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In vitro multiplication of *Coffea arabica* F_1 hybrid (S.2800) and its performance in the field

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Coffee (*Coffea arabica*) is one of the most important plantation crops which is traditionally propagated through seeds. However, due to segregation, off-types are noticed among the seedling progenies. Micropropagation can be an efficient method for multiplication of individual lines from segregating populations for perennial crop like coffee. Crop improvement programmes in coffee may result in superior performing F_1 hybrid plants, as seen in the case of F_1 Arabica hybrids (4n=44), propagated in Central America (Bertrand *et al*., 1999), yielding 25 to 30 per cent more, combined with resistance to various diseases, pests and nematodes. Economically viable asexual propagation protocol is being sought for large scale production of these plants. Micropropagation through somatic embryogenesis is a promising and ideal technology for large scale multiplication of elite plant material, also useful for commercial production. The superior coffee genotypes with disease tolerance, high yield, good cup quality can be multiplied in large numbers, maintaining the clonal fidelity of the regenerants.

Many of the crop improvement programmes have aimed at evolving hybrid lines with tolerance to pests and diseases. One of the spontaneous hybrids of Arabica and Robusta, Hybrido-de Timor (HDT), spotted in Timor Islands, is highly resistant to leaf rust. Bourbon coffee is known for its superior cup quality, but is highly susceptible to leaf rust. In many breeding programmes, HDT was used as a donor for rust resistant genes and hybrids were generated between HDT and other Arabica cultivars. Among the lines generated, S.2800 (3/1), a derivative of cross between Bourbon and HDT was found promising with disease resistance and good cup quality.

In coffee, propagation through tissue culture is useful for multiplication of superior coffee genotypes with disease resistance, high yield and good cup quality to evolve true to type plant materials. The progress in coffee tissue culture have been reviewed by Sondahl and Lauritis (1992); Sreenath and Muniswamy (2000); Ganesh *et al.* (2004) and Vinod Kumar *et al*. (2006). The stability of coffee somatic embryo derived plantlets produced *via* solid and liquid media was evaluated in Brazil using *C. arabica* cv. Bourbon with young tissue plants along with seedlings of the same line produced as control plants (Sondahl *et al*., 1999). Robusta clones produced by micropropagation were field tested in five coffee producing countries of Philippines, Thailand, Mexico, Nigeria and Brazil. All the micropropagated clones being evaluated in Philippines were found to have normal vegetative aspects with normal flowering and fruit set, two years after field planting (Sondahl and Baumann, 2001). A total of 20,000 plants from F_1 hybrids were produced and field test plots were established in four Central American Countries (Etienne *et al.,* 1999).

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Etienne and Bertrand (2001) reported the trueness-to- type and agronomic characteristics of *C. arabica* micropropagated by embryogenic cell suspensions. Muniswamy *et al*. (2002) reported the field performance of tissue cultured plants of *C. arabica* (Cauvery and Sln.9). Bertrand *et al.* (2010) reported the performance of *C. arabica* F_1 hybrids in comparison with American pure lines varieties. The use of somatic embryogenesis technology for large scale propagation of coffee on a commercial scale was first highlighted by Georget *et al.* (2010).

Though, there are several reports on *in vitro* multiplication of coffee through somatic embryogenesis, their field establishment and performance especially the F_1 hybrids, are very much limited. The present study deals with the successful multiplication and field establishment of the superior F_1 hybrid line S.2800 (3/1), through the process of somatic embryogenesis. Leaf ex-plants were cultured and plants were generated through somatic embryogenesis. The tissue culture protocol followed and the performance of plants in comparison with the seedling progenies in two different agroclimatic locations are discussed.

Superior F_1 plant of S.2800 (Bourbon x HDT) line, S.2800 (3/1), was selected from Central Coffee Research Institute (CCRI) experimental plot and used for *in vitro* multiplication through callus and subsequent somatic embryogenesis. The F_1 plant was developed at CCRI with an objective for combining disease resistance and good cup quality. The moderately matured leaves from F_1 plant of S.2800 (3/1) were selected, washed in running water for 15 minutes with Tween-20 and treated with 0.1 per cent Bavistin for 5 minutes. The leaves were sterilized with 1 per cent sodium hypochlorite solution for 6 to 7 minutes and then washed thoroughly in sterile Milli RO water. The leaves were cut under laminar air flow into small pieces (about 5-6 mm sided square pieces) in sterile water. The leaf explants were placed with abaxial side up on MS medium supplemented with 1 mg L^{-1} of 2,4-D and 5 mg L^{-1} of Kinetin (Kn) for callus induction. Further, the calli were sub-cultured on the same medium. The leaf explants were kept in dark, under room temperature for initial three months and later transferred to well-lit sterile room maintained at 23 ^{0}C. Somatic embryogenesis was induced after a period of 8-9 months. The embryogenic calli and somatic embryos were transferred onto half strength MS medium with 0.1 mg L^{-1} of Kn for further multiplication of somatic embryos and development of plantlets. Figure 1B shows the explants with somatic embryos showing initiation of plantlets.

The *in vitro* grown plants were planted in net pots containing commercially procured soilrite mixture. The well grown plants in net pots were hardened in a partially shaded area under polytunnels (Fig. 1D). Hardened plants with shoots of 20 cm length and stem girth of 1.5 cm having 4 to 6 pairs of leaves and well grown roots were used for field planting. Seedlings of the same plant were raised simultaneously in the nursery and planted in field for comparative studies.

The somatic embryo and seedlings derived plantlets of the F_1 hybrid plant of S.2800 3/1 at the same stage of development were established at two different agroclimatic locations, in an on farm trial at Regional Coffee Research Station (RCRS), Thandigudi (Tamilnadu) and in M/s. Subramanya Estate, Coorg (Karnataka), to study the field performance. Tissue cultured plants and seedlings were planted during the year 2003 in both the locations and general plant protection methods and nutrient application, as recommended for coffee plantations, were followed.

Growth parameters like stem diameter, bush spread, number of primaries, length of primaries and number of nodes per primary were recorded for four consecutive years after fourth year of planting and the CV of the parameters were analysed to study the significant differences. The fruit yield was recorded from 7 to 8 years old plants established in the field in two locations. Flowering started from 2nd year but debudding was practiced during initial years, to allow healthy vegetative growth of the plants. Cropping was allowed from 3rd year onwards. Yield was recorded for four consecutive years in both somatic embryo derived plants and seedlings. The yield data was subjected to paired 't' test. The seed samples of the somatic embryo derived plants and seedling plants were graded and analyzed for cup quality at the Coffee Board's Quality Evaluation Centre, Chickmagalur, Karnataka.

In vitro propagation

All the cultured explants produced calli and the callusing response of the leaf explants depended on

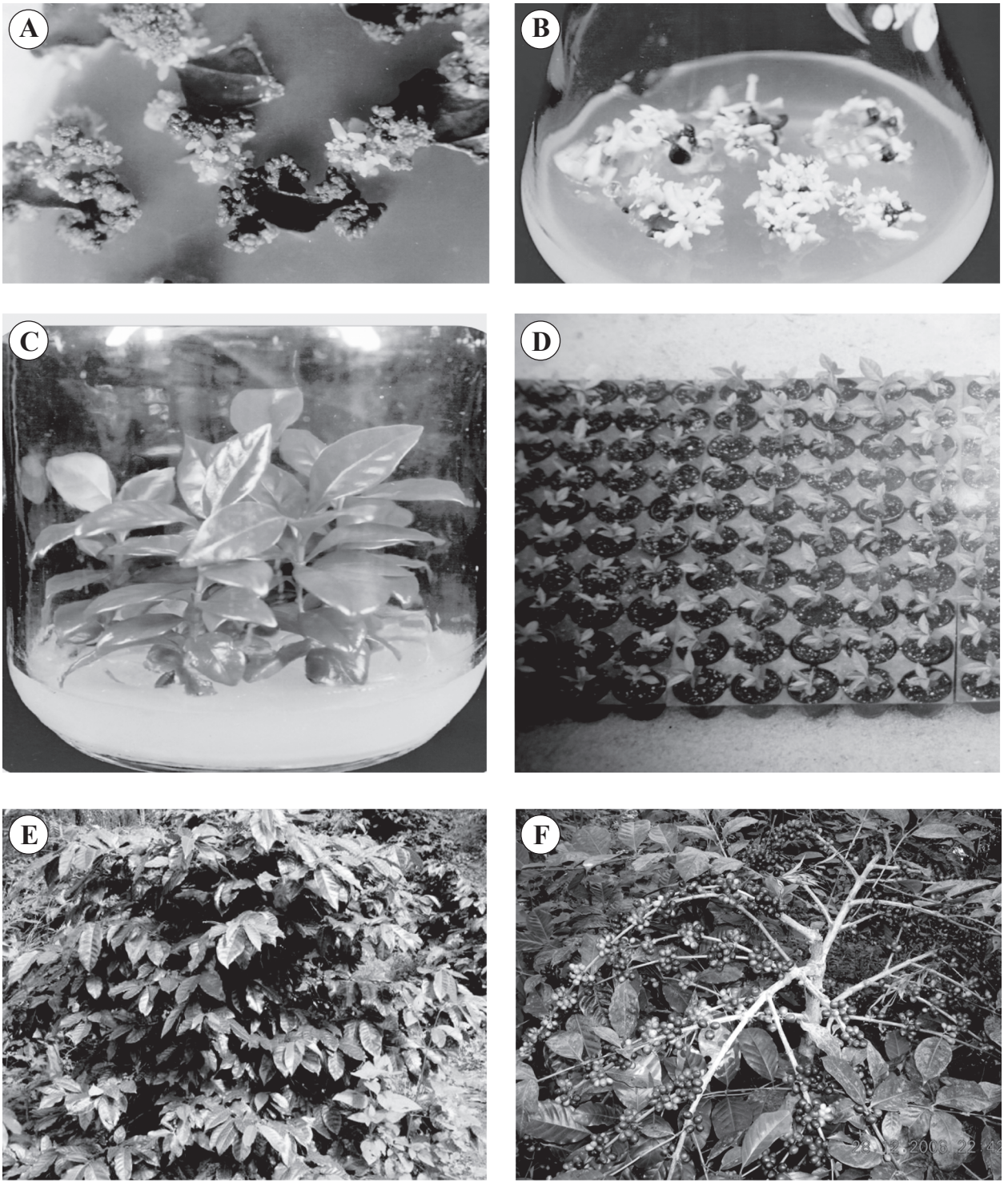

Fig. 1. Different stages of plant regeneration through somatic embryogenesis of S.2800. A) Somatic embryogenesis induced from the cultured leaf explants. B) Multiplication of somatic embryos, C) Regenerated plantlets reached for hardening, D) Hardening of regenerated plants in netpots containing soilrite mixture, E) Somatic embryo derived plant in the field, F) Yielding stage of somatic embryo derived plants

the stage of the explant and on the level of auxin present in the medium. Callus proliferation started 15-20 days after culturing, from the cut edges of the leaf tissues. However, the percentage of induction of somatic embryogenesis was very poor initially (Fig. 1A). However, after first few months, the embryogenic calli multiplied and high frequency somatic embryogenesis was induced on half strength MS medium supplemented with 0.1 mg L^{-1} of Kn (Fig. 1B). The somatic embryos were further multiplied by sub-culturing onto fresh nutrient medium of the same composition. About 60 per cent of the somatic embryos germinated into plantlets (Fig. 1C). However, the conversion of somatic embryos into healthy plantlets was one of the major hurdles observed in coffee (Jayashree *et al.,* 1995).

Field establishment of regenerated plants

The survival of TC plants was found to range from 20 to 25 per cent based on the stage and size of regenerated plants used for hardening. Morphologically, the somatic embryo derived plants of S.2800 genotype were found to be more vigorous and uniform than seedlings in both the locations. They produced thicker stems, better bush size, more number and longer primaries (Fig. 1E & 1F). They were observed to have luxuriant bush with thick vegetative growth having closer internodal length and more number of primaries with well developed dark green leaves. The data recorded in both the experimental locations is detailed in Table 1.

The growth of somatic embryo derived plants and the seedlings were found to be similar in appearance in both the locations. Though plant height was restricted to 3 feet by topping in both somatic embryo derived plants and their seedlings, there was a clear difference between the stem diameters which was highest in somatic embryo derived plants (5.4 cm) than the seedlings of S.2800 (4.5 cm) in the trial plot established at RCRS, Thandigudi. However, there was no difference in stem diameter observed between individual plants at M/s. Subramanya Estate in Coorg, Karnataka. The bush spread was higher in somatic embryo derived plants (180.1 cm) as compared to the seedlings (167.7 cm) at RCRS, Thandigudi, whereas, the bush spread was almost similar at Subramanya Estate, in both the plants. Number of primaries, number of nodes per primary and number of fruits per node was similar in both the plants in the two locations studied (Table 1).

Coefficient of variation (CV) was slightly higher for stem diameter, bush spread and length of primaries and lowest for number of primaries in TC plants of S.2800 in both the locations (Table 2 & 3). However, the CV for fruit yield was observed to be lower in both the locations. No significant variation was observed in stem diameter, bush spread and number of primaries in TC plants compared to seedlings. Leaf rust incidence was low initially in somatic embryo-derived plants (21%) compared to the seedling progenies (49%) at RCRS, Thandigudi. While, 56 per cent leaf rust incidence was observed in TC plants, 61 per cent incidence was recorded in seedlings at Subramanya Estate.

Flowering behavior and fruit yield

The flowering with healthy induction of buds and uniform blossom showers was recorded in both the progenies. All the TC plants have yielded indicating, no sterility factor in these plants. The fruit set was normal and the average fruits per node were 10 to 25 fruits in somatic embryo derived plants compared to their seedlings with 9 to 23 fruits at Subramanya Estate. Weight of 100 fruits

Table 1. Data on growth parameters recorded from TC and seedling plants of S.2800 established at two locations

Location	Materials	Stem diameter (cm)	Bush spread (cm)	No. of Primary pairs	Ave. no. of fruits per node (range)	100 fruit wt. (g)	Floats (%)	Leaf rust (%)
RCRS, Thandigudi	TC plants	5.4	180.1	6	10-25	250	5.0	41
(TN)	Seedlings	4.5	167.7	6	9-23	248	5.1	49
Subramanya Estate,	TC plants	5.1	192.5	7	9-25	170	5.3	56
Coorg (Kar)	Seedlings	4.9	188.1	7	9-25	171	5.3	61

*Values are average of data recorded in experimental plants available in the plot

Table 2. Coefficient of variation (%) of growth parameters of TC plants and seedlings of S.2800

Location	Materials	Bush spread (m)	Stem diameter (cm)	No. of primaries	Length of the primaries (m)
RCRS, Thandigudi (TN)	TC plants	10.0	14.4	15.6	10.7
	Seedlings	8.9	9.9	20.9	9.9
Subramanya Estate, (Karnataka)	TC plants	12.3	14.5	16.6	11.8
	Seedlings	11.9	13.8	16.0	10.9

was similar in both the somatic embryo derived plants and seedlings at both locations. The percentage of floats was very less and similar in both the types of plants (Table 1).

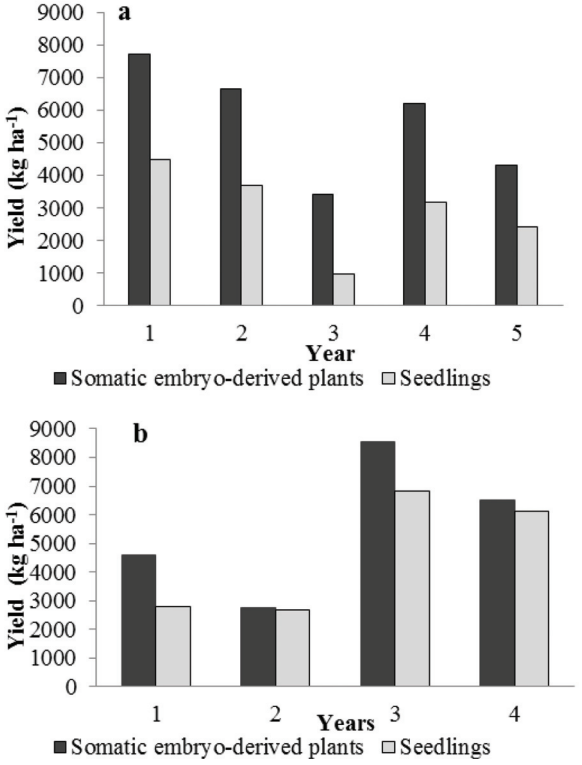

Fig. 2. Comparison of fruit yield (kg ha^{-1}) of S.2800 tissue culture plants and seedlings in two locations a) RCRS, Thandigudi and b) Subramanya Estate

At Subramanya Estate, highest fruit yield of 8564 kg ha^{-1} was recorded in third year of evaluation from somatic embryo derived plants. While, in RCRS, highest fruit yield of 7703 kg ha^{-1} was harvested in first year of evaluation in somatic embryo derived plants (Fig. 2). In statistical analysis, the yields of tissue cultured plants were found to be significantly higher with bold fruits and closer fruit clusters (Fig. 1F).

Bean grade percentage and cup quality

High percentage of pea berries (PB) was recorded in seedlings than in the somatic embryo derived plants at both the locations. However, the percentage of 'AA' (49.4%) and 'A' (37.9%) grade beans were higher in somatic embryo derived plants at RCRS, Thandigudi as compared to the seedlings ('AA' grade: 11.9% and 'A' grade: 38.7%). There was very less 'C' grade beans and triages (3%) in somatic embryo derived plants at RCRS, Thandigudi. At Subramanya Estate, high percentage of PB, AA and A grade beans were obtained in TC plants compared to seedling progenies with 19.6, 18.4 and 35.3 respectively. The cup quality ratings observed in the two locations is detailed in Table 3. An above average to good cup quality rating was obtained in somatic embryo derived plants from both the locations as against average to good cup rating in the seedlings.

The results obtained indicated that, *in vitro* multiplication of leaf rust resistant F_1 Arabica hybrids can be achieved through somatic

Table 3. Percentage of bean grade and cup quality rating in TC plants and seedlings established at two different locations

Place of planting	Materials	PB	AA	A	B	C & triage	Cup quality rating
RCRS Thandigudi (TN)	TC plants	9.1	49.4	37.9	3.6	3.0	Above average to good
	Seedlings	17.8	11.9	38.7	26.3	5.2	Average to good
Subramanya Estate (Kar)	TC plants	31.1	31.4	25.0	2.4	10.0	Above average to good
	Seedlings	19.6	18.4	35.3	17.7	9.0	Average to good

embryogenesis so as to maintain the uniformity and true-to-type nature of the plant similar to the elite plant material. The growth of TC plants was found to be almost on par with the seedlings of the respective mother plant without any segregation of traits as seen from the data on growth parameters. Further, a significant increase in yield was observed in TC plants at RCRS, Thandigudi. However, at the other location, the mean fruit yield in TC plants was slightly higher compared to seedling progenies though statistically there was no significant variation. In earlier study, similar result was obtained in studies conducted in somatic embryo derived plants (Deshayes, 2000). The present study also indicated no somatic variations in somatic embryo derived plants at both the locations of study though some earlier studies on molecular analysis of somatic embryo derived plants of *C. canephora* revealed chloroplast and mitochondrial DNA polymorphism (Rani *et al*., 2000). The present study clearly indicated that, superior performance of F_1 hybrid plants, multiplied and established following tissue culture techniques also being uniform, true-to-type progenies could be generated.

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