Chapter-4

Trachyspermum ammi (L)

INTRODUCTION:

The practice of plant tissue culture has changed the way some nurseryman approach plant propagation. In the recent past, the applicability of this technology to the propagation of trees and shrubs has been well documented. ***Micro-propagation*** is the term which best conveys the message of the tissue culture technique most widely in use today. The prefix ***Micro*** generally refers to the small size of the tissue taken for propagation, but could equally refer to the size of the plants, which are produced as a result. Micropropagation offers several distinct advantages not possible with conventional propagation techniques.

The wide spread use of micropropagation has many advantages-

- When classical methods of *in vivo* vegetative propagation prove inadequate, *in vitro* cloning is and important tool in speeding up the propagation.
- Adult plant material, which often cannot be cloned *in vivo*, can be rejuvenated *in vitro* and then propagated.
- Growth of *in vitro* propagated plants is often stronger then those cloned *in vivo* mainly due to rejuvenation).
- By *in vitro* propagation, expensive and laborious methods such as grafting and budding on a root stock can be proved absolute.
- It also enables us to isolate and clone the spontaneous and induced mutants.
- It is immune to variable environmental conditions, therefore round the year production is feasible.
- *In vitro* storage and propagation facilitated the creation of gene-bank (preservation of valuable plant material) and storage under pathogen free conditions in a relatively small area. By use of low temperature storage and freezing, the time spent on cloning as well as space required can be drastically decrease.
- This technique allows us to do genetic manipulations, which would be impossible, when no methods for regeneration protoplast, cells and tissue exist.

A single explant can be multiplied into several thousand plants in less than one year. Once established actively dividing cultures are continuos source of micro cuttings, which can result in plant production under greenhouse conditions without seasonal interruption.

Micropropagation through somatic embryogenesis from cultured cells is a useful system for propagation of plant material *in vitro*. Somatic embryogenesis is a developmental process which results in the formation of large number of embryo like structures that recapitulate the typical stages in the embryogenesis of fertilized cells, produced from the somatic cells of the plants. Somatic embryogenesis has been reported in almost all plant families.

Table: 4.1 Reports on somatic embryogenesis in members of Apiaceae

Plant	Explant	Authors	Year
Ammi majus	Ovary	Sehgal	1972
	Hypocotyl	Grewal *et al.*	1976
Ammi visnaga	Hypocotyl	Fathy K, El Fiky *et al*	1989
Apium graveolens		Reinert *et al*	1966
Carum carvi	Petiole	Ammirato	1974
Coriander sativum	Leaf, fruit	Steward *et al*	1970
Daucus carota	Root,Hypocotyl, leaf,peduncle, Stem,root	Halperin & Watherell Tisserat *et al*	1964 1979
Foeniculum vulgare	Stem	Maheshwari & Gupta	1965
Petronolium hortense	Petiole	Vasil and Hilderbrandt	1966
Pimpinella anisum	Fruit Hyoocotyl	Huber *et al* Ernst et al	1978 1984
Sium svave	Embryo	Steward *et al* Ammirato & Steward	1970 1971
Trachyspermum ammi	Hypocotyl	Jasrai *et al*	1992

Trachyspermum: *Trachyspermum ammi* (L) Sprague belonging to the family-Apiaceae (Umbelliferae), commonly known, as ***Ajwain*** is an aromatic herb cultivated through out in India. But mostly in distinct of North Gujarat and also in and around Indore and Hyderabad. The fruits are being used as spices and are also carminative, stimulant antispasmodic and tonic. They are given in colic flatulence, dyspepsia, indigestion, diarrhea, cholera, tympanities, hysteria etc. The fruits have an aromatic smell and a pungent taste. They yield 2 to 4 percent of a colorless to brownish essential oil in which thymol is present to the extent of 35-60%. The annual production of Ajwain oil from the seeds of *T. ammi* is over 35 metric tons, which is not sufficient to meet demands. The oil is used in perfumery industries, as insecticide and in indigenous medicine being an important source of thymol, if the yield of oil could be

substantially improved and the yield of fruit per acre increased, there is every chance of reviving the industry (Chopra *et al* 1958). The main processors of Ajwain oil are Devi Industries in Madurai (Tamil Nadu), Siva Industries in New Delhi and Ashok Thymol in Indore (Madhya Pradesh).

The main economic and medicinal uses of *Trachyspermum ammi* are:

1. The fruits are widely used as spice.
2. Fruits of *T. ammi* are stimulant antispasmodic, tonic and carminative, administered in flatulence, dyspepsia and diarrhea, and also recommended in cholera.
3. Ajwain oil is used for sore throat and bronchitis and is a common ingredient of cough mixtures.
4. A paste of crushed fruit is applied to the abdomen externally for relief from colic, dyspepsia.
5. It shows, antibiotic activity and is used in lotions and ointments applied for checking chronic discharge.
6. Essential oil, yielded by fruits is called Ajwain oil, principal consistent of the oil is thymol, which can be easily crystallized from the oil and is known as flowers of Ajwain.
7. Ajwain oil is employed as an aromatic, carminative and antiseptic also expectorant in emphysema bronchial pneumonia and some other respiratory ailments.
8. Aqueous solution left after separation of essential oil is called omum water; used as carminative in flatulence and gripe.
9. Extracted fruits may be used as cattle feed.
10. Fruits also yield fatty oil used externally on rheumatic swellings, also used in soaps and in the preparation of epoxy derivatives used as plasticizers in vinyl industry.
11. Deoiled cake used as a cattle feed and fertilizer.
12. Roots are carminative diuretic and used in febrile conditions and stomach disorders.

Hence somatic embryogenesis was studied for the production of artificial seeds in *Trachyspermum ammi* L Sprague. Earlier, somatic embryogenesis on *Trachyspermum ammi* was reported from our Laboratory (Jasrai *et al* 1992).

MATERIALS AND METHODS:

Plant material:

Viable seeds of *Trachypermum ammi* (L) Sprague were procured from Research Station, Gujarat Agricultural University, Jagudan. Seeds were washed under running tap water (2 hrs) and then with teepol detergent. The detergent was removed by several washings with tap water and finally rinsed with distilled water. After surface disinfestation with 0.1 % (w/v) $HgCl_2$ (3 min) and 5-6 rinses in sterile distilled water, the seeds were incubated on Murashige and Skoog's (MS) basal medium (1962) without any growth regulators for germination. After 15 days hypocotyl segments along with cotyledonary leaves (5-10 mm) were excised from such aseptically raised seedling (Fig-3 a) and transferred to MS medium with sucrose (3 % w/v) and following two combinations for callus induction.

Throughout the present studies with *T. ammi* friable and yellowish green callus raised on MS medium containing 2.4-D (2.22 uM) and KIN (4.45 uM) was used (Table 4.2) (Fig 4 a & b). After two subcultures callus was transferred to liquid media having same hormonal combinations to generate cell suspension.

Table- 4.2: Induction of callus from hypocotyl explants of *T. ammi*

Concentration of Growth Regulators (uM)		Callus Response
2,4-D	**Kinetin**	
2.22	4.65	Yellowish-green, friable
4.45	9.3	White, compact

* data collected after two weeks of incubation

A growth pattern was studied using this cell suspension by transferring a known amount of callus to fresh medium and growth was recorded in terms of fresh and dry weight. At every 5 days interval cell suspension was harvested by filtration through Whatman filter paper (No. 1) under vacuum and fresh weight was noted. The harvests were then kept in oven (60 ^{0} C) and dry weights were taken after 15 days. Maximum growth was achieved on the 16th day with almost 6-fold increase.

Fig-3 Induction of callus

a) In vitro germinated seedlings

b) slight callus formation of hypocotyl explants innoculated

c) proliferating callus


a
b
c

Fig-4 Callus combinations tried

a) 2,4-D 2.22 uM & KIN 4.65 uM

b) 2,4-D 4.45 uM & KIN 9.3 uM


a
b

Induction of somatic embryogenesis:

After 18 to 20 days the suspension was subjected for induction of somatic embryogenesis by transferring to hormone free liquid medium. And the cell suspension was screened at every 4 days for somatic embryos.

Synchronization:

Synchronization of somatic embryos was induced through –

1) physical method of sieving- The generated cell suspension was sieved through different grades of sieves (stainless steel mesh of various sizes: 4.8×10^7 um^2, 1.1×10^5 um^2 & 1.8×10^5 um^2).
2) chemical method- involving treatment with different concentrations of ABA (0.1 to 1.0 uM). ABA was incorporated in the medium after filter sterilization (0.22 u filter, Sartorius).

Maturation:

Maturation of somatic embryos was achieved by transferring the developing embryoids to different concentration of sucrose (3-6 %). The embryos for maturation were isolated by sieving through 1.8×10^5 um^2 mesh sieve.

Germination:

The mature somatic embryos, with cotyledonary leaves were transferred on to a germination medium (MS) containing 3 % sucrose and low levels of KIN (0.1 uM.). After the plantlets attained a height of 3-5 cm, they were transferred to the soil and mixture of vermiculite and perlite in a rate of 1 : 1 and subsequently to the field.

Encapsulation:

The mature somatic embryoids were suspended in MS basal medium containing sodium alginate (4 %). The embryoids were then picked up and dropped into a solution of $CaCl_2.2H_2O$ (0.06 uM). The resultant beads were kept in the same solution (30 min) for hardening on a gyrotary shaker (100 rpm). The encapsulated beads were removed by decanting the $CaCl_2$. $2H_2O$ solution, transferred to petridishes (3 cm dia) and sealed with parafilm.

Storage:

The sealed petridishes containing encapsulated embryoids were stored under desiccation at low temperature (5° C).

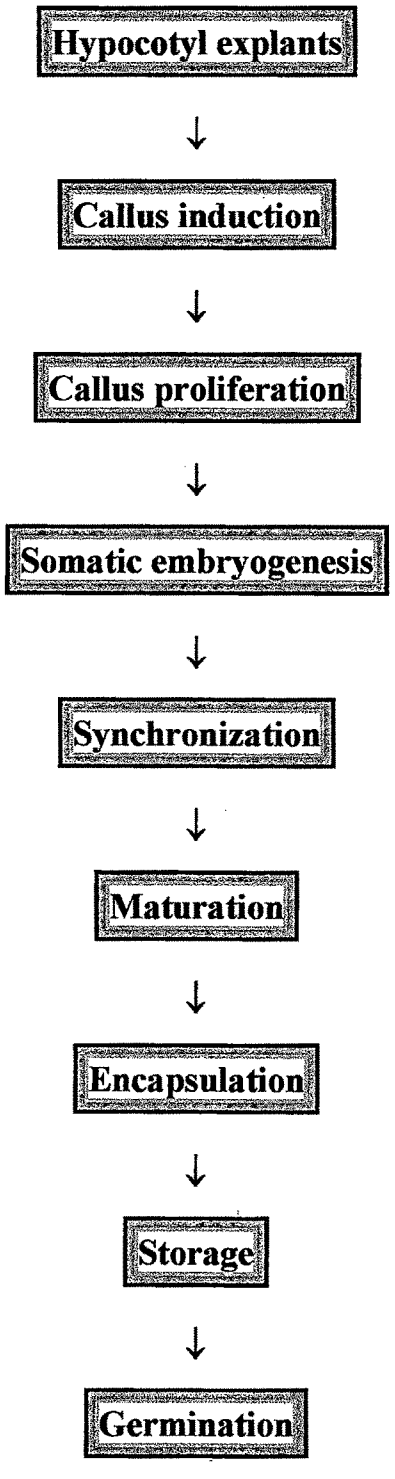
Germination:

At every fifteen days interval the stored artificial seeds were removed and transferred to –

(1) agar based MS medium supplemented with 0.1 uM of KIN
(2) mixture of vermiculite and perlite (1 : 1) irrigated with half strength basal medium for germination
(3) soil and irrigated with distill water

The substratum in the later two cases was irrigated with distilled water at every alternate day.

Fig-2: Basic protocol followed for the production of artificial seeds of *Trachyspermum ammi* L Sprague.



RESULTS AND DISCUSSION:

Plant regeneration on a large scale from tissue culture system is often the most critical step in the success of various biotechnological techniques for any plant improvement program. Rapid regeneration and high multiplication rates for propagules are now possible through somatic embryogenesis. This method produces somatic embryos with root and shoot meristems that originate from a single cell with or without a callus stage (Mc Williams *et al* 1974; Street & Wither 1974; Flaccius 1978).

In the present system of *Trachyspermum ammi* (L) Sprague yellowish-green friable callus was induced within two-week period from hypocotyl of *in vitro* raised seedlings (Fig-3 b). For best results the hypocotyl segments were gashed. After one or two subcultures the proliferating callus (Fig-3 c) was used to generate cell suspension.

A typical sigmoid pattern of cell growth was evidenced with the increase in the fresh and dry weights (Fig.4) with a short lag phase (8 days). Normally, after 20 days of incubation the cell growth showed a stationary phase (Fig-1).

Somatic Embryogenesis:

The removal of plant growth regulator mainly 2,4-D from the medium triggered the formation of somatic embryogenesis. The globular (5^{th} day) and heart shaped embryos (15^{th} day) were seen till 10 days of the culture (Table-4.3)(Fig 5 a & b). Generally, the basic protocol followed for embryogenesis is induction of callus growth in a auxin enriched medium followed by a transfer to a medium devoid of auxin for somatic embryogenesis (Reinert 1967). Most studies clearly show the requirement of auxin (2,4–D) being essential for the induction of somatic embryogenesis either from the callus or from explants directly. However for further growth and development of embryos into plantlets auxin is not required (Raghavan and Nagmani, 1983). There has been a report on the effect of cytokinin on enhanced embryogenesis. Jha *et al* (1981) reported embryogenesis in celery in the presence of a high levels of kinetin.

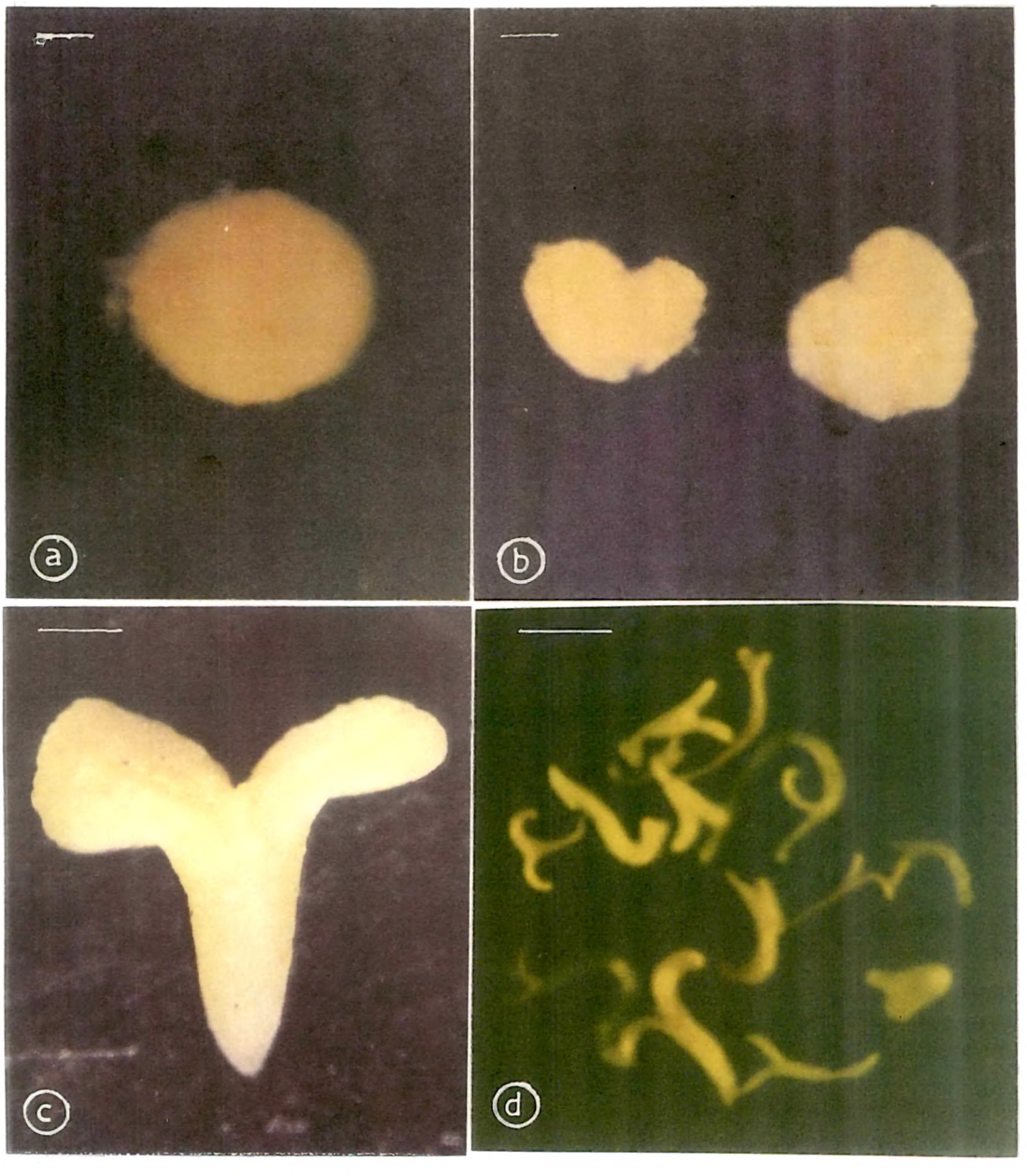
Fig-5 Different stages of Embryoids of *Trachyspermum ammi*

a) globular

b) heart shaped

c) full grown embryoid

d) synchronised embryoids


a
b
c
d

Growth Curve

Figure no. 4

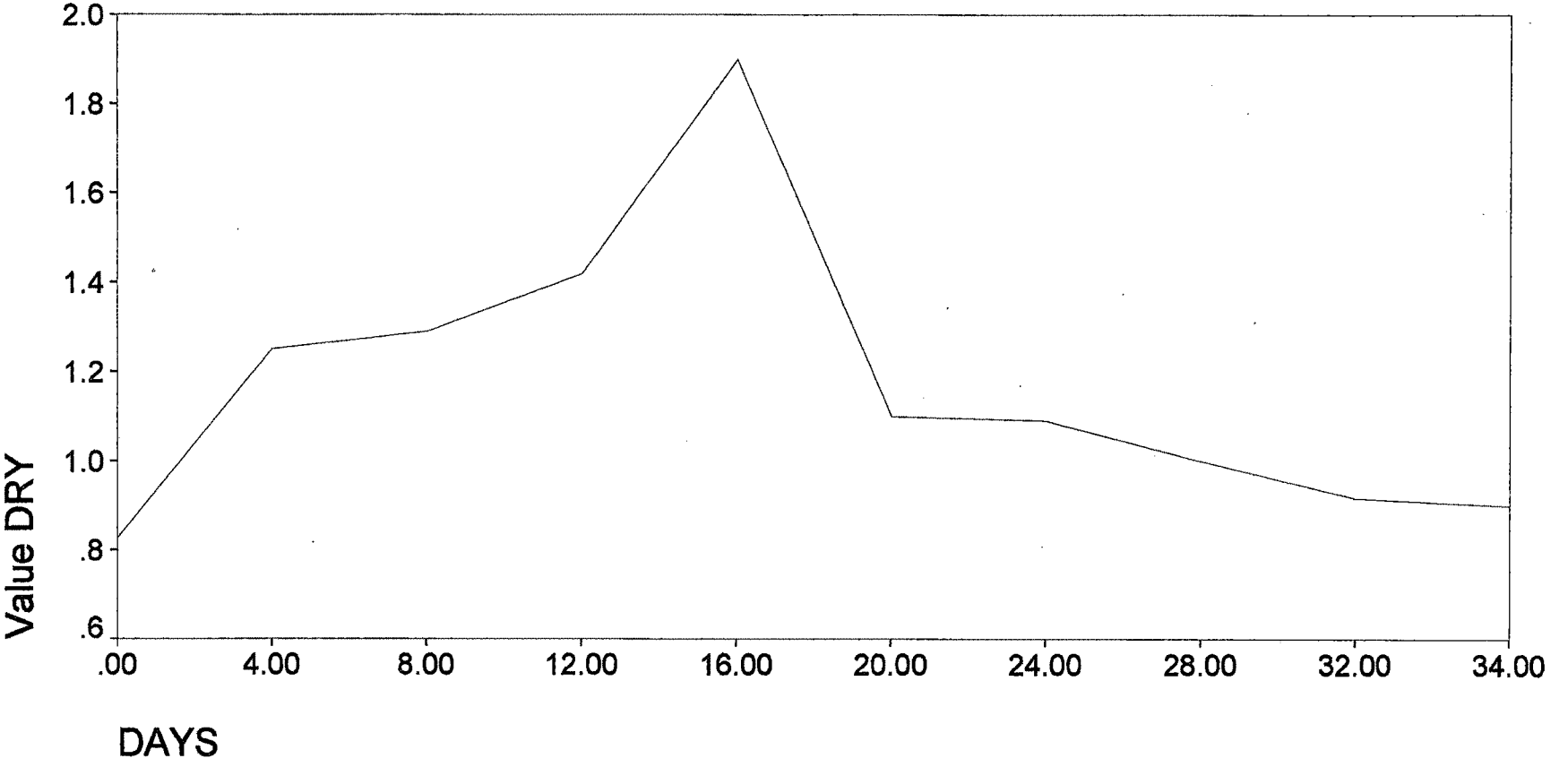


Table- 4.3: Time schedule for various stages of somatic embryogenesis in *T. ammi*

Days of Incubation	Nature of suspension culture
0	Fine suspension
5	Suspension with cell aggregates, globular
10	Globular
15	Heart shaped
20	Heart shaped
25	Torpedo
30	Plantlets

Synchronization:

Somatic embryogenesis from cultured cell is a useful system for propagation of plant material *in-vitro* and for the production of artificial seeds. If developing embryos obtained by somatic embryogenesis are used directly for propagation of plants or are encapsulated in a suitable material that promotes germination, plant clones with the same genes as the mother plant can be obtained with much ease and standard laborious plant bulking procedures can be avoided. Somatic embryogenesis usually occurs at a low frequency and, when somatic embryos are induced from embryogenic cells of embryogenic cell clusters, always embryos at different developmental stages are present in a given culture. Hence there should be a defined system in which embryogenesis occurs synchronously and at a high frequency (Osuga & Komamine 1994). There are reports on establishment of synchronous system for carrot cell suspension culture (Fujimura & Komamine 1997; Nomura & Komamine 1985; Osuga *et al* 1993). Synchronous cultures of *Catharanthus roseus* cells, induced by starvation and refeeding of phosphate (Amin *et al* 1983; Ando *et al* 1987) or auxin (Nishida *et al* 1992).

Synchronization helps in understanding of the molecular mechanisms involved in plant cell cycle. It helps not only in elucidating the nature of basic cell function, namely, cell division, a fundamental aspect of plant biology and an essential feature of the production of plant biomass, but for studies on plant development since the plant development is determined mainly by the organized division of cells.

Hence synchronization of cell division is very important for *in vitro* culture. It is essential in studying the cell cycle, for obtaining large volumes of cells in the came metabolic state, and for synchronizing embryo morphogenesis.

Synchronization attempts to regulate embryogenesis in the present studies, two approaches were undertaken

1) physical separation of embryogenic culture to produce uniform-sized cell masses so as to favor more uniform development and
2) use of growth regulators to physiologically synchronize the development.

Table-4.4: Effect of ABA on synchronization of somatic embryogenesis in *T. ammi*. The data shows no. of embryos obtained.

ABA Conc (uM)	No. of days	Synchronization (%) No. of embryos		
		Grade-1	Grade-2	Grade-3
Control	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	15	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	20	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	10	14.0 ± 3.0	10.0 ± 0.0	8.33 ± 1.66
	15	23.3 ± 3.3	13.0 ± 3.3	10.0 ± 0.0
	20	26.6 ± 3.3	23.0 ± 3.3 ^{c}	10.0 ± 0.0
3	10	40.0 ± 5.7 ^{bc}	43.0 ± 3.3 ^{c}	23.3 ± 3.3 ^{cd}
	15	30.0 ± 5.7 ^{bc}	36.6 ± 6.6 ^{c}	33.3 ± 3.3 ^{cd}
	20	43.3 ± 3.3 ^{bc}	33.3 ± 3.3 ^{c}	33.3 ± 3.3 ^{cd}
5	10	50.0 ± 5.7 ^{bc}	46.6 ± 3.3 ^{b}	33.3 ± 3.3 ^{cd}
	15	63.3 ± 3.3 ^{b}	56.6 ± 3.3 ^{b}	36.6 ± 3.3 ^{c}
	20	60.0 ± 5.7 ^{b}	53.3 ± 3.3 ^{b}	50.0 ± 0.0 $_{b}$
7	10	66.6 ± 3.3 ^{b}	56.6 ± 3.3 ^{b}	56.6 ± 3.3 ^{a}
	15	56.6 ± 3.3 ^{b}	46.6 ± 3.3 ^{b}	46.6 ± 3.3 ^{b}
	20	60.0 ± 5.7 ^{b}	50.0 ± 5.7 ^{b}	43.3 ± 3.3 ^{c}
9	10	63.3 ± 6.6 ^{b}	80.0 ± 0.0 ^{a}	63.3 ± 3.3 ^{a}
	15	76.6 ± 3.3 ^{ab}	83.3 ±3.3 ^{a}	73.3 ± 3.3 ^{a}
	20	80.0 ± 0.0 ^{ab}	90.0 ± 5.7 ^{a}	80.0 ± 5.7 ^{a}
10	10	63.3 ± 8.8 ^{b}	53.3 ± 3.3 ^{b}	43.3 ± 3.3 ^{c}
	15	56.6 ± 3.3 ^{b}	50.0 ± 0.0 ^{b}	46.6 ± 3.3 ^{b}
	20 days	63.3 ± 3.3 ^{b}	53.3 ± 0.0 ^{b}	40.2 ± 2.7 ^{cd}

- Means within a column followed by the same superscript letter are not significantly different ($P \leq 0.05$; Duncan's multiple range test)
- 10 days indicates globular embryo
- 15 days indicates heart shaped embryo
- 20 days indicates cotyledonary embryo
- Grd.1- 4.8 x 10^5 um^2, Grd.2- 1.8 x 10^5 um^2 and Grd.3- 1.1 x 10^5 um^2

The cell suspension of *T.ammi* was sieved with different grades of sieves and transferred to MS basal liquid media supplemented with different concentrations of ABA (1-10 uM) respectively. Preliminary experiments were tried to optimize the range of ABA concentration (0.1,1,10 and 100 uM). It was noted that ABA at 100 uM concentration completely inhibited the embryo development including germination of embryos and of 0.1 uM of ABA showed no significant effect. Since, concentrations range of 1–10 uM of ABA showed response, this range of concentration of ABA was tried for further experiment. Different concentrations of ABA (1-10 uM) were incorporated in the graded cell suspension generated through sieves of different pore size (ie 4.8 x 10^5 um^2, 1.8 x 10^5 um^2 and 1.1 x 10^5 um^2) respectively. The cell mass that was collected from the first grade (4.8 x 10^5 um^2) of sieve showed presence of large number of embryos at various stages of development along with the presence of cell clumps and polyembryonic cell initials which developed further to form globular embryos respectively. Whereas suspension collected from second grade (1.8 x 10^5 um^2) of sieve showed cell clusters of almost 20-45 um size which developed synchronously into embryos (globular, heart and cotyledonary stages) (Fig-5 a-d) (Fig-6). The cell suspension collected from the third grade (1.1 x 10^5 um^2) of sieve showed comparatively less number of embryos compared to suspension sieved through the second grade (Table-4.4). In carrot, initial cell clusters of 31–47 um in diameter were the smallest clusters of carrot cells in which embryogenesis could be induced. Embryogenesis rarely occurred when clusters were smaller than 31 um in diameter, whereas somatic embryogenesis could be induced from clusters of more than 47 um in diameter. However since multiple embryos were formed in this size range, large clusters were not suitable as material for propagation *in vitro* or production of artificial seeds (Osuga & Komamine 1994). Incorporation of ABA at 9 uM concentration was most effective in optimizing the synchronous embryo development (Fig-5 d)from cell suspension collected through 1.8 x 10^5 um^2 second grade sieve, whereas supplementation of medium with 1–5 uM concentration of ABA showed comparatively little response (Table-4.4).

In the recalcitrant mango, maximum number of normal somatic embryos were recovered by treatment with 5 uM ABA, whereas 100 uM ABA completely arrested maturation, elongation and germination of the embryos (Fernando *et al* 1996). In the present system with *T. ammi*, 9 uM ABA concentration was most effective during 10-

20 days (after the development of heart shaped embryo) of its exposure during their culture. In order to maximize the effect of ABA during orthodox somatic embryo maturation, it is necessary to apply it at a critical stage of embryo development. In *Medicago sativa*, after attaining the early cotyledonary stage, the response of somatic embryos to ABA declines rapidly. So according to Mc Kersie *et al* (1989), ABA should be applied to alfalfa somatic embryos during the early cotyledonary stage for synchronization of somatic embryos.

Maturation:

Maturation is a transitory, frequently indispensable stage between embryo development and embryo germination phases. The water relations between the embryo and its environment *in vitro* or *in vivo* play a determinant regulatory role in embryo development and in particular, its maturation (Adams & Rinne 1980). Precocious germination usually occurs in cultured embryos when the maturation phase has been ***short-circuited***. To stimulate the maturation of somatic embryos and to prevent precocious germination, increasing the concentration of sucrose in the medium is sufficient to circumvent the abnormal response. The production of dry somatic embryos for use as artificial seeds promises to revolutionize several aspects of vegetative propagation and germplasm storage (Mc Kersie *et al* 1989).

Table-4.5: Effect of different concentrations of sucrose on inhibition of precocious germination of embryoids

Sucrose concentration (%)	% Germination (Mean)
3 (control)	30.0 ± 5.7
4	50.0 ± 5.7
5	73.3 ± 8.8
6	46.6 ± 3.3

- values are the mean percentage of three independent experiments
- The data was collected after 15 days

The somatic embryos of *T. ammi* at the cotyledonary stage which were generated by exposure to 9 uM of ABA were further subjected to different concentrations of sucrose

(3–6 %) for one week. For stimulating maturation of somatic embryos and to prevent precocious germination 5 % sucrose concentration proved to be the optimum (Table-4.5) (Fig-7 b & c). Higher concentration of sucrose in the medium and, exogenous ABA application increases the number of embryos expressing desiccation tolerance and improves percentage germination of dry somatic embryos. Desiccation probably completes the developmental process and initiates the first biochemical events that prepare embryos for germination (Kermode & Bewley 1885). The use of such an ABA treatment followed by desiccation has been found effective in improving the germinability of somatic embryos of *Picea glauca-engelmanii, Picea sitchensis* (Roberts *et al* 1991) and oil seed rape (Senaratna *et al* 1991).

Germination:

The artificial seeds of T. ammi were stored at low temperature 5^0 C and subjected to germination at every 15 days and the percentage germination was recorded. Comparing different substrata for germination demonstrated maximum germination on semisolid MS medium (Fig-8 a-c) supplemented with 0.1 uM KIN, however germination in soil was also good compared to vermiculite and perlite mixture (Table-4.6) (Fig-10 a-c). Storage of artifical seeds for about 3 months showed 13.3 % germination on medium, whereas they failed to grow further in mixture of vermiculite and perlite (1:1) (Fig-9 a & b) and showed almost 6.6 % germination in soil.

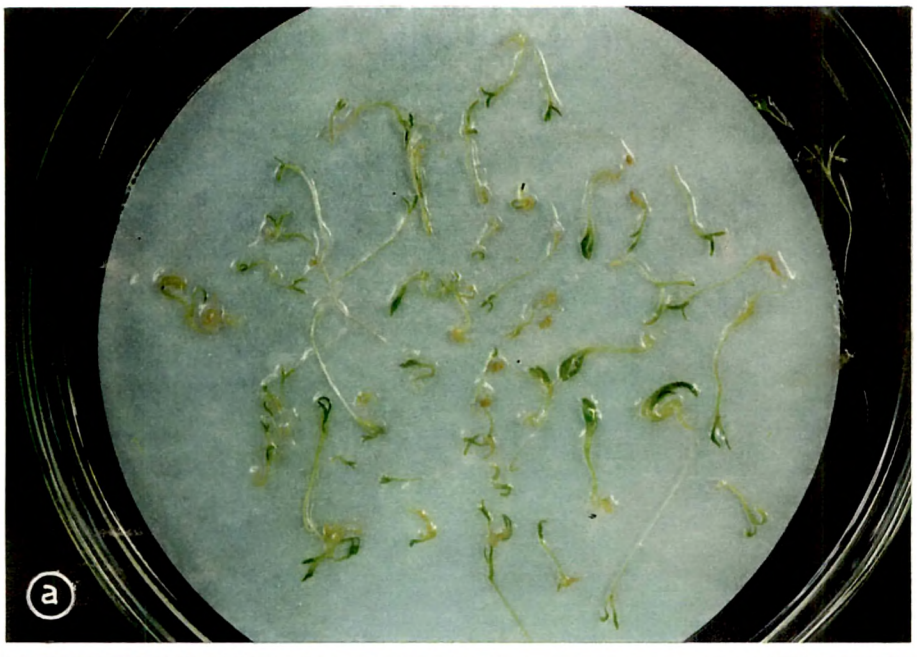
Drew (1979) obtained *ex vitro* germination of carrot somatic embryos on carbohydrate-free, gelled medium if the embryos were first subcultured on 10 % sucrose, but only three plants survived. Redenbaugh *et al* (1978,1980) encapsulated alfalfa somatic embryos within sodium alginate and obtained conversion into plants at frequencies of 7 % to 10 % in sand trays or in transplant plugs, Gupta and Durzan (1987) encapsulated loblolly pine in alginate, but conversion of embryos into seedlings was not achieved after storage at low temperature (4^0C) for 4 months. Nitzsche (1978.1980) reported that desiccated carrot callus pretreated with increased sucrose and ABA could be stored for 1 year. Gray et al (1987) reported that somatic embryos of orchard grass desiccated at 70 % RH had 12 % survival.

Fig-6 Normal germination of somatic embryoids in *T. ammi*

a) harvested and germinating embryoids

b) Seedlings at various stages of development.

Note the normal seed germinated seedling on the extreme left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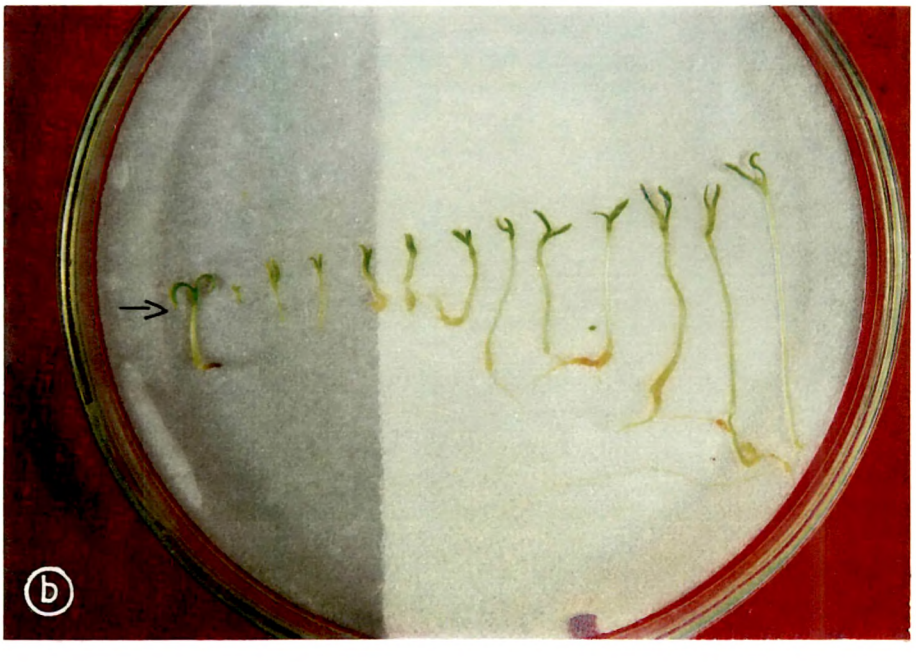
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Fig-7 Large scale production of somatic embryogenesis

a) Maintainance of cultures in different culture vessels

b) Precocious germination of somatic embryos

c) Further growth of somatic embryogenesis raised seedlings

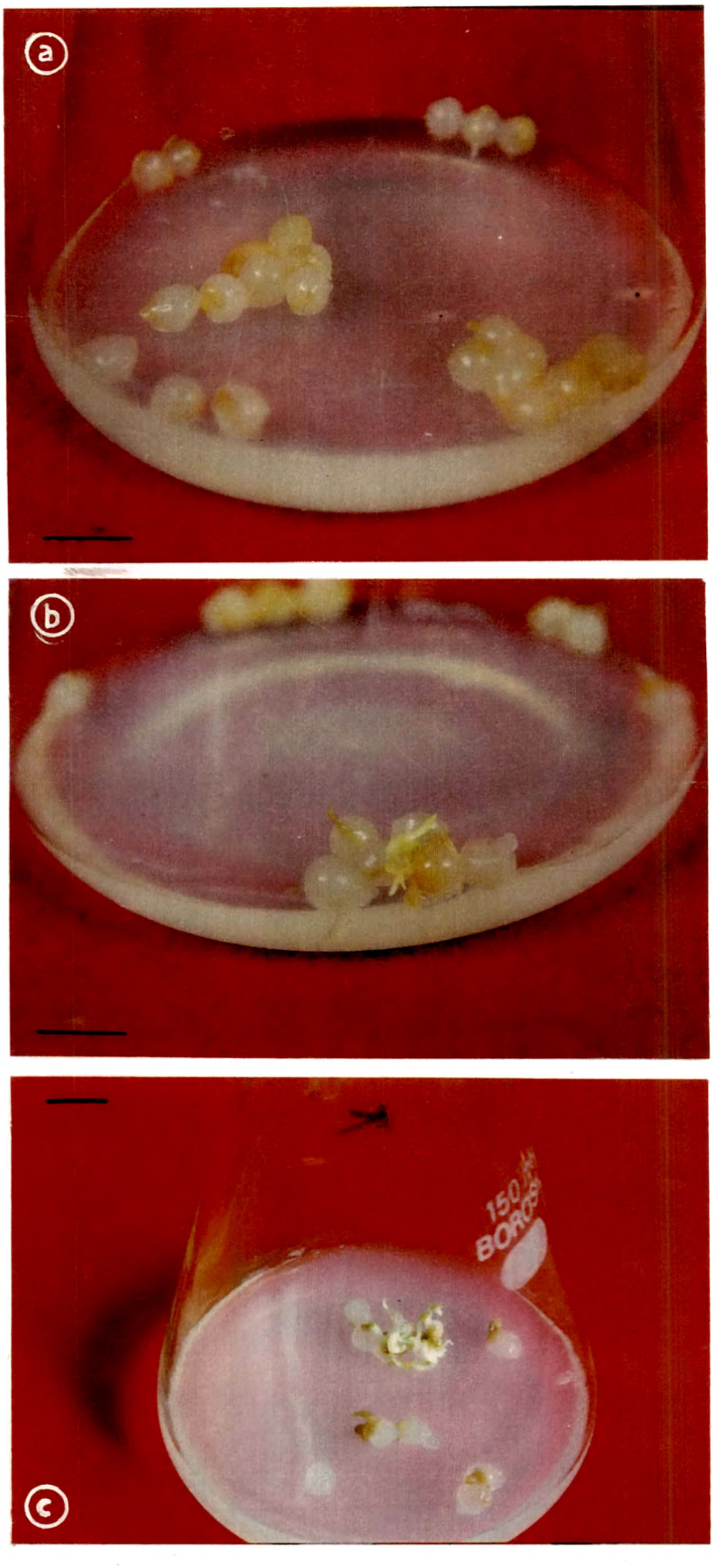

a
b
c

Fig-8 Synthetic seed production in *T. ammi*

a) somatic embryos encapsulated in alginate

b) Germination of synseeds on MS medium

c) Further growth of the germinated synseeds


a
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150
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Fig-9 Germination and growth of synthetic seeds

a) germination on perlite

b) Further growth

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a
b

Fig-10 Growth of Tissue culturally raised plants

a) Hardening of Tc plants in plastic pots containing vermiculite

b) Hardened plants

c) Transfer to the earthen pots containing garden soil


a
b
c

Table-4.6: Germination of artificial seeds of *T. ammi* after storage

No. of days	% Germination		
	On MS Medium + 0.1 KIN	Perl : Verm (1:1)	Soil
0 (control)	100.0 ± 0.0 [a]	100.0 ± 0.0	100.0 ±0.0 [a]
15	100.0 ± 0.0 [a]	86.0 ± 3.3	100.0 ± 0.0 [a]
30	90.0 ± 5.7 [a]	73.0 ± 3.3	83.3 ± 8.8 [b]
45	73.0 ± 3.3 [b]	50.0 ± 5.7	76.6 ± 6.6 [b]
60	50.0 ± 5.7 [b]	36.0 ± 3.3	43.3 ± 8.8 [c]
75	36.6 ± 3.3 [b]	13.3 ± 3.3	30.0 ± 5.7 [c]
90	13.3 ± 3.3 [c]	00.0 ± 0.0	6.6 ± 6.6

* means within the column followed by the same superscript letter are not significantly different ($P \leq 0.005$; Duncan's multiple range test)
# values are the mean percentage of three independent experiments

In Mulberry, the encapsulated shoot buds did not loose their capacity of germination even after their storage at 4^0 C for 45 days without nutrients (Bapat *et al* 1987). The encapsulated embryos of *Asparagus cooperi* germinated with 30.3 % to produce complete plantlets after 15 days storage, whereas eventually the conversion frequency was reduced to 14.6 % and 11.3 % after 60 and 90 days of storage (Ghosh & Sen 1994). The encapsulated embryos of *Eleusine coracana* showed 100 % germination when stored for one or two days and thereafter a marginal decrease in the germination. Clusters of encapsulated somatic embryos failed to germinate after 14 days of storage (George & Eapen 1995). For the potential application of somatic embryogenesis the frequency should be high for embryo production within a short span for the encapsulation. In *T. ammi*, on synchronization and maturation of somatic embryos, high frequency regeneration of somatic embryos was established. Almost 580-600 plantlets were obtained from one gram of callus used as inoculum within three months of culture. Hence the present system fulfils the criteria for the defined system in which embryogenesis occurs synchronously and at high frequency (Fig-7 a), for the encapsulation technology wherein not only high regeneration of somatic embryos is expected but production of desiccated, mature embryos which ensures germination after encapsulation and storage.