A STUDYON THE MICROPROPAGATION OF *ACACIA SINUATA*(LOUR.) MERR, *CASSIA ANGUSTIFOLIA*(LINN.) AND *PSORALEA CORYLIFOLIA*(LINN.) OF FABACEAE.

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Abstract: In this investigation a protocol was developed for to standardize a micropropagation protocol that can enhance mass production of *Acacia sinuata*(lour.) merr, *Cassia angustifolia*(Linn.), *Psoraleacorylifolia*(Linn.) of Fabaceae. Explants of *Acacia sinuata* in BM_2, *Cassia angustifolia*(Linn.)and*Psoraleacorylifolia*(Linn.) in BM_3, were found to be the best initiation medium in terms of bud breakage, and lower contamination. *Acacia sinuata*(Lour.) merr, *Cassia angustifolia (Linn.), and Psoraleacorylifolia*(Linn.) explants showed that highest number of shoots originated and average number of shoots formed in BM_1 medium. RT_5medium is the best media to get highest S/R in all explants of *Acacia sinuata*(Lour.) merr, *Cassia angustifolia (Linn.), and Psoralea corylifolia (Linn.).*RT6 medium is suitable to get highest length of root. HM_1 medium is best hardening mixtures to higher number of roots and high shoot/root ratio.

Introduction: Medicinal plants have been identified and used throughout human history. Medicinal plants were existing even before the origin of human beings on the earth (Gurib-Fakim 2006). Man's existence on this earth has been made possible only because of the vital role played by the plant kingdom in sustaining life (Bhushan Patwardhan*et al*., 2009). Medicinal plants have been regarded as sacred and used by early civilizations to treat sickness and to embellish man's well being (Dickson *et al*., 2004). In India the earliest records referring to curative properties of certain herbs are referred to in the Rigveda (3500-1800 BC).

In the beginning of 19^{th} century there was continuous activity in this area and many of the well-known medicinal plants were chemically analyzed and their pharmacological active principles were characterized. Those who live in remote places depend on traditional healers, whom they know and trust. The Indian medicine system is a Traditional System of Medicine (TSM) which encompasses 3 systems namely Ayurveda, Siddha and Unani, practiced by Vaidyas, Siddhars and Hakims respectively. The medicines that come under Ayurveda, Siddha and Unani system of treatment are called as Indian System of Medicines (ISM). The drug and Cosmetic Act defines the ISM as "Ayurvedic, Siddha and Unani drug includes all medicines, intended for internal or external use in diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals" (Sampath*et al*., 2001, Chatopadhyaya P. K, 2013)

Traditional Medicine in India: India possess an extremely rich biodiversity and these provide numerous plants with medicinal value.

From few decades, traditional system of medicine has become a topic of global importance (FawziMahomoodally 2013). India is a unique country because of the presence of active stream of traditional system of medicine which has indigenous Ayurveda. Many of the medicinal plants have been scientifically evaluated for their possible medical applications. The researchers can take a random approach to plant selection or can limit their search to plant of a certain species or genus (the taxonomic approach), or to plant that contains specific chemicals (the chemotaxonomic approach), or to plants that are already known as traditional medical cure (the ethno botanical approach).

There are many bioassay systems to evaluate the plant chemicals such as in vitro antimicrobial, anti proliferative, antioxidant and radical scavenging test (Attaur-Rahman and Choudhary, 2001). Herbal remedies have attained popularity among the common people, due to increasing awareness of personal health maintenance through natural products. The developed nations are also looking for eco-friendly means for treatment of various diseases through plant source. Studies said that the toxic effects of radiations and chemotherapy in cancer treatment could be reduced by Ayurvedic medications and similarly surgical wound healing could be accelerated by Ayurvedic medicines.

Propagation of Medicinal Plants: Traditionally, plants have been collected for medicinal use from wild areas. In natural product research, the presence of a large amount of plant biomass is necessary to provide enough bioactive compounds from the plant tissue. This presents an unfeasible solution due to the lack of reliable and abundant supply of plant material. Natural habitats for medicinal plant are disappearing fast and together with environmental instabilities, it is increasingly difficult to acquire plant-derived compounds. This has prompted industries, as well as scientists to consider the possibilities of using cell cultures as an alternative supply for the production of plant natural products (Dicosmo and Misawa, 1995). Plant cell cultures have the potential of providing a low cost route to numerous plant derived natural products that are very expensive to synthesize chemically or that occur naturally at very low concentration.

Tissue culture technology offers an alternative method for the conservation of germplasm as well as micro propagation of medicinally important plant resources. Presently there is great demand for the use of plant based medicinesin place of synthetic drugs. As a result of non-scientific exploitation, most of the medicinal plant resources are being endangered and are on the verge of extinction. Therefore, application of this technology provides the raw materials required for the isolation of drugs by the pharmaceutical industries without depleting natural plant resources. (Naika*et al.*, 2008).

Tissue culture is the technique of maintaining plant tissue in an artificial medium in vitro under control condition. The continuous supply of sterile plantlets will overcome the contamination problem and reduce the time for sterilization process.

All living cells of a plant are capable of differentiating and dedifferentiating into whole plants. This inherent property of the cells

called “cellular totipotency” has led to the concept of tissue culture studies. Plant tissue culture was originally developed as a research tool in order to study the biochemistry and physiology of plants. Plant tissue culture has advanced the knowledge of fundamental botany, especially in the field of agriculture, horticulture, plant breeding, forestry, somatic cell hybridization, phytopathology and industrial production of plant secondary metabolites etc. Plant tissue culture has turned into a standard procedure for modern biotechnology and has become one of the cornerstones of present day agriculture.

Micropropagation: Most of the plants raised through seeds are highly heterozygous and show great variations in growth, habit and yield may have to be discarded because of poor quality of flowers and fruits for their commercial release. Likewise majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Moreover many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items. Due to extensive utilization of medicinal plants for medicine and scientific research, many of them are facing extinction. Therefore, it is imperative to adopt alternative methods for rapid multiplication. In the recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under in vitro conditions. in vitro propagation also called micropropagation is in fact the miniature version of conventional propagation, which is carried out under aseptic conditions. Micropropagation provides a fast and dependable method for production of a large number of uniform plantlets in a short time.

Objective and Scope of Work: A single method is not sufficient to carry out micropropagation of plants.Afteranalyzing the above aspects, the author has considered ethno-pharmacological uses for the selection of three important medicinal species belongs to Fabaceaelike*Acacia sinuata*(lour.) merr,*Cassia angustifolia*(Linn.), *Psoraleacorylifolia*(Linn.) to standardize a micropropagation protocol that can enhance mass production of them.

a. To standardize micropropagation protocol of selected medicinal plants

b. Standardization of surface sterilization protocol ™

c. Standardization of suitable media for aseptic culture, establishment and multiplication

d. Standardization of a suitable media for organogenesis and a suitable protocol for hardening in order to achieve quality transplant.

e. Transplantation and acclimatization of the plantlets

Systemic position of *Acaciasinuata*:

Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae

Order: Fabales
Family: Fabaceae
Sub family:Mimosaceae
Genus: *Acacia*
Species: *sinuata*
Common Names: Chikaka, Shikakai, Banritha, Reetha, Kochi, Ritha, Sige, Shikai, Shika
Habitat:
Throughout India, grows wildly in forests especially in Peninsular region.
Useful Parts: Leaves & Fruits Pods
Description: Shikakai is a climbing, most well-known for the natural shampoo derived from its fruit. Thorny branches have brown smooth stripes - thorns are short, broad-based, flattened. Leaves with caducous stipules are not thorn-like. Pods are thick, flattened, stalked, 8 cm long, 1.5 cm wide.

Fig: 1 *Acacia sinuate* (Lour.) merr. Plant

Medicinal uses:
- It is popularly referred as "fruit for the hair" as it has a naturally mild pH that gently cleans the hair without stripping it of natural oils.
- Shikakai is used to control dandruff, promoting hair growth.
- Preventing premature greying of hair.
- Extracts of the ground pods have been used for various skin diseases.
- An extract of the Shikakai leaves is used to cure malarial fever.
- A decoction of the pods relieves biliousness and acts as a purgative.

Systemic position of *Cassia angustifolia:*
Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Fabales
Family: Fabaceae
Subfamily: Caesalpinioideae
Tribe: Cassieae

Genus: Cassia
Species: Angustifolia
Common names: Seena, Indian Senna, TinnervellySenna, Cassia Senna.
Habitat: Cultivated in dry lands of Southern & Western India,
Parts Used: Pods, Stems and leaves
Description:
A small erect shrub, Indian senna attains a height of about 2-3 feet. Its stem is green, smooth and erect. The spread out branches possess around 4-5 pairs of leaves. Flowers are yellow. The brown pod contains 5-7 seeds that are dark brown in color.

Fig: 2 *Cassia angustifolia* (Linn.) Plant

Uses & Benefits of Indian Senna

- Indian senna decreases pita and allows free movement of vata in the body.
- The herb stimulates liver for proper secretion of enzymes in the body.
- It helps in lowering bowels and increasing the peristaltic movement of the intestines.
- Indian senna purifies blood and restores the metabolic imbalance lost due to indigestion.
- The powder made from leaves and fruit is helpful in treating constipation and indigestion.
- It is useful in the treatment of osteoarthritis, gout and rheumatoid arthritis.
- The herb is used as an expectorant, wound dresser, antidysentric, carminative and laxative.
- Indian senna is handy in treating loss of appetite, hepatomegaly, spleenomegaly, malaria, skin diseases, jaundice and anemia.
- The herb has purgative, anthchiintic, antipyretic, laxative, vermifuge and diuretic properties.

Systemic position of *Psoralea corylifolia*
Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida

Subclass: Rosidae
Order: Fabales
Family: Fabaceae
Genus:*Psoralea*
Species:*corylifolia*
Common Names: Psoralea, Malay Tea, Cot Chu, Ku Tzu Malaysia, Scurf-pea, Malaysian Scurfpea. In India it is commonly called 'Babchi'. In Telugu it is called Bavanchalu.
Useful Parts: All parts are useful particularly seeds are more useful.
Description:
It is an endangered herbaceous medicinal plant distributed in the tropical and subtropical regions of the world. It grows mainly in winter season. It is an erect annual herb with an average height of 150 cm, with densely gland-dotted branches. Leaves are round, dotted with black glands on both surfaces. Flowers are small, bluish purple, 10-30 in a bunch, arising in axillary racemes. Fruits (pods) are 4 mm x 2.5 mm in size and they are black, roundish or oblong, closely pitted, one seeded, smooth. It is a slow growing species mainly cultivated by seeds.

Fig: 3 *Psoralea corylifolia* Plant

Medicinal Uses:
- According to Ayurveda, root is useful in carries of teeth and leaves are good for diarrhoea.
- Fruit is diuretic, and causes biliousness.
- It is useful in treatment of vomiting, piles, bronchitis, inflammation, anaemia etc. It improves hair growth and complexion.
- Seeds are refrigerant, alternative, laxative, antipyretic, antihelmintic, alexiteric and good for heart troubles.
- Seed oil is used externally for the treatment of elephantiasis.
- The seed is antihelmintic, antibacterial, aphrodisiac, astringent, cardiac, cytotoxic, , diaphoretic, diuretic, stimulant, stomachic and tonic.
- It is used in the treatment of febrile diseases, premature ejaculation, impotence, lower back pains, frequent urination, incontinence, bed wetting etc.
- The seed and fruit contain Psoralen. The root is used for treating dental caries.

- The plant yields a useful medicinal oleoresin, it treats kidney disorders, impotence, lumbago.
- It is also used externally to treat various skin ailments loike leprosy, leucoderma and hair loss.
- The antibacterial action of the fruit inhibits the growth of Mycobacterium tuberculosis.
- According to Unani system of medicine, its seed is purgative, stomachic, anthelmintic, vulnerary, stimulant, aphrodisiac and cures blood related troubles. It is applied externally in treatment of skin related troubles (Agharkar, 1991 and Bhandari, 1990).

Micropropagation Studies: Micro propagation techniques offer new avenuesfor the improvement of selected medicinal plants of the Fabaceae. For this entitled work efforts were made to standardize an efficient protocol for micro propagation of this valuable plants with enhanced in vitro regeneration of *Acacia sinuata*(Lour.) merr*, Cassia angustifolia* (Linn.) and *Psoralea corylifolia (Linn.).*

It was observed that bud breakage occurred in most of the explants of three plant species. Which were compared in terms of bud breakage (percentage), average shoot length, cluster formation. Explants of *Acacia sinuata*(Lour.) merr in BM_2 (MS + 1.0 mg/l BAP + 0.2 mg/l NAA), *Cassia angustifolia* (Linn.) in BM_3 (MS + 4.0 mg/l BAP + 0.4 mg/l NAA) and *Psoralea corylifolia* (Linn.) inBM_3 (MS + 4.0 mg/l BAP + 0.4 mg/l NAA), were found to be the best initiation medium in terms of bud breakage, and lower contamination. All explants of three plant species, showed highest shoot length with higher percentage of cluster in BM_1(MS + 0.5 mg/l BAP + 0.5 mg/l NAA) medium.

After 25-30 days of first subculture, established cultures were transferred to culture jars having respective media combinations. Multiplication of shoot cultures was carried out by culturing nodal segments/clusters excised from *in vitro*-raised plants. Observations were taken for evaluating the growth of explants by taking parameters like inter nodal distance average shoot length and number of nodes (15 shoots randomly selected per medium). From these results it was observed that *Acacia sinuate* (Lour.) merr*, Cassia angustifolia (Linn.), and Psoralea corylifolia* (Linn.) explants showed that highest number of shoots originated and average number of shoots formed in BM_1 medium, while MS medium showed lowest number of shoots. The texture of leaf was succulent and fleshy. During each passage, the number of leaves/shoots has increased substantially along with the height of shoots.

After three cycles of multiplication subculture, elongated shoots of 2 cm in length were excised and cultured on MS basal medium having different combinations of sugar and agar with MS basal (MS+ Sugar 30 gm/l +Agar-0. 8 gm/l) as control. The experiments were conducted twice, with 3 replications (with 3 shoots per bottle). During multiplication the rooting in plants in vitro culture is fairly spontaneous and no addition of growth regulators is further necessary. Hence the experiment is designed to study the rooting response with different treatment combination with two variations of agar (7 and 8

gm/l) and sugar (0, 10, 20, 30 gm/l). The results were postulated that highest shoot and root ratio (S/R) and biomass accumulation may indicate positive responses. It was observed in RT 5 (MS+agar 7gm/l+sugar 20gm/l) recorded highest S/R in all explants of *Acacia sinuata*(Lour.) merr*, Cassia angustifolia (Linn.), and Psoralea corylifolia (Linn.)* (3.01, 3.10 and 3.06 respectively). RT6 of three plants showed highest length of root.

Transplantation was done by using different soil mixtures in portrays and polybags. These trays and bags were then kept inside the poly house, where humidity maintained is approximately 80% and temperature was 28 –30°C and kept there for 15 days. In the next step they were transferred to the shade house (75%) with overhead sprinkler system for irrigation for next 10 days. Finally they were transferred to the open area for 9-10 days before transferring them to the field. Success of hardening protocol was determined by calculating survival percentage. Other parameters included were Root Length, Shoot Length, Shoot/Root ratio and number of roots. Survival percentage for all the mixtures used was >95 also reported that survival was 100% in sterilized soil rite. The observation was taken 30 days after transplantation. However in terms of root length and shoot length, HM_8 potting mixture (4 soil: 1agropeat) was found to be the best combination. The current micropropagation protocol is quite successful and recommended for further necessary work.

Media Preparation: The nutritional requirements for *in-vitro* micropropagation vary with species to their optimal growth. As such, no single medium composition is suggestible for all types of plant tissues and organs. When starting with a new plant, it is essential to work out a medium that would fulfil the requirement of micro propagation of such plants. In order to formulate a suitable medium for micro propagation it would be better to start with a well known medium such as MS medium. By making minor changes in media composition, through a series of experiments, a new medium may be evolved to accommodate specific requirements for the optimal micropropagation (Mishra *et al.,* 2015).

The stock solutions of the major salts, minor salts and growth regulators were prepared as per the composition and stored under refrigeration. Auxins were prepared by dissolving in 1N Potassium hydroxide and cytokinins in 1 N Hydrochloric acid before making up the final volume with distilled water. Auxins are generally used in plant tissue culture at a concentration range of 0.01-10.0 mg/l.

Cytokinins are generally used in plant tissue culture at a concentration range of 0.1-10.0 mg/l. When added in appropriate concentrations they may regulate cell division, adventitious shoot proliferation, stimulate auxiliary, regulate differentiation, inhibit root formation, activate RNA synthesis, and stimulate protein and enzyme activity.

They were then autoclaved at 121°C for 20 minutes at 15 psi pressure to eliminate the contaminants and transferred to the media storage room where they were kept under aseptic conditions till their further use.

Murashige and Skoog (1962) Medium is used for this purpose.

Collection of Plant Material: The plant material was collected from the Seshachalam a part of Eastern Ghats of Southern Andhra Pradesh, India. The authentication was checked by taxonomic expert Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University (SVU), Tirupathi, Andhra Pradesh. Required quantity of plant material i.e. *Acacia sinuata*(Lour.) merr, *Cassia angustifolia (Linn.)* and *Psoralea corylifolia*(Linn.) were collected and maintained in the university garden for our research work on tissue culture.

Explant Selection and Sterilization: Nodal explants were selected from disease free, young and healthy plants for carrying out our study as young and healthy cells are supposed to have retained their totipotency. Then explants were soaked in an aqueous solution containing 0.2 % Bavistin (BASF, India Limited) and 0.03% Streptomycin (Ambistryn – S, Sarabhai Piramal) for 10 minutes in Laminar flow hood. This was followed by gentle wash with sterile double distilled water for 5 minutes for 2 cycles.

Establishment of Cultures: After 9-10 (approximately) days of inoculation, the axillary bud break was seen in some explants. When the explants attain bud proliferation, these cultures were then transferred to jars containing fresh nutrient medium.

After 25- 28 days of incubation, the initiated plants were taken out from the jar under aseptic condition with the help of a clean and sterilized forceps. Medium adhered to the plants was removed, undesirable/brownish leaves were removed from the plants and were transferred to the culture bottles containing autoclaved semi-solid media having the same combinations as that for the culture initiation.

Axillary Shoot Proliferation:

Multiplication of shoots by repeated sub-culturing in multiplication media.

The preparation and sterilization steps for the medium, instruments and chamber were repeated as before.

Multiple shoots/cluster were transferred from the culture bottle to a sterile glass plate using flamed sterilized forceps, the brown leaves were removed from the primary shoots and sectioned into one node piece after removing the leaves.

These culture bottles were then incubated in the growth (incubation) room. These steps were repeated every 25-30 days for the next sub-culturing (Mathur and Kumar 1998, Tiwari *et al*, 1998 and Tiwari*et al*, 2000).

Rooting of the Shoots : Axillary shoots developed in cultures in the presence of cytokinin generally lack of roots. To obtain complete plant the auxillary shoots must be transferred to a rooting medium to initiate the growth of root, which is different from the shoot multiplication medium, especially in its hormonal composition. A low salt medium is satisfactory for rooting of shoots in large number of plant species (Mathur and Kumar 1998; Tiwari*et al.*, 1998; Tiwari*et al.*, 2000).

Rooting Protocol: In the laminar flow, under sterile conditions the Klin film and cap was removed from the culture bottles in sterile condition (laminar flow chamber) and with the help of sterile forceps the multiplied shoots were removed from the medium and placed on the sterile glass plate. With the help of sterile scalpel elongated shoots up to 1-2 cm in length were cut and placed into the rooting medium.

Protocol Followed: Plantlets were taken out of the culture bottles (multiplication subculture) with the help of forceps and washed thoroughly with sterilized water to remove remains of the medium.

0.1% Bavistin treatment was given to the plantlets in order to protect them from the fungal attack in the near future. Plantlets were separated into single shoots by cutting their bases gently with the help of blade. Single shoots were dipped in IBA solution (200 ppm) before planting into a hardening mixture. After this the single shoots are carefully planted in the trays containing soil and agropeat (M/s Varsha Enterprises, Bangalore, India) mixture in 1:1 ratio.

Transplantation and Acclimatization of the Plantlets: The transfer of plants from the culture vessel to the soil requires a careful, stepwise procedure. The roots of the plants are gently washed to remove the agar sticking to them. The most essential requirement for the successful transplantation is to maintain the plants under a very high humidity (90 – 100%).For the first 10 – 15 days by keeping them under moist or covering them with clear plastic. Some small holes may be poked in the plastic for air circulation.

After planting, plantlets are thoroughly watered and kept in polyhouse under humidity range of approximately 80%. The plantlets are then transferred to open area after 9-10 days and kept there for ten days prior transferring them to the field.

Aseptic Culture Establishment: Shoot initiation and establishment from *Acacia sinuate* (Lour.) merr, *Cassia angustifolia (Linn.) and Psorale acorylifolia* (Linn.) nodal explants cultured on MS basal and MS medium supplemented with various combinations of plant growth regulators i.e. BAP in combination with NAA and Kn is described. Most of the earlier research studies for other medicinal plant species have shown the use of cytokinin alone or in combination with other in different concentrations.

All the experiments were performed thrice with 3 replicates per treatment. Shrivastava and Rajani, 1999, also reported that shoot bud initiation was observed visually on the eighth to ninth day of incubation in all replicates in the media having different concentrations of BAP and Kn. After 3-4 weeks thick mat of shoot buds spread over 90-100% of the surface of explants in the presence of 2µM BAP and 4µM Kn.

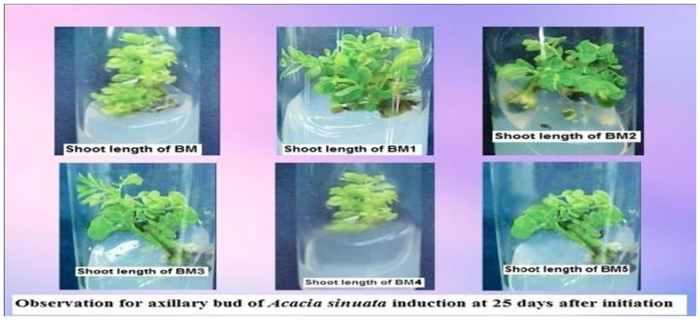

Fig: 4 Studies on Observation for axillary bud of *A.sinuata*(Lour.) merr. induction at 25 days After Initiation

Fig: 5 Observation on axillary bud of *C.angustifolia* induction at 25 days after initiation

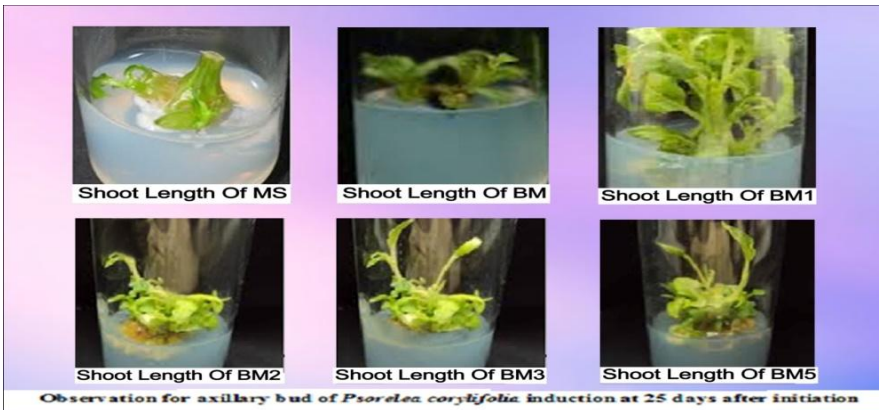

Fig: 6 Observation of axillary bud of *P. corylifolia* induction at 25 days after initiation

Multiple Shoot Proliferation: After 25-30 days of first subculture, established cultures were transferred to culture jars having respective media combinations. Multiplication of shoot cultures was carried out by culturing nodal segments/clusters excised from *in vitro*-raised plants.

Observations were taken for evaluating the growth of explants by taking parameters like inter nodal distance (inter-nodal distance was measured form the third node and measured up to sixth node from the shoot tip), average shoot length and number of nodes (15 shoots randomly selected per medium). The experiment was carried out in seven mediums having different concentrations of growth regulators

each with 3 replications, only results of best medium are given in Table 4.2.4 to 4.2.6, and Fig 4.2.4 to 4.2.6.

Fig : 7 Observation on Multiple shoot Proliferation of *A. Sinuate* (Lour.) merr.

Fig : 8 Observation of Multiple shoot Proliferation of *Cassia angustifolia* (Linn.)

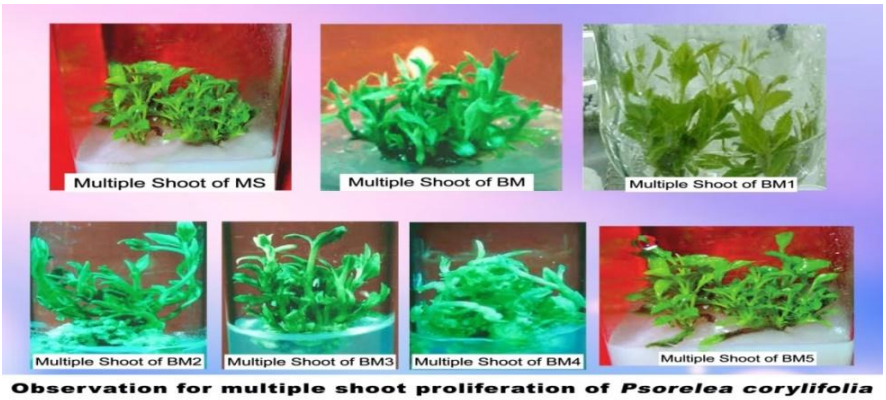

Fig : 9 Observation on Multiple shoot Proliferation of *Psorele acorylifolia* (Linn.)

Transplantation: Explants used for carrying out hardening experiment using different soil mixture were grown in MS basal medium as reported previously. After sufficient rooting, *Acacia sinuate* (Lour.) merr, *Cassia angustifolia (Linn.) and Psoralea corylifolia* (Linn.) plantlets were transferred to the polybags having different soil mixtures in different concentrations for hardening.

Potting mixtures of desired combinations were prepared and mixed properly and filled in the protrays and polybags. The polybags and protrays were sprinkled with water. The roots of each plant were dipped in 0.1% Bavistin solution to avoid future fungal attack and then roots of explants were inserted carefully into the soil mixture. The plants were then again sprinkled with water and kept inside the polyhouse at Vejendla, Chebrolu Mandal, Guntur District. Then plants were transferred to the polyhouse with misting/pad fan cooling system where humidity maintained is approximately 80% and temperature was 28 –30°C and kept there for 15 days. Finally they were transferred to the open area for 9-10 days before transferring them to the field. Success of hardening protocol was determined by calculating survival percentage. Other parameters included were Root Length, Shoot Length, Shoot/Root ratio and number of roots (Table : 4.2.8). Survival %age for all the mixtures used was >95. Tiwari*et al* (2000) also reported that survival was 100% in sterilized soil rite.

The following sterilization protocol was found satisfactory for the surface sterilization..

- Washing of nodal explants under running tap water for 30 minutes (2 cycles).
- Explants were soaked in an aqueous solution containing 0.2 % Bavistin and 0.03% Streptomycin for 10 minutes in Laminar flow hood followed by sterile water wash for 5 minutes for two cycles.

It was observed that bud breakage occurred in most of the explants of three plant species. Which were compared in terms of bud breakage (percentage), average shoot length, cluster formation. Explants of *Acacia sinuate* (Lour.) merr in BM_2 (MS + 1.0 mg/l BAP + 0.2 mg/l NAA), *Cassia angustifolia* (Linn.) in BM_3 (MS + 4.0 mg/l BAP + 0.4 mg/l NAA) and *Psoralea corylifolia* (Linn.) inBM_3 (MS + 4.0 mg/l BAP + 0.4 mg/l NAA), were found to be the best initiation medium in terms of bud breakage, and lower contamination.

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After three cycles of multiplication subculture, elongated shoots of 2 cm in length were excised and cultured on MS basal medium having different combinations of sugar and agar with MS basal (MS+ Sugar 30 gm/l +Agar-0. 8 gm/l) as control. The experiments were conducted twice, with 3 replications (with 3 shoots per bottle). Rooted shoots were taken after 2 weeks, shoot length, root length and no of roots per

explants (total 9 explants per treatment each time), were measured and tabulated. Initiation of rooting took place after 5-6 days of inoculation. Single and multiple roots were formed from the base and the nodal portions and the length of the roots were 1-2 cm within 8-10 days. During multiplication the rooting in plants in vitro culture is fairly spontaneous and no addition of growth regulators is further necessary. Hence the experiment is designed to study the rooting response with different treatment combination with two variations of agar (7 and 8 gm/l) and sugar (0, 10, 20, 30 gm/l). The results were postulated that highest shoot and root ratio (S/R) and biomass accumulation may indicate positive responses. It was observed in RT 5 (MS+agar 7gm/l+sugar 20gm/l) recorded highest S/R in all explants of *Acacia sinuata*(Lour.) merr*, Cassia angustifolia (Linn.), and Psoralea corylifolia (Linn.)* (3.01, 3.10 and 3.06 respectively). RT6 of three plants showed highest length of root. Minimal media (RT 1 and RT 2) i.e. having 0% sugar and lower concentration of agar also showed positive results so this combination can also be used for rooting of explants. Our results indicate 100% root formation in all the mediums (MS basal without any additional growth regulator). Ex-vitro rooting was also carried out for *Acacia sinuate* (Lour.) merr*, Cassia angustifolia (Linn.), and Psoralea corylifolia* (Linn.) using single shoots of 1-2 cm in height derived from MS medium and then transplanted in Soil: agro peat mixture in the ratio of 4:1which gave 100% survival rate.

From the Micro propagation studies we, have concluded that -

- Explants of *Acacia sinuata* in BM_2, *Cassia angustifolia* (Linn.) and *Psoralea corylifolia* (Linn.) in BM_3, were found to be the best initiation medium in terms of bud breakage, and lower contamination.
- All explants of three plant species, showed highest shoot length with higher percentage of cluster in BM_1 medium.
- *Acacia sinuata*(Lour.) merr*, Cassia angustifolia (Linn.), and Psoralea corylifolia*(Linn.) explants showed that highest number of shoots originated and average number of shoots formed in BM_1 medium. Whereas MS medium showed lowest number of shoots.
- RT_5medium is the best media to get highest S/R in all explants of *Acacia sinuata*(Lour.) merr*, Cassia angustifolia (Linn.), and Psoralea corylifolia (Linn.).*RT6 medium is suitable to get highest length of root.
- HM_1 medium is best hardening mixtures to higher number of roots and high shoot/root ratio.

In this entitled work we have successfully completed the micropropagation studies of selected plants of Fabaceae. But further pharmacological, and purification studies are needed to know the complete medicinal properties and to prepare new natural drugs from these plants.

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