

## ***In vitro* retrieval via embryo rescue of ‘Mohachao Narel’, a sweet endosperm coconut from Maharashtra**

**Anitha Karun<sup>1</sup>\*, K.K. Sajini<sup>1</sup>, M.K. Rajesh<sup>1</sup>, K.S. Muralikrishna<sup>1</sup>, K. Samsudeen<sup>1</sup>, P. Ajith Kumar<sup>1</sup> and D.D. Nagwekar<sup>2</sup>**

<sup>1</sup>ICAR-Central Plantation Crops Research Institute, Kasaragod 671124, Kerala, India

<sup>2</sup>Regional Coconut Research Station (RCRS), Bhatye, Ratnagiri, Maharashtra, India

\*Corresponding author: anithakarun2008@gmail.com

### **ABSTRACT**

*Mohachao Narel* is a variant in coconut with sweet and soft endosperm found in Ratnagiri district, Maharashtra, India. Embryos from sweet endosperm type nuts were found to be tiny and elongated and does not germinate naturally necessitating the embryo rescue technique as a sole method of propagation. Efforts were made to raise tiny slender embryos of *Mohachao Narel* type, which lack vigour and natural germination, *in vitro*. Embryos were cultured in Eeuwens's Y3 medium supplemented with 60 g/l of sucrose and subsequently transferred to medium containing lower concentration of sucrose. Additives such as thidiazuron and glutamine were included to improve the vigour of the plantlets. Germination percentage varied in the embryos from *Mohachao Narel* type, with a highest germination percentage of 50% recorded in the third collection. The plantlets resulted through *in vitro* cultured could be successfully planted in the field.

**Keywords:** *Mohachao Narel*, sweet endosperm, embryo rescue.

Coconut (*Cocos nucifera* L.) is a perennial monocot grown mainly in the West and East coast of India. Solid endosperm, the major economic part of the coconut, is white in colour and hard to touch. Special traits in coconut include *Makapuno* in the Philippines and *Thairu* or *Nei Thengai* in India where the endosperm is soft and jelly like (De Guzman *et al.*, 1971; Arunachalam, and Rajesh, 2008, 2017). Another variant with sweet and soft endosperm, named '*Mohachao Narel*' has been reported from Guhaghar taluk of Ratnagiri district of Maharashtra State in India (Samsudeen *et al.*, 2013). *Mohachao Narel*, mainly used for edible purposes, fetches premium price in markets. Twenty nine palms of '*Mohachao Narel*' were located in Guhaghar Taluk of Ratnagiri district (N17° 28'55" to N17° 29'50" and E73° 11'03" to E73° 19'50") and one palm in Ratnagiri city (N16° 58'15.3" and E73° 19'50.4"). The palms, aged 40 to 80 years, were randomly distributed in the population and were all tall types with the height ranging from 7 to 27 m. Nuts with sweet endosperm in a bunch varied from 10 to 77% in different palms. Forty six percent of all the nuts collected from 28 palms during winter season were of sweet endosperm type. The percentage came down to 39% during summer season. Four palms had less than 25% sweet endosperm nuts, 13 palms had 25 to 50% sweet endosperm nuts and 11 palms had more than 50% sweet endosperm nuts (Samsudeen *et al.*, 2013).

Nuts of *Mohachao Narel* will not germinate naturally which necessitates embryo rescue technique as a sole method of propagation. When compared to embryos from normal nuts, these embryos were found to be tiny and elongated. The weight of the *Mohachao Narel* embryos ranged from 29-100 mg where as it was 90 to 120 mg for normal nuts. Palm to palm variation was observed for the percentage of sweet endosperm type coconuts. Among the palms, three palms (palm nos. 14, 23 and 28) showed above 60 % of *Mohachao Narel* with an average embryo fresh weight of 84, 69 and 83 mg, respectively.

For rescuing and *in vitro* culturing of *Mohachao Narel* embryo, nuts were collected from Guhagar, Maharashtra State in three spells. In first two collections, the harvested nuts were transported to ICAR-CPCRI, Kasaragod where as in the third one surface sterilized endosperm plugs with embryo were transported.

In the first collection, a total of 309 nuts were harvested from 26 mother palms, of which 131 nuts possessed sweet endosperm amounting to 42.1%. In second collection, out of 303 nuts harvested from 19 palms, 41.9% of nuts found to possess sweet kernel. Collected nuts (175 nuts from 25 palms) were tested for sweet endosperm type in the field itself in case of third collection. About 36% of the nuts

were found to be *Mohachao Narel* type. Endosperms along with the embryo (endosperm plugs) were scooped and were surface sterilized by washing in 0.1% mercuric chloride for three minutes in the field and directly collected in the sterile polythene bag and transported to ICAR-CPCRI, Kasaragod, Kerala in the ice box (Table 1).

Endosperm plugs were split open and embryos were excised in laminar air flow chamber. Embryos were surface sterilized by rinsing with sodium hypochlorite solution (0.8% available chlorine) for 15 minutes followed by washing in sterile distilled water for four times. Embryos were inoculated into Eeuwens's Y3 medium (Eeuwens, 1976) supplemented with 60 g/l of sucrose. The inoculated embryos were incubated in dark till the germination initiation, *i.e.*, protuberance of plumule. Embryo culture protocol developed at ICAR-CPCRI (Karun *et al.*, 1999) was followed for culturing *Mohachao Narel* type embryos *in vitro*.

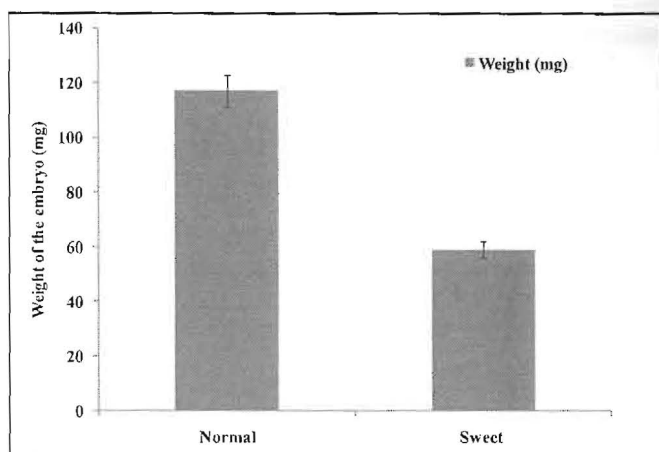
The time period between nut harvest and inoculation of embryos plays a crucial role since the embryos were weak and sweetness of the endosperm makes it vulnerable for decay and contamination. The average fresh weight of *Mohachao Narel* type embryos were significantly lower (60 mg) than that of normal nuts (117 mg) (Fig. 1). In case of the first and second collections, the germination percentage of *Mohachao Narel* type embryos were found to be 35 and 11%, respectively where as it increased to 50% in third collection. The contamination percentage in the cultures was reduced to 50% in third collection when compared to first and second collections (Fig. 2).

The germination of the embryo took around 45-60 days. *Mohachao Narel* type embryos resulted in weak and slender plantlets which took a long time for *in vitro* retrieval. To improve the growth and development of the germinated embryo, the cultured were transferred to basal medium supplemented with additives such as thidiazuron (1 mg/l)

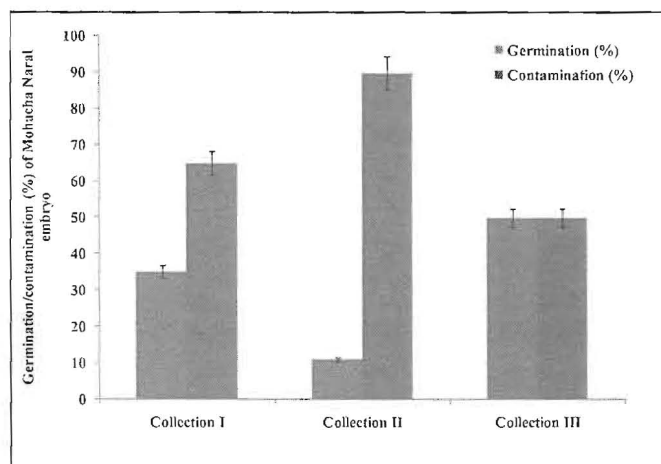
**Table 1:** Detail of the nuts collected from *Mohachao Narel* type palms during different periods from Guhagar, Maharashtra, India. The percentage of the nuts with sweet endosperm from each palm is represented.

Palm no.	Collection I			Collection II			Collection III		
	TN	SE	SE (%)	TN	SE	SE (%)	TN	SE	SE (%)
NSD1	25	13	52.0	15	7	46.7	-	-	-
NSD2	8	4	50.0	9	4	44.4	5	3	60.0
NSD3	16	7	44.0	-	-	-	2	1	50.0
NSD4	20	10	50.0	-	-	-	27	11	40.7
NSD5	8	4	50.0	-	-	-	10	1	10.0
NSD6	11	5	45.0	12	5	41.7	7	3	42.9
NSD7	12	5	45.0	15	6	40.0	6	1	16.7
NSD8	12	7	54.0	0	0	0.0	1	0	0.0
NSD9	11	6	50.0	45	23	51.1	8	5	62.5
NSD10	14	3	21.0	11	2	18.2	9	5	55.5
NSD11	8	2	25.0	19	7	36.8	-	-	-
NSD12	12	6	50.0	17	9	53.0	10	6	60.0
NSD13	3	1	33.0	4	1	25.0	1	0	0.0
NSD14	10	6	60.0	10	3	30.0	9	3	33.3
NSD15	28	8	29.0	41	22	53.7	8	2	25.0
NSD16	9	4	44.0	20	9	45.0	-	-	-
NSD17	14	5	36.0	-	-	-	1	1	100.0
NSD18	4	1	25.0	-	-	-	-	-	-
NSD19	-	-	-	-	-	-	2	1	50.0
NSD20	-	-	-	-	-	-	3	0	0.0
NSD21	11	5	45.0	-	-	-	18	4	33.3
NSD22	7	3	43.0	15	7	46.7	7	3	42.9
NSD23	3	2	66.0	7	5	71.4	3	2	66.7
NSD24	10	1	10.0	17	4	23.5	4	1	25.0
NSD25	11	5	45.0	8	3	37.5	-	-	-
NSD26	13	6	46.0	8	3	37.5	5	1	0.0
NSD27	17	3	18.0	21	2	9.5	14	0	0.0
NSD28	12	9	75.0	9	5	55.5	2	2	100.0
NSD29	-	-	-	-	-	-	6	1	16.7
NSD30	-	-	-	-	-	-	7	1	14.3
	309	131	42.1	303	127	41.9	175	58	36.2

TN, Total number of nuts; SE, number of nuts with sweet endosperm, SE (%), percentage of nuts with sweet endosperm



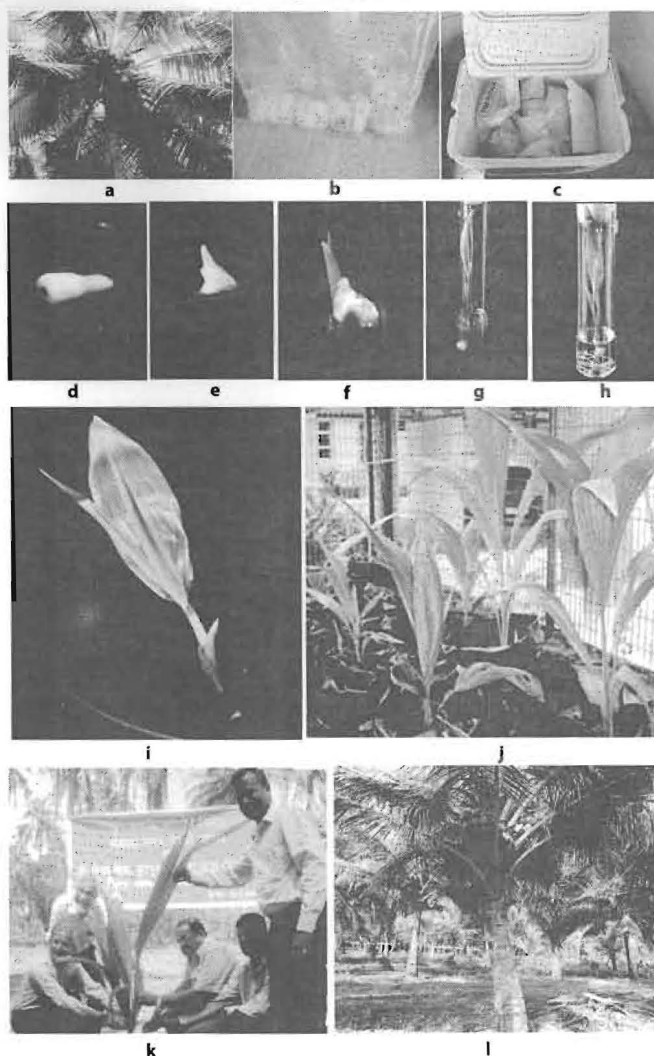
**Fig. 1:** Average fresh weight of the embryo from normal and sweet type *Mohachao Narel* coconut



**Fig. 2:** Percentage of germination and contamination observed in *in vitro* cultured embryos collected during different periods. In collection I and II nuts were transported and embryos were rescued in lab while in III, endosperms plugs were sterilized and transported

and L-glutamine (5 mg/l) and incubated for two weeks. The plantlets were later transferred to the same basal medium supplemented with lower levels of sucrose (50 g/l) and subsequently to 40 g/l and finally to 30 g/l. The hardened plantlets were handed over to Regional Coconut Research Station (RCRS), Bhatye, Ratnagiri, Maharashtra and planted in the field. The field planted palms have started flowering and showed similar character as that of mother palms for the occurrence of *Mohachao Narel* type nuts (Fig. 3).

In order to avoid contamination and high *in vitro* recovery of these small sized embryos of *Mohachao Narel* type, it is better to transport the surface sterilized endosperm plugs with the embryo from the field of collection to the distantly located culture lab. *Mohachao Narel* type might have evolved from inbreeding resulting in tiny slender embryos which lack vigor and natural germination. Further characterization by morphological and molecular markers



**Fig. 3:** Field collection and *in vitro* culturing of *Mohachao Narel* type coconut. (a) Harvesting of matured coconut; (b) Surface sterilization of endosperm plug with embryo; (c) Transportation of packed endosperm plug in ice box; (d) Embryo of *Mohachao Narel* type; (e-h) *In vitro* germination of embryo; (i, j) Hardening in pot and poly bag; (k) Field planting of embryo cultured *Mohachao Narel* type plantlet and (l) Bearing embryo cultured coconut palm at Regional Coconut Research Station (RCRS), Bhatye, Ratnagiri, India

is warranted in these *in vitro* raised plants of *Mohachao Narel* type. Possibility of increasing the sweet kernel nuts by selfing in these palms may also be explored.

## REFERENCES

Arunachalam, V. and Rajesh, M.K. 2008. Breeding of coconut palm (*Cocos nucifera* L.). In: CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources No. 053.

Arunachalam, V., Rajesh, M.K., 2017. Coconut genetic diversity, conservation and utilization. In: Biodiversity and Conservation of Woody Plants. (Eds.). Ahuja, M.R., Jain, S.M. Springer International Publishing. pp. 3-36.

De Guzman, E.V., Del Rosario, A.G. and Eusebio, E.C. 1971. The growth and development of coconut 'makapuno' embryo *in vitro*. *The Philippine Agricultural Scientist* **53**: 566-579.

- 
- Eeuwens, C.J. 1976. Mineral requirement for growth and callus initiation of explants excised from mature coconut palms (*Cocos nucifera* L.) and cultured *in vitro*. *Physiologia Plantarum* **36**: 23-28.
- Karun, A., Sajini, K.K. and Shivashankar, S. 1999. Embryo culture of coconut: The CPCRI protocol. *Indian Journal of Horticulture* **56(4)**: 348-353.
- Samsudeen, K., Rajesh, M.K., Nagwaker, D.D., Raghavan Reshmi, Ajith Kumar, P., Devadas, K. and Karun, A. 2013. Diversity in *Mohachao Narel*, a sweet endosperm coconut (*Cocos nucifera* L.) population from Maharashtra, India. *National Academy of Science Letters* **36** (3): 319-330.