Somatic Embryogenesis and Plant Regeneration of Papaya (*Carica papaya* L. cv. Shahi)

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Abstract

To develop an efficient protocol for somatic embryogenesis of *Carica papaya* cv. Shahi, special attention was paid to regenerate plantlets from the somatic embryos. *In vitro* grown young leaf segments were used as explants. Within 18 days 85% explants induced embryogenic callus on MS supplemented with 1.5 mg/l 2, 4-D. Best somatic embryos differentiation was achieved by culturing embryogenic callus on MS fortified with 1.0 mg/l Kin, 0.5 mg/l NAA and 200 mg/l glutamine. Addition of 6% sucrose in the medium increased the number of somatic embryos up to 78 per culture. The highest percentage (68%) of somatic embryo germination was observed on MS supplemented with 1.0 mg/l GA_3 and 10% CW. Proper shoot and root system was developed when germinated somatic embryos were cultured on half-strength MS fortified with 4.0 mg/l IBA. Regenerated plantlets were successfully acclimatized and survived 80% in the experimental field.

Keywords: somatic embryogenesis, regeneration, C*arica papaya* L. cv. shahi

1. Introduction

Papaya (*Carica papaya* L.) is an important fruit plant of Bangladesh, having commercial importance for its high nutritive and medicinal value [1]. It is also a good source of vitamin A and C and the proteolytic enzyme papain and chymopapain [2, 3].

C. papaya cv. Shahi is a popular local variety which is widely grown and contributes to major sources of income for the farmers in Bangladesh. Most commercial plantations are established from seed derived plants, resulting in crops with low profitability because of heterogenecity and genetic variation displayed by the plants after cross-pollination [3]. Besides, this production method leads to increased susceptibility to disease and hence increasing production loss. The lack of true to type high yielding cultivar is one of the major problems faced by the papaya growers in Bangladesh.

Biotechnological techniques have been used alternatives to conventional seed propagation including somatic embryogenesis, which represents a potential mean for the successful of more homogeneous plantlet that are free from diseases and have desirable agronomic characteristics [4].Thus somatic embryogenesis may help to increase the overall papaya production.

Many studies on the somatic embryogenesis of *C. papaya* can be found in literature [5 -12]. But there is no report in our country on somatic embryogenesis of papaya. Therefore, the present study was undertaken to develop a suitable protocol for somatic embryogenesis and plant regeneration of the *Carica papaya* L. cv. Shahi.

2. Materials and Methods

For somatic embryogenesis, young leaves were excised from *in vitro* grown shoots of selected superior genotype of female papaya plant, *Carica papaya* cv. Shahi. Young leaf segments were used as explants. Explants were cultured in MS medium with different concentrations (0.5-2.5 mg/l) of 2, 4-D for induction of embryogenic callus. Explants with embryogenic callus were sub-cultured for somatic embryogenesis in MS medium supplemented with different concentrations and combinations of cytokinins (BAP, Kin) and auxin (NAA) with glutamine (100-400 mg/l). Cultures were incubated in the dark at 27^0C for 10 days. After 10 days cultures were transferred to normal light-dark conditions at 25 ± 2^0C. Different concentrations of sucrose (3-8%) were used in the medium to increase the somatic embryos. For germination of somatic embryos, mature embryos were cultured in MS medium with GA_3 (0.5-2.0 mg/l) and coconut water (CW) (5-15%). *In vitro* plantlets were taken out from the test tubes and gently washed to remove medium from the root system. Plantlets were then transferred to small earthen pots containing a mixture of soil, sand and compost (2:1:1) and covered with polyethylene bag to maintain high humidity. After 7 days polyethylene bag was removed and after 20 days plants were planted in the experimental field.

3. Results and Discussion

To induce embryogenic callus, *in vitro* grown young leaf segments were used as explants. Explants were cultured in MS medium with different concentrations (0.5-2.5mg/l) of 2, 4-D. Among the different concentrations of 2, 4-D, the best response was observed in MS+1.5 mg/l 2, 4-D (Fig. 1). Within 18 days 85% explants induced embryogenic callus in the cut end. These explants with embryogenic callus were transferred to MS medium with 3% sucrose for the development of somatic embryos.

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The medium was supplemented with various concentrations and combinations of BAP, Kin, NAA and L-glutamine (Table 1). Cultures were incubated in the dark at 27±2^{0}C for 10 days. After 10 days these were transferred to normal light-dark conditions (16h photoperiod) at 25±2^{0}C. In the present study, MS +1.0 mg/l Kin + 0.5 mg/l NAA + 200 mg/l glutamine was found optimal to induce somatic embryo after culture of six weeks. The highest number (60±5.25) of somatic embryos per culture was obtained in this medium (Table 1, Fig. 2a). Somatic embryos were sub-cultured in the same medium for the maturation. After sub-culture of 4 weeks somatic embryos gradually became mature (Fig. 2c). A researcher [13] obtained maximum somatic embryos from hypocotyl section of ten-days-old papaya seedlings cultured on half-strength MS medium containing 4.5 µM 2, 4-D, 400 mg/l glutamine and 6% sucrose. Somatic embryogenesis of papaya was also described by [14-15]. They observed that the somatic embryos was promoted by transferring the embryogenic callus on MS containing 0.25 µM IAA and 8.0 µM Kinetin. Somatic embryos were obtained from hypocotyl cultured of papaya on MS medium supplemented with 5.0µM NAA and 5.0 µM Kinetin [11].

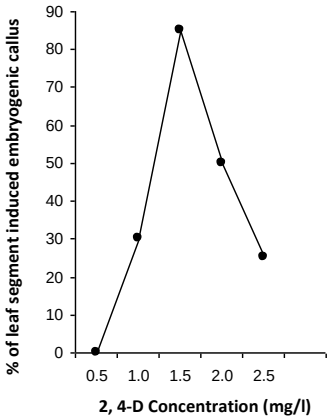

Fig. 1: Effects of 2, 4-D concentration on embryogenic callus induction

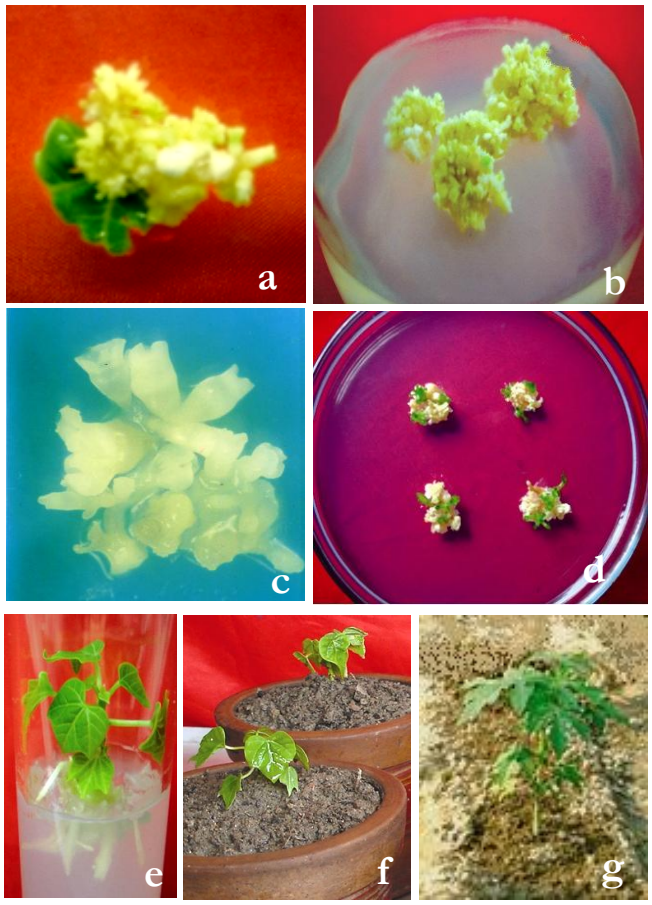

Fig. 2: Regeneration of *Carica papaya* L. cv. Shahi via somatic embryogenesis a) Somatic embryo formation in MS+1.0 mg/l Kin+0.5 mg/l NAA+200 mg/l glutamine, b) Positive effect on somatic embryos development by using 6% sucrose, c) Microscopic view of mature somatic embryos, d) Somatic embryo germination in MS+1.0 mg/l GA_3+10% CW, e) Well developed shoot and root system of germinated somatic embryo in half strength MS+4.0 mg/l IBA, f) Acclimatized plants and g) Survived plant in experimental field

To increase the number of somatic embryos per culture, different concentrations (3-8%) of sucrose were used with the selected medium (MS+ 1.0 mg/l Kin+0.5 mg/l NAA+200 mg/l glutamine). In the present investigation, it was observed that by using of 6% sucrose in the determined medium the number of somatic embryos increased up to 78 per culture (Figs. 2b, 3). Increasing the concentrations of sucrose from 3% to 6% showed better result for somatic embryos multiplication, while further addition of sucrose decreased the rate of somatic embryos proliferation.

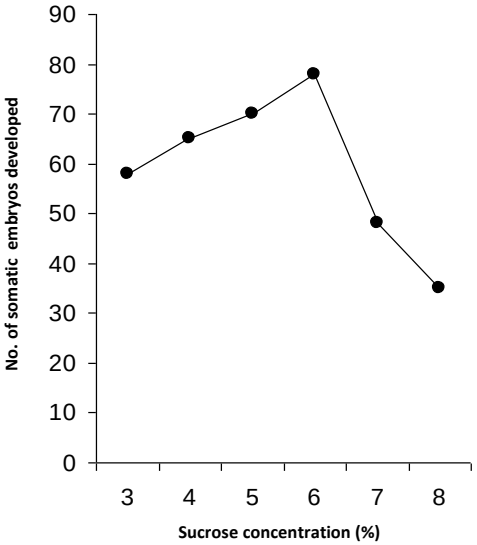

Fig. 3: Effects of different concentrations of sucrose (3-8%) on number of somatic embryos development in MS+1.0 mg/l Kin+0.5 mg/l NAA+200 mg/l glutamine

Mature somatic embryos were transferred for germination onto MS medium. Attempt was made in first step for germination of mature somatic embryos in MS medium without growth regulators, which resulted in very poor germination rate. In the second phase, MS with different concentrations and combinations of GA_3 and CW were tried for somatic embryo germination. The cultures were

maintained at 25±2^{0}C under 16h photoperiod with a light intensity of 3000 lux from philips cool white fluorescent tubes. The best response was obtained in medium with 1.0 mg/l GA_3 +10% CW (Table 2, Fig. 2d). In this case, the germination rate was found maximum (68%). In this medium, the somatic embryos enlarged and became green. Effects of GA_3+CW on conversion of somatic embryos derived from mature cotyledon of *Camelia vietnamensis* X *C. chrysantha* to plantlets was also reported by [16]. Whereas, [17] reported the conversion of somatic embryos to plantlets in MS medium with 1.0 mg/l GA_3+1.0 mg/l IAA+400 mg/l glutamic acid. A tissue culturist [13] observed that MS medium without any growth regulator was suitable for somatic embryo germination in papaya. Similar results were observed by [14] in somatic embryo germination of papaya.

Table 1: Effects of different growth regulators and organic supplement in MS medium on induction of somatic embryos from embryogenic callus (Data were recorded after six weeks of culture)

Growth regulators (mg/l)			Organic supplement (mg/l)	No. of somatic embryos/culture (±SE)
BAP	Kin	NAA	L- glutamine	
0.5	0	0	100	-
1.0	0	0	100	-
1.5	0	0	100	20 ± 3.14
2.0	0	0	100	25 ± 3.32
0. 5	0	0.5	100	-
1. 0	0	0.5	100	-
1.5	0	0.5	100	30 ± 4.22
2.0	0	0.5	100	-
0	0.5	0	100	28 ± 4.32
0	1.0	0	100	32 ± 4.42
0	1.5	0	100	40 ± 4.18
0	2.0	0	100	35 ± 3.12
0	0.5	0.5	200	15 ± 3.14
0	1.0	0.5	200	60 ± 5.25
0	1.5	0.5	200	52 ± 5.18
0	2.0	0.5	200	50 ± 5.24
1.5	0	1.0	300	45 ± 4.32
2.0	0	1.0	300	50 ± 5.15
2.5	0	1.0	300	40 ± 4.22
0	1.5	1.0	400	52 ± 5.24
0	2.0	1.0	400	40 ± 4.12
0	2.5	1.0	400	32 ± 4.18

For the well development of shoot and root system, the germinated green somatic embryos were cultured in half-strength MS medium supplemented with 4.0 mg/l IBA. Within 25 days of culture proper shoot and root system developed in this medium (Fig. 2e). Germinated somatic embryos were maintained *in vitro* until they were 5 - 7cm long. *In vitro* grown plantlets were acclimatized (Fig. 2f) and successfully transferred to experimental field (Fig. 2g) where survival rate was 80%.

Table 2: Effects of different media formulations on the somatic embryo germination (Data were recorded after six weeks of culture)

Basal medium	GA_3 (mg/l)	CW (%)	% of germinated somatic embryos
MS (Full strength macro and micro nutrients, vitamins and 100 mg/l myo-inositol, 3% sucrose)	0.5	5	35
	1.0	5	50
	1.5	5	42
	2.0	5	38
	0.5	10	45
	1.0	**10**	**68**
	1.5	10	55
	2.0	10	48
	0.5	15	40
	1.0	15	58
	1.5	15	50
	2.0	15	45

4. Conclusion

Through this experiment, an efficient protocol for somatic embryogenesis and plant regeneration of *Carica papaya* L. cv. Shahi was developed. It will also be possible to produce a large number of true to type high quality planting materials of described papaya cultivar round the year and will be helpful to fulfill the farmers demand.

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