within 1 to 2 hrs from the copra units situated around Port Blair. The endosperm cylinders containing the embryo were scooped out using a cork borer. After all the endosperm cylinders are extracted, these were washed in tap water and in 95% ethanol quickly to remove the fats and then disinfected with 100% commercial bleach for 20 minutes. These were then washed 3 times with sterile water to remove the bleach. The embryos were then taken out by splitting the cylinder and the embryos sterilized with 10% bleach for 2 minutes and washed several times with sterile water. The embryos were then inoculated in semi-solid Y3 inoculation media without addition of any growth hormones meant for coconut embryo culture. The embryos were sub-cultured every month till a very balanced shoot and root growth was established. The prolific rooting of the seedlings was initiated by inoculating in Y3 liquid media. When the sprout emerged, these were sub-cultured once in 15 days. Embryo culture was maintained by serial sub-culturing.

In addition to the 15 palms identified and confirmed through the key informants such as coconut climbers and progressive farmers, at least 45 unidentified palms (Table 1) exist in different localities in South Andaman. The area under coconuts in the South Andaman island is about 3514 ha [1]. This approximates about 0.6 million coconut palms under the recommended density of planting of 7.5 x 7.5 meters. Therefore, the frequency of occurrence of the 60 identified and unidentified Andaman Ordinary Tall (ADOT) palms having the dahi nariyal trait in the South Andaman Island is estimated to be about 1×10^{-4} which is around 0.0001 %. More than 80% of the palms produced only A type nuts even at eleven months of age and varietal differences play an important role in generating particular type of macapuno. To substantiate varietal effect on macapuno frequency in Andaman Tall variety, this mutation was also found at relatively high frequency in the Laguna Tall cultivar in Philippines [10].

Among the areas where coconuts were cultivated, Tirur locality on the western side of Port Blair was found to harbour maximum number of mutant coconuts. In mainland India, West Coast Tall (WCT) cultivar is cultivated under a large expanse of area and so far only one dahi nariyel palm was identified to be located near Kozhikode locality in Kerala (personal communication- Augustine B. Jerard) and the frequency of occurrence in the West Coast Tall is almost negligible. These unusually high frequency of occurrence of such mutant dahi nariyal palms in the local Andaman Ordinary Tall cultivar may be due to the introduction of genetic mutation for soft endosperm from the source population that was once brought from the Nicobar group of Islands. Moreover the farmers of South Andaman produce their own coconut seedlings and as a result less vigorous seedlings, as expected for the heterozygote for the α-D-galactosidase deficiency (α-D galactosidase metabolizes the endosperm and supply nutrients contributing to the

Table 1. List of Macapuno coconuts collected from various copra making units located in South Andaman

S.No.	Copra units situated around Port Blair	No. of dahi Nariyel nuts collected during April 2012-Mar 2013	Place of coconuts procurement	No. of unidentified trees
1.	Ograbraj	22	Ograbraj Local	8
2.	Burmanella (Unit-1)	41	ChidiyaTapu, Burmanalla,	18
3.	Burmanella (Unit-2)	4	Birchgunj	1
4.	Port Mount	7	Port Mount Local,	1
5.	Mithakhaadi	9	Wandoor	2
6	Maccapahad (Unit-1)	8	Brichgunj	3
7.	Maccapahad (Unit-2)	2	Garacharma	1
8.	Maccapahad (Unit-3)	4	Tirur	1
9.	Burmanella (Unit-3)	32	Tirur, Kadakatchal Pema (Calicut)	9
10.	Bidnabad	1	Bidnabad local	1
	Total	130		45

young coconut seedling's vigour) are also selected and planted. This reason may also contribute to the high frequency of occurrence of such mutants in these Islands.

In our study we used Y3 liquid and solid media for embryo culture. The germination of embryo started in a month's time and the seedlings got developed into a three leaf stage plantlet in about 9 months (Fig. 1). The fastest sprout emergence was observed in 1 month time. However the percentage of embryos converting to plantlets was quite low. Only about 10-20% of the embryos got converted into plantlets. About 12 plantlets were obtained and among them a plantlet with 3 leaf stage and ready for transfer was obtained.

The first successful development of an *in vitro* embryo cultured macapuno seedling was reported with White's basal media [11]. However, Y3 embryo culture media has been successfully used since then for the embryo culture of coconut. In our experiments we made slight modifications by doubling the iron source but no improvement in rate of germination was observed. In Y3 media [12] high sucrose was added for the development and proliferation of primary and secondary roots of the embryo cultured plantlets. The embryos cultured plantlets showed slender adventitious roots in the inter-zone between stem and root in media supplemented with NAA whereas the adventitious roots in normal and embryo cultured seedlings were as thick as the primary root [13]. However, like previous study [14], we also observed

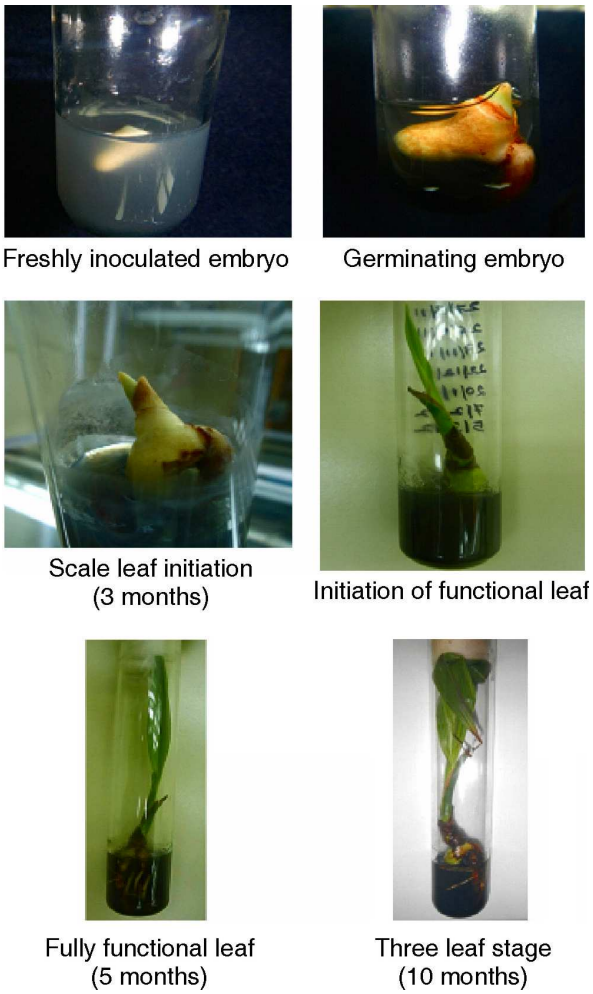

Fig. 1. Different stages of macapuno coconut embryo development into plantlets

normal root and shoot growth in our plantlets even without adding any hormone. We also followed the earlier method [15] for individual inoculation in each test tube for both solid and liquid Y3 media. Plumule culture has also been tried by previous researchers on coconut [16,17] and favorable results were obtained by the researchers with locally adapted cultivars.

As embryo culture technique is the only method at present to propagate these type of coconuts, the homozygous plantlets derived out of embryo rescue technique may be planted together in isolated locations such as selected islands to inter-breed among themselves to produce only pure dahi nariyel types. Since the embryo cultured plantlets are grown under asceptic conditions, the *in vitro* propagules of this novel coconut type can also be conveniently transported to mainland for further development even without quarantine considerations [18]. Such efforts may bring new products from this coconut type resulting in product diversification and economic prosperity.

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