Chapter IV

MICROPROPAGATION OF *Syngonium podophyllum* Schott. var Butterfly

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Introduction:

Origin and distribution: *Syngonium* made up of two words syn, joined; gone, womb; the ovaries being united, belonging to the family-Araceae and includes about 20 species of tropical vines that closely resembles certain climbing Philodendrons. It is a native of tropical South and Central America, distributed from Mexico to Panama.

General description: The leaves are sagittate but becoming pedatly parted with age on long petioles with a persistent accresent sheath. Peduncles short, spathe yellowish or whitish green; tube small ovoid, persistant; with spadix shorter than the spathe. Staminate flowers with 3-4 stamens, where as pistillate flowers with oblong, ovoid 2 or abortively 1-loculed ovary. The seed is solitary in each locule, obovoid or globose, black in colour (Anonymous, 1977).

Ornamental value: *Syngoniums* are among the most sought houseplants. The houseplants belonging to different families come from varied habitats. They succeed indoor under different growing conditions, but the most successful kind are those that can be kept more or less permanently in average shade, these plants have decorative foliage rather than attractive flowers (Kindersley, 1979).

Syngoniums have normal green-variegated arrow shaped foliage, which is of ornamental value. The leaves of most *Syngoniums* are leathery and glossy, with sheathed stalks that tend to be equal in length to the leaves. In young plants, each leaf is usually undivided, though deeply lobed. Mature plants normally, have leaves divided into distinct segments. As *Syngonium* ages, it carries both types of leaves, and it also gradually develops a climbing or trailing stem. Stems of older plants get hardened and thickened to a diameter of up to 3-4 cm, and an indoor length of 6 feet. The stems of indoor *Syngonium,* therefore, can either be trained up on thin stakes pushed into potting mixture or be permitted to grow in hanging baskets. Flowers are calla lily-shaped, but are rarely

produced indoors. This tropical-American, slightly woody climbing or creeping plants contain milky juice. The leaf bearing nodes also harbour roots. There are 20 species of *Syngonium*; few of them being more preferred are listed below:

***Syngonium erythrophyllum*:** from Panama, **Copper Syngonium** daintily creeping plant with small, arrow-shaped, waxy leaves. Leaf blade is metallic coppery-green and covered with tiny pink dots and reddish beneath.

Syngonium macrophyllum: growing in the region from Mexico to Panama, **Big Leaf Syngonium** is a climber with large, heart shaped, emerald-green, fleshy leaves with velvet sheen, becoming divided during the mature stage, which is much larger than *S. podophyllum.*

***Syngonium wendlandi*:** from Costa Rica, is a creeper with tri-lobed, deep green, velvety leaves and sharply contrasting white veins in the juvenile foliage, the leaves are divided at maturity stage and plain green in colour.

***Syngonium xanthophyllum*:** origin – Mexico, it is considered to be a variety of *S. podophyllum,* the cultivated plant called **Green Gold.** The juvenile arrow shaped leaves are suffused and marbled with yellow-green. At maturity stage the foliage becomes large and dissected.

Syngonium venulosa: Climbing, branches of young plants being slender, old with shorter brownish green internodes. Leaves are broadly sagittate, adult leaves become 5-sect segmented; stalk about as long as the leaf blade.

***Syngonium augustatum*:** called **Arrow Head Vine,** a variety whose leaves change in both shape and color from the juvenile stage of the plant to its maturity. The juvenile leaves are about 3 inches long, 2 inches wide and lobed, with the large middle lobe shaped like an arrow-head, as plants age, the leaves produced are long and wide. Each leaf is divided into three or five distinct segments, and leaf colour being pale green with white mid rib in each segment.

***Syngonium auritum*:** five fingers, has fleshy, dark green leaves that are 6 inches long, 3 inches wide, and triple-lobed when the plant is young. The mature leaves are up to 15 inches long and 12 inches wide, and each leaf is composed of five separate segments. There is pair of very small opposite leaflets at the base of the leaves.

***Syngonium podophyllum*:** Arrow-Head Plant or vine usually produces medium green, rounded leaves which are 6 inches long and 4 inches wide with three deep-cut lobes when the plant is young. Mature plants produce leaves upto 12 inches long and wide divided into seven segments.

Conventional propagation: *Syngoniums* are traditionally propagated through seeds or leafless stem cuttings, depending upon the cultivars. However, most of the highly variegated cultivars must be propagated vegetatively to retain the characters.

Potting mixture and time: Equal parts of soil-based potting mixture and coarse leaf mold or peat moss are used. Repotting of each *Syngonium* is mandatory every spring, moving the plants into pots one size larger than the current pots. Alternately every spring when the roots have filled the pots, top-dress them with fresh potting mixture. These plants require 12-15 cm pot or 15-20 cm hanging basket (Anonymous, 1979; Conover et al, 1990).

Cultural Requirements:

Light: They require bright but filtered light throughout the year. The direct sunlight is harmful.

Temperature: Normal room temperatures are ideal. However, they grow best between 21° to 32° C, although night temperature can be dropped even up to 15.5°C without the loss of quality.

Irrigation: For actively growing plants, moderate water is needed, allowing the top half-inch of the potting mixture to dry-out before watering again. Irrigation

procedure is important with this plant as certain bacterial pathogens spread with overhead - applied water, splashing off from the foliage of infected plants (Anonymous, 1990).

Physiological problems in *Syngonium*: there are various physiological problems occurring in *Syngonium* like:

Water-soaked leaves:

Symptoms and Causes: Portions or entire leaves appear wet or water-soaked. This almost occurs on young leaves and occurs primarily in early morning during winter, when sunlight first strikes the foliage and warms the leaf tissue, and the potting medium is still cold. This temporary condition causes a water imbalance in the plant.

Control: Maintain root temperature at 18°C or above or increase air temperature slowly.

Long, thin internodes:

Symptoms and Causes: Internodes are elongated with wide spaces between leaves. This condition is caused from lack of sufficient light as the stems elongate more in darker places.

Control: Increased light levels are recommended.

Loss of cutting through non-pathogenic means:

Symptoms and Causes: Single node (eye) cuttings rot and not responding in the propagating beds.

Control: Selecting only healthy, mature node cuttings and place them in a well-aerated propagating medium. Maintain water and nutrients applications to stock plants at appropriate levels for the production of high quality cuttings. This problem is rare today because most of the finished product is delivered from tissue-cultivated plants (Poole et al, 1987).

Bacterial problems:

Bacterial leaf spots (*Erwina spp* or *Pseudomonas cichorii*):

Symptoms and Causes: Symptoms first appear as small water-soaked lesions, which can be translucent. Sometimes, confined between leaf-veins and other times they get exposed irregularly across the veins. The colour of the spots is usually tan to dark-brown depending upon moisture conditions and activity.

Control: Plant multiplication must be based on the use of pathogen - free cuttings or tissue cultured plantlets, since bacteria can be carried on the surface or within stems or through asymptomatic plants. Minimizing overhead water splashing is also very important, since bacteria need water to spread and infect other plants.

Erwinia cutting rot (*Erwina spp*):

Symptoms and Causes: Rapid decay of *Syngonium* occurs when cut ends getcontaminated with *Erwinia spp* or the pathogen moves from an infected leaf into stem.

Control: Use of pathogen - free cuttings is paramount.

Xanthomonas blight (*Xanthomonas compestris*):

Symptoms and Cause: Symptoms first occur on leaf margins, where the bacterium enters through hydathodes, lesions are first translucent, yellowish and water soaked and later become papery and tanned and can be surrounded by a bright yellow halo. If the plant becomes systematically infected, it will show signs of yellowing, shirting, loss of water and leaves. Eventually, these systematically infected plants die.

Control: Avoidance of this disease is the most effective control. Only limiting overhead irrigation to reduce pathogen spread and one must keep in mind that most of the commonly produced aroids *(Dieffenbachia, Agalonema and Anthurium*) are also hosts of this pathogen (Chase, 1983).

FUNGAL PROBLEMS:

Black cane rot or Ceratocytis blight (*Ceratocyctis fimbriata*):

Symptoms and Causes: The disease appears as a black, water soaked area, sometimes even girdling the stem. Leaves gradually become chlorotic and die.

Control: Hot water treatment of infected stem cuttings (30 min) at 49° C has been effective in eradicating this pathogen. Use of pathogen-free cuttings is also recommended.

Cephalosporium leaf spot (*C.cinnamomeum, Syn. Acremonium crolocinigeum*):

Symptoms and Causes: Small Lesions on leaves and petioles, reddish-brown, circular to irregularly shaped, and have a slightly yellowish border. Disease is more common where plants are grown in ground beds and exposed to rainfall or overhead irrigation.

Control: Use of systemic fungicides.

Myrotheium leaf spot and petiole rot (*M. roridum*):

Symptoms and Causes: It occurs on small plantlets. Lesion starts as small, water soaked areas, which appear greasy. These spots are generally circular and when mature contain black and white fruiting bodies on the under sides of the leaves at the petiole bases.

Control: Minimizing wounding and foliage wetting greatly reduce the severity of this disease. Additionally, avoid applications of higher than necessary amounts of fertilizer, since this can increase the plant susceptibility to the pathogen.

Rhizoctonia aerial blight and leaf spot (*R. solani*):

Symptoms and Causes: *Rhizotonia* aerial blight of *Syngonium* appears as small, irregular shaped, water-soaked lesions on lower leaves or leaf edges. Lesions are brown or reddish-brown.

Control: Since the pathogen is soil-borne, plant roots must be treated for disease control. Optional use of pathogen-free potting media (Chase et al., 1983).

Insects and mite problems: The major anthropod pest of this plant species includes aphids, moth (worms), fungus gnats, mealybugs, mites, scales, thrips and whiteflies. Mealybug, mite and scale infestation are typically the result of bringing infected plant material.

Aphids:

They are pear-shaped insects, which vary in colour from light green to dark brown, infestation may go undetected until honeydew or sooty mold is observed. They cause distortion of new growth and in extreme cases infected plants show stunted growth.

Caterpillars (Worms):

They are voracious eaters, the worms cause holes in the centre or along the edges of leaves.

Fungus gnats:

They are small black flies observed running around the soil surface or on leaves. Damage is caused by their larvae feeding on roots, root hairs, leaves in contact with the soil and the lower stem tissues (Price et al, 1989).

Mealybugs:

Appears as white, clothy mass in leaf axils, on the lower surface of leaves and on the roots. Honeydew and sooty mold are often present and infected plants become stunted. With severe infections, plant parts begin to die. For control of mealybugs soil drenching with an insecticide is recommended.

Mites:

Mites are very small and go unnoticed until plants become severely damaged. Damaged foliage begins to turn yellow or become speckled due to feeding by mites.

Scales:

Scales can be found feeding on leaves, petioles or stems. Infected plants become weakened or stunted and begin to die.

Shore flies:

They are small black flies and are frequently observed sitting on the tips of leaves or on the soil surface feeding on algae. The insect is believed to feed only on algae. Adults do not cause any direct damage but may be responsible for spreading plant pathogen as vectors.

Snails and slugs:

Damages by snails, slugs and caterpillars are similar; they are voracious feeders, with smaller stages feeding on surface tissue and larger ones eating into irregular holes in the foliage. These pests are generally nocturnal.

Thrips:

They are small thin insects feed on the leaves, which eventually become curled or distorted, with silver-grey coloured scars where feeding has occurred. They transmit the tomato spotted virus to many different ornamentals.

White flies:

Infected leaves have small yellow spots where adults or immature white flies have fed (Short et al, 1991).

Due to all these above-mentioned problems, micropropagation of *Syngonium* is mandatory for disease-free, mass propagation for ultimate commercial exploitation. The *in-vitro* raised plants have following advantage over the conventially propagated plants:

1. Production time is minimized.
2. The *in-vitro* raised plants established as plugs are usually multi-branched and in vigorous condition as received by the finished plant grower.
3. Micro-cuttings from tissue culture do benefits from bottom heat of approximately 21° C in the root-zone.

4. Many physiological problems, bacterial and fungal infections are eliminated from *in-vitro* regenerated plants.
5. Soil-borne pathogen is very much controlled due to the use of pathogen free potting media.

Florida Foliage Nurserymen (1970) listed several cultivars of *S. podophyllum* which includes **Cream, Emerald Green, Green Gold, Variegatum, White Butterfly** and *Xanthophyllum.* By 1990, the cultivar emphasis had shifted considerably to include **Compacta, Jenny, Lemon Lime, Maxima, Maya Red, Patrica, Pink Allusion, Pixie, Robusta** and **Variegatum.** Many producers consider **Compacta, Lemon Lime** and **Maxima** being essentially the same with compact, self-branching selections made from **White Butterfly** (Henley et al, 1990). Therefore, *Syngonium podophyllum* Schott., **White Butterfly** variety was selected for *in vitro* studies on multiplication.

Materials and methods:

Plant material: Mature healthy plants of *Syngonium podophyllum* Schott. **Butterfly** with beautiful varigated foliage growing in the botanical garden of the MS University of Baroda were selected (Fig 8-a). The selected shoots containing 8 to 9 nodes were cut from the parent plants.

Sterlization of explants: The single node segments with axillary buds were obtained from fast growing juvenile shoots. They were kept under running tap water (15 min). The shoots were then washed with Teepol (1%; v/v) and kept again under running tap water (20 min) similar to the sterilization procedure adopted (Misra et al, 1997). The single nodal explants of *Syngonium* were pre-treated (45 min) with a mixture containing Bavistin (1%; w/v) and Rifampcin (0.5%; w/v) on shaker (100 rpm) and then washed thrice with single distilled water. Later, they were sterilized with $HgCl_2$ (0.1%; v/v) or NaOCl for different time intervals. Different explants such as terminal and axillary buds (1.5 to 2 cm) were isolated and inoculated on MS basal medium with 3% sucrose and 0.8% agar. All these cultures were incubated at 25° C ± 1° C at 16 hr photoperiod with 50-60 μEm^2 sec^{-1} light intensity.

Initiation of cultures: Contamination free cultures were obtained on hormone free MS basal solid medium. The shoot apices were initially incubated on MS medium supplemented with high concentration of BA (44.39 μM) and then transferred to the multiplication medium. For bud sprouting, elongation and multiple shoot induction cytokinins BA and KN were used whereas for apical shoot tip culture for multiple shoot induction cytokinin BA alone or in combination with IAA was tried. As roots were induced in the multiplication medium therefore no rooting medium was used.Acclimatization and hardening procedure of regenerated plants was followed as described in Chapter 2.

Results and discussions:

Contamination problem: It was observed that there was a great difficulty in the establishment of sterile cultures. To overcome this problem the single node explants were subjected to treatment of sterilants ($HgCl_2$ or NaOCl) for various time intervals at 0.1% concentration. On comparing the effect of both the sterilants at (0.1%) for different time intervals with respect to the contamination rate (Fig 9)and number of cultures survived (Table-4.1), it was concluded $HgCl_2$ (0.1%) treatment for 6 minutes was found to be most suitable for the establishment of exenic cultures.

Table-4.1: Effect of treatment of two surface sterilants $HgCl_2$ and NaOCl (0.1%) on survival rate of explants of *Syngonium podophyllum* Schott. var Butterfly at different exposure time.

STERILANT NAME	SURVIVAL RATE AT DIFFERENT EXPOSURE TIME (min)* MEAN ± S.E.						
	½	1	2	3	4	5	6
$HgCl_2$	45 ± 17.6	30 ± 7.0	55 ± 3.5	60 ± 0.0	60 ± 14.1	60 ± 14.1	55 ± 3.5
NaOCl	65 ± 3.5	50 ± 7.0	50 ± 7.0	65 ± 10.6	55 ± 17.6	45 ± 10.6	40 ± 0.0

±S.E. represents Standard Error
*Values are the percentage means of 3 independent experiments

Seasonal effect: It is well known that season in which buds are isolated and cultured has a profound effect on their response in culture. In present studies with

Fig 8: Establishment and growth of axillary buds of *Syngonium podophyllum* Schott. var **Butterfly**.

(a) Plants growing in the Botanical garden, M S University of Baroda and selected for *in vitro* multiplication.

(b) Sprouted axillary bud on MS solid medium containing BA (2.21 μM).

(c) Elongation growth of shoots from nodal explants on MS solid basal medium supplemented with BA (8.87 μM).

(d) Bud sprouting on MS liquid medium containing BA (2.21 μM).
(Note: poor growth of shoots and leaves did not open).

(e) Maximum three multiple shoots produced by incision in the sprouted bud.

In figures, the bar = 1 cm

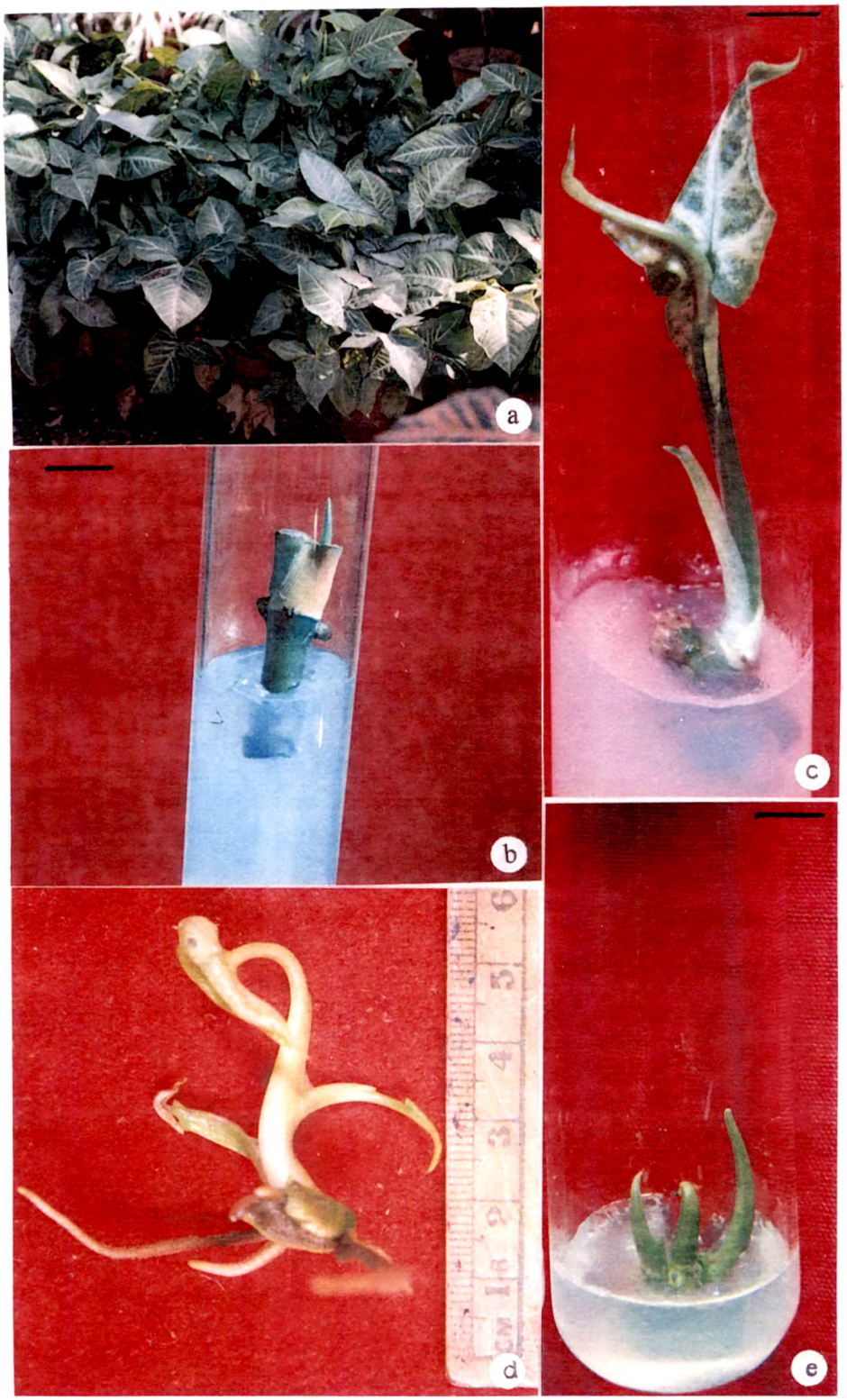
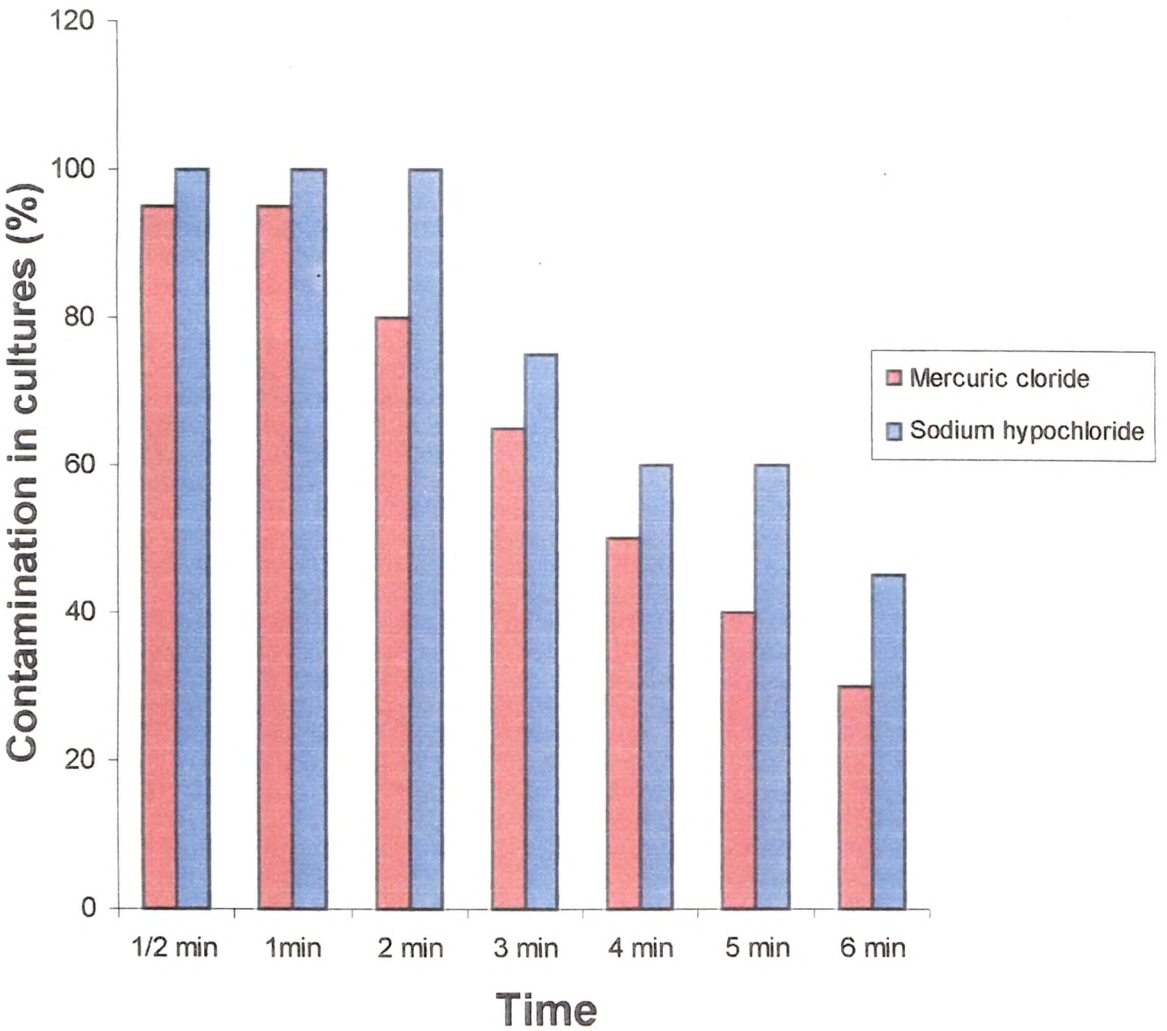

a
b
c
d
e

Fig 9: Effect on the contamination rate of the explants of of *Syngonium podophyllum* Schott. var :Butterfly by treatment of mercuric chloride and sodium hypochloride for different time intervals at 0.1 % concentration



Syngonium, seasonal effect was observed in terms of percent bud break response. As such vegetative buds are available throughout the year on this plant but remain covered within the sheathing leaf bases. Cultures were initiated from explants collected in different months of the year on MS basal medium supplemented with BA (2.21 μM). Plant material collected during August and September gave maximum response with 69% and 75 % of bud break respectively (Table- 4.2).

The response showed declining trend from October onwards. During the months of April and May the bud break response was the least (10-14%). However, this seasonal effect was not observed in the subsequent subcultures.

Table-4.2: Seasonal effect on bud break response of axillary buds of *Syngonium* on MS medium containing BA (2.21 μM) after 10 days of incubation

MONTHS	BUD BREAK RESPONSE (%) MEAN ± S.E.
January	16.3 ± 1.9
February	22.6 ± 0.9
March	13 ± 1.2
April	10.6 ± 0.9
May	12.3 ± 1.7
June	46.3 ± 6.0
July	64 ± 3.7
August	69 ± 3
September	75.6 ± 3
October	46.3 ± 5.7
November	21.6 ± 1.3
December	26.6 ± 3.5

± S.E. – Standard error

*Values are the percentage means of 3 independent experiments.

Bud sprouting: The increasing demand for the large-scale production of ornamentals can be best met by tissue culture techniques over the conventional method (Holdgate, 1997; Evans, 1990). Mass production of these plants of desired quality has

been possible through micropropagation. Apical dominance can be broken by removing the apical buds and separating the sub-tending nodes, resulting in the release of dormancy of the axillary buds. For *in vitro* propagation, the axillary bud proliferation method is frequently applied, since it is relatively simple and quite safe with reference to the appearance of variation and relatively high / rapid rate of propagation (Woodward and Thompson, 1996).

The maximum bud break induction occurred on agar-based BA supplemented MS medium (Table-4.3).

Table-4.3: Comparison of liquid and solid MS medium supplemented with cytokinin (BA / KN) for bud-break response in *Syngonium podophyllum* Schott. var Butterfly Data recorded after 12 days of incubation.

CYTOKININS (μM)	BUD SPROUTING (%) MEAN ± S.E.	
	AGAR BASED SOLID MEDIUM	LIQUID MEDIUM
Control	-	-
BA 0.44	23.3 ± 2.7	48.8 ± 6.9
1.10	60.4 ± 9.7	28.9 ± 1.9
2.21	64.4 ± 8	58.2 ± 5.8
4.43	70.0 ± 4.1	66.9 ± 2.6
8.87	16.5 ± 6.8	63.8 ± 2.2
KN 0.46	10 ± 5.7	37.1 ± 7.9
1.16	23.3 ± 7.2	49.0 ± 4.0
2.32	60.0 ± 4.7	59.0 ± 4.6
4.64	46 ± 5.4	51.6 ± 5.1
9.29	20 ± 12.4	50.2 ± 5.3

± S.E. – Standard error

* Values are the mean percentage of three independent experiments

SOURCE	df	SUM OF SQUARES	MEAN SQUARES	F RATIO	SIG.**
Between groups	4	12.8509	3.2127	18.9252	.000
Within groups	198	33.6122	0.1698		
Total	202	46.4631			

(ANOVA TEST)

**Significant at 0.05 levels (probability is ≤ 0.05).

It took 10-12 days of incubation for bud-break and resumption of growth from nodal explants. BA (2.21 μM) was found to be better (Fig 8-b) as compared to the other cytokinin KN tested for bud break response. The solid media responded better compared to the liquid medium with both the cytokinins tested. More precisely the liquid medium did not favour the opening of the leaves and further growth (Fig 8-d).

Elongation and growth: The highest concentration of BA (8.87 μM) tested (Table-4.4) produced maximum 3-4 leaves with a growth of 4.5 ± 0.2 cm length within 2 weeks (Fig 8-c). When BA was tried with addition of low concentration of auxin to the same medium, was unable to give enhanced response for multiple shoot induction and their growth, compared to BA tried alone. The cytokinin-BA has been used for initiation and growth of species like *Catalpa ovata* (Lisowska and Wysokinska, 2000) and *Centalla asiatica* (Tiwari et al, 2000).

Table-4.4: influence of cytokinins (BA / KN) on the growth and elongation of *Syngonium podophyllum* Schott. var: Butterfly. Data recorded after 15 days of incubation.

CYTOKININ (μM)		NUMBER OF LEAVES / PLANT* MEAN ± S.E.**	PETIOLE LENGTH (cm)* MEAN ± S.E.**
Control		1.8 ± 0.2	3.9 ± 0.4^{ab}
BA	0.44	1.4 ± 0.15^{ab}	4.5 ± 0.2^{c}
	1.10	1.4 ± 0.15^{ab}	3.7 ± 0.5^{a}
	2.21	2.1 ± 0.2^{b}	4.0 ± 0.3^{b}
	4.43	2.0 ± 0.1^{a}	3.9 ± 0.3^{ab}
	8.87	3.7 ± 0.2	4.5 ± 0.2^{c}
KN	0.46	1.5 ± 0.1^{a}	3.8 ± 0.4^{ab}
	1.16	1.4 ± 0.1^{a}	4.0 ± 0.3^{b}
	2.32	1.7 ± 0.2^{b}	4.4 ± 0.2^{cb}
	4.64	1.4 ± 0.2^{ab}	4.5 ± 0.3^{c}
	9.29	2.1 ± 0.2^{b}	4.3 ± 0.3^{cb}

± S.E. – Standard error

* Values are the mean percentage of three independent experiments

**Means within a column followed by the same superscript letter are not significantly different ($p \leq 0.05$; Duncan's Multiple Range test).

Multiple shoot induction: The rate at which cultures grow and multiply during micropropagation is influenced by the physical nature of the growth medium. The most common support for plant tissue cultures is agar-solidified medium. The advantages of using solidified media are well known (George, 1993).

1. Gels or solidifying agents provide a support to tissues growing in static conditions.
2. Agar gels do not react with media constituents.
3. They are not digested by plant enzymes.
4. Remain stable at all feasible incubating temperatures.

In order to get number of multiples the incubated sprouted buds were given incisions. The incisions made with sterile blade were of two types:

- ⇨ Single vertical incision in the plane of bud (E1) ()
- ⇨ Two vertical incision intersecting each other in the bud (E2) ()

Buds with two types of incisions were transferred to fresh medium supplemented with BA (2.21 μM). The buds with E2 incision proved better than E1 type of incision for the induction of multiple shoots and gave maximum 3 multiples (Table-4.5) on transferring to BA (2.21 μm) containing MS medium (Fig 8-e). The E1 type of incision could produce only two maximum numbers of multiples.

Earlier, Kang et al (1994) also reported multiple shoot induction with low concentration of BA in *Fragaria ananassa*. However, the results indicated that higher concentration of BA were inhibitory to the shoot production. Similarly, Hu and Wang (1983) also reported reduction in the number of shoots with high concentration of cytokinin.

Table-4.5: Effect of Multiple shoot induction on the type of incision made on the sprouted nodal explants on semi solid medium containing BA (2.21 μM) in *Syngonium podophyllum* Schott. var Butterfly

TYPE OF INCISION	NO. OF MULTIPLES* MEAN± S.E.
C	-
E1	2.0 ± 0
E2	3.3 ± 0.2

C- No incision made
*Values are the mean percentage of three independent experiments

Apical shoot tip culture: It was not until the early 1970's that the potential of shoot meristem as reproductive material was realized with reports of the successful culture of the herbaceous strawberry (Boxus, 1974) and the ornamental *Gerbera jamesonii* (Murashige et al, 1974). The growing apices of plants show potential for unlimited growth because of the presence of apical meristems. This technique of shoot tip culture has been applied for many ornamental foliage species such as *Cordyline, Dracaena, Scindapus, Syngonium* with great success (Miller and Murashige, 1976). Shoot tip culture technique has the advantage of producing genetically stable and true-to-parent type plantlets. Further, as meristems are supposed to be pathogen free, the plants produced by meristem culture are pathogen free which have been confirmed by adequate indexing (Boxus, 1979; Stone, 1978).

The isolated white-yellowish coloured shoot-tip (Fig 10-a) took a week's incubation in the culture, to become green (Fig 10-b). Induction of multiples was observed after greening of the meristem only. Lack of greening lead to the stunted growth and ultimately senescence of the explant. The induction of multiple shoots from the exposed shoot apices with a stem base (3 to 4 mm) along with 2 to 3 mm, thin fragile pale yellowish pointed exposed meristem was observed on the 7th day onwards of their inoculation.

Prior to the multiplication stage, the shoot apices were initially incubated on MS medium supplemented with high concentration of BA (44.39 μM) and then transferred to the multiplication medium. This may be due to the fact that as the shoot tips are already rich in auxin, it required exogenous supply of high cytokinin in the medium for multiple shoot induction. A continuous presence of cytokinin in the medium is utmost important for the formation of new shoots (Nordstorm and Eliasson, 1986). When cytokinin treated shoot tips were transferred to the semi-solid MS medium supplemented with BA alone or in combination with IAA (Table-4.6) for multiple shoots, the maximum number of shoots (6.1 ± 0.3) were produced on IAA (1.42 μM) and BA (33.29 μM) after 21 days of incubation (Fig 10-c). In contrast, the maximum length of multiples (0.94 ± 0.1) was observed on the medium containing IAA (1.42 μM) with less concentration of BA (11.6 μM). BA alone in MS medium could not produce satisfactory results. Initially, the highest number of multiples (2.5 ± 0.1) was observed after incubation for a week on MS medium with IAA (1.42 μM) and BA (11.1 μM). However, after 3 weeks of incubation, the number of multiples produced was directly propotional to the concentration of BA in the medium. Contrastingly, shoot multiplication through shoot tip culture of *Helianthus annuus* resulted in rosette shoots with poor survival, when BA concentration was increased (Paterson, 1984). Similarly, in *Catalpa ovata* for shoot multiplication, the shoot tips were incubated on Shenk and Hilderbrandt media containing BA alone or in combination with IAA (Lisowska and Wysokinska, 2000).

After 3 weeks, the maximum number of multiples generated from the apical shoot was 9 on MS medium supplemented with IAA (1.42 μM) and BA (33.29 μM) with almost 2.2 cm elongation growth. At every 12 to 15 days interval, these multiples (Fig 11) were further subcultured to MS medium containing same level of IAA with reduced level of BA without agar (Fig 10-d). After 5 subcultures, maximum 123 multiple shoots were produced on solid medium (Fig 10-e) compared to only 12 shoots in the liquid medium. Thus solid medium proved to be better for shoot multiplication from the apical meristem.

Fig 10: Apical bud culture of *Syngonium podophyllum* Schott. var **Butterfly**.

(a) 2-3 mm, thin fragile pale yellowish apical bud isolated for establishment.

(b) Greening of the apical bud after a week of inoculation.

(c) Multiple shoots (6 in number) produced on MS solid medium supplemented with IAA (1.42 μM) and BA (33.29 μM) after 21 days of incubation. (Note: root induced in the same medium).

(d) Multiple shoots produced on MS liquid medium after 5 subcultures (each subculture after 21 days) containing IAA (1.42 μM) and BA (33.29 μM). (Note: root produced in the same medium).

(e) Multiple shoots produced on MS solid medium containing IAA (1.42 μM) and BA (33.29 μM) after 5 subcultures.

In figures, the bar = 1 cm


a
b
c
d
e

Fig 11: Influence on the rate of the multiplication of shoots derived from apical meristem of Syngonium podophyllum Schott. on MS basal medium containing BA (1.42 mM) with and without agar after five subcultures.

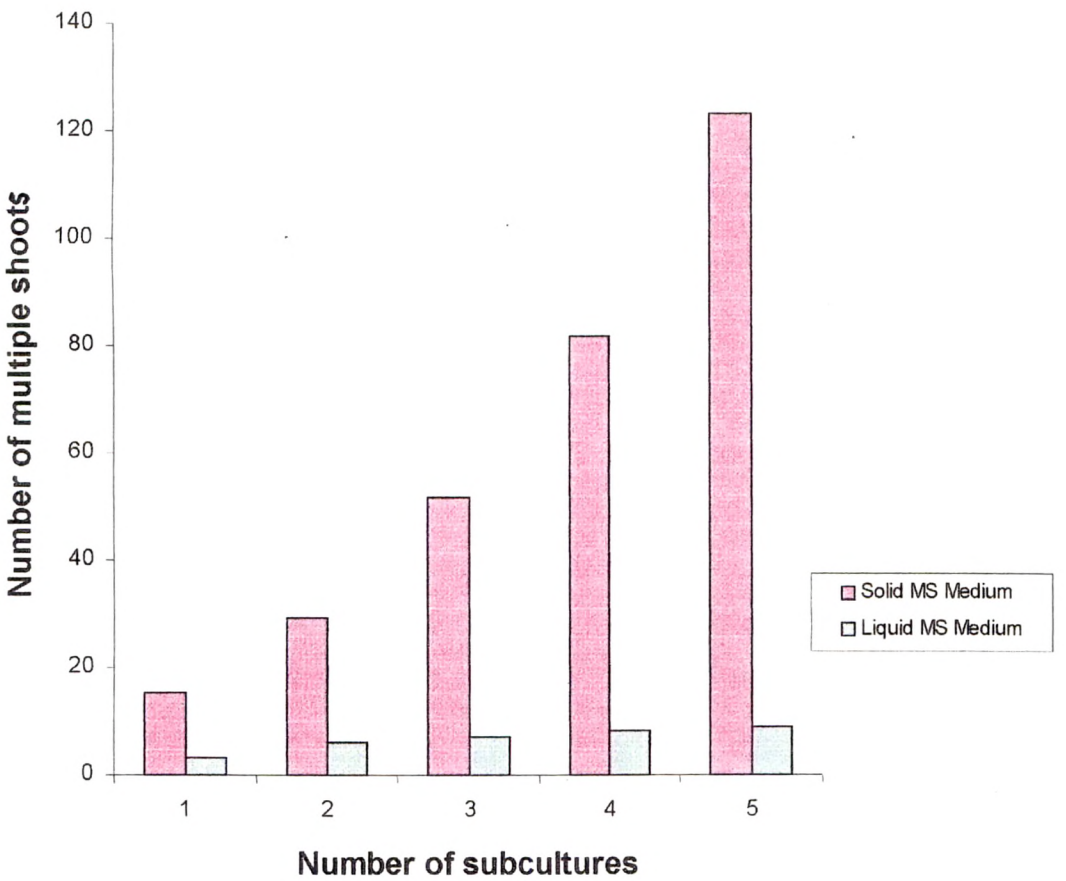


Table-4.6: Influence of BA alone or in combination with IAA on apical meristem for multiple shoot induction after 1st, 2nd and 3rd week of incubation on MS medium in *Syngonium podophyllum* Schott. var: Butterfly

GROWTH REGULATOR (μM)		7 DAYS		15 DAYS		21 DAYS	
		Number of multiples Mean±SE	Length of multiples (cm) Mean±SE	Number of multiples Mean± SE	Length of multiples (cm) Mean±SE	Number of multiples Mean±SE	Length of multiples (cm) Mean ± SE
BA	IAA						
1.42	-	-	-	-	-	-	-
2.21	-	0.3 ± 0.2	0.4± 0.16	1.3 ± 0.3	0.35±0.05	1.5± 0.25	0.46± 0.05
4.43	-	-	-	0.75± 0.29	0.3 ± 0.1	1.1 ± 0.2	0.6 ± 0.1
8.87	-	-	-	1.7± 0.1	0.47± 0.06	1.6 ± 0.2	0.5 ± 0.08
4.43	1.42	1.3 ± 0.2	0.24± 0.04	1.8 ± 0.1	0.64± 0.07	2.2 ± 0.1	0.7 ± 0.07
11.6	1.42	2.5 ± 0.1	0.28 ± 0.04	2.8 ± .1	0.6 ± 0.06	3.2 ± 0.2	0.94 ± 0.1
22.2	1.42	1.5 ± 0.2	0.23 ± 0.09	2.8 ± 0.2	0.8 ± 0.09	5.3 ± 0.7	0.9 ± 0.09
33.29	1.42	0.5 ± 0.2	0 ± 0	1.25 ± 0.2	0.1 ± 0.8	6.1 ± 0.3	0.36 ± 0.12

± S.E. – Standard error
- Indicates no response
*Values are mean of three independent experiments.

Root induction: Root induction was achieved in the same medium, which induced shoot formation with all the concentrations of BA individually and in combination with IAA tried. The root induction was observed after 7th day of incubation and the growth of roots occurred simultaneously with the shoots. This is because of the fact that axillary explants always harbour one root primordium. Healthy and sturdy growth of roots was noted when well grown plantlets were transferred to plastic net-pots.

Acclimatization and field transfer of the plants: The plantlets growing in net pots (2 cm diameter) in a tray containing vermiculite, perlite and sand (2:1:1; v/v) covered with polythene bag (Fig 12-a), showed healthy growth. Regular

irrigation and exposure of plantlets outside culture conditions (Fig 12-b) easily helped them to adapt to the field conditions resulting in high survival rate of plantlets (90%). Plants showed vigorous and sturdy growth similar to their parent plant after nine months of their field transfer to botanical garden in the earthen pots containing a mixture (2:1:1; v/v) of garden soil, compost and sand (2:1:1; v/v) (Fig 12-c).

Growth comparison of *in vitro* and *in vivo* raised plantlets: The morphological appearance of tissue-cultured plantlets was compared with conventionally propagated plants after 9 months of field transfer. The leaves were arrowhead-saggitate. The attachment of the petiole to the leaf midrib and the shape was such that it gives an appearance of two lobes at the back. The maximum number of leaves produced in regenerated plants were 11.75 $\pm$ 1 with 19.7 $\pm$1.79 petiole length, compared to 12.8 $\pm$ 1.20 number of leaves with 20.7 $\pm$ 2.3 cm petiole length. The length and the breadth of the leaves and the growth pattern were found to be similar with the parental stock (Table-4.7). Statistically also the characters for both the number and size (petiole length, length and breadth) of leaves of regenerated and parental stocks were significantly similar indicating that in vitro raised plants were true-to-type to the parental stock and clonally propagated.

Table 4.7: Comparison of *in vitro* raised plants and conventionally propagated plants of *Syngonium podophyllum* Schott. var: Butterfly, after 9 months of their field transfer.

TYPE OF PLANTS	NUMBER OF LEAVES / PLANT* MEAN $\pm$S.E.	PETIOLE LENGTH(cm)* MEAN $\pm$S.E.	LENGTH OF LEAVES (cm)* MEAN $\pm$S.E.	BREADTH OF LEAVES(cm)* MEAN $\pm$S.E.
In vivo raised	12.8 $\pm$ 1.2	20.7 $\pm$ 2.3^{a} **	11.3 $\pm$ 0.8^{b} **	7.9 $\pm$ 0.5^{c} **
In vitro raised	11.75 $\pm$ 1.0	19.7 $\pm$ 1.7^{a} **	11.2 $\pm$ 0.7^{b} **	6.2 $\pm$ 0.3^{e} **

$\pm$ S.E. – Standard error
*Values are the mean percentage of three independent experiments.
**Means within the column followed by the same superscript letter are not significantly different ($p < 0.05$, Fisher-Behranes test)

Fig 12: Acclimatization of regenerated plantlets of *Syngonium podophyllum* Schott. var **Butterfly**.

(a) Plantlets in the plastic pots covered with polythene sheets containing vermiculite perlite and sand (2:1:1).

(b) Hardened plants in the plastic pots.

(c) Plants in the earthen pots (8 cm diameter) after 2 months of their transfer to the Botanical garden.


a
b
c

From the apical shoot tip culture on an average six shoots were produced on the multiplication medium containing BA and IAA after 21 days of incubation. These shoots were subsequently subcultured 5 times each of 21 days duration increasing approximately 1 ½ times the original number of shoots after each subculture. Total 123 shoots were obtained after 5 subcultures. These shoots rooted in the same multiplication medium from the axils and later acclimatized (15 days) for field transfer. Thus within 4 months' cycle 123 shoots were produced from a single shoot tip.

Flow chart (Fig 13) for the clonal multiplication of *Syngonium podophyllum* Schott. var **Butterfly** is included in the following page:

Fig 13: Flowchart for clonal multiplication of *Syngonium podophyllum* Schott. var Butterfly.