Research and Reviews: Journal of Botanical Sciences e-ISSN: 2320-0189

An updated overview of Atropa acuminata Royal ex Lindl: A critically Endangered Medicinal plant of Kashmir Himalaya

Azra Nahaid Kamili*[1] Shabeer Ahmad Dar[1],Irshad Ahmad Nawchoo[2], Sumira Tyub[1]

[1]Department of Research Development, university of Research for Development, Kashmir, Srinagar, JammuKashmir, India

[2]Department of Botany, university of Kashmir, Srinagar, JammuKashmir, India

Research Article

Received: 02/11/2021

Accepted: 16/11/2021

Published: 23/11/2021

***For correspondence:**

Azra Nahaid Kamili, Department of Research Development, university of Research for Development, Kashmir, Srinagar, JammuKashmir, India

E-mail: azrakamili@gmail.com

Keywords: Atropa acuminate; therapeutic uses; in vitro culture; Mutation; sodium azide; EMS; Overview

ABSTRACT

Studies revealed that Atropa acuminata is used for different purposes around the world from ancient times, including medicine. The plant shows diverse medicinal properties such as antispasmodic, anticholinergic, anodyne, analgesic, hallucinogenic, Parkinsonism, carcinoma, encephalitis and spastic dysmenorrhoea, narcotic, mydriatic and sedative. Atropa has so far yielded a significant number of bioactive compounds. The plant is rich source of alkaloid atropine ($C_{17}H_{23}NO_3$) and scopolamine ($C_{17}H_{21}NO_4$) which plays an essential role in the investigation of autonomic pharmacology. The drug Atropine acts as a stimulant to the sympathetic nervous system, respiratory system, circulatory system and is used as an antidote to opium. This highlights the importance of conducting a literature review in order to report on additional details about the plant. An overview on all important aspects is documented so as to make information available for additional study of the plant for human reimbursement.

INTRODUCTION

Plants are important to all forms of life on the planet. The plants have provided and will continue to provide daily food, animal fodder, wood, shelter, entertainment, and medicines to the majority of the world's human population. One of the most essential sources of medicine is plants. The biological active principles of medicinal plants are what make them so valuable. Plant chemicals are classified into two main groups: primary metabolites and secondary metabolites. Primary metabolites include chlorophylls, sugars, amino acids, carbohydrates, proteins etc Which can be found in all kinds of plants, medicinal and non-medicinal alike? While secondary metabolites such as alkaloids, terpenoids, phenolics, and other secondary metabolites are compounds that do not play significant role in plant metabolism and their distribution varies from plant to plant. Secondary metabolites are accumulated in

smaller amounts by plant cells than primary metabolites. They are known as active principles of that plant because they are synthesized in specialized cells at specific developmental stages and have a profound physiological impact on the mammalian system. These active concepts could be found in storage organs such as leaves, roots, seeds, bark, etc. These active principles physiological effects are used to treat illnesses [1].

Atropa acuminata R commonly known as maitbrand is herbaceous perennial plant belonging to the family Solanaceae. The genus atropa consists of four species, Atropa acuminata Royle, Atropa belladonna L., Atropa baetica Willk, Atropa pallidiflora Schönb, Tem. The name Atropa is derived from the Greek goddess Atropos; while as the name acuminata means a long tapering pointed leaves. The leaves and light blackberries of the plant contain enormously poisonous tropane alkaloid Scopolamine causing unusual delirium and hallucinations. The plant is also a rich source of atropine (C17H23NO3) which plays a crucial role in medical industry. Atropine also acts as a stimulant to the sympathetic nervous system, circulatory system, respiratory system and is used as an antidote to opium. In medicine and cosmetic field, Atropa acuminata is continuously playing an important role Traditionally the rhizome of Atropa acuminata has been used to cure arthritis, joint and muscle pain, muscle spasms. Keeping the significance of this medicinal herb in consideration, an overview on Atropa acuminata is conducted covering all most all important characteristics with an object to make available entire storeroom of references to researchers for operative examination and utilization in the welfare of human beings [2].

Distribution and habitat

Atropa acuminata is indigenous to the Himalayan mountain ranges found in eastern Afghanistan, the north of India and Pakistan from Balochistan in the west to Kashmir in the east in a region including Himachal Pradesh, Uttarakhand. Extending from Kashmir, the plant is mostly found at an altitude of 1800-3600 meters above sea level (asl) to the neighboring hilly ranges of the Himachal Pradesh at 2500 meters (asl). The species is growing under the shade of alpine forests, with well-drained moist calcareous or chalky soil, with an altitudinal range of 1800-3600 meters. The plant also prefers to grow in alkaline soil [3].

Plant description:

Atropa acuminata is a branched sub-alpine medicinal herb growing approximately 1.6 m tall, with a light purplish stem that is undivided at the base and divides into three to four branches a little above the ground. The stem is also glabrous during the young stage. The thick, fleshy, whitish roots about 5-6 inches long grow into loamy well-drained soils. Simple leaves (3-10 cm long), ovate, dull, darkish green in color with entire margins. Lower leaves are existing solitary while upper ones arise in pairs alternately on the opposite side of the stem. The apex of leaves is acute with short petioles; veins are prominent on the abaxial surface while as depressed on the adaxial surface. Flowers occur solitary in the axial leaves [4]. The flowers are yellow-colored, dingy, tinged with green, bell-shaped, having long narrow shaped depressions. The petals are generally five in number having five large teeth or lobs. The flowering occurs from June to ending august. Black-colored berries are full of purple inky juice, consumed by grazing animals, and help in the dispersion of seeds. The seeds contain toxic alkaloids like scopolamine, hyoscyamine. The seeds are small, the light showing long periods of dormancy.

Cultivation and harvesting:

Atropa acuminata grow mostly in well-drained, loamy, permeable, and chalky soil in full sun under the shade of alpine trees. for the cultivation, the soil should be kept moist at all times. Under in vitro conditions, the seeds take almost 48 days to germinate because of long periods of dormancy. However, the treatment of seeds with concentrated sulphuric acid or chilling treatment decreases the dormancy period. Atropa acuminata may also be propagated from tips of green branches. Plant species growing in natural habitats have a high percentage of alkaloids like scopolamine, hyoscyamine, and atropine [5]. The growing range of plant species is between 45° and 55° N. Latitude and an altitudinal range of 1800-3600 meters, but it may down from sea level where calcareous soil is available with good drainage and tolerable shade. Use of manure or soda nitrate mixture increases the crop yield. The newly grown plants species are approximately 1.6 feet high and flowers in late September, the foliage and tops are collected to avoid overcrowding in the following year. In the following year, plants are cut down 1 inch above the ground during the flowering stage in June so that another crop will be available for harvesting in September. Generally, roots are harvested in autumn of the quarter year. The plant parts harvested should be dried up immediately in the sun because faded foliage and other parts yield a little number of alkaloids like scopolamine, hyoscyamine.

Yield:

Atmospheric conditions play a significant effect in the alkaloid contents of Atropa acuminata. Plants growing in sunny and dry conditions have the highest percentage of alkaloids. In May and June, about 0.67% alkaloid has been reported in A. acuminata in bright sunlight and dry conditions slightly differing to 0.35% in the same month lacking bright light and dry conditions. August and September being wet, only 0.34% to 0.38% alkaloid content was reported. Soils treated with a mixture of soda nitrate showed high percentage of alkaloids present in dry foliage and stems of next year plants accounted about 0.85%. In the second and third years, the average crop of the fresh plant is 5-7 tons per acre, and about 5 tons of fresh foliage and tops yield approximately 1 ton of dried plant. The yield in the first year of growth is average per acre and increases accordingly in the succeeding year.

Chemical ingredients:

The roots of Atropa acuminata contain a total of 13 alkaloids following great resolution GLC and GLC-MS, while 7 alkaloids have been found in the above-ground parts except for hygrines. Among all the 13 alkaloids, scopolamine being synthesized only in roots but hyoscyamine is frequently synthesized in shoots also. Hyoscyamine N-oxide was reported to be found in all plant parts. A natural constituent N-Methylornithrine was reported from Atropa acuminata as an important tropane alkaloid precursor following radioactive [5- 14C and 5- 3H] nurturing of ornithine to the plant species. Several essential compounds from leaf extracts of Atropa acuminata like norhyoscyamine, apoatropine, hyoscyamine, 6β-hydroxyhyoscyamine, and scopolamine have been isolated by using rapid and easy CE-electrospray interface (ESI)-TOF-MS procedure. Among the other alkaloids, the presence of cuscohygrine has also been reported by using sodium acetate-2-14C. From methanolic extract of Atropa acuminata seeds, eight important steroidal glycosides have been reported named atroposides A, B, C, D, E, F, G, and H by using Sephadex gel filtration and chromatography on silica gel-filled with silver nitrate Among all these atroposides the structure of A, C, E, G are explained as 3-O-alpha-D-galactopyramoside; 3-O-beta-D-glucopyranosyl (1-->4)-beta-D-galactopyranoside; 3-O-beta-D-glucopyranosyl (1-->2)-beta-D-glucopyranosyl (1-->4)-beta-D-galactopyranoside; and 3-O-alpha-L-rhamnopyranosyl (1-->4-beta-D-glucopyrasol (1-->2)-beta-D-glucopyranosyl (1-->4)-beta-D-galactopyranoside.

Factors affecting growth and chemical ingredients:

Seasonal differences in total alkaloid content and alkaloid patterns in A. acuminata by HPLC analysis was studied in June-July from berries and seeds and it was found that maximum alkaloid percentage were noticed at early morning and early night in berries while as highest peak was found in the afternoon in mature seeds. Hyoscyamine and scopolamine was the chief product examined. reported the concentrations of ammonium and nitrate in the medium holds a strong impact on the ratio of scopolamine/ hyoscyamine calculated the effect of inoculant morphology on growth of A. acuminata roots and manufacture of total tropain alkaloids and it was found that of hyoscyamine concentration was more in mature root tissues (2.1± 0.2 mg g-1) while as in tips it was (1.1± 0.3 mg g-1). has made whole sequence of amino acid of (2Fe-2s) ferredoxin from Hyoscyamus niger and Atropa acuminata by automatic Edman degradation of full S- carboxymethylcysteinyl peptides and proteins obtained by digestion of enzymes. These two ferredoxins showed 1-8 difference in the sequence of amino acids as compared to all other tropain alkaloid containing plants like D. stramonium, D. arborea, D. metel. And 8-24 difference among other ferredoxins of solanaceous, therefore suggesting that taxonomically the tropain- alkaloid containing plants are closely related. Also suggested that the rapid increase in the number of hairy root tips (3 to 9) in shaking flasks containing 50, 75 and 100 ml of medium reduced 40% growth rates in A. acuminata, thereby representing the result of medium conditions on growth of roots.

Growth and tropain content in transmuted root cultures of Atropa acuminata (strain M8) grown in 250 ml flasks up to one month, the biomass of cultured roots was increased 15 times and extended 5 g dry wt. 1-1; the estimated alkaloid content was 0.02% for littorine, 0.1% for 6βhydroxy hyoscyamine and 0.7% for hyoscyamine. Seven chemicals were added (individually to 20 day old cultures) chitin (0.1%), chitosan (0.1%), glutathione (5Mm) and yeast extract (0.1%) has shown no effect on release of alkaloids into the medium but cultures treated with 5mM H2O2 result a brief release of tropain alkaloids from transmuted roots and highest amount was noticed 0.35 mg per flask. Improved excretion of alkaloids into the medium was also achieved by Cu2 and Cd2 (5mM) however, led to dissolution of transmuted roots. studied the influence of putrescine on seed germination and early growth of Atropa acuminata under the influence of sodium chloride (NaCl) and the result was found that seeds presoaked in 10-2 mM putrescine can ease the adverse effect of NaCl during seed germination and growth of Atropa species however, increase the content of alkaloids and endogenous putrescine. The effect of nitrogen fertilization and water stress on the content of secondary metabolites (Hyoscyamine and Scopolamine) in the roots Atropa species was noticed by and the results were found that highest content of alkaloids was achieved with about 90% depletion of available soil water and a nitrogen supply of 1.63 g/pot.

MATERIALS AND METHODS

Medicinal uses

Have informed that the roots of A. acuminata have sedative properties. In case of mushroom or toadstool poisoning, A. acuminata is used as an antidote .The plant parts also have the properties of pain-relieving, antispasmodic, hallucinogenic, mydriatic, narcotic and sedative. Drops prepared from the extracts of plant in the past by women are used to dilate the pupil of eye and to look more beautiful. In traditional treatments, A. acuminata is used for variety of conditions including menstrual symptoms, headache, swelling, motion and peptic ulcer diseases. A. acuminata is used as a sedative, to cure bronchial spasms in whooping cough and asthma and as a remedy for cold and hay fever. Apart from these, Atropa acuminata is also used to treat psychiatric ailments (hyperkinesis), plasters, hyperhidrosis and hemorrhoid suppositories amid others. The ointments obtained from A. acuminata are used to cure Parkinson's disease, joint pain, nerve pain and leg pain stated that A. acuminata is used to cure sunstroke and painful menstruation have found that the leaves and roots of A. acuminata in India are used as diuretic, anodyne and mydriatic, narcotic. A. acuminata is used to dilate the eyes in eye operations, alleviate intestinal colic, and treat peptic ulcers.

It is also used to treat Parkinson's disease symptoms, such as tremors and rigidity, while also enhancing expression and mobility. It is used to treat sunstroke and painful menstruation. Atropa acuminata contains tropane alkaloids and highly oxygenated triterpenes measured total biomass, tropane alkaloid percentage, total alkaloid per plant, ornithine decarboxylase activity (ODC), arginine decarboxylase (ADC) activity, and putrescine levels separately in A. acuminata roots, stems, and leaves containing changing potassium ion concentrations which are widely used in medical field. A. acuminata is used to dilate the pupils in eye operations, alleviate intestinal colic, and treat peptic ulcers. It can also be used to treat Parkinson's disease symptoms, such as tremors and rigidity, while also enhancing expression and mobility. According to all parts of this plant contain varying quantities of tropane alkaloids. The leaves contain 0.4 percent active alkaloids on average, while the root contains 0.96 percent. The alkaloid content varies depending on the stage of the plant's growth, from low when it's flowering to very high when it's bearing green berries.

The parasympathetic nervous system, which regulates involuntary body movements, is inhibited by these alkaloids. Saliva, gastric, intestinal, and bronchial secretions, as well as urinary tubule, bladder, and intestine activity, are all decreased. It is used to treat conjunctivitis, fever, encephalitis, muscle and joint pain, acute inflammation, pancreitis, peritonitis, and scarlet fever, according to. A 10 fold increase in nitrogen fertilization of Atropa acuminata resulted in an increase in the content of hyoscyamine and scopolamine in the entire plant and stems, while their contents in leaves and roots remained unchanged, according to. The aerial parts of this plant have been used in traditional medicine to treat a number of illnesses, including acute infections, anxiety, asthma, and chicken pox, according to according to it is also used to treat sore throats, ulcerative colitis, and whooping cough. Atropa acuminata has been used in folk medicine for inflammatory disorders such as arthritis, asthma, conjunctivitis, encephalitis, pancreatitis, peritonitis, acute infections, and neuroinflammatory disorders, according to.

Other uses

Atropa acuminata also plays an important role in cosmetic industry; however, it may cause slight visual misrepresentation and additional adverse effects.

Adulterants

Various other plant species such as Scopolia japonica (Japanese belladonna- rhizome part), Inula helenium (Elecampane-root), Scopolia carniolica (Scopolia-rhizome), Medicago sativa (Medicago-root), Arctium lappa (Lappa-root) are used as adulterants of Atropa acuminata.

Clinical trials

Analyzed the mechanical properties of skin lesions of rats' after application of Atropa acuminata (24 rats were haphazardly divided into 3 groups; two symmetrical skin cuts were given on each rat and quickly sealed) and lesion

tensile power of each group (Group A – plant extract not given, considered as control; Group B- plant extract given only two after surgery; Group C- plant extract give 5 days' continuously after surgery) was measured 120 hours after the surgery. Atropa acuminata extract preserved groups (Group B: 244 ± 50 g; 2.09 ± 0.43% of unwounded skin; Group C: 255 ± 68g; 2.18 ± 0.57% of unwounded skin) was meaningfully higher (P< 0.05) than controlled group (A: 193 ± 32g; 1, 66 ± 0.25 of unwound skin), therefore, signifying an optimistic effect on aseptic medical skin cut healing. used the rat model to recommend the capacity of atropine and Atropa acuminata for urinary retention and Atropa acuminata has shown more effective capacity as compared to atropine examined the effect of Atropa acuminata on skin cut healing following histological and biochemical study in rats and in vitro study in human umbilical vein endothelial, keratinocytes and 3TC fibroblasts. The in vitro experimental results has shown that Atropa acuminata treated skin cuts shortened inflammation process and increased collagen formation while as strongly increasing the wound stiffness as compared to non-treated ones. Besides this in vitro study showed that maximum Atropa acuminata extract concentration uttered CK19; in addition, all concentrations were tending to stimulate cell proliferation of human umbilical vein endothelial. A case study of 48-year old man was reported by who consumed three handful of Atropa acuminata and showed disorientation, tachycardia and aggressiveness was treated with diazepam and hospitalized. The blood samples of patient were analyzed and showed muscarinic receptor total binding equal to binding of 135µg/l atropine using radio receptor technique. carry out single-blind placebo controlled analysis to investigate the dose-dependent vagotonic and vagolytic effects. Noninvasive arterial finger blood pressure and heart rate like parameters were studied after an oral administration (5 hours after) of Atropa belladonna solution (ABS: 0.1mg/ml alkaloid concentration. Scopolamine/Atropine 1:20; prescribed amount =day 1: 2ml, day 2, placebo, day3: 5ml, day4: 2ml) the results were found that ABS helps effectively in parasympathetic activities in humans. ABS also showed no side effects on blood pressure.

Cytology

It was reported that the Atropa species contain true haploid chromosome number n=36, which were large in size.

Cell Biology

Studied the developmental changes of Atropa acuminata seed in subcellular structural stage. The results were found that young cells mostly contain spherosomes while as protoplasts, mitochondria and ribosomes are present in cytoplasm. The protein bodies are formed during ripening from endosperm of embryo and vacuole. The protein bulk also contains spherical or crystalline body (stain with periodic acid Schiff reagent). The endosperm and embryo of ripe seeds of Atropa acuminata were similar having presence of spherosomes and protein bodies also grew pollen mother cells (PCMS) of Atropa acuminata without germs in a nutrient medium in micro-culture. The results were found that a multicellular structure is giving rise 5-8 microspores which were released during maturity due to bursting of callose wall caused by high internal pressure.

Toxicology

The rooted part of Atropa acuminata is most poisonous followed by leaves, flowers and berries stated a case when a boy about 8-9 years old mistakenly ate 15-24 berries of A. acuminata and serious Atropine poisoning was reported in the boy who commenced with mental illness; however, the boy stays alive due to intravenous administration of physostigmine. Belladonna have been reported to cause obstruction of nervous system, urinary retention, paralytic ileus, fast heartbeat, stenosis, constipation, intestinal blockage, dry mouth, fever, red dry skin, unclear vision, mental problems, spasms and other toxic effects of Atropa species reported intoxication of Atropa species on about 49 children's and observed results showed symptoms and signs which were meaningless speech. Mydriatic, tachycardia and blushing but not death. reported simple poisoning of Atropa species in two adults who ate few berries. Atropine level was found high in urine and treatment of the two adults with physostigmine was unsuccessful.

Diseases of Atropa acuminata

The insects that attack the leaves of Atropa acuminata are called as "flea- beetles" and it pierces the leaves to such a range that it makes the sale of Atropa acuminata neglect able in the market in a dried state (naphthalene balls kept in the Atropa growing soils may keep the beetles off) reported virus contamination in the Atropa acuminata plant and it was found that virus occurs properly high concentration surviving for 5-11 days in extracted fluid but within the temperature range of 75-80ºC, the virus gets inactivated defined belladonna mottle virus (belonging to TMV group) as minor round virus particles containing 180 subunits of proteins which are arranged in a T=3 icosahedral superficial lattice. The uppermost and lowermost viral particles crystallize isomorphously in a hexagonal space assembly R3 (a=B=296Å, C= 729Å) experimentally proved that Atropa acuminata mosaic virus is transferred by nematodes into sandy soils.

Hybrid analysis

Magnificently created intertribal cross cell lines of Atropa acuminata × nicotiana chinensis by duplicating single protoplast union products and hybrid nature of copy lines (thirteen formed) were confined by cytogenetic (chromosome morphology and size), biochemical (amylase isozyme studies) and physiological (in vitro peculiarities of cell growth) analysis. The results obtained were understood as new signs for the opportunity of using non sexual hybridization for the creation of such new plant hybrids which cannot be formed by sexual journeys make possibility of selection of somatic hybrids by fusing protoplasts of Atropa belladonna (2n=72) and Datura innoxia (diploid: 2n=24, tetraploid: 4n=48- chlorophyll deficient mutant) and the thirteen new hybrids found produce chromosomes at metaphase stage varying from 84-175 and chromosomes of any hybrid species distinguishable by their sizes prepared protoplast fusion of Atropa acuminata foliage mesophyll and nicotina tabacum (B6S3) top lesion cells to obtain 55 hybrid lines and verified them by using cytogenetic, biochemical and molecular studies. Achieved asymmetric hybrids between Atropa acuminata (n=36) and Nicotiana plumbaginifolia (n=10) by 'gamma merging 'and cytogenetic study of new hybrid plants exposed that the plants retain many (tetra or hexaploid) sets of N. plumbaginifolia along with 5-29 chromosomes of Atropa. In majority of hybrids, rDNA genes of the both plants were found but chloroplast DNA of only Nicotonia species was present found nucleo- cytoplasmic incompatibility in hybrid plants owning genome of Nicotiana plastome and Atropa acuminata exposed elimination of spontaneous chromosomes of Atropa acuminata in somatic crossbreed lines developed between Nicotiana tabasum and Atropa acuminata (2n=72). Among all lines only three (3) possess one small chromosome similar to Atropa acuminata and 48 large chromosomes similar to Nicotiana tabacum persuaded intergenetic somatic cross plants between Hyoscyamus muticus (2n=28) and Atropa acuminata (2n=72). The observed results showed that the plants were varying in chromosome number (100-178) and in overall morphology; genetic material complementation as well as eradication (Atropa) were found in hybrids established useful cybrid (mesophyll protoplast fusion) between Atropa belladonna and Nicotiana tabasum (tryptomycin resistant, chlorophyll deficient) using polyethylene glycol/high pH/high Ca+-/dimethyl sulfoxide technique.

Three sets of reagents were recognized–(a) nuclear hybrids; (b) Nicotiana/Atropa cybrids containing tobacco genome as well as Atropa plastome and also phenotypically the cybrids were similar to tobacco bearing seeds and morphologically diploid; (c) Atropa plants ascending from rare living protoplast. For genetic manipulation and propagation of medicinal plants, biotechnological approaches play important role through callus stimulations, cell suspension in bioreactors, in-vitro regeneration, in vitro mutagenesis, and genetic transformations As a consequence of the asexual process, propagation mediated by plant cells and tissue culture is also known as 'clonal propagation,' implying that all offspring are produced under in vitro conditions known as 'clones.' McClintock. Plant tissue culture is an efficient technique and has opened up a spacious range of fields for clonal propagation research, biodiversity conservation, somaclonal variation generation, and bioactive compound manufacture and also offers exclusive advantages over methods, i.e. all time available opportunities for uninterrupted mass propagation, production, and isolation of drugs as well as in vivo production of plant drugs. Environmental problems such as climatic and soil conditions, pathogen attacks, and herbivores may influence bioactive compounds. Therefore, biotechnological techniques need to be used to meet the commercial requirements of medically important active compounds, i.e. an alternative method has been reported as secondary metabolites produced by plant and plant tissue culture for this purpose. The success of Morel with orchids, followed by which succeeded in micro-propagating Convolvulus arvensis, resulted in interest clonal plant propagation. Datura species are known as the tropane alkaloid treasure and this medicinal plant genus has witnessed all aspects of haploid production plant tissue culture technique to protoplast fusion to lift interspecific and intergeneric hybrids raised Atropa belladonna plantlets from the pollen, while gained ground through somatic embryogenesis in opium spp. Some of the endangered taxas for which in vitro culture protocols have been standardized are Picrorhiza kurroo, Podophyllum hexandrum Rheum emodi Gentiana kurroo, Similarly, Amaranthus hybridus, Prunella vulgaris, Artemisia annua, Rumex dentatus, Cichorium intybus, Hyoscyamus niger, Crocus sativus, Artemisia amygdalina, Juglans regia, Lupinus polyphyllus.

For the application of genetic engineering techniques to improve and boost secondary metabolite content, the development of a reliable in vitro rejuvenation protocol is a requirement. The first seedlings of A. belladonna and A. belladonna cultivar lutea Doll were raised by placing seeds in Petri dishes having 2 moistened sheets of filter paper with distilled water and incubating at 25°C in the dark. attempted and productively germinated seeds of A. baetica on MS medium containing 3 percent sucrose and 0.7 percent agar after a long gap. A. belladonna shoot tips on Wood and Braun's medium supplemented with NAA and Kn, then moved them to MS medium supplemented with LS vitamins and BAP. After 35 days of incubation, the cultures were shaken at 25°C with illumination, and the tissues were harvested, dried, and powdered before extraction with organic solvents for chemical analysis. They also discovered that shoot cultures developed lower levels of alkaloids than intact plant shoots used shoot tip culture to produce sterile A.

belladonna plants that were held in hormone-free MS medium. Hairy roots appeared 2-5 weeks after Agro bacterium rhizogenes strain 15834 with Ri plasmid pRi 15834 was inoculated onto plant stems, and segments were extracted and cultured on hormone-free MS agar. HPLC and TLC were used to detect atropine and scopolamine, and GLC was used to determine the quantities. After one month of history, the axenic cultures of these hairy roots derived from the stems multiplied60-fold based on the initial fresh weight. The levels of hyoscyamine and scopolamine in these cultures were equivalent to or even higher than those found in field-grown plants grew several shoot cultures from A. belladonna shoot tips on MS liquid medium supplemented with various combinations of plant growth regulators, including BAP (5.0ppm); BAP (1.0ppm) + NAA (0.1ppm); Kn (0.2ppm) + IAA (0.1ppm); Kn (0.05ppm) + 2,4 – D (0.2ppm); and Kn (0.2ppm) + NAA (0.1ppm) (0.1ppm). On MS basal medium supplemented with 1 mg/l BAP and Kn alone, as well as IAA, NAA, IBA, and 2,4-D, observed shoot proliferation from shoot tip explants. The use of BAP and IBA together resulted in a large increase in the number of responding shoot tip explants. These excised elongated shoots were transferred to rooting media; they showed 80–100% rooting in the various treatments. In complete RT medium with IBA (1mg/l), the best rooting answer in terms of length and number was obtained. On half and full-strength MS, RT, and B5 medium, replacing IBA with NAA or IAA resulted in less or no rooting. developed a micropropagation protocol for A. belladonna from nodal segments. On MS medium supplemented with BAP (1 mg/l), GA3 (0.5 mg/l), and IBA (0.1 mg/l), around 82 percent of bud sprouting was obtained. Multiple shoot formation was induced by BAP (0.5 – 2.0 mg/l). BAP (1.5 mg/l) + IAA (0.5 mg/l) produced the highest number of shoots. Micro shoots are easily rooted in growth regulator-free MS medium or medium with low auxin levels. On MS basal medium supplemented with 1 mg/l each of BAP and Kn alone, as well as IAA, NAA, IBA, and2, 4-D, observed shoot proliferation from nodal segments. BAP and IBA (1 mg/l) each produced the most multiple shoots. Callus induction was recorded by from A. acuminata leaves cultured on a medium containing2, 4 – D (0.1mg/l). Root initiation was also observed in some explants from the midrib. 62.5 percent of 1-year-old callus cultures grown in the presence of Kn (1.0 mg/l) had roots regenerate.

Table1: achievements made in Atropa spp. from 1969-2020

S. NO.	Species	Explants Used	Culture media/ other supplements	Phytohormones	Response
1	A. belladonna	Seeds	-	-	Seedling formation
2	A. belladonna	Root segments	Standard synthetic medium	NAA(2mg/l) Kn(0.1mg/l)	Callus
3	A. belladonna	Protoplast	Modified MS liquid medium and 0.5 M sorbitol	NAA (2mg/l) Kn (0.1 mg/l)	Complete plantlets
4	A. belladonna	Mesophyll Protoplast	Modified MS liquid medium and 0.5 M sorbitol	NAA (2mg/l) Kn (0.1 mg/l)	Complete plantlets
5	A. belladonna	Anthers	Agar and liquid medium	-	Callus
6	A. belladonna	Leaves	W and B medium	NAA (2mg/l) Kn (0.5mg/l)	Shoot buds and callus
			Lin and Staba vitamins		Shoot bud regeneration
			MS medium	BAP (1mg/l)	
7	A. belladonna	Shoot tips	W and B medium	NAA and Kn	Shoot multiplication
8	A. belladonna	Shoot tips	MS medium	-	Hairy roots
9	A. belladonna	Shoot tips and nodal segments	MS liquid medium	BAP (5.0), BAP(1.0) + NAA (0.1),	Shoot multiplication
				Kn (0.2) +IAA (0.1)	

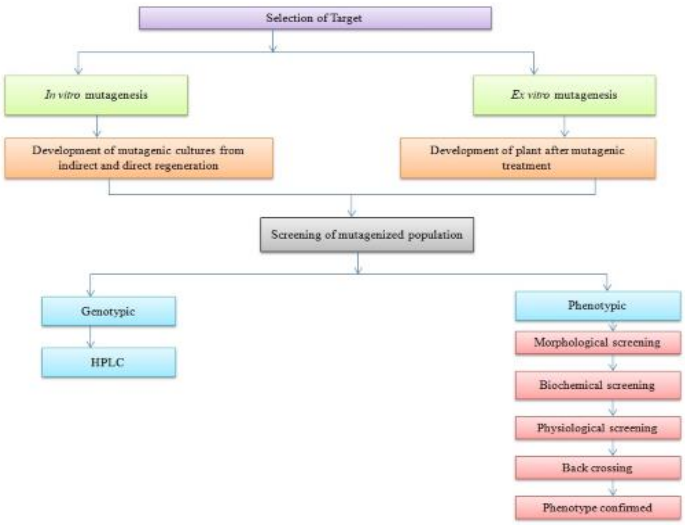
				Kn (0.05) + 2, 4 –D (0.2), and Kn (0.2) + NAA (0.1) ppm.	
10	A. belladonna	Stem and root fragments	MS medium	BAP (1 - 2mg/l)	Callus
		Anthers and ovaries			
			Nitsch and Nitsch medium	IAA (0.1mg/l)	
11	A. acuminata	Leaves	-	2, 4 – D (0.1mg/l)	Callus and rooting
12	A. baetica	Seeds	MS medium	-	Seedling formation
13	A. baetica	Nodal segments	MS medium	BAP (0.5 – 2.5 mg/l), GA_3 (0.5mg/l) IBA (0.1 mg/l)	Rooting of micro shoots
14	A. belladonna	Nodal segments	MS medium	BAP (0.5 – 2.5 mg/l), GA3 (0.5mg/l) IBA (0.1 mg/l)	Shoot multiplication
				IAA (0.5mg/l) Low levels of auxins	
					Rooting of micro shoots
			MS medium		
15	A. belladonna	Leaf discs	MS medium	NAA(0.5mg/l) TDZ(1.5mg/l) BAP(3.5mg/l) Rooting	Callus and multiple shoots
16	A. acuminata	Shoot tips and nodal segments	MS medium	BAP(1 mg/l), and Kn (1mg/l) each alone and with IAA, IBA, NAA and 2,4 – D(1mg/l each)	Shoot multiplication
17	A. belladonna	Leaf, stem & root segments	MS medium	BAP and NAA (1mg/l) each	Callus
18	A. acuminata	Nodal segments	MS medium	BAP and IBA(1mg/l) each	Shoot regeneration
19	A. acuminata	Seeds	MS medium	IAA(1mg/l)	Multiple rooting
20	A. acuminata	Leaf segments	MS medium	2-4-D, IAA,NAA(2mg/l)	Callus
				Each.	

21	A. acuminata	Leaf segments	MS medium	BAP (3mgl)+	Shoot regeneration
				IAA (2mg/l).	

Mutagenesis

Induced mutagenesis is an important method to cause genetic variation and unpredictability at a faster pace and is continuously being used in the development of economically and medicinally fundamental traits like high yield content, metabolite content, environmental stress resistance, etc in several crops. With the aid of the induced mutation approach, more than 3000 mutant varieties had been developed so for. There are numerous methods and techniques to display these mutant cultivars like phenotypic screening which includes: Morphological, Biochemical, Physiological, and Genotype screening: which includes: High-Performance Liquid Chromatography (HPLC), Quantitative PCR (qPCR), DNA Sequencing, Gel, and Capillary electrophoresis, Next-generation sequencing (NGS), etc. preserved seeds of A. belladonna with ethylene imine (EL-0.05 and 0.025%) nitrosoguanidine (NG-1 and 2mM) results were found a significant increase in height of the plant, foliage number and length, tiller number, and metabolite content. It was also reported that all treated plants resulted in a significant gain in variability in metabolite content as related to controlled ones (except NG-1mM treatment). Carried out an analysis to increase germination and alkaloid percentage in A. acuminata seeds following treatments with gibberellic acid and gamma irradiations and results were found that improvement in germination occurs up to 100 ppm GA treatment and 150 Gy irradiation and greatest was recorded at 100 ppm GA and 110 Gy irradiation. Seed color and evaluated alkaloid-containing mutants were selected. There are numerous methods and techniques to display these mutant cultivars like phenotypic screening which includes: Morphological, Biochemical, Physiological, and Genotype screening: which includes: High Performance Liquid Chromatography (HPLC), Quantitative PCR (qPCR), DNA Sequencing, Gel and Capillary electrophoresis, Next generation sequencing (NGS) etc. (Fig.1). Induced mutation supply raw materials for genetic development of crucial plant species and is also an important tool to create genetic variability in lesser times in qualitative as well as quantitative traits. Agronomically significant characters such as shortening of growing period suitable for alternation, stress tolerance etc are developed with the tool of induced mutations. **(Figure.1)**

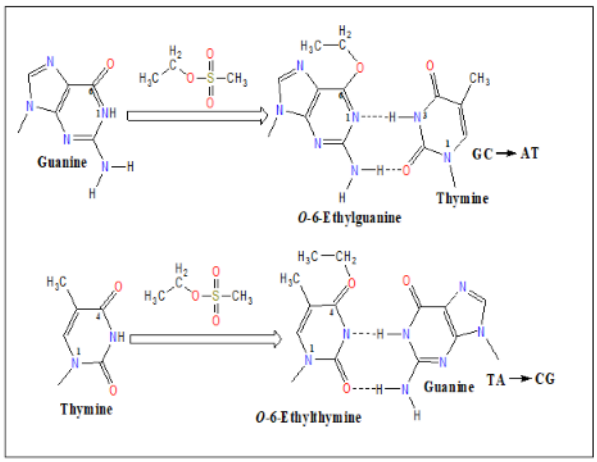
Figure1: Summary of different methods to screen a mutagenized population



To increase genetic diversity in crop plants, various chemical mutagens such as Ethyl Methane Sulfonate (EMS), Methyl Methane Sulfonate (MMS), N-ethyl-N-nitrosourea (ENU), and physical mutagens such as X-ray or fast-neutrons, gamma radiations have been used such as Chickpea Solanum lycopersicum Phoenix dactylifera Rosa hybrida Solanum xanthocarpum Asteracanth. But chemical mutagenesis, on the other hand, produces the bulk of the modifications in a plant system. These chemicals may induce insertions, point mutations and deletions in genomic strands, resulting in phenotypic and genotypic variations. One such important method to induce genetic variation is in vitro mutagenesis using EMS (Fig.2.2) is a popular method for inducing genetic variation in plants

since it has a low effect on biological processes and thus can be used to create new and novel mutants with desirable traits such as increased metabolite content, resistance to abiotic stress, phenotypic trait heterogeneity, and genotypic changes in a wide range of genetic test systems. Point mutations, which are single-base substitutions, are the most common cause of these variants. They can occur as a result of transformations (purine to purine or pyrimidine to pyrimidine) or transversions (pyrimidine to a purine) **(Figure2).**

Figure 2: Mechanism of mutation by Ethyl Methane Sulfonate

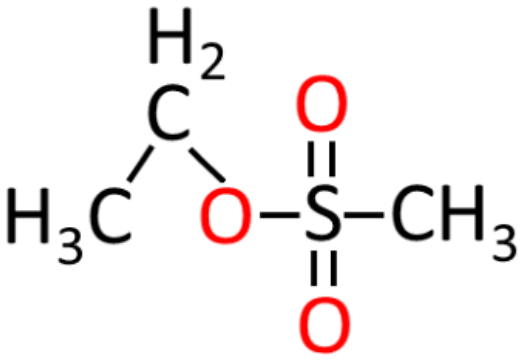


Mechanism of Action of Ethyl methane sulphonate

Many studies have focused on the interactions between DNA and chemical mutagens, as DNA is thought to be the most important target for the induction of mutations by chemical agents. EMS is widely used as a chemical mutagen for DNA lesions and induce base changes or nucleotide substitution, which alter codon sequences, resulting in either non-synonymous or synonymous effects, and induces a skewed range of G/C-to-A/T transitions, which occur due to alkylation at the O6 or N7 position of guanine, This allows the cytosine base pairing to be substituted with thymine. Originally N-7 of guanine was discovered to be the first site ethylated by EMS and is now considered the most common site of EMS attack in DNA. Since 7-alkylguanine residues are typically produced when alkylating agents, such as EMS, react with DNA, mispairing of this modified purine was once thought to be a major cause of mutations. Furthermore, depending on the location of the mutation, EMS can result in small deletions and rearrangements were the first to suggest that mutagenicity could be linked to the formation of O6-alkylguanine as opposed to N-7-alkylguanine.

The signal for mutation at O6-alkylation by EMS and MNU (Methyl Nitroso Urea) was found to be higher, but MMS (Methyl Methane Sulfonate) and DMS (Dimethyl Sulphate) showed very little mutation. Other researchers discovered that chemicals reacting via the SN mechanism produce more O6-alkylguanine than chemicals reacting via the SN2-type mechanism. As a consequence, EMS, which can react via an SN1 as well as an SN2 mechanism, is expected to produce more O6-ethylation than MMS and DMS, which only react via an SN2-type mechanism. EMS exposure caused a high frequency of G/C-to-A/T changes in Arabidopsis thaliana Caenorhabditis elegans Lotus Japonicas and Solanum lycopersicum. As a result of these properties, EMS has been widely used in many plants to cause variability, which can increase the quality and quantity of crop plants. Thus in the present work, A. acuminata is selected for mutagenesis with special references to secondary metabolite pathway because A. acuminata is being considered as an important source of alkaloid production mainly Atropine and scopolamine. Thus for a mass propagation and improvement of this plant, mutagenesis was required **(Figure 3).**

Figure 3: Molecular structure of Ethyl methane sulphonate



RESULTS AND DISCUSSION

Similarly, sodium azide is another powerful mutagen in microorganisms and a very useful mutagen in barley and other crop plants, but it is only partially mutagenic in mammals and non-mutagenic in Neurospora, and Arabidopsis thaliana, a model plant species. Many plant species, including barley, rice, maize, and soybean, are metabolized in vivo to a strong chemical mutagen. SA's mutagenic effect is highly dependent on the pH of the treatment solution, and, like MNU (N-methyl-N-nitrosourea), can be enhanced further by seed pre-germination prior to NaN3 exposure. used sodium azide for the first time as a mutagen in barley, finding a dose-dependent increase in the frequency of chlorophyll mutations in the concentration range 1-4 mM at pH3, with the highest frequency, 17.3 percent, recorded for 4 mM. In many barley genotypes, combined treatment with NaN3 and MNU resulted in a wide range of gene mutations, resulting in dwarf and semi-dwarf characters or alterations in root system growth and structure.
Sodium azide has been shown to have beneficial effects on pollen embryogenesis in Solanum nigrum and Hordeum vulgare, in addition to being cell division suppressor. During germination, cellular metabolism can affect SA's mutagenicity. When applied during the DNA synthesis stage of the first cell division of the germination process, sodium azide is more effective at inducing mutations. When applied at this stage to rice seedlings at a dosage of 10 mM for six hours, sodium azide produced the highest proportion of mutations (11.2 percent and 1.23 percent, respectively) based on M1 panicles and M2 seedlings. Differentiation of callus structures derived from sugar cane leaf segments was delayed by sodium azide in a concentration-dependent manner (0.07 to 0.7 mM). In sodium azide-treated callus, the number of regenerated plantlets was reduced. Additionally, necrosis in meristematic sugar cane callus was observed at a concentration of 0.9 mM.

Effect of mutation under in vivo conditions

Mutational analysis in Abelmoschus moschatus was documented by using gamma radiations. For the mutant screening process, both qualitative and quantitative traits were examined. At 20 kR, a mutant was observed with a light yellow corolla and a light purple eye at the flower's base, as well as differences in oil constituents and morphometric traits when compared to the control. Under in vivo conditions, an attempt was made to create large-scale mutant screens using EMS and NaN3 as a mutant in Solanum lycopersicum. On the basis of morphological characteristics, 3,837 M2 families were derived, which were then screened and putative mutants were categorized into 15 primary and 47 secondary groups using the SOL database developed tomato mutants and split them into various varieties. There were 1,047 variants identified, with 548 pleiotropic and 499 non-pleiotropic. Backcrosses confirmed further mutations, indicating that mutations were successfully induced. By exposing seeds of Abelmoschus moschatus to different concentrations of sodium azide (NaN3) ranging from 0.05 percent to 0.10 percent and gamma radiations ranging from 20 kR to 80 kR, when compared to the control, the mutants showed differences in germination percentage, survival rate, and chlorophyll content. in tomato, investigated the mutagenic effects of EMS and gamma rays alone, as well as the in combination (EMS + Gamma rays). It was observed that in the M1 generation, there was a gradual decrease in germination percentage, seedling height, and pollen fertility as mutagen concentrations increased.

However, as opposed to EMS and gamma treatment alone, the combined treatments proved to be more deadly. investigated the impact of gamma rays on breeding programs in Solanum melongena L. Different concentrations of gamma rays (0, 80, 120,160, 180and 200 Gy) were used to treat eggplant seeds. Germination rate, plant fresh weight, plant dry weight, shoot fresh weight (mg/plant), shoot dry weight, shoot length, hypocotyls length, leaf width and leaf length were among the morphological characteristics measured. The findings revealed that gamma rays

can be used to grow crop varieties with desirable traits. In Lycopersicon esculentum, investigated the mutagenic effects of EMS and NaN3 on seed germination, meiotic cell division and pollen fertility. Seeds were given five separate doses of EMS and NaN3 ranging from 0.02-0.10%. Meiotic anomalies were discovered, including univalents, multivalents, stickiness, bridges, uneven separation, stray chromosomes and disrupted polarity. Furthermore, increased mutagenic treatment resulted in a decrease in seed germination and pollen sterility in the M1 generation. Results were found that lower EMS concentrations caused less harm, reduced pollen fertility and created valuable mutations that could be used in breeding programs. recently studied the mutagenic effects of EMS on cytological characteristics, morphology and biochemical elements in Coriandrum sativum L. It was discovered that high concentrations of EMS had a toxic effect on photosynthetic pigments like chlorophylls, caroteoids and proline. investigated the usefulness of EMS in Physalis peruviana. Seeds were treated with EMS at various concentrations (0.01, 0.02, 0.03, 0.04, 0.05 percent), as well as a control. Characters such as chlorophyll content, viability, germination, and mutation frequency were all measured in the M1 and M2 generations. In terms of increased chlorophyll content, 0.02 percent EMS was found to be the most effective therapy. Higher concentrations were lethal and resulted in sterility, but lower concentrations were advantageous in terms of different growth parameters that can be used in the future.

Effect of mutation under conditions

To cause artificial mutagenesis in crop plants, a lot of work has been done. For example, seed mutagenesis has been used to induce male sterility in cucumber, improved pollen viability and fruit rot resistance in bell paper, and herbicide tolerance in soybean used EMS at various concentrations(0,1,3, 5, 8, 10, and 30 mM) to treat somatic embryogenic suspension cultures of soybeans (Glycine max L.). The mean survival rate of embryogenic cultures varied depending on the EMS concentration used, with major differences observed for cultures treated with 30mM relative to all other EMS concentrations. published on the induction of mutations in grown callus from common bean leaf petiole explants by EMS and N-ENU. Callus was given various doses of EMS and ENU for 30, 60, and 90 min., with concentrations varying from 2.5 to 6.5 mM and 6.5 to 10 mM, respectively. With both mutagens, a short mutagenic time period (30 minutes) resulted in the highest callus biomass, whereas a longer time period resulted in an inhibitory effect. Under settings, 500 and 1000 gamma rays were used to induce the mutation in Chrysanthemum morifolium ray florets. Mutation induction reduced direct shoot regeneration, as well as plant height, leaf size, and ray floret morphology Effect of mutation under conditions on seed germination was observed in four pea varieties: Sprinter, Winner, Karina and Bolero.

However, gamma ray treatment showed no effect on seed germination percentage. At 60 Gy and 140 Gy therapies, the rooting percentage of the Winner and Karina cultivars decreased. 0.5 percent EMS was applied to Ipomoea batatas callus for 0,1,1.5,2,2.5, and 3 hours. Salt-tolerant callus were produced and sub-cultured on MS medium containing 150mM Nacl. The callus was put in a shoot and root regeneration medium containing 4 mg/l abscisic acid (ABA) and 10 mg/l gibberellic acid (GA). Plants that were formed as mutants were more salt tolerant than control plants. Dracaena sanderiana callus was regenerated on MS medium supplemented with2, 4-D after being treated with different concentrations of EMS. Lower EMS treated lines (ET1 and ET2) showed a considerable increase in callus induction percentage and biomass performance as equivalence to control and other EMS treatments (ET3, ET4 and ET5). On MS medium with BAP, Callus of the ET1 line showed high rejuvenation potential. On MS medium supplemented with IBA, a considerable increase in rooting was observed as the EMS concentration was increased. Examined the embryogenic ability of four soybean cultivars at various EMS concentrations and pre-culture durations. Cultivars treated with different concentrations of EMS showed genotypic variations in terms of somatic embryogenesis performance from immature embryos. Different doses of EMS, NaN3 (0.5, 2.5, 3.5and 5.0%) and cold treatments were given to seeds of Hydrangea macrophylla and Hydrangea paniculata. Under conditions, seed germination found to be increased in most H. macraphylla cultivars at lower dosages. When subjected to the same doses of EMS and NaN3 however, cold-treated seed showed higher levels of mutagen tolerance than controls. Fang and Traore conducted mutagenic experiments using EMS in Saintpaulia cv. Crystobal. leaf of Saintpaulia cv. Crystobal was exposed to different EMS treatments at 0%, 0.2%, 0.3%, and 0.5% for 30, 60, 120, and 200 min. A total of ten mutants were discovered among the 1838 plantlets that were grown to flowering level. The ability of EMS to induce mutation in Saintpaulia under conditions was demonstrated by mutation induction.

Solanum tuberosum nodal explants when grown under conditions and exposed to varying doses of gamma rays, with mutants obtained using selection media containing50, 100, and 125,150 mM NaCl. The treated and control plants were analyzed at the molecular level using the RAPD-PCR method; the polymorphism rate according to the selected primers was calculated to be 88.66 percent. In the same year conducted mutagenic experiments in Asteracantha longifolia L. MS medium supplemented with BAP (8.88 M) + NAA (2.69 M), leaf segments were

cultured under sterilized conditions. On the basis of morphological, molecular and phytochemical approach, 4 dwarf, 7 leaf, and 13 flower mutants were found. The height of a plant (18.6 to 42.3 cm), length of an internodes (2.2 to5.72), inflorescence number (3 to10), flower colour (white to violet), and phytosterol content (0.033 to 0.0467 mg/g) all varied morphologically and biochemically. Using gamma radiations, mutagenesis in Rosa hybrida L.cultivar 'Pusa Mohit' was recorded. Nodal segments were exposed to varying doses of gamma rays(0,5,10,15,25,40,55,65,70, and 80 Gy), and then cultured on MS medium supplemented with BAP (15.5 µM) + NAA (0.55 µ M)+ GA3 (1.45 µM).

It was observed higher doses of 65, 70, or 80 Gy were found to be toxic and had deleterious effects. At high gamma radiation doses, morphological anomalies were observed. Four separate flower colour mutants were isolated from raised mutant plants and control plant. investigated genetic heterogeneity in Etlingera elatior and devised a method for inducing mutations and regenerating shoots. The findings were assed using 9 primers, and out of 59 reproducible bands, 8 regenerants showed variance in their banding patterns, with 35 (55.31 percent) being polymorphic. The PL1 mutant had the highest similarity value (0.814), while the PL7 mutant had the lowest (0.537) examined the effects of gamma rays, EMS, and MMS in rough lemon (Citrus jambhiri) seeds and epicotyls on various parameters in the same year. Different doses of EMS (0.2, 0.3 and 0.4%), MMS (0.1, 0.2 %) and gamma rays (0, 40, 60, 80, 100 and 120 Gy) were given to seeds and grown epicotyls and were grown into regenerated MS medium. The best response was observed at 60 Gy and 0.2 percent (EMS and MMS) based on survival and regeneration potential. Cultured sterilized Petunia hybrida seeds. Leaf segments from grown plants were inoculated on MS media supplemented with BAP (0.5 mg/l) after being treated with various concentrations of EMS (1 %, 2%, and 3%) for different periods (15,30, 45, and 60 minutes). Leaves treated with EMS (2%) for 30 and 60 minutes were found to be lethal, and leaves treated with EMS (2%) for 15 minutes showed a substantial reduction in fresh and dry weight of callus. Sugarcane was subjected to mutagenesis and selection for salt (NaCl) tolerant varieties (Saccharum officinarum L.) grown callus were irradiated (10 to 80 Gy) and exposed to various NaCl concentrations (0, 50, 100,150, 200, mmol/l). Increases in NaCl concentrations resulted decrease in overall growth.

Biochemistry

Provided complete [2Fe-2S] ferredoxin amino acid sequences from A. acuminata Via the automated Edman degradation of all S-carboxymethylcysteinyl proteins and peptides obtained through enzymatic digestion from A. acuminata and Hyoscyamus niger. Compared with other tropane-alkaloid-containing plants (Scopolia japonica, Datura stramonium, D. metel and D. arborea), the two ferredoxins demonstrated 1-8 variations in their amino acid sequences and 9-23 differences between other solanaceous ferredoxins. These scientific findings suggest that the tropain-alkaloid containing plants are taxonomically closely related.

Alkaloid production and isolation

Atropine and scopolamine presence in hairy roots of Atropa acuminata with Agrobacterium rhizogenes by HPLC and TLC method was reported by. However, their amounts were calculated by GLC, and it was found that the amount of atropine and scopolamine in the cultures was same as normal plants growing in field. recommended an integrated method of extraction and liquid membrane separation (di-isopropyl ether as liquid membrane and sulfuric acid as a strip off agent) of atropine from roots (85% field, about 45 times higher than natural extract). Had determined the tropane alkaloid content from different parts like leaves, roots of the wild and grown plants of Atropa acuminata Royle by high-performance liquid chromatography (HPLC) method. Higher amounts of atropine and scopolamine was found in leaves and roots For profiling alkaloids expressed in the hairy root clone of Atropa acuminata DART spectrometric technique has been applied by The effect of two surfactants (Tween 20 and Tween80-0.2%) on removing belladonna plant by the mechanical agitation process using ethyl alcohol and water as solvents showed that the drug was fully extracted. In comparison to tween20, tween 80 obtained better performance.

Molecular genetics

Molecular analysis is useful for determining plant genetic diversity, mutational studies, gene expression, gene action, and the development of genetic maps. Used a molecular technique to gain a deeper understanding of the potential and limits of mutation breeding and to characterize genetic variability. Studied the ribosomal behavior of genes of RNA in the procedure of somatic hybridization within Atropa acuminata and Nicotiana tabasum. Complete hybridization of parent species DNAs to 32p-r DNA probes exposed two classes which were repeats of ribosomes (A. acuminata – 9.4kb, 10.3kb; N. tabacum – 11.2kb, 10.5kb) and in parent species, ribosomal DNA repeat was absent but it was present in new hybrid line- NtAa-1 [prompted genetic alteration in A. acuminata enriched biomass

and alkaloid production by root bringing (Ri) T-DNA from A. rhizogenes. testified kanamycin- resistant transgenic by converting through a CaMV 35S-rol C chimeric gene (co-induced NTP-II) of Ri plasmid of A. rhizogenes and the transmuted plant species showed dramatic promotion of blossoming, small flowers, pale and lanceolate leaves, apical dominance. persuaded transgenic A. acuminata by using agrobacterium aided conversion system (Hyoscyamine 6β hydroxylase which catalyzed oxidative reaction in this pathway from hyoscyamine to scopolamine in the regulator of cauliflower mosaic virus 35S promotor) J attained transgenic A. belladonna doubly transferre with diverse A. rhizogenes (MAFF-03-01-724 and ATCC-15824) dyes in hairy root cultures.

The transformants were confirmed by polymerase chain reaction and opine assay (alkaloid content remain intermediate while IAA level gets low in transformed root cultures). separated gene of Hyoscyamine 6β-hydroxylase from A. acuminata and the results were found that this gene was differentially expressed in the anthers and root pericyclic. Used binary method to induce root cultures (integration of neomycin phosphotransferase and beta-glucuonidase genes) from haploid and diploid varieties of A. acuminata using Ri plasmid of A. rhizogenes (A4 strain). The haploid plantlets exhibited dwarfing features while as, diploids exhibit hairy root disorder. Treated hairy root cultures of Atropa species along with salicylic acid carboxyl methyltransferase and the gene was developed to a high concentration of exogenously supplied Salicylic acid and after 12 hours, expression and exposure of gene occurs sequenced the plastid genetic material of Atropa acuminata (circular DNA of 156, 688 bp) and related with the available sequence of N. tabacum in order to understand nuclear-plastid mismatches and to speciation. It was found that (1) genes were extremely preserved, with reformed residing mainly in low regions of practical significance; (2) signal element promotors and translational replications were preserved in both A. acuminata and N. tabacum (3) RNA editoypes greatly differ between the species initiating rapid reproductive segregation of populations. testified six differently genes expressed in crown gall leaf of A. acuminata along with the infection Rhodococcus fascians (confirmed by RT-PCR). Make comparison between plastid genome of nightshade plants and tobacco and suggests that sequences in the loci were possible to distinguish the huge number of species for barcoding resolution introduced a retroposon in the gene granule bound starch synthesis I (GBSSI), which discloses the nonexistent diploid forefather in the polyploid origin of A. acuminata. Thereby, signifying that retroposons were best molecular markers for studying polyploid evolution.

Biotechnology

Detected organogenesis of cell suspension cultures obtained from excited root cultures of Atropa species. Started callus and cell suspension cultures from plantlets of A. acuminata. Plants raised from shoots introduced on callus cultures and results were showing presence of hyoscyamine, atropine, hyoscine and cuscohygrine. These alkaloids were found absent from callus and cell suspension cultures when started without roots on callus. Investigate the variety of morphogenesis in cell suspension cultures of A. acuminata. Observe the factors (time taken by root cultures to maintain, medium composition, subsequent culturing) affecting morphogenesis in excised roots and cell suspension cultures of A. acuminata. Isolated protoplast from growing cell suspension cultures of Atropa acuminata, which divide continuously and undergo embryogenesis and develop plantlets. Maximum protoplast yield was reported 80% in 4-6h by treating cell suspension cultures with mixture of enzyme cellulose R10 (1%) and macrozyme R10 (0.5%) in 0.5M sorbitol at 30°C. established tissue cultures from haploid and diploid plants of Atropa species and investigate morphogenetic and biosynthetic ability. It was reported that haploid tissues regenerate plantlets much quickly than did the diploids. The obtained regenerates were successfully transplanted into soil and showed growth up to flowering stage. Isolated protoplast from leaf mesophyll of Atropa belladonna and

CONCLUSION

Cultured in liquid culture media regenerate, divide and form callus. Following induction of shoot and root organogenesis leads to the development of plantlets which grows at maturity stage after successfully transplanted to soil. Reported effective growth improvement for stem callus cultures of Atropa belladonna by using glutamate and aspartate derived amino acids alone or along with inorganic nitrogen. However, retorted growth was found by using ornithine, arginine, threonine, lysine and methionine. Persuaded crown gall and hairy root culture of grown plantlets of Atropa belladonna by different strains of A. tumefaciens and A. rhizogenes. Results were obtained that root culture induced by R1 plasmid A4 produced measurable quantity of alkaloids. Have shown that A. acuminata suspension cultures have a small capacity to control the transport of phenylalanine (the atropine precursor) into cells during the stationary growth process. After the start of the suspension culture, the rate of phenylalanine uptake was fastest from 2 to 7 days (increase from 50 to 300% depending on cell line). By repressing phenylalanine synthesis, the enhancement can be blocked by cyclo- hexamide as well as glutamine. From one young root and from the stems of 12 plants of a. acuminata initiated callus cultures (n=156). In their growth reaction on modified Wood and Braun's nutrient medium and in their alkaloid development, the callus derived from

the same piece of stem showed large variations. In the callus cultures of intact mother plants containing a high level of hyoscyamine and scopolamine in seeds, roots or leaves, alkaloid levels were not significantly higher than in callus cultures originating from plants with low alkaloid development. The highest content of hyoscyamine (0.2-0.3g/Kg dry wt.) was typically contained in the 7th and 9th passages in the stem callus lines. The alkaloid content decreased rapidly after the 9th passage, and synthesis repression could not be prevented by lower temperature (14°C as against 260C) or by lower or higher auxin level of the medium.

The best protocol for sterilisation in A. acuminata is 10 minutes of neutral scour marinating 30 minutes of tap water over-washing, 6 minutes of 0.1 percent mercuric chloride sterilization, 3 to 5 times sterile water rinsing. Appropriate medium for inducing the differentiation on bud of the first culture is MS + 0.7mg/L 6-Benzyl adenine + 0.2mg/L NAA + sucrose 3.0%. MS + 0.5 mg/L 6-BA + 0.2 mg/L NAA + sucrose 3.0 percent is the optimal medium for phase generation and bud propagation, while MS + 0.2 mg/L IBA + sucrose 2.0 percent was the best medium recommended for rooting. The highest incidence (45%) of polyploidy91 is caused by axillary buds dipped into 0.1 percent colchicine. labelled U-14C arginine (Arg), ornithine (Orn) and phenylalanine (Phe) and incorporated both the dissected roots of intact plants and the homogeneous or aggregating suspension culture of A. acuminata into and found that hygrine, tropinone and tropanol were present only in roots, and alkaloid production proceeded as far as to scopolamine thereafter. Ornithie was used more successfully than Arginine in the synthesis of the tropane skeleton. In both ethanol insoluble compounds (particularly proteins) and ethanol soluble compounds (particularly phenolics). It was reported that neither hyoscyamine nor scopolamine were found in heavily root suspensions, but traces of tropanol were observed, indicating that at the later biosynthetic stages the synthesis of tropane alkaloids appears to be controlled. Studied growth and uptake of minerals ions transformed root cultures of A. acuminata and other related species ((examined during batch culture over 28 days in modified 14 litre stirred tank reactors containing Gamborg's B5 salt medium; all cultures completely removed NH4+ and PO43- from the medium). NO3- than any other ion, and concomitant absorption of sucrose and glucose release into the medium was consumed by each of the cultures. The roots were found to contain low free sugar levels, and the production of hyoscyamine ranged from 115 mg to 633 mg per reactor. Among several clones of hairy roots, this suggests that the tropane-alkaloid containing plants are taxonomically closely related and regenerated plants. Studied variation of alkaloid productivity. Showed an increase in the production of tropane alkaloids (1490 mg developed in a 30-1 tank) by transforming Belladonna root cultures. Following SEM analysis in floral organogenesis and growth in A. acuminata and other members of the Solonaceae family. Fused pmt (Putrescine N- methyltransferase) gene from N. tabacum with CaMV 35S promoter and integrated into A. acuminata genome and the transgenic plants derived from root cultures showed over expression of the gene. It appears that the expression level of pmt alone does not restrict the formation of tropane alkaloids in A. acuminata.

REFERENCES

1. J R Stepp, et al. The role of weeds as sources of pharmaceuticals. J Ethn. 2004;92:163-166.
2. T Hartmann, et al. Reinvestigation of the Alkaloid Composition of Atropa belladonna Plants Root Cultures and Cell Suspension Cultures. Planta Med. 1986;52:390-395.
3. S H Hedges, et al. δ-N-Methylornithine: a natural constituent of Atropa belladonna. Phytochemistry. 1981; 20:2064-2065.
4. F E Baralle, et al. Biosynthesis of cuscohygrine in Atropa belladonna from sodium acetate-2-14C*1. Phytochemistry. 1969;8:853-855.
5. S A Shvets, et al. A chemical study on the steroidal glycosides from Atropa belladonna L seeds. Adv Exp Med Biol. 1996;404:475-483.