Development and Increasing the Efficiency of Hybrid Macapuno Coconuts Tissue Culture in Thailand

Supaporn Sachati[1]*, Yokthip Sudaree[2], Orathai Tananchai[3], Siriluck Kaewsuralikhit[4], Parinda Hrunheem[5], Lawan Junamporn[6], Supattra Lertwatanakiat[7] and Wilaiwan Twishsri[8]

[1]Horticulture Research Institute, Department of Agriculture, Phahonyothin Road, Ladyao Subdistrict, Chatuchak District, Bangkok 10900, sachati08@hotmail.com
[2] Chumphon Horticultural Research Center, Sawi District, Chumphon 86130, yokthips@hotmail.com
[3] Chumphon Horticultural Research Center, Sawi District, Chumphon 86130, otananchai@hotmail.com
[4] Agricultural Production Sciences Research and Develoment Division, Department of Agriculture, Phahonyothin Road, Ladyao Subdistrict, Chatuchak District, Bangkok 10900, kaew1611@hotmail.com
[5] Suratthani Seed Research and Development Center Tha Chana District, Suratthani 88170, hr_farida@hotmail.com
[6] Loei Agricultural Research and Development Center, 81 M. 8, Na Pong Subdistrict, Mueang District, Loei 42000, lawan1st@yahoo.com
[7] Horticulture Research Institute, Department of Agriculture, Phahonyothin Road, Ladyao Subdistrict, Chatuchak District, Bangkok 10900, supattra_120@yahoo.com
[8] Horticulture Research Institute, Department of Agriculture, Phahonyothin Road, Ladyao Subdistrict, Chatuchak District, Bangkok 10900, wilaiwan_hri@hotmail.com

Introduction

Coconut (*Cocos nucifera* L.) is a major Thai economic crop. At present, the major growing areas are still in the south, especially in Prachuap Khiri Khan, Chumphon, and Surat Thani provinces. in 2008 – 2013, productive area and yield decreased with age and plant conditions, because most of the area is the old coconut plantation. And in 2010, an outbreak of coconut pests and the drought during the relatively dry weather to suitable for the infestation of such insects. As a result, the coconut yield is less. Conduce to a shortage of raw materials, high price per fruit. As a result, farmers have increased demand for good coconut varieties. But the government has insufficient production capacity.

Embryo culture is a technique that has been practiced by breeders for a long time. The key benefit is Helping the embryos of plants that cross-species or cross-genus and become sterile to grow into a complete plant. Kathi coconut cannot germinate in nature, so the embryo rescue technique was used. But the efficiency of seedling production is still low. Using the Kathi coconut embryo rescue technique, the zygotic embryos were successfully cultured in several laboratories. (Ashburner, 1991, Assy-Bah, 1989, Karunaratne *et al*., 2009, Rillo and Paloma, 1990) In Thailand, Somchai *et al*. (2008) successfully made Kathi coconut embryo culture and this technique is currently used as a good Kathi coconut production system by the Department of Agriculture. The result showed that medium and embryo placement characteristics affected 5 varieties, NamHom x Kathi (NHK), Malayan Dwarf x Kathi (RDK), Thungkled x Kathi (TKK), West African Tall x Kathi (WAK), and Malayan Yellow Dwarf x Kathi (YDK), of hybrid Kathi coconut embryo germination.

In addition to trying to increase the number of seedlings produced from a single zygotic coconut embryo by somatic embryogenesis. The propagation efficiency can also be increased by developing higher embryo culture techniques, this percentage can be increased up to 95%. Including the development of techniques at each stage of embryo culture; Germination, suitable recipes for each stage of development, and increasing the number of new shoots from a single embryo, etc., which is the purpose of this activity.

Materials & Methods

Medium and Embryo Placement Characteristics Affected 5 varieties of Hybrid Kathi Coconut Embryo Germination

Embryos of hybrid Kathi coconut 5 varieties, NamHom x Kathi (NHK), Malayan Dwarf x Kathi (RDK), Thungkled x Kathi (TKK), West African Tall x Kathi (WAK) and Malayan Yellow Dwarf x Kathi (YDK) were isolated from 11 months old fruit

The experiment has a completely randomized design with 3 treatments, consisting of hybrid Kathi coconut 5 varieties' embryo that the fruiting age 11 months and culture medium with Embryo Placement Characteristics; modified Y3 liquid medium (Parinda, 2018) (Figure 1A), modified Y3 solid medium with placed upward (Figure 1B) and Murashige and Skoog (MS) solid medium with the addition of 2,4-Dichlorophenoxyacetic acid (2,4-D) 1 mg L^{-1} (Orathai, 2019) with placed upward. Each embryo was cultured in the dark and taken for 8 weeks. The number of embryo's germination and development were observed and recorded every 2 weeks from 2 months after culturing.

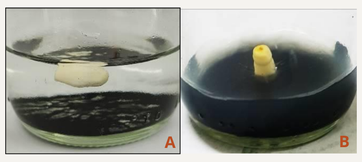

Figure 1 Embryo in liquid medium (A) and in solid medium with placed upward (B)

Effect of Appropriate Medium on Propagation of 5 Varieties Macapuno Using Plant Tissue Culture Technique

Step 1: Induction of embryo germination to form roots and shoots.

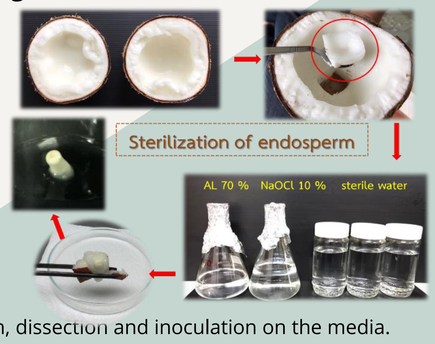

Figure 2 Sterilization of endosperm, dissection and inoculation on the media.

Step 2: Embryo development in the bright room

The seedlings with shoots and roots were taken from the dark room and transferred to solid media Y3 and B2 media. Placed in a room with light 14 hours a day, temperature 25-30 °C. Embryos were cultured for 12 weeks, the pulp was removed. and sub-cultured by placing in the original solid media and placed in a bright room. Embryos were cultured for 16 weeks, sub-cultured solid medium to liquid medium. using the original recipe and sub-cultured every month. Seedling survival rate and plant height were recorded.

The effect of coconut aging and culture medium with cut in half of shoot to plant of Chumphon 84-2 hybrid macapuno coconut.

The experiment design has a completely randomized design (CRD) with 6 treatments, consisting of the age of fruit being 9, 10 and 11 months and culture medium namely modified Eeuwens medium (Y3) (Parinda, 2018) and Murashige and Skoog (MS) medium supplemented with 0.4 mg L^{-1} Indole-3-butyric acid (IBA) and 3.2 mg L^{-1} kinetin (referred from Sisunandar *et al*., 2015). The embryo was cultured on modified Y3 solid medium in the dark condition for 2 months to develop into germination stage (Figure 3A). Cut in half of shoot (Figure 3B) were cultured on modified Y3 and MS medium supplemented with 0.4 mg L^{-1} IBA and 3.2 mg L^{-1} kinetin in the light condition, Light Intensity 4,000-5,000 Lux and photoperiod 12 hours per day, for 2 months. percent of embryo development in the light was recorded.

Figure 3 Embryo begins to germinate after 2 months and is ready to be halved (A) The pieces of halves (B) 2 halves of embryo after 2 months of culture (C).

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Results

Medium and Embryo Placement Characteristics Affected 5 varieties of Hybrid Kathi Coconut Embryo Germination

Table 1 Embryo germination percent of five varieties hybrid Kathi coconut after 8 weeks of culturing in medium with embryo placement characteristics in the dark.

Treatment	Embryo germination in the dark (percent)				
	NHK	RDK[1/]	TKK[1/]	WAK	YDK
modified Y3 liquid medium	51.0	53.3 b	50.7 b	60.0	72.3
modified Y3 solid medium with placed upward	86.7	74.0 ab	86.7 a	69.0	74.0
MS solid medium with 2,4-D 1 mgl^{-1} with placed upward	80.0	86.7 a	99.7 a	93.3	82.3
C.V. (%)	44.8	20.0	18.5	29.5	22.7

[1/] The averages in the same column that follow with the same letter were not statistical difference at 95% confidence level by DMRT

Table 2 Embryo development percent of five varieties hybrid Kathi coconut, from were cultured medium with embryo placement characteristics in the dark, after 8 weeks of sub-culturing in modified Y3 solid medium and transferring to the light.

Treatment	Embryo development in the light (percent)				
	NHK[1/]	RDK	TKK[1/]	WAK[1/]	YDK
modified Y3 liquid medium	28.7 b	45.0	35.7 b	53.3 b	45.3
modified Y3 solid medium with placed upward	80.0 a	62.7	86.7 a	69.0 ab	62.7
MS solid medium with 2,4-D 1 mgl^{-1} with placed upward	73.3 a	86.7	99.7 a	93.3 a	82.3
C.V. (%)	35.5	34.8	27.8	27.5	30.6

[1/] The averages in the same column that follow with the same letter were not statistical difference at 95% confidence level by DMRT

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Figure 4 Embryo development percent of 5 varieties hybrid Kathi coconut after 8 weeks of sub-culturing in modified Y3 solid medium and transferring to the light.

The effect of coconut aging and culture medium with cut in half of shoot to plant of Chumphon 84-2 hybrid macapuno coconut.

Figure 5 Characteristics of halved fragments (A) embryo halves No root development (B) and root emergence of embryo halves when cultured in Root medium (C).

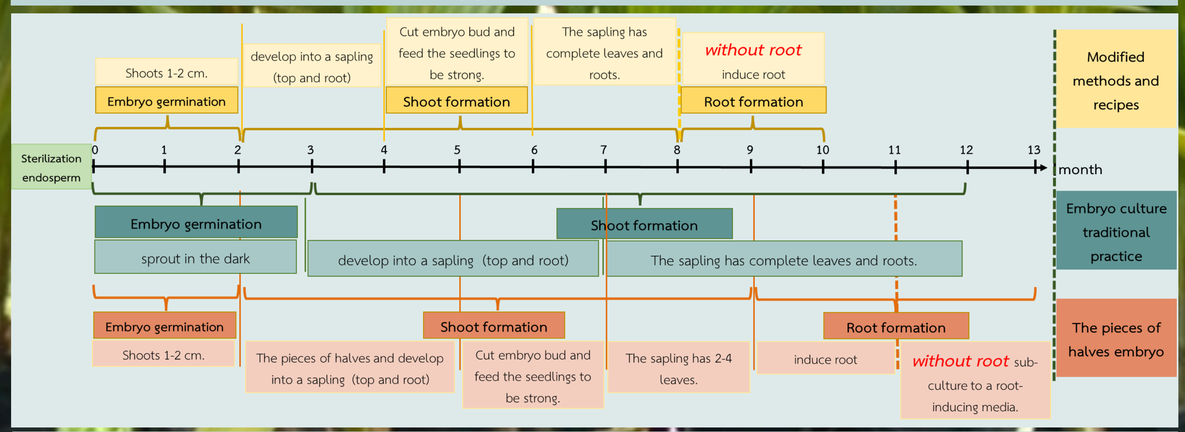

Figure 6 Comparison of the time period of the traditional practice of macapuno embryo culture, modified methods and the pieces of halves embryo

Conclusion

1. medium and embryo placement characteristics affected 5 varieties of hybrid Kathi coconut embryo Germination, it was found that the embryos were cultured in solid medium in the dark showed the best embryo germination and development to plantlet.

2. Different varieties of macapuno hybrids affect the response to different recipes. The suitable medium for propagating NHK macapuno hybrids were MS formula with 2,4-D or B2 formula in the dark and Y3 formula in light. RDK is MS formulation in the dark and B2 liquid medium in light. The embryo develops at most 70 percent of the seedling maturity, and the TKK, WAK and YDK strains are B2 formula in the dark and B2 liquid medium in the light.

3. Embryo incision can be applied to produce double seedlings of Hybrid Macapuno Coconuts. The best protocol is to first incise the germinated embryos at the meristem site, followed by cutting half the embryo into two and then recovering the embryos in Murashige and Skoog (MS) medium supplemented with 2 µM IBA and 15 µM kinetin.

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