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Short Communication

Potential of Encapsulated Somatic Embryos for Production of Quality Planting Material in Papaya

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Papaya (*Carica papaya L.*) is an economically important fruit crop belongs to the family Caricaceae. The crop was grown in the tropical and sub-tropical regions of the world. Major papaya producing countries are Brazil, Mexico, Nigeria, Indonesia, India, Ethiopia, Congo, Peru, China and the Philippines. A small dicotyledonous family consists of four genera. Carica papaya is the largest genus with 22 described species. There are three primary sex types which include pistillate, staminate and hermaphroditic type plants of indeterminate sex type. It is rich source of carbohydrates, minerals, vitamin A and C, pectin, alkaloid and carpine. Papaya is used as fresh, ripened fruit as well as raw vegetable. This fruit is gaining more importance due to extraction of latex which is known to contain an enzyme papain and chymopapain [1]. The papain is used in tenderizing of meat, manufacture of cosmetics, curing of leather, brewing, chill proofing of beer and treatment of digestive disorders.

Besides its quick and continuous yielding habit, generating early income to the growers and gaining popularity among tropical fruits worldwide. However, its cultivation is hindered by problems due to the inherent heterozygosity and dioecious nature production of non-true-to-types seeds of open-pollinated flowers exhibit considerable variation in shape, size and flavour and susceptibility to papaya ring spot virus [2]. Papaya cultivation is mainly hampered by the presence of papaya ring spot virus and virtually no resistance has been found in cultivar available for conventional breeding. Regeneration of papaya plants has been reported from protoplast, cotyledon, petiole, hypocotyls, root, anther, ovule and immature zygotic embryo cultures significant progress has been achieved using organogenesis and somatic embryogenesis [3]. Hence, several studies have been conducted on micropropagation of papaya over the past several years. “Protocols for regeneration of papaya *via* shoot organogenesis, somatic embryogenesis and enhanced auxiliary bud proliferation have been reported’’ [4].

Somatic embryogenesis is a developmental process where a plant somatic cell can dedifferentiate to a totipotent embryonic stem cell that has the ability to give rise to an embryo under appropriate conditions. This new embryo can further develop into a whole plant. In woody plants, somatic embryogenesis plays a critical role in clonal propagation and is a powerful tool for synthetic seed production, germplasm conservation, and cryopreservation. A key step in somatic embryogenesis is the transition of cell fate from a somatic cell to embryo cell. Although somatic embryogenesis has already been widely used in a number of woody species, propagating adult woody plants remains difficult.

Synthetic seed is the encapsulated plant tissue or somatic embryo which will give rise to a plant. Plants are unique in its ability to produce somatic embryos. Somatic embryos are structurally same as zygotic embryos. They can develop into a complete plant. The difference of somatic embryos from zygotic embryos is that the somatic embryos develop from somatic cells. Quality seed is the basic input for agriculture. It should be physically, genetically pure, physiologically sound and pathologically clean. Normal seed production encounters problem in certain cases like hybrid varieties, vegetatively propagated crops and transgenic plants. Alternate method is the production of synthetic seeds because desirable attributes of synthetic seed is non - damaging to the somatic embryos, durable during storage, transportation and planting, protect the embryos while

allowing for germination and conversion contain nutrients, micro-organisms necessary for germination, enable the formation of mono-embryogenic synthetic seeds, sow able using existing farm machinery, direct delivery of tissue cultured plants to the field, propagation of desirable genotypes with genetic uniformity, reduction in cost of vegetative propagated elite plant, preservation of germplasm and convenience in germplasm exchange, reduction in dormancy period, large production identical embryos in short time, produced throughout the year. Keeping all these facts in view, the present investigation was under taken in popular papaya *cv.* 'Arka Surya'.

The seeds of *Carica papaya L. cv*. Arka Surya, the hybrid derivative of Sunrise Solo X Pink Flesh Sweet, were collected from Indian Institute of Horticultural Research, Hesargatta, Bangalore and raised in the nursery of the University of Horticultural Sciences, Bagalkot, Karnataka. It is gynodioecious, smooth skinned, flesh has attractive red colour, soft, crisp, free from typical papaya odour with high TSS which is used for explants for the present investigation. Plant materials, namely lateral buds and young leaves were collected in a beaker containing tap water to avoid desiccation. Surface sterilization was done under aseptic conditions in laminar air flow cabinet. They were surface sterilized with 70% ethyl alcohol (1 min) followed by 0.1% $HgCl_2$ for 2 min. After surface sterilization, lateral buds and young leaves were divided into small pieces (approx. 1.0 - 1.5 cm). These were used as explants and cultured onto culture medium. MS containing 3% sucrose was used for all shoot and for *in-vitro* root formation.

The various stock solutions and plant growth regulators were dispensed separately. The pH of the medium containing all the above components was adjusted to 5.8 using 0.1 N HCl or NaoH. Culture bottles containing the medium were steam sterilized in autoclave (pressure: 1.06 Kg/cm^2; temperature 121°C) for 15 to 20 minutes. After sterilization, the culture tubes with the prepared media were allowed to cool at room temperature. All the cultures were grown at 25±2°C under a 16-h photoperiod. The illumination was provided by cool white fluorescent tubes at a light intensity of 30-40 µmol m-2 S-1 PAR. The influence of BAP and NAA were evaluated in various experiments as described below.

Somatic Embryo Production

The study was carried out to investigate the efficiency of different growth regulators for inducing somatic embryos, for somatic embryo production, the friable callus was sub cultured on different growth hormone medium (BAP and Kinetin). The best suited medium was determined, for early and good embryo development. The response in each treatment was recorded. For somatic embryo production embryos were transferred to the MS medium with BAP and Kinetin at different concentrations and the 16 hours light and 8 hours dark with 4000 lux of light was maintained in the growth chamber. At an interval of 7 days the culture was sub cultured to avoid deviation in growth conditions, since in older media as nutrients goes on exhausting; the pH becomes unfavourable for growth of the culture. The response in each treatment was recorded. The somatic embryo production was carried out with medium of different concentrations of BAP and kinetin. The medium that produced maximum number of embryos was standardized and used subsequently. The response in each treatment was recorded.

The use of somatic embryogenesis, for studying early phases which are regulating the plant embryogenesis and also advantage of large scale production of plantlets respectively with less labour, by combining embryo-genesis with mechanized culture systems. *In vitro* development of somatic embryogenesis is not controlled by the environmental factors, unlike zygotic embryonic development, which is closely influenced by the environment of the ovule and embryo sac and more responsive to environmental conditions [5]. On the basis of these assumptions, the number of studies were conducted to standardize the process of somatic embryogenesis in papaya.

For somatic embryo production well-grown whitish friable callus was inoculated on MS medium containing different growth regulators like, BAP and kinetin, for production somatic embryos from callus.

Influence of BAP on Somatic Embryos Production from Callus

Different levels of Benzylaminopurine (BAP) were tried in the experiment and observed for embryo production at 7th, 14th, 21st, and 28th day interval. The results of the study were depicted in table 1. While comparing all the levels of BAP, 0.30 mg/l shown good embryos production with 60 per cent when compared with 0.20 mg/l BAP (37.78%) and no somatic embryo production in rest of the treatments. 0.10 mg/l and 0.20 mg/l BAP has shown spongy tissue development, but fail to express within 28 days after inoculation. At 1.0 mg/l and 2.0

Table 1. Effect of BAP and kinetin on production of somatic embryos from callus (%)

Tr. No.	Treatment	Stage-I (7 DAI)	Stage-II (14 DAI)	Stage-III (28 DAI)
1	MS + BAP (0.0 mg/l)	0	0.00(0.00)	0.00 (0.00)
2	MS + BAP (0.1 mg/l)	0	2.22 (8.57)	14.44 (22.33)
3	MS + BAP (0.2 mg/l)	XX	8.89 (17.34)	37.78 (37.91)
4	MS + BAP (0.3 mg/l)	XXX	16.67 (24.09)	60.00 (50.75)
5	MS + BAP (1.0 mg/l)	0	2.22 (8.57)	5.56 (13.63)
6	MS + BAP (2.0 mg/l)	0	1.11 (6.05)	2.22 (8.57)
7	MS + Kinetin(1.0 mg/l)	0	2.22 (8.57)	4.44 (12.17)
8	MS + Kinetin (2.0 mg/l)	0	1.11 (6.05)	4.44 (12.17)
	Mean	-	4.31 (7.88)	16.11 (18.98)
	SEm(±)	-	2.51	1.98
	CD (p=0.01)	-	9.72	7.66

*Values in the parentheses are transformed values
No Response – 0; Very poor response – x; poor response – xx; Good response – xxx

mg/l BAP and 1.0 mg/l & 2.0 mg/l kinetin recorded delayed response with production of only spongy tissues.

Influence of Kinetin on Somatic Embryos Production from Callus

Maximum somatic embryos were produced at 0.2 mg/l kinetin (58.89%), when compared to kinetin at 0.3 mg/l and 0.1 mg/l which are recorded 38.89 and 14.44 per cent, respectively (Table 2). The rest of the concentration of kinetin had shown no response of embryo production. The results indicated that more 0.3 mg/l kinetin produced no any somatic embryo with further increase in kinetin concentration.

It is observed that, the level of response in differentiation of callus cultures into pro-embryogenic masses (PEMs) was found superior in MS medium with 0.3 mg/l BAP, but fail to produce further somatic embryos, from PEMs, at increased levels of Benzylaminopurine (BAP) even at the end of 42 days after incubation. This leads to the doubt that, addition of higher levels of BAP in the inoculation medium may hinder the process of transformation of PEMs into induction of somatic embryos. Therefore, the PEMs were transformed in to culture media with low and different levels of BAP and kinetin, without any auxins.

The results were interesting as there was good transformation of pro-embryogenic masses into somatic embryos on medium with lower levels of both BAP and kinetin; yet, kinetin was more influenceable than BAP. Kinetin with the concentration of 0.2 mg/l recorded maximum production of somatic embryos in to plantlets (58.89 percent); recommends kinetin (0.3 mg/l) in developing embryo maturation and conversion of plantlet

Table 2. Effect of kinetin on production of somatic embryos from callus (%) Days

Tr. No.	Treatment	Days after inoculation (DAI) of callus for induction of SEs		
		(7 DAI)	(14 DAI)	(28 DAI)
1	MS + Kinetin (0.0 mg/l)	0	0.00 (0.00)	0.00 (0.00)
2	MS + Kinetin (0.1 mg/l)	XX	2.22 (8.57)	14.44 (22.33)
3	MS + Kinetin (0.2 mg/l)	XXX	17.78 (24.93)	58.89 (50.10)
4	MS + Kinetin (0.3 mg/l)	XXX	10.00 (18.43)	38.89 (38.56)
5	MS + Kinetin (0.4 mg/l)	XX	1.11 (6.05)	6.67 (14.96)
6	MS + Kinetin (0.5 mg/l)	0	2.22 (8.57)	3.33 (10.52)
7	MS + Kinetin (1.0 mg/l)	XX	5.56 (13.63)	5.56 (13.63)
8	MS + Kinetin (2.0 mg/l)	0	1.11 (6.05)	4.44 (12.17)
	Mean	-	5.00 (9.12)	16.53 (19.80)
	SEm(±)	-	2.28	2.14
	CD (p=0.01)	-	8.85	8.29

*Values in the parentheses are transformed values
No Response – 0; Very poor response – x; poor response – xx; Good response – xxx

[6]. These reports suggest the support of concept that, "the media components and culture conditions used in the present context were restricting the transformation of pro embryogenic masses into somatic embryos on the morphogenic medium itself". Thus the findings reported that, somatic embryos in the cultivar 'Surya' of papaya crop requires a period of change on medium having the low levels of cytokinin preferably kinetin (0.2 mg/l) for vigorous conversion and production of plantlet. These findings are similar with the reports [7-10].

The incubation of PEMs at lower levels of Benzylaminopurine (BAP) and kinetin for 28 days period had shown maximum transformation. This gives for to study one more experiment with increased concentrations of growth regulators with different period of incubations. Here, the PEMs incubated with various levels of BAP and kinetin for 7-14 days period, were just transferred to MS medium containing zero growth hormones. The results on development of somatic embryos and plantlets reported that, like in the earlier study, more conversion of somatic embryos shown on with 0.3 mg/l (BAP) and 0.2 mg/l (kinetin). But, period of development of embryo was reduced from 21 to 14 days. Along with this, there was decrease in the use of nutrients and plant hormones, as for good development of somatic embryos and plantlets occurred on MS medium without plant hormones. Usman *et al.* [11] concluded that "the largest qualities of embryogenic callus of papaya were produced which transferred to somatic embryos when the media is free from hormones with full strength MS medium".

Encapsulation of Somatic Embryos

Mature somatic embryos, washed with sterile water, were dehydrated in petri dishes at room temperature. Encapsulation was done by inserting individual dry embryos into drops of 3% Na-alginate and complexed with 100 Mm Calcium Chloride ($CaCl_2$) for 30 min followed by a rinse in water (Figure 1). The dry somatic embryos

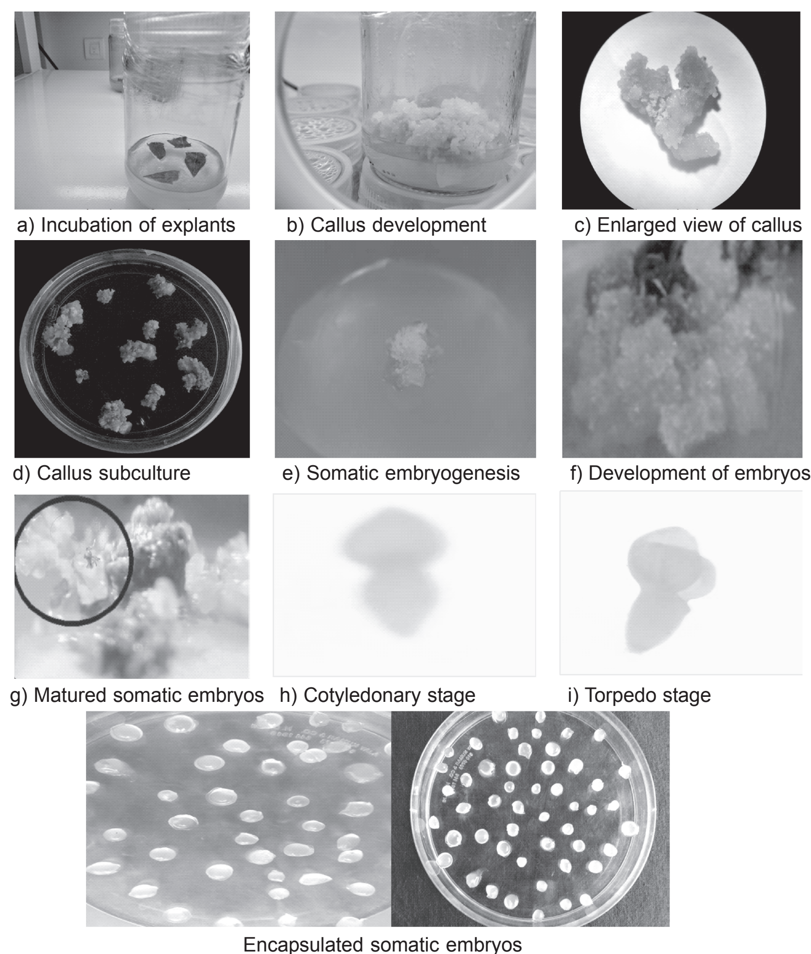

Figure 1. Procedure of production synthetic seeds

Table 3. Effect of different media's on germination of synthetic seeds

Sr. No	Type of media for germination of synthetic Seed	Total no. of seeds inoculated	No of Days after sowing (DAS) the synthetic seeds			Total no. of seeds germinated
			5 DAS	10 DAS	15 DAS	
1	MS nutrient media	10	1 (10)*	3 (30)	4 (40)	8 (80)
2	Directly to soil	10	0 (00)	0 (00)	0 (00)	0 (00)
3	Directly to sterilized soil	10	0 (00)	0 (00)	0 (00)	0 (00)
4	Directly to peat	10	0 (00)	0 (00)	0 (00)	0 (00)
5	Directly to sterilized peat	10	0 (00)	0 (00)	0 (00)	0 (00)
6	Sterilized peat + Nutrients	10	0 (00)	1 (00)	2 (20)	3 (30)

*Figure in parentheses are percentage germinated

and artificial seeds were tested for germination on hormone-free MS medium or sterile soil, peat soil and in direct field condition. All cultures were placed under a 12h photoperiod (1200 lux) at 25±l°C, except in direct field and observed for seed quality parameters.

Establishment and Germination of Synthetic Seeds

The germination of synthetic seeds directly in field and green house were evaluated. No germination was observed in direct planted to soil and green house sowing of seeds. In sterilized peat soil with MS nutrients showed 30 per cent germination, whereas without nutrients there was only 10 per cent germination. The details on the survival of the synthetic seeds in poly bags placed in the poly house, with respect to cultivar 'Arka Surya' has been furnished in table 3. The frequency of survival of plants in glass house was good with high frequency. Establishment of plants was found similar as compared to micro propagated 72 and 68 per cent, respectively. The encapsulated synthetic seeds were tested for various seed quality parameters like germination (80%), root length (5.90cm), shoot length (3.83cm), seedling vigour index (588.90), 100 seed weight (11.09g), and field emergence (0.00%).

CONCLUSION

The study suggests that, by employing "encapsulated seeds", the "micro propagated plants" can be raised on a simplified medium eliminating "subcultures", thus minimizing the "cost of production. Development of procedure for direct recovery of plants from "synthetic seeds" under non sterile conditions may have a higher impact. Although more plants can be produced *"in vitro* cultures through embryogenesis/ multiple shoot cultures in *Carica papaya,* their delivery is cumbersome. Embryos or shoots have to be divided singly and transferred for rooting to achieve root shoot balance" and the plantlets have to be hardened in green house before field planting".

For large scale commercialization in encapsulated seeds, enhanced production of propagules is necessary. Current tissue culture methods do not generate adequate propagules and are not sufficient to meet the demand in papaya. Standardization of methods for synchronization of developing propagules followed by automation of the whole process of sorting, harvesting, encapsulation and germination"of the coated propagules can enhance the pace in the production of artificial seeds".

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