INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
Available online www.irjponline.com

ISSN 2230 – 8407
Review Article

AN UPDATED OVERVIEW ON *ATROPA BELLADONNA* L.

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Article Received on: 12/09/11 Revised on: 10/10/11 Approved for publication: 03/11/11

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ABSTRACT
Atropa belladonna L. (Family: Solanaceae; commonly known as belladonna, deadly nightshade, devil's berries amongst others), a perennial herb (2n=72) is native of Europe, North Africa and Western Asia, possesses a long tradition as one of the classic poisons of antiquity. The species is also the source of atropine alkaloid (dl-hyoscyamine) and is important in the study of autonomic pharmacology. Considering the therapeutic uses of *A. belladonna* as well as its significant toxic effects (due to tropane alkaloids including scopolamine and hyoscyamine), an overview on all necessary aspects is documented to provide information for further exploration of the species for human benefits.
KEY WORDS: *Atropa belladonna*, Overview

INTRODUCTION

Atropa belladonna L. (commonly known as belladonna, deadly nightshade, divale, dwale, banewort, devil's berries, naughty man's cherries, death cherries, beautiful death, devil's herb, great morel and dwayberry[1]), a perennial herb (Family: Solanaceae) hallowed by long tradition as one of the classic poisons of antiquity. The name Atropa is said to be derived from Greek goddess Atropos; while, the name belladonna means 'beautiful lady' in Italian language[1]. The foliage and berries of the species are extremely toxic containing tropane alkaloids which includes scopolamine and hyoscyamine causing a bizarre delirium and hallucinations[1]. The plant species is the source of alkaloid atropine ($C_{17}H_{23}NO_3$, dl-hyoscyamine) which has been proven to be a cornerstone in the study of autonomic pharmacology[2]. *A. belladonna* is also significant for use in medicine and cosmetic[3]. Considering the significance of the medicinal plant species an overview on *A. belladonna* is conducted covering nearly all essential aspects with an objective to provide unabridged repository of references to researchers for its effective exploration as well as utilization in human welfare.

Distribution and Habitat

The plant species is native to Europe, North Africa and Western Asia[1] but not common in England (occurs locally as an apparent native in open vegetation on calcareous soil, also sub-spontaneously and as a relic of former cultivation[3]) and Scotland[4]. *A. belladonna* is a weed species in parts of the world, where it colonizes in areas with disturbed soils[5]. The species grows most luxuriantly forming bushy habit in shade of trees, on wooded hills, on chalk or limestone[4]; while, plants exposed to too much sun become stunted[6]. Growing under natural conditions the species is more subjected to the attacks of insects[4].

Plant description

A. belladonna[1,4] is a branched herbaceous perennial (about 5 ft tall, rare often it is 6.0 ft in height) with purplish coloured stem, stout, undivided at the base but dividing a little above the ground into three to more, rarely 2 or 4 branches each of which branched freely; roots thick, fleshy, whitish, branched, about 6 inches long or more; leaves 3 to 10 inches long, ovate, dull, darkish green in colour, the lower leaves solitary, the upper ones in pairs alternately from opposite sides of the stem, one leaf of each pair much larger than the other, acute at apex, entire with short petioles, veins prominent in undersurface and depressed on upper surface; plant glabrous though soft downy hairs may occur in stem and leaves when they are quite young; flowers solitary in the axil of the leaves, dark and dingy purplish colour, tinged with green, about an inch long, pendent, bell shaped, furrowed, the corolla with 5 large teeth or lobes, slightly reflexed, flowering time from June to early September; fruits berry and five-cleft calyx spreads round the base, shining black colour, full of dark inky juice, sweet and consumed by animals that disperse seeds, even though the seeds contain toxic alkaloids[7].

Cultivation and Harvesting

The species[4,6] prefers a well-drained, well-limed soil in full sun or part shade; however, light, permeable and chalky soil is most suitable for the crop. The soil should be kept moist all times. Belladonna is most frequently propagated by seed sown in flats and the seeds take 4-6 weeks to germinate and when the seedlings are an inch or so high they are set out 18 to 20 inches apart, watered after transplantation and shaded for several days. Belladonna may also be propagated by cuttings of the green branch tips.

Belladonna cultivation in the slope of a hill gives especially good results in regards of high percentage of alkaloids. The limits of growth of the species are between 50^0 and 55^0 N. latitude and an altitude of 300 to 600 ft, though it may descend to sea level where the soil is calcareous with good drainage and adequate shade. The crop may be appreciably increased by the use of farmyard manure, or a mixture of nitrate of soda, basic slag and kainit.

First year plants are generally 1.5 feet high and flowers in September, the leaves and tops are only collected and the plants are thinned to 2.5 to 3.0 feet apart at the approach of winter to avoid overcrowding in the following year. In June, the second year plants are cut to 1 inch above the ground at flowering and in good years a second crop will be ready for harvesting in September. The roots may be harvested in autumn of the fourth year. The parts harvested should be dried quickly in the sun as faded leaves and plant parts yields small amount of alkaloids.

Yield

Atmospheric conditions show marked influence on the alkaloid contents of Belladonna, the highest percentage of alkaloid being yielded in plants grown in sunny and dry seasons. About 0.68% alkaloid has been reported in Belladonna in the months of May and June in sunny and dry seasons in contrary to 0.34% in the same months lacking sunshine. August and September proving very wet season yields about 0.35 to 0.38% alkaloids. English growers found excellent results when soil treated with basic slag and the percentage of total alkaloids in dry leaf and stem from third-year plants accounted to 0.84. The average crop of fresh herb in the second and third years is 5 to 6 tons per acre, and 5 tons of fresh leaves and tops yield 1 ton of dried herb. The yield per acre in the first year of growth is average about 6 cwt. of dry leaves. The greatest loss of plants is in wet winters[4].

Chemical constituents

Hartmann *et al.*[8] found a total of 13 alkaloids from roots of *A. belladonna* following high resolution GLC and GLC-MS; while, the above ground parts of the plant revealed 7 alkaloids (hygrines completely absent). Scopolamine has been reported to be synthesized in roots only but Norhyoscyamine has been frequently found in shoots of the plant species. Hyoscyamine N-oxide was found in various plant parts. Hedges and Herbert[9] reported a natural plant constituent δ-N- Methylornithrine from *A. belladonna* as a tropane alkaloid precursor following radioactive [5- ^{14}C and 5- ^{3}H] feeding of ornithine to the plant. Arraez- Roman *et al.*[10] developed a rapid and easy CE- electrospray interface (ESI)-TOF-MS procedure to isolate several important compounds such as tropine, belladonnine, norhyoscyamine, apoatropine, hyoscyamine, 6β-hydroxyhyoscyamine and scopolamine from leaf extract of *A. belladonna*. Baralle and Gros[11] reported the presence of cuscohygrine (using sodium acetate-2-^{14}C) among other alkaloids. Van Haga and Reinouts[12] reported that cuscohygrine as a normal constituent alkaloid of *A. belladonna*. Shvets *et al.*[13] isolated eight steroidal glycosides tentatively named atroposides A, B, C, D, E, F, G and H from the methanolic extract of *A. belladonna* seeds by Sephadex gel filtration and column chromatography on silica gel impregnated with silver nitrate. The structure of atroposides A, C, E, G are elucidated 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-glucopyranosyl (1 -->2)-beta-D-glucopyranosyl (1-->4)-beta-D-galactopyranoside.

Alkaloid production and Isolation

Kamada *et al.*[14] reported the presence of atropine and scopolamine in hairy roots (hairy roots induced by inoculation of stem of sterile plants of *A. belladonna* with *Agrobacterium rhizogenes*) by TLC and HPLC but their amounts were quantified by GLC, and the results showed that the amount of two alkaloids in the axenic cultures was same as those of normal plants grown in the field. Dimitrov *et al.*[15] suggested an integrated process of extraction (applying pertraction in a rotating film [RF] contractor) and liquid membrane isolation (di-isopropyl ether as liquid membrane and sulfuric acid as a stripping agent) of atropine from roots (87% field, about 48 times higher than in the native extract. Srivastava and Chadha[16] studied the effect of two surface-active agents (Tween 20 and Tween 80 - 0.2%) on the extraction of belladonna herb by percolation and mechanical agitation process using 70% alcohol and water as solvents leading to near complete and expedient extraction of the drug (Tween 80 gave better result).

Biochemistry

Mino *et al.*[17] made complete amino acid sequences of [2Fe-2S] ferredoxin from *A. belladonna* and *Hyoscyamus niger* by automated Edman degradation of the entire S-carboxymethylcysteinyl proteins and of the peptides obtained by enzymatic digestion. The two ferredoxins exhibited 1-8 differences in their amino acid sequences compared to other tropane-alkaloid -containing plants (*Scopolia japonica, Datura stramonium, D. metel* and *D. arborea*) and 9-23 differences among the other solanaceous ferredoxins, thereby suggesting that the tropane-alkaloid containing plants are closely related taxonomically.

Factors affecting growth and chemical constituents

Salonen and Simola[18] suggested that growth and NRA (nitrate reductase activity) can be stimulated by NH_4^+ and by proline, by proline plus ornithine, but not by glutamate, in NO_3^- containing medium. Amino acids tested were not inhibiting. Bensaddek *et al.*[19] also reported that nitrate and ammonium concentrations in the culture medium possess a strong influence (dose dependent) on the scopolamins/hyoscyamine ratio. Phillipson and Handa[20] found marked fluctuations in N-oxide content in the species during organogenesis, highest being found in ripe fruit. Sporer *et al.*[21] studied the seasonal variation in total alkaloid content and alkaloid patterns (by HPLC analysis; examined in June and July from berries and seeds) in belladonna (hyoscyamine was the main product examined), and two peaks were found significant: maximal alkaloid yields were detected at early night and at early morning while in matured seeds, it was highest in the afternoon. Falk and Doran[22] assessed the influence of inoculum morphology on growth of *A. belladonna* hairy roots and production of tropane alkaloids and it was inferred that although hyoscyamine was found to accumulate at higher concentrations in more mature root tissues (2.1 ± 0.2 mg g^{-1}) than in the tips (1.1 ± 0.3 mg g^{-1}), hyoscyamine content was however independent of inoculum morphology. Kanokwaree and Doran[23] suggested that increase in the number of hairy root tips (3 to 9) in shake flasks (50 and 100 ml of medium) reduced growth rates by 40% in belladonna, thereby indicating the influence of medium conditioning and oxygen mass transfer on root growth. Lee *et al.*[24] analyzed growth and tropane content in transformed root cultures of *A. belladonna* (strain M8) grown in 300 ml flasks and after one month in culture, the biomass of the transformed roots increased 15 times and reached 5 g dry wt l^{-1}; the alkaloid contents were 0.7% for hyoscyamine, 0.1% for 6β-hydroxyhyoscyamine, 0.02% for scopolamine and 0.02% for littorine. Out of the seven chemicals added (individually to 18-day-old cultures) glutathione (5 mM), chitin (0.1%), chitosan (0.1%) or yeast extract (0.1%) were with no effect on the release of alkaloids into the medium but treatment with 5 mM H_2O_2 induced a transient release of tropane alkaloids from transformed roots and maximum amount was 0.35 mg per flask. Cu^{2} and Cd^{2} (5 mM) also improved the excretion of tropane alkaloids into the medium; however, led to lysis of transformed roots. Baricevic *et al.*[25] noted the effect of water stress and nitrogen fertilization on the content of hyoscyamine scopolamine in the roots of nightshade, and experimental results indicated that maximal content of alkaloids was achieved with 95% depletion of available soil water and a nitrogen supply of 1.60 g/pot. Ali[26] studied the effect of putrescine on germination and seedling growth of *A. belladonna* under the influence of NaCl and it was found that presoaking seeds in 10^{-2} mM putrescine can alleviate the adverse effect of NaCl during germination and early growth of the species but increase alkaloids as well as endogenous putrescine.

Apart from these stage of development[27], Gibberellic Acid[28], heavy water[29], Tiron[30] (a water-soluble radical scavenger) were the factors amongst others in relation to alkaloid content, growth and morphology of *A. belladonna*.

Therapeutic uses

Drops prepared from the belladonna plant are used to dilate pupils of an eye[31,32]. *A. belladonna* is used in traditional treatments for an assortment of conditions including headache, menstrual symptoms, peptic ulcer disease, histaminic reaction, inflammation and motion sickness[33] as well as in homeopathic drug[1]. Belladonna is used as a sedative, to stop bronchial spasms in asthma and whooping cough and as a cold and hay fever remedy[33]. It is also used for Parkinson's disease, painkiller and ointments of belladonna is applied for join pain (rheumatism), leg pain (sciatica), and nerve pain (neuralgia)[6]. Apart from these, belladonna is also used in plasters, treating psychiatric disorders (hyperkinesis), excessive sweating (hyperhidrosis), and hemorrhoid suppositories amongst others[6]. Ramoutsaki *et al.*[34] reported belladonna as an analgesic and emetic against miscellaneous diseases or ailments and as an antidote for snakebite. Bousta *et al.*[35] reported that *A. belladonna* possesses significant neurotropic and protective effects on behavioral and gastric alterations induced by experimental stress.

Adulterants

Different plants species like *Scopolia japonica* (Japanese Belladonna - rhizome part), *Scopolia carniolica* (Scopolia - rhizome), *Inula helenium* (Elecampane - root), *Medicago sativa* (Medicago - root), *Althaea officinalis* (Marshmallow - root), *Arctium lappa* (Lappa - root) are used as adulterants of *A. belladonna*. (Source: Felter HW, Lloyd JU. King's American Dispensatory 1898.)

Other uses

Cosmetic industry; however, its use is limited as it may cause minor visual distortion and other adverse effects[1].

Clinical trials

Tita *et al.*[36] used rat model to suggest the ability of *A. belladonna* and atropine for urinary retention, and *A. belladonna* was found to be more effective. Bettermann *et al.*[37] performed single-blind placebo-controlled study to investigate the dose-dependent vagolytic and vagotonic effects (parameters studied : heart rate and noninvasive arterial finger blood pressure) after a oral administration (4 hour after) of *A. belladonna* tincture (ABT : 0.1 mg/ml alkaloid concentration, atropine / scopolamine = 20:1; doses = day 1: 2ml, day 2 : placebo, day 3: 5ml, day 4: 1 ml) and found that ABT can be effectively used to stimulate parasympathetic activity in man. The mode of vagal activation changes between 2 and 5 ml ABT from vagotonic to vagolytic. ABT was found to possess no or very little effect on blood pressure control. Toporcer *et al.*[38] studied the mechanical properties of skin wounds after *A. belladonna* application in rats (24 animals were randomly divided into 3 groups: two symmetrical skin incisions were given on the back of each animal and immediately sutured) and wound tensile strength of each group (Group A – plant extract not given, served as control; B – extract given for first two days after surgery; C – extract given for five consecutive days after surgery) was measured 120 hours after surgery. *A. belladonna* extract treated groups (B group: 244 ± 48g; 2.09 ± 0.42% of unwounded skin; C group: 254 ± 67g; 2.18 ± 0.58% of unwounded skin) was significantly higher ($P<0.05$) than untreated group (A: 194 ± 31g, 1.67 ± 0.26% of unwound skin), thereby suggesting a positive effect on aseptic surgical skin wound healing. Gal *et al.*[39] studied the effect of *A. belladonna* (AB) on skin wound healing following biomechanical and histological study in rats and *in vitro* study in keratinocytes, 3TC fibroblasts, and human umbilical vein endothelial cells. Results of *in vivo* experiments showed that AB treated wounds shortened the process of inflammation and accelerated collagen formation as well as significantly increased wound stiffness as compared to control tissues. In *vitro* examination showed that highest AB extract concentration expressed CK19; in addition all concentrations were stimulatory to human umbilical vein endothelial cell proliferation. In addition only the AB extract at the lowest tested concentration increased fibroblast growth, but higher concentrations decreased cell survival. Bogan *et al.*[40] reported a case study of 48-year old man (ingested three handful of Belladonna) who experienced disorientation, aggressiveness and trachycardia was treated initially with diazepam (and intravenous infusion of physostigmine and activated charcoal) and hospitalized. Blood sample analyzed showed that a muscarinic receptor total binding equivalent to binding of 130 µg/l atropine (determined by a radio receptor technique).

Toxicology

The root is the most poisonous, the leaves and flowers less so, and the berries, except to children, least of all[4]. Lange and Toft[41] reported a case where a boy (9 years old) ate 20-25 berries of belladonna plant and serious atropine poisoning was caused which commenced with psychosis; however, the boy survived with intravenous administration of physostigmine Belladonna has been reported to induce blockage of body nervous system, gastrointestinal blockage, urinary retention, paralytic ileus, atony, stenosis, fast heartbeat, constipation, esophageal reflux worse apart from dry mouth, enlarged pupils, blurred vision, red dry skin, fever, inability to sweat, hallucination, spasms, mental problem, convulsions, coma amongst other as a part of toxic effect of the plant species. Southgate *et al.*[42] reported severe poisoning by deadly nightshade in two adults who mistakenly ate berries. Atropine levels were reported in urine and treatment with physostigmine was ineffective. Caksen *et al.*[43] analyzed intoxication of deadly nightshade on 49 children and observed symptoms and signs were meaningless speech, tachycardia, mydriasis and flushing but not death.

Diseases of Belladonna

Smith[44] reported virus infection in the plant species and it occurs in fairly high concentration withstanding for 6-11 days in extracted sap but was inactivated at a temperature between 75 and 80^{0}C. Sol *et al.*[45] reported that *A. belladonna* mosaic virus is transmitted by nematodes in sandy soil. Heuss *et al.*[46] described belladonna mottle virus (belonging to turnip mosaic virus group) as small spherical virus particles containing 180 protein subunits and arranged in a T=3 icosahedral surface lattice. The top and bottom viral components crystallize isomorphously in hexagonal space group R3 (a = B = 296 Å, C = 729Å). The insect pest that attack belladonna leaves is so called 'fleabeetle' and it perforates the leaves to such an extent that it makes it unfit for sale in a dried state (naphthalene kept in soil may be useful to keep the beetles off)[4].

Cytology

The chromosome number in the species was reported to be 2n=72 (krumbiegel and Schiender[47], Gleba *et al.*[48], Babiychuk *et al.*[49]).

Cell biology

Bajaj[50] grew isolated PMCS (pollen mother cells) aseptically in a nutrient medium in microculture and found a multicellular structure giving rise to 6 to 8 microspores which were released at maturity due to rupturing of thick callose wall caused by inside pressure. Simola[51] studied the developmental changes (4 stages) in the subcellular structure of the seeds. The young cells contain spherosomes and the cytoplasm with ribosomes, proplastids and mitochondria and during ripening the vacuoles of endosperm and embryo developed into protein bodies with globoid cavities and the protein mass contains a roundish or crystalline body (strain with periodic acid Schiff-reagent). The embryo and endosperm of ripe *Atropa* seeds were similar containing protein bodies and small spherosomes.

Hybrid analysis

Krumbiegel and Schieder[47] made selection of somatic hybrids after fusion of protoplasts from *Datura innoxia* (diploid - 2n=24, and a tetraploid - 4n=48 – chlorophyll deficient mutant) and *A. belladonna* (2n=72), and the 13 selected hybrids produced chromosomes at metaphase varying from 84 to 175 and the chromosomes of either species were distinguishable from their sizes. On the contrary, sexual incompatibility exists between the two plant species (Krumbiegel and Schieder[52]). Gleba *et al.*[53] successfully produced intertribal hybrid cell lines of *A. belladonna* + *Nicotiana chinensis* by cloning individual protoplast fusion products and the hybrid nature of the clone lines (thirteen obtained) in confirmed by biochemical (studies of amylase isozymes), cytogenetic (size and morphology of chromosomes) and physiological (peculiarities of cell growth *in vitro*) analyses. Results obtained were interpreted as new evidence for the possibility of using non-sexual hybridization for the production of such plant hybrids which cannot be obtained by sexual crossings. Gleba *et al.*[54] made ptotoplast fusion of *N. tabacum* (B6S3) crown gall cells and *A. belladonna* leaf mesophyll to obtain 57 hybrid lines which were verified from biochemical, molecular and cytogenetical studies. Kushnir *et al.*[55] developed functional cybrid (mesophyll protoplast fusion) between *N. tabacum* (chlorophyll deficient, treptomycin-resistant) and wild *A. belladonna* using the polyethylene glycol/high p^H/high Ca^{++}/dimethylsulfoxide method. Three groups of regenerants were identified - (a) nuclear

hybrids; (b) *Atropa* plants arising from rare surviving parental protoplast; (c) *Nicotiana/Atropa* cybrids possessing tobacco genome and *Atropa* plastome and the cybrids were diploid morphologically and phenotypically similar to tobacco yielding viable seeds. Gleba *et al.*[48] obtained asymmetric hybrids between *Nicotiana plumbaginifolia* (n=10) and *A. belladonna* (n=36) by "gamma-fusion" and cytogenetic analysis of hybrid plants revealed that the plants possessed multiple (tetra-or hexaploid) sets of *N. plumbaginifolia* along with 6 to 29 *Atropa* chromosomes some of which were deleted. rDNA genes of both parental species were present and amplified in majority of the hybrids but chloroplast DNA was only from *Nicotiana* parent. However Kushnir *et al.*[50] reported nucleo-cytoplasmic incompatibility in cybrid plants possessing an *Atropa* genome and a *Nicotiana* plastome. Babiychuk *et al.* (1992) showed spontaneous extensive chromosome elimination of *A. belladonna* in somatic hybrid lines developed between *N. tabacum* and *A. belladonna* (2n=72). All lines (3) possess 48 large chromosomes similar to *N. tabacum* and one small fragment of *A. belladonna*. Ahuja *et al.*[57] induced intergeneric somatic hybrid plants between *A. belladonna* (2n=72) and *Hyoscyamus muticus* (2n=28) and the plants varied in chromosome number (100 to 178) and gross morphology, chromosome complementation as well as elimination (*Atropa*) were noted in hybrid plants.

Mutagenesis

Kaul and Choudhary[58] treated seeds of *A. belladonna* with nitrosoguanidine (NG- 1 and 2 mM) and ethylene imine (EI- 0.05 and 0.025 %) resulted in significant increase in plant height, tiller number, leaf number, leaf length, leaf width and alkaloid content. It was also observed that all the treatments (except NG, 1 mM) resulted in a considerable increase in variability in alkaloid content as compared to the control. Abdel-Hady *et al.*[59] carried out investigation to improve germination percentage and alkaloid production in Belladonna seeds following treatments with gamma irradiations (50, 80, 110 and 150 Gy) and gibberellic acid (20,40,60,80,100 and 120 ppm) and it was found that enhancement in germination occur upto 150 Gy (irradiation) and 100 ppm GA treatments and best was recorded at 110 Gy irradiation and 100 ppm GA. Seed colour (red seeds) and three promising high alkaloid containing mutants (M-11-1, M-11-2 and M-15-1) were selected.

Molecular genetics

Jung and Tepfer[60] induced genetic transformation in *A. belladonna* enhanced biomass and tropane alkaloid production by root inducing (Ri) T-DNA from *A. rhizogenes*. Borisjuk *et al.*[61] studied the behavior of ribosomal RNA genes in the process of somatic hybridization between *N. tabacum* and *A. belladonna*. Blot hybridization of parental species DNAs to ^{32}P-rDNA specific probes revealed two classes of ribosomal repeats (*N. tabacum* – 11.2 kb, 10.4 kb; *A. belladonna* – 9.4 kb, 10.2 kb) and a new class of ribosomal DNA repeat absent in parental species was found in hybrid line NtAb-1. Mathews *et al.*[62] developed transgenic *A. belladonna* via *A. tumifaciens* (C58 C1, harboring the plasmid pGV 3850::1103 containing the coding sequences of neomycin phosphotransferase; explants cultured overnight with or without acetosyringone), and Southern hybridization showed the integration of foreign DNA into the plant genome. Of the progeny tested, 2 out of 3 showed monogenic dominant segregation for kan super (R). Kurioka *et al.*[63] reported kanamycin-resistant transgenic by transforming with a CaMV 35S-rol C chimeric gene (co-introduced NPT-II) of the Ri plasmid of *A. rhizogenes*, and the transformed plants exhibited dramatic promotion of flowering, reduced apical dominance, pale and lanceolate leaves and smaller flowers. Saito *et al.*[64] also raised herbicide- resistant *A. belladonna* using Ri binary vector (pRi15834 and pARK5) and integration of T-DNAs from pRi15834 and pARK5 were confirmed by DNA – blot hybridization. The transgenic plants showed resistance towards bialaphos and phosphinothricin. Yun *et al.*[65] induced transgenic *A. belladonna* (hyoscyamine 6 beta-hydroxylase which catalyzes the oxidative reactions in the pathway leading from hyoscyamine to scopolamine under the control of the cauliflower mosaic virus 35S promoter) by the use of an *Agrobacterium* – mediated transformation system. Jaziri *et al.*[66] obtained transgenic *A. belladonna* doubly transformed with different *A. rhizogenes* (ATCC 15834 and MAFF-03-01724) strains in hairy root culture. The transformants were tested by the opine assay and polymerase chain reaction (alkaloid content intermediate while IAA level decreased in transformed roots). Yoshimatsu *et al.*[67] induced root cultures (integration of beta-glucuonidase and neomycin phosphotransferase genes by binary vector method) from diploid and haploid *A. belladonna* using Ri plasmid of *A. rhizogenes* (strain A4). Except for the rol b and rol c insertion mutants, the diploid plantlets were with hairy root syndrome; while, the haploid exhibited dwarfing features. Suzuki *et al.*[68] isolated hyoscyamine 6β-hydroxylase gene from *A. belladonna* and showed that the gene was differentially expressed in the root pericycle and anthers. Bonhomme *et al.*[69] raised transformants of *A. belladonna* hairy root lines via *A. tumefaciens* (2 series: rol C and npt-II; rol ABC and npt-II) and were confirmed by PCR analysis. Possible role of rol C gene in hairy root growth rate and tropane alkaloid in hairy root culture was studied and found to be enhanced several folds. Schmitz-Linneweber *et al.*[70] sequenced the plastid chromosome of *A. belladonna* (circular DNA of 156, 688 bp) and compared with the published sequence of *N. tabacum* to understand nuclear-plastid incompatibilities and to speciation. It was noted that (1) promoters as well as translational and replicational signal elements in both species were well conserved; (2) genes were highly conserved, with differences residing predominantly in regions of low functional importance, and (3) RNA editotypes differ between the species causing rapid reproductive isolation of populations. Fukami *et al.*[71] induced Salicylic Acid Carboxyl Methyltransferase in hairy root cultures of the species and the gene was expressed to a high concentration of exogenously added salicylic acid, expression begins 12h after the exposure and continued over 144h. Nonar *et al.*[72] reported six differentially expressed genes (confirmed by RT-PCR) in *A. belladonna* leafy crown gall following *Rhodococcus fascians* infection. Kress *et al.*[73] made comparison of the total plastid genome of tobacco and deadly nightshade and suggests that the sequences in the loci (nuclear internal transcribed spacer region and plastid trnH-psbA intergenic spacer) were potential to discriminate the large number of species for barcoding purposes. Yuan *et al.*[74] inserted a retroposon in the nuclear gene granule-bound starch synthase I (GBSSI or waxy), which reveals the extinct diploid ancestor in the polyploid origin of belladonna, thereby suggesting that retroposons were promising molecular markers to study polyploid evolution. Kwon *et al.*[75] studied the expression of *A. belladonna* salicylic acid (SA) carboxyl methyltransferase gene, AbSAMT1, encoding S-adenosyl-L-methionine (SAM): SA carboxyl methyltransferase (SAMT) in *A. belladonna* after application of biotic and abiotic stresses to plants. Results suggested that AbSAMT1 may play a dual regulation role of distinct signaling in *A. belladonna* plants, namely the signaling pathway of the SA-dependent response and also a jasmonic acid dependent response in local regions.

Biotechnology

Rajbhandary *et al.*[76] initiated root, callus and cell suspension cultures from seedlings of *A. belladonna*. Cultured roots and plants raised from shoots initiated on cultured callus were shown to contain atropine (hyoscyamine) and hyoscine and cuscohygrine. The alkaloids were found to be absent from cultured callus and cultured cell suspensions and from leaves when initiated without roots on callus. Thomas and Street[77] observed organogenesis in cell suspension culture from excised cultured roots of the species.

Thomas and Street[79] noted the factors (time that the root cultures were maintained, composition of the medium, subsequent culturing, elevated amount of ammonium ions) influencing morphogenesis in excised roots and suspension cultures of Belladonna. Konar *et al.*[79] studied the diversity of morphogenesis in suspension cultures of *A. belladonna*. Rashid and Street[80] studied the development of haploid embryoids from anther culture of *A. belladonna*. Rashid and Street[81] further studied growth, embryogenic potentiality and stability of a haploid cell culture of *A. belladonna*. Gosch *et al.*[82] isolated protoplast from actively growing cell-suspensions of *A. belladonna*, which divide repeatedly and to undergo embryogenesis eventually developed into plantlets. Optimum protoplast yield was upto 80% in 4-5h by treating cell suspension with enzyme mixture of cellulose R 10(1%) and macerozyme R10 (0.5%) in 0.6M sorbitol at 30°C. Eapen *et al.*[83] investigated morphogenetic and biosynthetic ability of tissue cultures established from haploid and diploid plants of the species. It was observed that haploid tissue regenerated plantlets much more readily than did the diploids. The regenerants obtained *in vitro* were successfully transplanted to soil and grown to flowering stage (alkaloids in quantities comparable to the plants raised from seeds). The authors[83] established tissue cultures from leaves of one anther-derived haploid plant of *A. belladonna*, and the regenerants obtained from callus cultures were transferred to soil and reared to maturity. Callus cells (alkaloid content low) and regenerants (content variable) exhibited variable degree of ploidy. Lorz and Potrykus[85] isolated leaf mesophyll protoplasts of *A. belladonna* and when cultured in defined liquid culture media regenerate cell walls, divide and form calli. Subsequent induction of shoot and root organogenesis leads to plantlets which grow to maturity after transfer to soil. Heberle-Bors[86] noted the effects of different types of iron chelates and activated charcoal in anther culture of belladonna (Nitsch medium). FeEDTA was superior to FeDTPA (Sequestrene 330), FeEGTA, FeEDDHA (Sequestrene 138) or Fe citrate. Activated charcoal in combination with FeEGTA and FeEDDHA inhibited haploid formation due to the preferential adsorption of these iron chelates by activated charcoal. Iron chelates seemed to be active during the transition from the globular to the heart-shaped stage of embryogenesis; while, activated charcoal increased the amount of premitotic pollen after a week of culture. Saloneu[87] showed glutamate and aspartate derived amino acids were used alone, or with inorganic N, or in various combinations as N sources effectively enhance growth for stem callus cultures of *A. belladonna* but growth was found to be retarded by arginine, ornithine, hydroxyproline, lysine, threonine, isolencine and methionine. Ondrej and Protiva[88] induced crown galls and hairy roots in *in vitro* cultivated seedling of *A. belladonna* by different *A. tumifaciens* and *A. rhizogenes* strains. Only root cultures induced by Ri plasmid A4 synthesized detectable amounts of alkaloids. Nyman and Simola[89] showed that suspension cultures of *A. belladonna* possess limited capacity to regulate the transport of phenylalanine (precursor of atropine) into the cells at stationary phase of growth. The rate of phenylalanine uptake was fastest from 2 to 7 days (increase from 50 to 300% depending on cell line) after the start of the suspension culture. The enhancement may be inhibited by cyclohexamide as well as glutamine by repressing the synthesis of phenylalanine. Simola and Nieminen[90] initiated callus cultures (n=156) from one young root and from stems of 12 plants of *A. belladonna*. The calli derived from the same piece of stem showed wide variations in their growth response on modified Wood and Braun's nutrient medium and in their alkaloid production. Levels of alkaloids were not significantly higher in the callus cultures of intact mother plants possessing a high level of hyoscyamine and scopolamine in seeds, roots, or leaves than in callus cultures originating from plants with low alkaloid production. The maximum hyoscyamine content (0.2 – 0.3g/Kg dry wt.) was usually found between 7th and 9th passage in stem callus lines. After the 9th passage the alkaloid content decreased rapidly, and the repression of synthesis may not be prevented by lower temperature (15°C as against 25°C) or by lower or higher auxin level of the medium. The best sterilization protocol in *A. belladonna* is that 10 minutes marinating by neutral scour, 30 minutes over-washing by tap water, 6 minutes sterilization by 0.1% mercuric chloride, rinsing 3 to 5 times by sterile water. 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%. The appropriate medium on step-generation and propagation of bud is MS + 0.5mg/L 6-BA + 0.2mg/L NAA + sucrose 3.0%; while, for rooting the best medium recommended was MS + 0.2mg/L IBA + sucrose 2.0 % (100% rooting). Axillary buds dipped in 0.1% colchicine induce highest rate (45%) of polyploidy[91]. Nyman[92] labeled U-^{14}C arginine (Arg), ornithine (Orn) and phenylalanine (Phe) and incorporated into hyoscyamine and scopolamine of both dissected roots of intact plants and homogenous or aggregating suspension culture of *A. belladonna* and found that hygrine, tropinone and tropanol were present only in roots, and alkaloid synthesis proceeded as far as to scopolamine thereafter. In the synthesis of the tropane skeleton, Orn was used more efficiently than Arg. Phe both in roots and suspension cultures, and the label was incorporated in both ethanol insoluble compounds (particularly proteins) and ethanol soluble compounds (phenolics). It has been reported that in heavily root suspensions neither hyoscyamine nor scopolamine were found, but traces of tropanol were detected thereby suggesting that the synthesis of tropane alkaloids seems to be regulated at the later biosynthetic steps. Hilton and Wilson[93] studied growth and the uptake of sucrose and mineral ions by the transformed root cultures of *A. belladonna* and other related species and hybrids (examined during batch culture over 28 days in modified 14 litre stirred tank reactors containing Gamborg's B5 salt medium; all cultures completely removed NH_4^+ and PO_4^{2-} from the medium). Each of the culture consumed NO_3^- than any other ion, and concomitant uptake of sucrose and release of glucose into the medium. Roots were found to contain low levels of free sugars and hyoscyamine production ranged from 115mg to 633mg per reactor. Aoki *et al.*[94] studied variation of alkaloid productivity among several clones of hairy roots and regenerated plants. Lee *et al.*[95] showed enhancement in tropane alkaloid production (1490 mg produced in 30-l tank) by transformed root cultures of Belladonna. Yang *et al.*[96] studied floral organogenesis and development following SEM analysis in *A. belladonna* and in a Solanaceous member (*Anisodus tanguticus*). Rothe *et al.*[97] fused pmt (Putrescine *N*-methyltransferase) gene from *N. tabacum* with CaMV 35S promoter and integrated into *A. belladonna* genome and the transgenic plants derived from root cultures showed over expression of the gene. Expression level of pmt alone is apparently not limiting for tropane alkaloid formation in *A. belladonna*. Richter *et al.*[98] reported that the overexpression of tropinone reductases alters alkaloid composition in root cultures of the species. It has been found that strong expression of the tropine-forming reductase enhanced 5-fold scopolamine and 3-fold hyoscyamine compared with control roots.

CONCLUSION

Present and future researchers interested in the plant species may think of enhancing the existing gene pool (although the plant species is perennial but seed propagated) through the methodology of induced mutagenesis and, if possible, by biotechnological approaches to screen desirable variants with enriched alkaloid contents. Furthermore, sustainable cultivation of the crop under suited climatic conditions is also an essentiality for its effective utilization in human benefits.

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