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IN VITRO RAPID MULTIPLICATION OF *SOLANUM TRILOBATUM* L. FROM SHOOT TIP EXPLANT

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ABSTRACT

An efficient protocol was developed for *in vitro* regeneration of *Solanum trilobatum* (L.) is a perennial deciduous armed shrub belonging to the family solanaceae. Shoot tip explants of *Solanum trilobatum* were cultured on MS basal medium supplemented with different concentrations of BAP and KIN ranging from **0.5 - 2.5** µM/L for multiple shoot induction. The two cytokinins tested, BAP was found to develop in shoot multiplication and higher number of shoots from the shoot tip explants when compared to KIN. Higher number of shoots was produced from all the concentrations of both BAP and KIN. The highest frequency (**100%**) of shoot induction and maximum number of shoot (**8.4±1.51**) was observed on 2.0 µM BAP with shoot length of **4.94±0.20 c.m**. in KIN **1.5** µM/L to produce maximum number of shoots (**6.4±1.81**) in the shoot length of **4.66±0.37** and the shoot induction frequency is **100%.** The isolated shoots were transferred to MS basal medium supplemented with different concentrations of IBA and NAA ranging from 0.5 - 2.5 µM/l for root induction. The rooted plantlets were successfully transferred in soil through hardening and established in the field.

KEYWORDS: Micropropagation, *Solanum trilobatum*, BAP, IBA.

INTRODUCTION

Taxonomic classification of *Solanum trilobatum L.*

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus: *Solanum*

Species: *Solanum trilobatum* L.

Synonyms:

SynonymSolanum canaranum Miq.

Solanum fuscum B.Heyne ex Wall., nomen nudum

Solanum griffithii (C.B.Clarke) Kuntze

Solanum hainanense Hance

Solanum maingayi Kuntze

Solanum miyakojimense T.Yamaz. & Takushi

Solanum procumbens Lour.

Solanum prostratum Raeusch., nomen nudum

Solanum sarmentosum Nees

Solanum trilobatum var. griffithii C.B.Clarke

Common Names :

Kannada : Kakamunji,Ambusondeballi

Malayalam:

Tudavalam,Mothirangani,Puttacunta,Tutavalam,Thothuvala,Putricunta,Putharichunda

Marathi : Mothiringnee,Thoodalam

Oriya : Bryhoti

OthersThai : Nightshade,Purple Fruited Pea Eggplant,Thoodhuvalai

Sanskrit : Agnidamini,Achuda,Agnidamani,Vallikantakarika,Alarka

Tamil : Sandunayattan,Nittidam,Surai,Tuduvalai

Telugu : Mullamusti,Alarkapatramu,Kondavuchinta

Nature has a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. The World Health Organization (WHO) has also recommended the evaluation of plants for effectiveness against human diseases and for the development of safe modern drugs.[1] *Solanum trilobatum* Linn (Family: Solanaceae), a thorny creeper with bluish white

flower and grows as a climbing under shrub. It is one of the important medicinal plant, more commonly available in Southern India and has been used in herbal medicine to treat various diseases like respiratory problems, bronchial asthma and tuberculosis.[2] This plant is well known in Ayurveda and Siddha systems. In Sanskrit it is known as 'Alarka', in Telugu 'Alarkapatramu', in Tamil 'Tuduvalai' and in Malayalam 'Tutuvalam'. The roots, berries and flowers are used for cough.[3]

Botanical description

Solanum trilobatum Linn (Solanaceae), the nightshade, (order Solanales), with 102 genera and nearly 2,500 species. It is a prickly diffuse, bright green perennial herb, woody at the base, 2–3 m height, found throughout India, mostly in dry places as a weed along roadsides and waste lands. The plant having much branched spiny scandent shrubs. Leaves are deltoid or triangular, irregularly lobed. Flowers are purplish-blue, in cymes. Berry are globose, red or scarlet.

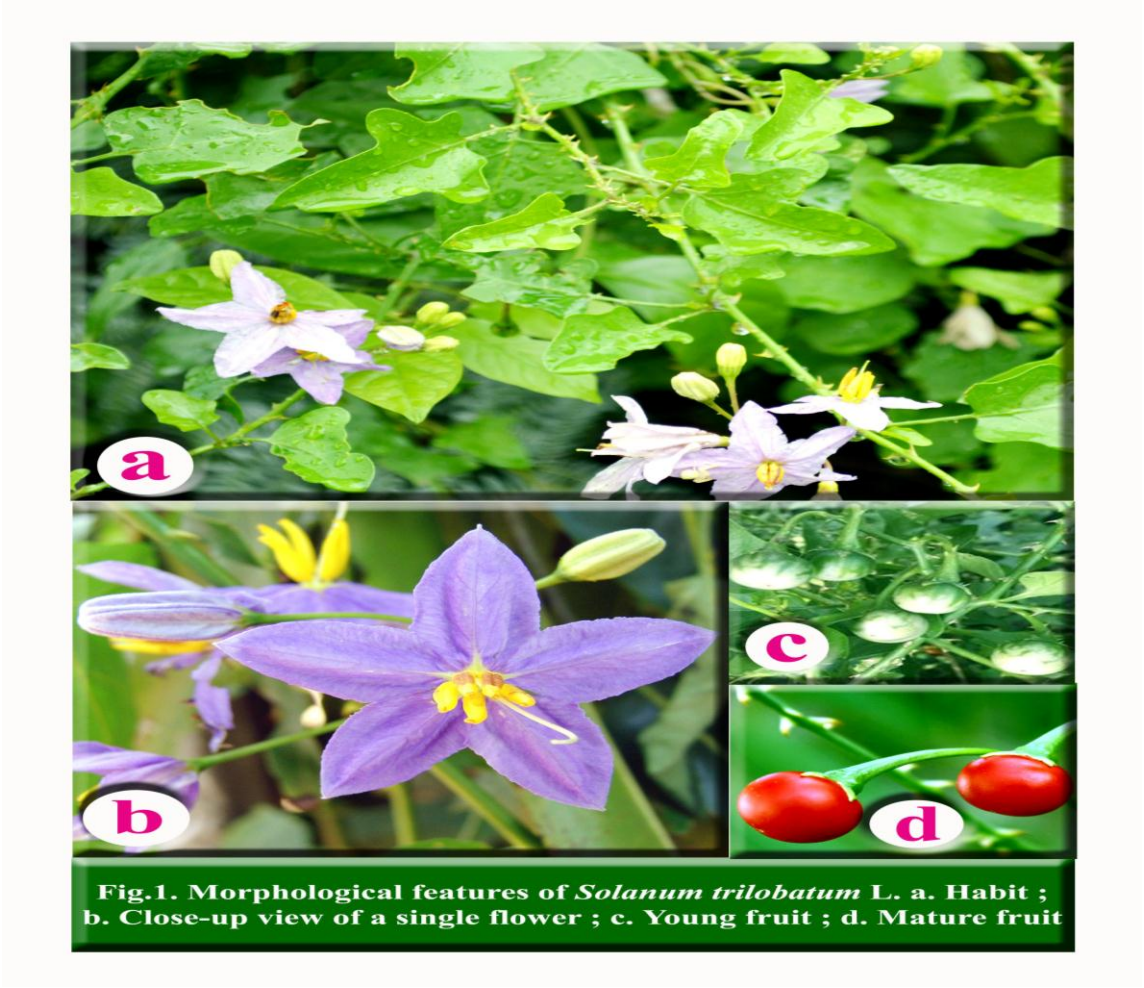

Fig.1. Morphological features of Solanum trilobatum L.

Traditional Uses

Plants are playing an important role in the health of millions of people's life in many villages of India in their day to day life by its traditional usage. *S. trilobatum* is reported to cure numerous diseases viz., respiratory problems and bronchial asthma. S. trilobatum was reported to harbour hepatoprotective activity, antimicrobial activity, larvicidal activity, antidiabetic activity, cytotoxic activity and anticancer activity. The leaves and stem of *S. trilobatum* are reported to possess antimitotic, anti-inflammatory and anti-ulcerogenic properties. The leaf extracts are used to increase male fertility and to cure snake poison.[4] It is used with ghee in siddha for treating tuberculosis, as decoction in case of acute and chronic bronchitis, root and berries for treating cough.[5] The major alkaloids identified in the alcoholic extract from leaves and stem part of S. trilobatum has been shown to possess antimitotic and antimicrobial activity against bacteria and fungi. Biological screening of the alkaloid mixture of this plant revealed anticancer activity against certain type of cancer and its effectiveness as an adjuvant in cancer chemotherapy.[6]

Distribution

The Plant *Solanum trilobatum* L. spread over in throughout India, growing wild.

Medicinal Properties

Plant pacifies vitiated pitta, kapha, cough, bronchitis, dyspnoea, anorexia, worm infestation, skin diseases, hemeplegia, edema, urinary calculi, amenorrhea, and urinary tract disorders. Whole plants are used for many medicinal purpose.[7,8]

Phytochemical Studies

Phytochemical screening of various extracts such as chloroform, ethanol, water of *S. trilobatum* revealed the presence of secondary metabolites such as Steroids, triterpenoids, sugars, Reducing sugars, phenolic compounds, tannins, anthroquinone, amino acids, Saponins.[9] Phytochemical analysis of dried powder of *S. trilobatum* leaves showed the presence of carbohydrates, saponins, phytosterols and tannins, where as the stem portion possess carbohydrates, saponins, phytosterols, tannins, flavonoids and cardiac glycosides. Alkaloides such as soladunalinidine and tomatidine(4) were isolated from the leaf and stem of Solanum species. *S. trilobatum* contains chemical compounds like Sobatum(1), β-solamarine, solasodine(2), solaine(3), glycoalkaloid and diosogenin(5).[10]

Micropropagation

Micropropagation is referred to as the true-to-type clonal propagation of any selected genotype under the in vitro condition by plant tissue culture technique. It is often associated with mass production of plants at a competitive price. This is one of the many plant tissue culture techniques wherein plants can be produced either through organogenesis or somatic embryogenesis at large scale. Practically any part of a plant can be induced to regenerate into complete plant under in vitro condition. They can be multiplied and rooted under in vitro or *ex vitro* condition or can be made into artificial seeds for automated sowing of these propagules under natural conditions.

These new aseptic propagation methods are reliable and present a new tool in the plant propagation industry for those with the inclination to use them. Grains with increased yield, trees with better form and faster growth, plants with disease resistance, uniform crops able to be harvested at an optimum time, plants with known characteristics that are able to be better marketed are the advantages accrue as the advance of technology goes on.

Micropropagation provides a fast and dependable method for production of large quantity of uniform plants in a short time throughout the year. This technique ensures round the year propagation with high multiplication rate that could be utilized in scaling up the production at commercial level. Hitherto plants impossible to propagate are now being done with ease.

Endangered species are being proliferated and taken off the 60 endangered list even though they are no longer found in their native habitat. Clonal propagation through tissue culture, popularly called, micropropagation, can be achieved in a short time and space. Thus, it is possible to produce plants in large numbers from a single individual. Use of plant tissue culture for micropropagation was initiated by Morel (1960) [11] who found that this is the only commercially viable approach for orchid propagation. Micropropagation technology owns unique distinction as the quick and easy method of deriving plants with identical genetic constitution.[12],[13] It has a significant impact on plant breeding, horticulture and medicine. This technique is an alternative method of propagation as there is an increase in the propagation rate of plants, availability of plants throughout the year, protection of plants against pests and pathogens under controlled conditions and the availability of uniform clones and uniform production of secondary metabolites.[14] Uniformity in the plantlets obtained through micropropagation technique and the year round availability of the plantlets surpassing field dormancy make tissue culture technique as attractive alternative to the

conventional methods of propagation. Somaclonal variations are observed in the plantlets when they are regenerated from the callus but when plants are regenerated from apical or axillary buds or meristems, these are morphologically almost identical among themselves and also the parent plant. The *In vitro* morphogenic processes are usually affected by physical environment, growth regulators,[15], [16] carbon source,[17],[18] gelling agent[19],[20] and explant type and origin.[21],[22] It is well known that cytokinins suppress the growth of apical meristems and instead induce excess formation of lateral meristems, resulting in multiple shoots. The cytokinin–auxin combination has also been used widely for shoot regeneration in various protocols.[23],[24],[25],[26], [43],[44],[45],[46],[47]

In recent years, several diseases and microbial infections such as respiratory infections, bacterial meningitis more developed population increased day by day. Plants have been an integral part of human civilization. Medicinal plants have also been relied upon by over 80% of the world population for their basic health care needs.

Medicinal plants have attracted global interest as they constitute a rich treasure improve of cultural information and are source of natural products, which provides health security to millions to in rural communities. Nowadays has been renewed interest in natural medicines that are obtained from plant parts or plants extracts. Nearly 40% or more of the pharmaceuticals currently used in Western countries are derived or at least partially derived from natural sources.

Indiscriminate exploitation coupled with lack of attention to the development of cultivation practices has resulted in considerable depletion of the wild stock of many medicinal herbs. Preservation of germplasam collections and micro propagation of economically important plants are of utmost important.

Traditional healers found more medicinal plants which are highly effective to treat various diseases and also it is necessary to prove scientifically in order to develop new drug molecules.[27] *Solanum trilobatum* Linn (Family: Solanaceae) is one of the important medicinal plant, more commonly available in Southern India and has been used in herbal medicine to treat various diseases like respiratory problems, bronchial asthma and tuberculosis.

Micropropagation is a plant tissue culture technique used for producing plantlets and implies the culture of aseptic small sections of tissues and organs in vessels with defined culture medium and under controlled environmental conditions and has become an increasingly important tool for both science and commercial applications in recent years.

Increased demand due to medicinal properties and depletion of natural sources has initiated the development of plants through micropropagation,.[28] Only limited success has been reported for *in vitro* micropropagation and organogenesis of *S. trilobatum*,.[29] There are only a few reports on *S. trilobatum* for micropropagation to propagate plants from leaf and node explants. Increasing human and livestock populations have already affect either status of wild plants, particularly those used in herbal medicine. In this present study an attempt was made to standardize the protocol for micropropagation of *S. trilobatum* by using different plant growth regulators and to evaluate the activity from *in vitro* growing plants.

MATERIALS AND METHODS

Source of Explants

The field grown *Solanum trilobatum* L. (Solanaceae) was selected for the source of explants in the present Study. Shoot tip regions with axillary bud of two weeks older plants were used as explants for micropropagation.

Culture Medium

The nutrient medium consists of inorganic salts, carbon source and organic supplements. In addition, vitamins and growth regulators are also added to the medium. In the present study, the basal medium consists of the mineral salts and organic nutrients of Murashige and Skoog (MS) salts with B5 vitamins are used. For convenience, throughout this chapter, MS medium with MS salts plus B_5 vitamins is being referred as MS medium. The basal medium is supplemented with various concentrations and combinations of different growth regulators.

Growth regulators

The prwsent study the hormone concentration was used in µM (micromole). These growth regulators were used as supplement to the basal medium individually as well as in different combinations.

Cytokinins : **BAP** (6-benzylaminopurine)
KIN (6-furfurylaminopurine)

Auxins : IBA (Indole butyric acid)
NAA (Naphthalene acetic acid)

The present study was performed in the basal medium with MS salts, B5 vitamins, 3% sucrose and 0.8% agar.

The basal medium was variously supplemented with factorial combinations of different growth regulators ranging from 0.5 – 2.5 µM/L BAP or KIN alone for shoot multiplication or in combination of both the cytokinins. After adding all the supplements (various concentrations of different hormones) to the basal medium, the pH of the medium was adjusted to 5.8. The molten medium was dispensed in culture tubes or culture bottles or conical flasks and was capped with cotton plugs.

Sterilization of Culture Medium and Glassware's

The culture medium containing high concentration of sucrose supports the growth of several microorganisms. These microbes generally grow much faster than the explants and finally spoil the culture. So it is very essential to maintain a complete aseptic environment inside the culture tube. Therefore, the culture medium, glassware's, forceps and scalpels was sterilized by autoclaving at 1.06 kg cm^{-2} and 121°C for 15 min. As well as the same procedure to follow the sterilization of. During this period much care was taken to avoid denaturation of growth regulators and vitamins that were incorporated into the medium. The culture tubes left free until agar in the medium become solidified. Then the tubes were transferred to inoculation chamber for inoculation.

Sterilization of explant

The explants were taken from the field grown mature plants. The explants consisting of the nodal regions with axillary bud were surface sterilized by rinsing in running tap water for 30 minutes. Then they were washed in an agitated solution of liquid detergent (Teepol) for 5 minutes and followed by distilled water for 2-3 times for removing the traces of liquid detergent. After thorough washing, the materials were taken in to the Laminar Flow Chamber where they were disinfected with 70% alcohol for 30-60 seconds followed by 0.1% mercuric chloride for 3-5 minutes. Finally, the materials were thoroughly rinsed with sterile distilled water for 4-5 times to remove the traces of mercuric chloride.

Inoculation Procedure

Before starting inoculation all the requirements such as culture tubes, containing media, spirit lamp, sterile water, glassware and explants, were placed in the laminar air flow chamber. The platform surface of the chamber was swapped with 70% alcohol. After swapping the chamber with 70% alcohol, the UV light was switched on for 30 minutes. After 30 minutes, the UV light was switched off and the white fluorescent light was switched on. Before inoculation, hands were rinsed with absolute alcohol. The instruments were sterilized by dipping in absolute alcohol followed by flaming and cooling.

The inoculation was carried out in the vicinity of flame. The surface sterilized explants were aseptically transferred to the respective culture media in the Laminar Flow Chamber. The explants were taken out from beaker and at the same time the cotton plug of the culture tube was slightly opened in front of the spirit lamp flame, the explant was put it the medium and immediately covered with cotton plug. The explants with nodal regions were inserted in the medium vertically. Cultures were transferred to fresh media with the same hormone concentration at 4 week intervals.

Culture Conditions

The cultures were maintained in a culture room at 25±2°C under 16 hr photoperiod with a light intensity of 30-40 µM m^{-2} s^{-1} supplied by cool white fluorescent tubes. These growth conditions were referred to as standard culture conditions for *in vitro* studies.

Culture Maintenance

The nodal explants regions, were initially cultured on MS solid medium in test tubes. After 4 weeks, the initiated shoot multiples were subcultured on MS basal medium fortified with the same growth regulator concentrations and combinations or whichever is the best for further multiplication. To facilitate higher number of shoot formation, the explants were also subcultured on conical flasks and/or culture bottles which can provide more space and more medium for growth and multiplication.

Rooting

In vitro raised shoots of 2 cm and above were excised from the culture tube or culture bottle and subcultured into MS medium fortified with 3% sucrose (w/v) and 0.8% agar (w/v). The medium was further supplemented with different concentrations (2.0-10.0 µM) of IAA, IBA or NAA. The root number and length were measured in each culture medium.

Hardening and Acclimatization

Plantlets with well-developed roots were dislodged from the culture medium and roots were washed gently under running tap water to remove the adhering medium. Plantlets were transferred to plastic cups (10 cm diameter) containing autoclaved garden soil, farmyard manure and sand (2:1:1). Each plantlet was irrigated with distilled water every 2 days for 2 weeks followed by tap water for one week. The potted plantlets were initially maintained under culture room conditions (3 weeks) and later transferred to normal laboratory conditions (2 weeks).

The potted plantlets were initially covered with porous polyethylene sheets to maintain high humidity and were maintained inside the culture room. The relative humidity was reduced gradually. After 30 days the plantlets were transplanted to the field under shade for 3 weeks and then transplanted to the soil for further growth and development.

Experimental Design, Data Collection and Statistical Analysis

The design of all the experiments was a complete randomized block and each experiment consisted of five explants per flask and five replicate culture flasks per plant growth regulator treatment. The parameters recorded were frequency (number of cultures responding in terms of multiple shoot proliferation and root development), number of shoots per explant, shoot length, number of roots per shoot, root length and survival rate (%). All of the experiments were repeated five times. The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance.[16]

RESULTS AND DISCUSION

Shoot tip explants of *Solanum trilobatum* L. were cultured on MS basal medium supplemented with different concentrations of BAP and KIN of both these cytokinins ranging from **0.5 - 2.5** µM/L for shoot multiplication. Multiple shoots were initiated within 12 days of inoculation. Maximum number of shoots was observed in 20-30 days. The data in respect of shoot induction frequency, number of shoots and length of shoots on different concentrations of each hormone on nodal explants were presented in **Table 1 and Fig.2**.

Table 1. Effect of different concentrations of cytokinins on shoot induction from the shoot tip explants of *Solanum trilobatum L.*

BAP	KIN	Percentage of response (%)	Number of shoots	Shoots length
0.5 µM	--	80	5.4±1.14	3.62±0.32
1.0 µM	--	85	5.2±1.64	3.96±0.59
1.5 µM	--	90	5.8±1.92	4.32±0.55
2.0 µM	--	100	8.4±1.51	4.94±0.20
2.5 µM	--	85	6.2±1.30	4.48±0.44
--	0.5 µM	85	6.2±2.38	3.98±0.21
--	1.0 µM	90	5.2±1.30	4.06±0.20
--	1.5 µM	100	6.4±1.81	4.66±0.37
--	2.0 µM	85	4.6±2.07	4.32±0.60
--	2.5 µM	90	5.8±1.48	4.22±0.28

Mean ±standard deviation of 5 replicates per treatment in three repeated experiments

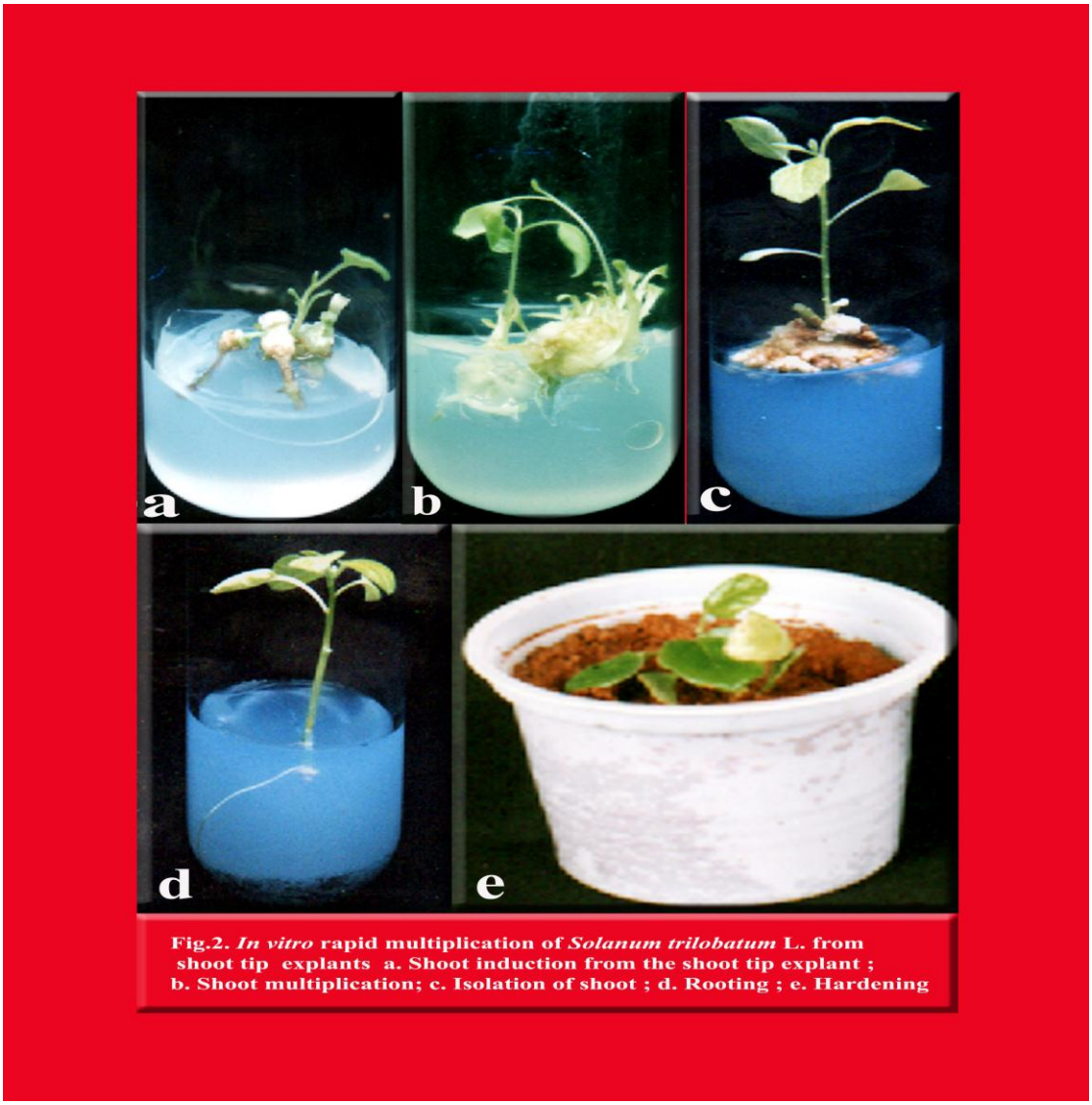

Fig. 2. *In vitro* rapid multiplication of *Solanum trilobatum L.* from the shoot tip explants

Higher number of shoots was produced from all the concentrations of both BAP and KIN. The highest frequency (**100%**) of shoot induction and maximum number of shoot (**8.4±1.51**) was observed on 2.0 µM BAP with shoot length of **4.94±0.20 c.m**. in KIN **1.5** µM/L to produce maximum number of shoots (**6.4±1.81**) in the shoot length of **4.66±0.37** and the shoot induction frequency is **100%.** The isolated shoots were transferred to MS basal medium supplemented with different concentrations of IBA and NAA ranging from **0.5 - 2.5** µM/L for root induction.

The basal medium fortified with different concentrations of KIN induced less number of shoots when compared to BAP. Maximum number of 10.6 shoots per explant was induced on MS basal medium containing 8 µM KIN and mean shoot length 5.78 cm. These results showed that both the cytokinins tested were found to initiate and proliferate shoots from the nodal explants. However, BAP was found to be more suitable than KIN for shoot multiplication.

In micropropagation technique, shoots are directly induced from the nodal explant with axillary buds where meristematic tissue is present. This technique is primarily used to produce pathogen free plantlets. Nowadays, it is widely used to get a mass propagation within a short period. Since the meristematic region is the very active site, the axillary buds are readily proliferated. The efficiency of shoot multiplication depends on plant growth regulators and types of explants.[30], [31]

In many plants, multiple shoots were obtained from the shoot tips or axillary buds by administering BAP or KIN.[32],[33],[34],[35],[36],[37] In the present study nodal explants with axillary bud were taken as explants source. The nodal explants showed active site of positive morphogenetic response and readily developed multiple shoots. The propagation rate and morphogenetic response significantly varied to a greater extent according to the explant type. Shoot tips have always been preferred for *in vitro* studies because they can be handled easily and restore their regeneration potential over other explants. Some earlier findings showed that more number of shoots were produced from the nodal explants.[36],[38],[39]

Shoot responses from node explants were tried in different concentrations; in this study it was found that BAP 2.0 mg/l with MS medium showed good response to shoot induction from node explants.[40] similar reported the BAP with MS medium, in this concentration the node explants of *S. trilobatum* showed better multiple shoot within 20 days after inoculation. BAP

and MS medium individually and in combination induced a higher frequency of adventitious shoots from single explants of *S. xanthocarpum*,.[41], [42]

CONCLUSION

Micropropagation was carried out from the nodal explants with axillary buds of *Solanum trilobatum* Linn. Nodal explants with axillary buds were grown on MS basal medium supplemented with different concentrations BAP or KIN. Of the two cytokinins BAP was found to induce more number of shoots from nodal explants when compared to KIN. The MS basal medium supplemented with 2.0 µM/L BAP showed the maximum number of **8.4±1.51** shoots per nodal explant and 1.5 µM/L KIN produced the maximum number of **6.4±1.81** shoots per node.

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