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Review

Phytochemistry, pharmacology, and biotechnology of *Withania somnifera* and *Withania coagulans*: A review

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Withania **(Family: Solanaceae) is a highly acclaimed genus in the Indian Ayurvedic system of medicine. In Ayurveda, *Withania* is known to promote physical and mental health and used to treat almost all the disorders that affect human health. *Withania somnifera* and *Withania coagulans* are the two most esteemed species of this genus having high medicinal significance. These species are natural source of withanolides (steroidal lactones) which are used as ingredients in many formulations prescribed for a variety of diseases. Many pharmacological studies have been conducted to investigate the properties of *Withania* as a multi-purpose medicinal agent. Advances in biotechnology, especially *in vitro* culture techniques, molecular biology and metabolite profiling provided new insights for conservation and management of plant genetic resources and better harvesting of drugs from medicinal plants. This review presents a consolidated account of the phytochemistry, pharmacology and biotechnology involving *in vitro* propagation, genetic transformation and metabolite profiling in *W. somnifera* and *W. coagulans.***

Key words: *Withania*, phytochemistry, pharmacology, withanolides, micropropagation, metabolite profiling.

INTRODUCTION

The genus *Withania* (Family: Solanaceae) is a highly acclaimed genus of medicinal plants in the Indian Ayurvedic system of medicine because of its valuable pharmaceutical and nutraceutical properties. It is a small group of herbs distributed from the Canary Islands, the Mediterranean region and Northern Africa to the South-west of Asia (Hepper, 1991; Bhandari, 1995). Among the twenty-three known species of *Withania*, only two (*Withania somnifera* and *Withania coagulans*) are economically significant (Negi et al., 2006) (Figure 1). *W. somnifera*, commonly known as 'Ashwagandha', is the most exploited species of the family Solanaceae; however, its counterpart *W. coagulans* has also received attention in the recent years due to its ethnomedicinal properties (Hemalatha et al., 2008). Table 1 shows the botanical description of both plants.

The commercial cultivation of both the species of *Withania* is mainly associated with two major problems: first, the plant to plant variation in the alkaloid quantity and yield, and secondly the long gestation period (4 to 5yrs) between planting and harvesting (Rani et al., 2003; Ciddi, 2010). The reproductive failure due to unisexual nature of flowers in *W. coagulans* is also a major bottleneck in this regard. The increasing market demand of the drug causes overexploitation of natural populations. Therefore, there is need to develop approaches to ensure the avail-ability of raw material of a consistent quality from regular and viable source, thus circumventting the need of harvesting plants from wild.

The previous reviews on *Withania* described the pharmacological properties of both the species (Budhiraja and Sudhir, 1987; Gupta and Rana, 2007; Hemalatha et al., 2008; Kulkarni and Dhir, 2008; Maurya and Akanksha, 2010; Uddin et al., 2012). This review presents the comprehensive information of the research work conducted

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Table 1. Botanical description of *W. coagulans and W. somnifera* (Bhandari, 1995).

S/N	Description	*Withania coagulans* (Stocks) Dunal	*Withania somnifera* (L.) Dunal
1	Habit	Herb	Undershrub
2	English Name	Vegetable Rennet, Indian Rennet	Winter Cherry, Indian Ginseng
3	Vernacular Name	Panir Bandh, Punir, Panir Dodi	Ashwagandha
4	Leaves	Alternate, elliptic lanceolate-coriaceous, obtuse, entire margins, glabrous, coated with minute stellate hairs on both the surfaces	Alternate, broadly ovate, sub-acute, entire margins
5	Inflorescence	Axillary	Axillary, umbellate cymes
6	Flowers	Dioecious	Monoecious
7	Calyx	Campanulate, gamosepalous with 5 sepals clothed with fine stellate grey tomentum	Accrescent, gamosepalous with 5 sepals
8	Corolla	Campanulate, greenish-yellow with 5 petals	Campanulate, greenish-yellow with 5 petals
9	Androecium	Anthers long and filamentous in male flowers, smaller in female flowers	Anthers 1.2 mm long, broadly ovate
10	Gynoecium	Ovary ovoid/globose, without style or stigma	Ovary ovoid/globose, glabrous
11	Style	Glabrous	Filiform
12	Stigma	Mushroom-shaped, 2-lamellate	Mushroom-shaped, 2-lamellate
13	Fruit (Berry)	Globose, smooth, closely girt by the enlarged membranous persistent calyx	Globose, enclosed in the persistent calyx, seeds yellow, reniform
14	Seeds	Globose, ear shaped, glabrous, enclosed in the persistent calyx yellow, reniform	Globose, enclosed in the persistent calyx, yellow, reniform
15	Flowering	November-March*	Throughout the year

*Rarely flowers.

on phytochemistry, pharmacology and biotechnology of *W. coagulans* and *W. somnifera.*

PHYTOCHEMISTRY

Several specific reactions operating temporally and spatially are responsible for the production of secondary metabolites in medicinal plants. Changes of the environmental factors or placing a plant into tissue culture may also produce a new, and sometimes unexpected, secondary metabolic profile (Cordell, 2011). The phytochemistry of *Withania* species has been studied extensively by several workers and several groups of chemical such as steroidal lactones, alkaloids, flavonoids, tannin etc. have been identified, extracted, characterized and isolated (Atta-ur-Rahman et al., 1993; Kapoor, 2001). At present, more than 13 alkaloids, 138 withanolides, and several sitoindosides (a withanolide containing a glucose molecule at carbon 27) have been isolated and reported from aerial parts, roots and berries of *Withania* species (Subramanian and Sethi, 1969, 1971; Budhiraja et al., 1983; Velde et al., 1983; Neogi et al., 1988; Atta-ur-Rahman et al., 1993; Choudhary et al., 1995; Atta-ur-Rahman et al., 1998, 1999, 2003; Nur-e-Alam et al., 2003; Mirjalili et al., 2009b; Xu et al., 2011).

The major chemical constituents of this plant, withanolides, are mainly localized in the leaves and roots and their concentration usually ranges from 0.001 to 0.5% dry weight (Kapoor, 2001). The withanolides are a group of C28-steroidal lactones built on an ergostane structure in which C-22 and C-26 are oxidized to form a six-membered lactone ring. The basic structure is designated as the 'withanolide skeleton' (Figure 2) (Tursunova et al., 1977; Glotter, 1991; Alfonso et al., 1993). The withanolide skeleton may be defined as a 22-hydroxyergostan-26-oic acid-26,22-lactone. Modifications of the carbocyclic skeleton or the side chain give rise to many novel structures variants of with anolides. It has been reported that plants accumulating these polyoxygenated compounds possess enzyme machinery capable of oxidizing all carbon atoms in the steroid nucleus. The characteristic feature of withanolides and ergostane-type steroids is one C8 or C9-side chain with a lactone or lactol ring. The lactone ring may be either six-membered or

Figure 1. *Withania coagulans* (Stocks) Dunal and *Withania somnifera* (L.) Dunal plants growing in the field. (a) *W. coagulans* plant; (b) *W. somnifera* plant; (c) *W. coagulans* fruits; (d) *W. somnifera* fruits.

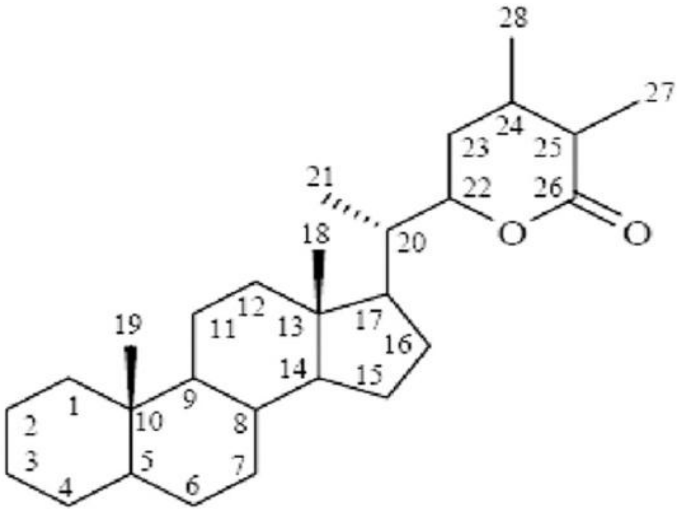

Figure 2. The basic withanolide skeleton.

five-membered and fused with the carbocyclic part of the molecule through a carbon-carbon bond or through an oxygen bridge. Appropriate oxygen substituents may lead to bond scission, formation of new bonds, aromatization of rings and many other kinds of rearrangements resulting in novel structures (Kirson et al., 1971; Glotter, 1991; Mirjalili et al., 2009b).

Though withanolides are the principal bioactive compounds found in both species, there are some withanolides specific to each of them. Withaferin A is a major compound found in *W. somnifera*, whereas, coagulin L has been found in major amounts in *W. coagulans*. A unique thio-dimer of withanolide named ashwagandhanolide has been found in *W. somnifera* (Subaraju, 2006). Withanolides containing a 14,20-epoxide bridge are specific to *W. coagulans* (Subaraju, 2006). Zhao et al. (2002) isolated five new withanolide derivatives from the roots of *W. somnifera* together with

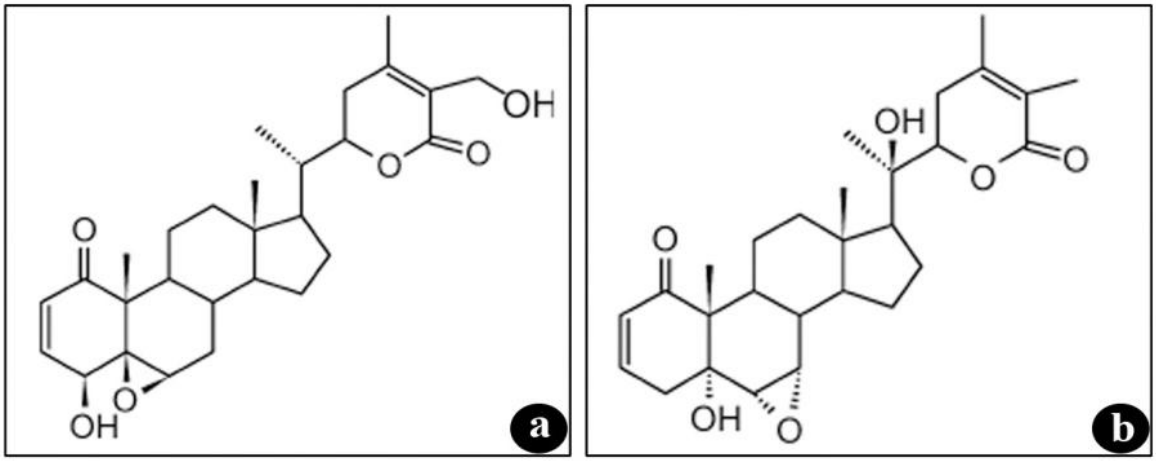

Figure 3. Structures of important withanolides: (a) Withaferin A and (b) Withanolide A.

fourteen known compounds and recently Tong et al. (2011) also reported a novel chlorinated withanolide, 6a-chloro-5b,17a-dihydroxywithaferin A (1), from *W. somnifera*.

Other compounds in *Withania*

Many other chemical compounds in *W. somnifera* and *W. coagulans* have also been reported. For example, Gupta et al. (1996) detected alkaloids in all the plant parts (roots, fruits, leaves), with the highest content found in leaves. Another study also detected nicotine, somniferine, somniferinine, withanine, withananine, pseudowithanine, tropine, pseudotropine, 3a-tigloyloxy-tropane, choline, cuscohygrine, dl-isopelletierine and new alkaloids anaferine and anhygrine in this medicinal plant (Gupta and Rana, 2007). The total alkaloid content varied between 0.13 and 0.31%, (Johri et al., 2005). Apart from these contents, the plant also contains chemical constituents like acylsteryl glucosides, starch, hantrea-cotane, ducitol, and a variety of amino acids including aspartic acid, proline, tyrosine, alanine, glycine, glutamic acid, cystine, tryptophan, and high amount of iron (Gupta and Rana, 2007; Hemalatha et al., 2008).

PHARMACOLOGY

The chemical constituents of *Withania* have always been of great interest to the scientific community. The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine, etc), steroidal compounds including ergostane-type steroidal lactones, withaferin A, withanolides A-Y, withasomniferin A, withasomidienone, withasomniferols A-C, withanone, etc (Gupta and Rana, 2007; Maurya and Akanksha, 2010). Withaferin A (4β,27-dihydroxy-5β,6β-epoxy-1-oxowitha-2,24-dienolide) (Figure 3a), and withanolide A (5α,20α-dihydroxy- 6α,7α -epoxy-1-oxowitha-2,24-dienolide) (Figure 3b) are the main withanolidal active principles isolated from the plant. These are chemically similar but differed in their chemical constituents (Sangwan et al., 2007; Hemalatha et al., 2008).

Anti-inflammatory activities

The anti-inflammatory potential of *W. coagulans* and *W. somnifera* has been studied in details by several workers. Budhiraja et al. (1984) showed that the aqueous extract of fruits of *W. coagulans* has significant anti-inflammatory activity at 10 mg kg^{-1} in subacute models of inflammation, such as granuloma formation and formalin-induced arthritis in rats. Anbalagan and Sadique (1981) reported that *W. somnifera* possesses efficient anti-inflammatory activity as compared with hydrocortisone, a common anti-inflammatory drug.

The effect of *W. somnifera* on glycosaminoglycan synthesis in the granulation tissue of carrageenin-induced air pouch granuloma was studied by Begum and Sadique (1987). Oral administration of 1000 mg kg^{-1} *W. somnifera* root powder decreased the glycosaminoglycan content by 92%, which was much higher than that of the hydrocortisone and phenylbutazone. Al-Hindawi et al. (1992) studied the granuloma-tissue formation inhibiting activity of various fractions of an extract of the aerial parts of *W. somnifera* using subcutaneous cotton-pellet implantation in rats. The methanolic fractions of the extract showed high anti-inflammatory activity as compared to that of a 5 mg kg^{-1} dose of hydrocortisone sodium succinate. The activity in both the species was attributed

to the high content of biologically active steroids in the plant, of which withaferin A is known to be a major component. Withaferin A is potent inhibitor of the pro inflammatory transcription factors and a promising agent for the treatment of the inflammatory cascade of cardio-vascular diseases (Kaileh et al., 2007).

Anticancer and chemoprotective activities

The anticancer effect of *Withania* has been studied extensively (Devi et al., 1995, 1996; Devi, 1996; Davis and Kuttan, 2000; Prakash et al., 2002; Senthilnathan et al., 2006; Winters, 2006; Widodo et al., 2007), and it was found that it is the most effective agent in preventing cancer through its ability to reduce the tumor size. Treatment of root extract of *W. somnifera* on induced skin cancer in mice exhibited significant decrease in the incidence and average number of skin lesions compared to control group (Prakash et al., 2002). Withaferin A showed tumor-inhibitory activity against cells derived from human carcinoma of the nasopharynx (Jayaprakasam et al., 2003) and it also inhibited the growth of roots of *Allium cepa* by arresting the cell division at metaphase (Palyi et al., 1969). In another study, *W. somnifera* was evaluated for its antitumor effect in urethane-induced lung adenomas in adult male albino mice. Simultaneous administration of *W. somnifera* extract (200 mg kg^{-1} body weight daily orally for seven months) and urethane (125 mg kg^{-1} biweekly for seven months) reduced tumor incidence significantly (Singh et al., 1986). Additionally, in a different study the aqueous extract of *W. coagulans* was used for anti-cytotoxic effect in chicken lymphocytes and remarkable inhibitory activity of dimethyl sulfoxide (DMSO)-induced cytotoxicity with a decrease in TNF-G production was reported (Chattopadhyay et al., 2007).

Hepatoprotective activity

The extract of *W. coagulans* roots exhibited hepato-protective activity against carbon tetrachloride (CCl_4)-induced hepatotoxicity in adult albino rats of either sex due to the presence of 3-β-hydroxy-2, 3-dihydro-withanolide F. The hepatoprotective effect of W. *somnifera* root powder was studied by Mohanty et al. (2008). The extract influenced the levels of lipid peroxidation and thereby provided the hepatoprotection. Verma et al. (2009) also examined the effect of *W. somnifera* aqueous root extract on the hepatic cell of *Clarias batrachus* and reported that the root extract contains different flavonoids and neurotransmitters that stimulated the neuroendocrine system, leading to hyper-activity of the endomembrane and the exit of molecules through the surface via exocytosis.

Immunomodulatory activity

Withaferin A has been reported in various studies to possess both immuno-activating and immunosuppressive properties. Withaferin A has specific immunosuppressive effects on human B and T lymphocytes viz. antigen recognition and proliferative capacity of B and T lymphocytes (Bahr and Hansel, 1982). In mice, the ashwagandha extract was able to suppress the cyclophosphamide-induced potentiation of delayed type hypersensitivity (DTH) reaction. A protective effect in cyclophosphamide-induced myelosuppression was observed in animals treated with this extract (Agarwal et al., 1999). In another study, the aqueous suspension of the *W. somnifera* root powder inhibited the mitogen induced lymphocyte proliferation and DTH reaction in rats (Rasool and Varalakshmi, 2006). The root extract of *W. somnifera* also enhanced total white blood cell count, inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages (Davis and Kuttan, 2002). Significant increases in hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight were observed in *W. somnifera*-treated mice compared to untreated control mice.

Huang et al. (2009) isolated novel withanolides, withacoagulins from the areal parts of *W. coagulans* and reported the inhibitory activity of the extract on T and B-lymphocyte proliferation in murine spleen cells. It was also observed that the ethanolic extract showed strong activities in inhibiting the T and B-lymphocyte proliferation. Coagulin H isolated form *W. coagulans* exhibited effects on the immune response, including an inhibitory effect on lymphocyte proliferation, and expression of interleukin-2 (IL-2) cytokine. A complete suppression of phytohaemagglutinin-activated T-cells was observed at ≥2.5 µg/ml coagulin H. The withanolides from both the plants are found to be useful as a general tonic, due to their beneficial effects on the cardiopulmonary system. These alkaloids had a prolonged hypotensive, bradycardiac, and respiratory-stimulant action in dogs (Budhiraja et al., 1983; Mohanty et al., 2004).

Antifungal and antibacterial activities

Antifungal and antibacterial properties have been demonstrated in the withanolides isolated from the ethanolic extract of the whole plant and leaves, respectively. The methanolic extract possessed maximum inhibitory activity against a spectrum of bacteria. Oral administration of the aqueous fruit extracts successfully obliterated *Salmonella* infection in mice as revealed by increased survival rate, as well as less bacterial load in various vital organs of the treated animals (Owais et al., 2005). The methanol, hexane and diethyl ether extracts from both leaves and roots of *W. somnifera* were evaluated for the antibacterial

/synergistic activity by agar plate disc-diffusion assay against *Salmonella typhimurium* and *Escherichia coli* (Arora et al., 2004).

Hypocholesterolemic and hypolipidemic activities

The aqueous extract of fruits of *W. coagulans* and the root powder of *W. somnifera* have been reported to decrease total lipid, cholesterol and triglycerides in hypercholesterolemic animals (Andallu and Radhika, 2000; Hemalatha et al., 2006). Visavadiya and Narasimhacharya (2007) carried out a study to investigate the hypocholesterolemic activity of *W. somnifera* in male albino rats and suggest that the hypocholesterolemic effect of *W. somnifera* could be mediated through an increased bile acid synthesis for elimination of body cholesterol. The hypocholesterolemic and hypolipidemic activities of *W. coagulans* were also reported by Hemalatha et al. (2006). Administration of an aqueous extract of fruits of *W. coagulans* to high fat diet-induced hyperlipidemic rats for 7 weeks significantly reduced elevated serum cholesterol, triglycerides and lipoprotein levels. This extract also showed hypolipidemic activity in triton induced hypercholesterolemia (Hemalatha et al., 2006).

Central nervous system (CNS) related activities

The bioactive metabolites isolated form *Withania* have been found to be effective in alleviating many central nervous system disorders such as epilepsy, anxiety, depression, catalepsy, and sleep (Subramanian and Sethi, 1971; Budhiraja et al., 1977; Bhattacharya et al., 1997; Dhuley, 2001; Jain et al., 2001; Naidu et al., 2006). The extracts for the different parts of both the plants have the capacity to modulate various neurotransmitters also. Bhatnagar et al. (2009) observed that the extract work as a suppressor of corticosterone release and activating choline acetyltransferase, which in turn increase serotonin level in hippocampus. Withanolide A and withanoside IV from *W. somnifera* roots promote neurite outgrowth in cultured neurons and in rodents injected with Aβ 25-35 and after oral administration of withanoside IV, sominone, an aglycone of withanoside IV, was identified as the main metabolite (Kuboyama et al., 2002). Recently Sehgal et al. (2012) revealed that the semi-purified extract of the roots of *W. somnifera* reversed behavioural deficits, plaque pathology, accumulation of β-amyloid peptides (Aβ) and oligomers in the brains of middle-aged Alzheimer's disease transgenic mice by enhancing low-density lipoprotein receptor-related protein in brain microvessels and liver.

Free radicle scavenging activities

The effect of the aqueous solution of root extract of *W. somnifera* on lipid peroxidation was investigated on stress induced rabbits and mice and (Dhuley, 1998). The oral administration of the extract prevented the elevation in lipid peroxidation by the free radical scavenging activity. The free radical scavenging activity of *W. coagulans* was detected by Hemalatha et al. (2004); it was concluded that administration of aqueous extract of *W. coagulans* to diabetic rats significantly lowered the liver and serum lipid peroxidation. The presence of free radical scavenging activity and lipid peroxidation lowering activity in aqueous extract of *W. coagulans* might have helped in providing protection to some degree against oxidative damage to beta cells of pancreas.

BIOTECHNOLOGY

Biotechnological approaches, specifically plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Namdeo, 2007). For plant cell culture techniques to become economically feasible, it is important to develop methods that would allow for consistent production of high yields of products from cultured cells (Berlin and Sasse, 1985). In order to obtain yields in high concentrations for commercial exploitation, efforts have been focused on the stimulation of biosynthetic activities of cultured cells using various methods (Dixon, 2001; Rao and Ravishankar, 2002).

Plant regeneration through micropropagation

Micropropagation of plants for the mass cultivation and production of plantlets in culture has been a useful vegetative propagation process for agriculture, horticulture and forestry, and plant biotechnology (Zhou and Wu, 2006). Other potential applications of plant tissue cultures are the production of novel compounds that are not normally found in the original plant, and biotransformation of low-cost precursors into more valuable compounds. In a number of medicinal plants there is little knowledge of *in vitro* culture, genetic and cellular network descriptions. The genetic diversity of medicinal plants is going to be endangered at an alarming rate because of ruinous harvesting practices and over harvesting for production of medicines. Hence, an efficient and most suited alternative solution to the problem faced by pharmaceutical industry is the development of *in vitro* systems for mass production of medicinal plants, conservation of germplasm, study and production of bioactive compounds and for genetic improvement (Nalawade and Tsay, 2004).

Different explants including cotyledons, hypocotyls, leaves, shoot tips, zygotic embryos, embryonal leaves, stems, internodes and roots and combinations of different plant growth regulators have been employed for plant regeneration in *Withania.* Sen and Sharma (1991) examined regeneration from cultured shoot-tip and nodal explants of *W. somnifera.* Teli et al., (1999) obtained analogous results. Roja et al. (1991) reported callus formation from axillary meristem on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 2 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), whilst Baburaj and Gunasekaran (1995) observed callus induction from leaf explants of *W. somnifera* using MS medium supplemented with 2 mg L^{-1} naphthaleneacetic acid (NAA) and 0.5 mg L^{-1} kinetin (Kn). Kulkarni et al. (1996) also described the direct regeneration of *W. somnifera* with sixteen shoots on an average from leaf explants in *in vitro* raised seedlings using MS medium fortified with indole-3-acetic acid (IAA) and 6-benzylami-nopurine (BA), while Abhyankar and Chinchanikar (1996) showed direct shoot regeneration from leaf discs grown on MS medium supplemented with IAA, BA, and Kn in various combinations. Furthermore, Rani and Grover (1999) initiated callus cultures from cotyledonary leaf, hypocotyl and root segments on MS medium supplemented with 2,4-D (2 mg L^{-1}) and Kn (0.2 mg L^{-1}). They also observed that MS medium with BA (2 mg L^{-1}) was best for multiplication of shoot bud on subculturing. Kulkarni et al., (2000) also reported regeneration of shoots from nodes, internodes, hypocotyls and embryos using BA and thidiazuron (TDZ) in the MS medium and found that the embryo derived plantlets failed to acclimatise.

Meanwhile, Manickam et al. (2000) reported successful development of a callus mediated indirect plant regeneration system using shoot tips and internodal segments cultured on MS medium supplemented with 0.5 mg L^{-1} 2,4-D. Wadegaonkar et al. (2006) developed a protocol for direct rhizogenesis and establishment of fast growing normal root organ culture of *W. somnifera* using MS medium fortified with 0.5 mg L^{-1} IAA and 2 mg L^{-1} IBA in a bubble column reactor. Dewir et al. (2010) used leaves as explants source and 2 mg L^{-1} BA and 0.5 mg L^{-1} IAA for indirect differentiation of shoots in *W. somnifera*. In addition, Vadawale et al. (2004) and Kannan et al. (2005) advocated use of higher cytokinin levels for shoot multiplication in *W. somnifera.* Joshi and Padhya, (2010) also studied the effect of cytokinins and revealed that the leaf explant in presence of individual cytokinin does not regenerate shoot buds. The synergistic effect of combination of two cytokinins in the medium induced shoot bud regeneration. Saritha and Naidu (2007) observed *in vitro* flowering on MS supplemented with 0.5-4.0 mg L^{-1} Kn and 0.1 mg L^{-1} IAA and *in vitro* fruiting on Kn (2.0 mg L^{-1}) and IAA (0.1 mg L^{-1}) in *W. somnifera*. Lee et al. (2007) analyzed the effect of photon flux density and light quality on *in vitro* morphogenesis in cultures of *W. somnifera*. Sivanesan and Murugesan (2005) studied the effect of various cytokinins on regeneration from axillary buds of *W. somnifera*. Khatun et al. (2008) examined the effect of copper on growth and antioxidant enzyme responses *in vitro* grown plants of *W. somnifera.* Sinha et al. (2010) optimised the level of micronutrient copper in the culture medium and found that the number of shoot buds incraesed 1.9-fold on MS medium supplemeted with 5 mg L^{-1} BA and 1 mg L^{-1} IAA with 5× the MS level of copper. Sivanandhan et al. (2011) developed an efficient protocol for *W. somnifera* from nodal explants of field-grown plants on MS medium supplemented with BA (1.5 mg L^{-1}), IAA (0.3 mg L^{-1}) and with the addition of polyamine, spermidine (20 mg L^{-1}), while Kanungo and Sahoo (2011) developed a short term protocol for *in vitro* propagation of *W. somnifera* using apical buds from the 6 week old *in vitro* grown seedlings.

In *W. coagulans*, Jain et al. (2009) for the first time reported the direct regeneration using shoot tip and nodal segments as explant culutred on MS+ BA (0.5 mg L^{-1}), Kn (0.5 mg L^{-1}) with histological details. Valizadeh and Valijadeh (2009) also developed an *in vitro* callus induction and plant regeneration protocol using leaf and internodal explants of *W. coagulans* cultured on 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} Kn, but the yield of shoot regeneration was only 18 - 33%. Adventitious shoot regeneration using leaf explants with a maximum of 17.6 ± 0.5 shoots per explant and determination of clonal fidelity of regenerated plantlets using randomly amplified polymorphic DNA (RAPD) in *W. coagulans* has also been reported by Jain et al. (2011).

Genetic transformation

The use of genomics and proteomics technologies to elucidate and characterize metabolite pathways in a holistic manner provided new directions in the metabolic engineering and metabolomics, in the past few years. Genetic manipulation of a metabolic pathway in medicinal plants requires accessibility of cloned genes and the development of basic gene transfer and expression technology to allow over-expression or down-regulation of genes involved in metabolic processes in the pathway (Capell and Christou, 2004). Although the establishment of transgenic cell lines and hairy root cultures is relatively simple, such systems have inherent drawbacks such as high cost of bioreactors and unstable nature of cell lines, which limit their usefulness and in turn the efficiency of the system to produce the target molecule. The development of protocols for successful and efficient genetic transformation in medicinal plants with exclusive metabolic pathways is important in terms of understanding the molecular basis and regulation of secondary metabolism in plants. There are many reports describing genetic transformation of a number of agriculturally

important plant species, however, such efforts on medicinally important plants have been very few (Gómez-Galera et al., 2007; Pandey et al., 2010).

In *W. somnifera,* transformation has been reported through *Agrobacterium rhizogenes* in the transformed root cultures by Ray et al. (1996) and Ray and Jha (1999), but these resulted in the formation of hairy roots, which required *in vitro* culture and produced only those chemicals that are synthesized in the roots such as withanolide D, but not withaferin A. Accumulation of withaferin A was detected in transformed root lines of *W. somnifera* (Bandyopadhyay et al., 2007). In another study carried out by Murthy et al. (2008), the cultured hairy roots synthesized withanolide A and its concentration in transformed roots was 2.7-fold more than in non-transformed cultured roots. A significant enhancement of the antioxidant activity was reported in transformed roots of *W. somnifera* (Kumar et al., 2005). High yielding transformed cultures of *W. somnifera* using *A. tumefaciens* and synergistic improvement of the withanolide was achieved by a dual elicitation strategy, comprising of the addition of selected abiotic and biotic elicitors cultures by Baldi et al. (2008). A synthetic gene encoding the fungal protein cryptogene has been transformed in *W. somnifera* through *A. rhizogenes* (Chaudhuri et al., 2009). The transformed root cultures of *W. somnifera* showed increased production of secondary metabolites, with increase in biomass production. Mirjalili et al. (2009a) developed a protocol for hairy root culture in *W. coagulans* in order to obtain information about the biosynthesis of withanolides in *W. coagulans* using *A. rhizogenes*. Pandey et al. (2010) also described *Agrobacterium tumefaciens* mediated transformation of *W. somnifera* using leaf segments and observed than leaf segments from 2½ month-old greenhouse grown seedlings were more efficient in transformation, as compared to those from the *in vitro*-grown shoots.

Metabolite profiling

The analysis of total metabolome of a plant is important to extend our understanding of complex biochemical processes within a plant. Significant technological advances in analytical systems like nuclear magnetic resistance (NMR), gas chromatography-mass spectroscopy (GC–MS) and high performance liquid chromato-graphy (HPLC) have opened up new avenues for plant metabolomics research aimed at rapidly identifying a large number of metabolites quantitatively and qualitatively. This has become an important area of investigations in pharmacology and functional genomics of medicinal plants. Comprehensive chemical analysis is required not only to establish correlation between complex chemical mixtures and molecular pharmacology, but also to understand complex cellular processes and biochemical pathways via metabolite-to-gene network (Nakabayashi et al., 2009). The metabolic constituents, particularly secondary metabolites differ with the variety in both *W. somnifera* and *W. coagulans*, tissue type and sometimes with growth conditions (Abraham et al., 1968). Such variations often lead to inconsistent therapeutic and health promoting properties of various commercial *Withania* preparations (Sangwan et al., 2004; Dhar et al., 2006) and the compositional standardization of herbal formulation becomes difficult. Deocaris et al. (2008) described cases where multi-component *W. somnifera* extracts showed better medicinal efficiency than the purified compounds.

Qualitative and quantitative analysis of withanolides in *Withania* has been reported by several workers. In general, undifferentiated calli and cell suspensions of *W. somnifera* do not produce withaferin A (Yu et al., 1974; Ray et al., 1996) although shoot cultures from axillary meristems in *W. somnifera* synthesize withanolides (Roja et al., 1991). Furmanowa et al. (2001) underpinned the importance of various physical and hormonal parameters for production of withanolides from shoot cultures of *W. somnifera*. Ray and Jha (2001) raised shoot cultures of *W. somnifera* and determined that multiple shoot cultures were promising source of withaferin A. Ganzera et al. (2003) and Khajuria et al., (2004) developed a direct and rapid protocol for separation, identification, and quantification of selected withanolides in plant extracts of *W. somnifera* by HPLC-UV (DAD). Sharma et al. (2007) established a validated and densitometric high-performance thin-layer chromatographic (HPTLC) method for the quantification of withaferin-A and withanolide-A in different plant parts of two morphotypes of *W. somnifera* and reported that both the morphotypes differ in their morphological and chemical characteristics.

Phytochemical variations and withanolide A biogeneration in *in vitro* shoot cultures of *W. somnifera* were also described by Sangwan et al. (2004, 2007). Later Sangwan et al. (2008) reported that withanolide A is *de novo* synthesized in roots of *W. somnifera.* The productivity of withanolide A was found to vary considerably with the change in the hormone composition of the media as well as genotype used (Sharada et al., 2007). Hairy roots of this species were able to produce withanolide D (Ray et al., 1996), and sporadically, some root lines also produced withaferin A (Bandyopadhyay et al., 2007) and withanolide A (Murthy et al., 2008). Chaurasiya and Sangwan, (2007) examined develop-mental patterns and secondary metabolism in relation to eco-physiology and phytopharmaceutical variability and *de novo* biosynthesizing capacity via incorporation with a radiolabeled primary precursor, [$^{2\text{-}14}$C]-acetate in *W. somnifera* and found that *de novo* biogenesis and accumulation of withanolides was most active in young leaves. More recently, Nagella and Murthy (2010a, b) established cell suspension cultures of *W. somnifera* for the production of

withanolide A and Dewir et al. (2010) performed a comparative analysis of withanolides in *in vitro* and green house grown plants and found that *in vitro* grown plants of *W. somnifera* contained greater contents of phenolics, flavonoids and polysaccharides while lower contents of withanolides than greenhouse grown plants. Chatterjee et al. (2010) presented a comprehensive metabolic finger-printing of leaf and root extracts of *W. somnifera* and identified a total of 62 major and minor primary and secondary metabolites from leaves and 48 from roots with a common set of 27 metabolites in both the tissues in *W. somnifera*. Sabir et al. (2011) developed strategy for biotransformation of withanolides in *in vitro* suspension cultures of *W. somnifera*. Alam et al. (2011) characterised the phenolic acids, flavonoids and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activities in methanolic extracts of *W. somnifera* fruits, roots and leaves using HPLC and found that the fruits and leaves provide potential benefits for human health because of its high content of polyphenols and antioxidant activities, with the leaves containing the highest amounts of polyphenols specially catechin with strong antioxidant properties.

Furthermore, Sivanandhan et al. (2011) compared the withanolide content in various parts of *in vitro* raised plants with field grown plants and observed that withaferin A and withanone content were 1.14 and 1.20 times higher in the leaves of *in vitro* derived plants than in the leaves of field-grown parent plants, respectively and the roots of *in vitro* shoots had shown 1.10 times increase in withanolide A production than the roots of field-grown parent plants. Significant level of withanolide B content was recorded in the leaves (6.5 times) and roots (3.3 times) of *in vitro* derived plants. Most recently, Sabir et al., (2012) studied the effect of various types of salts under *in vitro* culture conditions using tissue specific isozyme profiling and reported that the tissue could grow better under sodium chloride (NaCl) and potassium nitrate (KNO_3) compared to other salts and the *in vitro* shoots appeared healthy at 50 mM concentration of NaCl and KNO_3, while the total withanolide content increased with 50 mM NaCl and declined with all other salt treatments.

In vitro cultures of *W. coagulans* are also the convincing source of the active principle (withanolides) but there are only a few reports on withanolides production in tissue cultures of this plant. Recently, Abouzid et al. (2010) reported production of withaferin A in the root cultures of *W. coagulans.* Jain et al. (2011) for the first time confirmed the high accumulation of three important withanolides (withaferin A, withanolide A and withanone) in the shoot cultures of *W. coagulans*. Mirjalili et al. (2011) introduced the *Arabidopsis thaliana* squalene synthase gene in *W. coagulans* using *A. rhizogenes* and found that engineered hairy roots have a strong positive correlation between biosynthesis of phytosteroles and withanolides and expression level of the transgene.

FUTURE PROSPECTIVE

The use of *W. somnifera* and *W. coagulans* as multi-purpose traditional medicine has resulted into several commercial drugs and therefore *Withania* ranks a highly valued plant in the pharmaceutical industries. The phyto-chemistry and pharmacology of *Withania* has been widely investigated, but the studies on toxicology of the extracts of the plant parts in different solvents are very few. In the case of *W. somnifera*, the studies are at a primary level and there are no such reports on *W. coagulans*. Although it is required to identify the novel clinical properties of the plant, the identification and isolation of the particular compound responsible for the specific activity is more important. We believe that further advancements in the analytical and separation chemistry will provide valuable insights on the toxicology and isolation of novel compounds along with the chemotypic variation of these two ethnobotanically important species. The availability of micropropagation protocol will be supportive to conserve the elite germplasm of this genus. Further, the transgenic protocols for either the plants are well established but the efforts to enhance the withanolides or alkaloids content in plant parts using this approach are lacking. The progress in the transgenic biotechnology will further pave the way for metabolic engineering of useful compounds from *W. somnifera* and *W. coagulans.*

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