The Pharma Innovation Journal 2023; 12(7): 1928-1935

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 1928-1935

www.thepharmajournal.com
Received: 28-04-2023
Accepted: 04-06-2023

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The past, present and future aspects of *Withania somnifera* (L.) Dunal breeding: A review

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Abstract
Withania somnifera (L.) Dunal is known to be verging on all parts of the world since antiquity for its medicinal attributes contributed by the active constituents present in its roots and leaves. The foremost biochemical compositions of Ashwagandha are withanolide A, withaferin A, withanoside IV and V, 12-deoxy withanostamalide, protein, fibre, carbohydrate, iron, carotene, calcium and many more which are present in substantial amount and imparts medicinal value to it. Ashwagandha is low in natural regeneration and is high in demand by pharmaceutical industries and there is a requirement to bring this crop in cultivation in large area. Further the progress in novel breeding methods and molecular techniques is needed. This particular review deals with the past, present and future aspects of *Withania somnifera.*

Keywords: Ashwagandha, active constituents, biotechnological approaches, breeding behavior, omics

Introduction
The medicinal plants for a long duration reported to provide considerable resources with respect to preparing chemical solutions to intensify strategies for survival in the form of various natural products. The utilization of these products precisely occurs in pharmaceuticals, pesticides, fragrance, agrochemicals, food additives and flavor ingredients. Preponderance of these products derived from plants is based on many traditional medicines and extracts from these have been utilized by human beings for the treatment of varying diseases (Croteau *et al*., 2000; Rao and Ravishankar, 2002) [7, 47].

The chemical structure of the chemical products originated from plants plays one of the prime roles against several ailments. The Food and Drug Administration, during the period of 2005–2007 founded thirteen new drugs into the market which originated naturally and for clinical studies more than hundred drugs developed from natural product (Li and Vederas, 2011) [34]. Nowadays, mostly all drugs are utilized for making western medicine. The plant derived natural compounds in the form of dietary components gradually becoming the vital part of human nutrition research (Pandey and Rizvi, 2009) [46]. These chemical entities in the form of natural products that do not confer directly in development and growth of a plant are described as secondary metabolites.

Under specific ecological conditions such as biotic and abiotic stresses plant secondary metabolites *viz*. terpenoids, alkaloids and phenylpropanoids play valuable role in plant survival. In many instances secondary metabolite are often restricted to specific plant species.

These herbal drugs are prominent due to their low cost, potency, fewer side-effects and efficacy. The proliferating global demands for herbal medicine, there are not only requirement for huge quantity, but as well as quality of raw material of medicinal plants, in which active compounds should be present in desirable amount. Moreover, synthesizing these complex natural compounds synthetically is difficult due its complex chemical structures and the whole process of developing it is economically restricted.

Withania somnifera (L.) Dunal, is one such a medicinal plant of immense reputes and have the capability to boost immunity and health to fight against SARS-CoV-2 infection (Singh *et al*., 2022) [50]. *Withania somnifera* (L.) Dunal member of nightshade family, having chromosome number 2n=2x=48, has vernacular names such as Ashvagandha, Winter cherry, Asgandh, Ashvakandika, Balada, Ashwagandha, Indian ginseng, Balaja, Vajigandha, Vajikari, Singalese, Vajiini, Palashaparni, Gandhapatri, Falso Alchechengi, Aasoganda, Amukkara, Badzigandha (Kirtikar *et al*., 1993) [29]. The name Ashwagandha is made from two sanskrit words- ashwa means “horse” and gandha means “smell.” Whereas, many believe that name Ashwagandha is due to the horse-like smell that herb releases.

The nutrient composition of Ashwagandha root powder (Per 100 g) obtained by chemical analysis (Kumari and Gupta, 2016; Gulati *et al*., 2017) [31, 18] is as given in the table 1.

Table 1: Nutritional constituents of Ashwagandha root powder *per* 100 g

Nutritional composition	Root powder of Ashwagandha
Protein (g)	3.9
Iron (mg)	3.3
Calcium (mg)	23
Total carotene (µg)	75.7
Carbohydrate (g)	49.9
Fibre (g)	32.3
Vitamin C (mg)	5.8
Moisture (%)	7.45
Oil (%)	0.3
Ash (g)	4.41
Energy (Kcal)	245
Total alkaloids (%)	0.26-0.31
Tannins (mg/g)	0.39-0.82

Phylogeny, origin and history

The solanaceae family is rich in diversity and is worldwide distributed and imparts 93 genera and 2700 species (Mehmood *et al.*, 2020) [36]. The subfamily is solanoideae and the order is solanales of the genus *Withania*, holds near about 20 species. Globally, among the list of *Withania* species due to the therapeutic potential value, Ashwagandha [*W. somnifera* (L.) Dunal] and ashutosh booti [*W. coagulans* (Stocks) Dunal] are examined essential. *Withania* species are crucial in the Ayurvedic medicine in Southeast Asia. It is as ancient as Ayurveda itself, which is believed to be 5,000 years old. According to Charaka Samhita, Susruta Samhita and other ancient texts, Ashwagandha is called as Brusya, Balya, Kamarupini, Pustida and vajikari (Mukherjee *et al*., 2021) [38].

The monographs of WHO includes *Withania* (Marderosion, 2001) [35]. It has been broadly domesticated from the wild form and retains a natural occurrence, likely in the humid and drier areas, its habitat expanded from the Cape Verde Islands and Canary region to the Arabia and Middle East region like Pakistan, India, Southern China and Sri Lanka, Mediterranean region to all around tropical region of Africa and South Africa, from Baluchistan to Afghanistan (Gaurav *et al*., 2015) [14]. It is not present in the Northern Cape regions and western half of the Western. In India, extensively spread in the states such as Maharashtra, Gujarat, Himachal Pradesh, Rajasthan, Uttar Pradesh, Jammu, Madhya Pradesh and the Punjab plains (Indian Drug Manufacturer's Association, 2002). It is also cultivated in parts of Madhya Pradesh, Chhattisgarh and Rajasthan.

According to NMPB, the *W. somnifera* (L.) Dunal has occupied 3rd place among the list of most prioritized medicinal plant. In India around 3000 plants are recognized for their medicinal value and it is acknowledged as one of the 12 mega diversity centers in the whole world. It occupies 4th rank in Asia and 10th in the world and has around 20,000 plant species with medicinal value, out which 30 percent are reviewed as endemic to India (Sapra *et al*., 2020) [44]. The actual availability of ashwagandha is 1500 tons but the yearly demand is very high nearly 7000 tons. So in order to fulfill this demand, commercial cultivation of Ashwagandha has been started for high quality and production (Akshatha *et al*., 2018) [2].

Botanical and agronomic attributes

Ashwagandha is a perennial, dicot, hermaphrodite, chasmogamous, self-pollinated plant. The time from seed sowing to the seedling establishment is considered as a vital phase in the life cycle of any plant and it has a significant impact on the final yield and quality of seeds. As Ashwagandha is propagated by seeds and its seeds can withstand desiccation to low moisture contents and storage at very low temperatures and therefore it falls under the category of orthodox seeds (Thakur *et al.*, 2004) [55]. The germination of seeds is low due to dormancy, therefore many techniques are needed to breakdown seed dormancy in plant (through seed priming) and to increase germination and synchronise emergence of seed (Heydecker *et al*., 1975) [20].

Based on the standard descriptor, the plant habit of cultivated ashwagandha is perennial, sexual mode of reproduction, erect or semi-erect plant growth habit, leaf shape is ovate or ovate-rounded, and leaf color is greenish yellow or light green or pale green, root color is cream or either whitish cream, root fracture is either fibrous or non-fibrous, internal root color is either white or cream, inflorescence type is axillary fascicles or umbellate cymes, flower color is dull yellow or yellow or green and berry color is either red or orange.

The vegetation is found mostly on the river banks and in margins of forest (Govindaraju *et al*., 2003) [16], most presumably for its fleshy roots. Existence reported in both sunny and slightly dark places (Gaurav *et al*., 2015) [14]. Ashwagandha is not suitable for water logging conditions as there is no arenchyma or few arenchyma present in their parenchymates tissue of roots (Sahu, 2015) [40]. Flowering time of *Withania* is generally from mid of the October to June. The germination of the Ashwagandha seed takes more than 15-20 days.

Active constituents

A vast variety of metabolites, including steroidal lactones, alkaloids, flavones, glycosides, carbohydrates, quinones, saponins, tannins, terpenoids, and coumarin, can be found in Ashwagandha. Eight distinct polyphenols, including three types of flavonoids (naringenin, catechin, and kaempferol) and five phenolic acids (p-coumaric, vanillic, benzoic, syringic acid and gallic acid). This plant contains a variety of distinct metabolites, and the quantities of these metabolites differ amongst the various chemotypes. Withaferin A and withanone are the two main substitution patterns of its steroidal components. Withanones with oxidation at carbons 5, 6, and 7 rarely exhibit any action, whereas withaferin A with oxidation at carbons 4, 5, and 6 is regarded as an active kind, particularly as an anticancer (Joshi *et al*., 2014) [24]. *Withania somnifera* is the source of the three active ingredients, withasomnine, hydroxywithasomnine, and methoxywithasomnine. These pyrazole-containing compounds all exhibit a variety of pharmacological actions (Kandar, 2020). A new asteroid named 5,6-de-epoxy-5-en-7-one-17-hydroxy withaferin A was isolated from the leaves and roots of *Withania somnifera* L. Dunal along with several other known compounds, including 16-en-27-deoxy withaferin A, 16-en-27-deoxy withaferin A, 27-deoxy withaferin A, withaferin A, withanolide D, and 27-hydroxy withanone. The with anolides are a class of naturally occurring C28-steroidal lactones that are based on an unaltered or modified ergostane framework, where the oxidation of C22 and C26 results in the formation of a six-

member lactone ring. Due to the structural similarity between withanolides and the ginsenosides found in Panax ginseng, *W. somnifera* is often known as "Indian ginseng". In plants treated with a lengthy photoperiod, withanolides were found to be more abundant.

General health benefits

This is one of a select few key medicinal plants that is highly sought after both National and international (Hassannia *et al*., 2019) [19]. This plant is highly appreciated in the pharmaceutical industry since it has been acknowledged as the medicinal plant used in the production of more than 200 traditional medicinal herbal formulations (Jain *et al*., 2012) [23]. According to reports, this plant's leaf and root extracts contain a variety of secondary metabolites that have been used as treatments for a variety of illnesses, including cancer, neurological disorders, and immunological disorders, with only minor adverse effects (Gupta and Rana, 2007) [12]. According to Kujur *et al*. (2002) [30], the chemical nature of Ashwagandha roots, or the content of total alkaloids, which ranged from 0.16 to 0.66%, is what gives them their therapeutic qualities. *Withania somnifera* is a small herb that is common throughout India. It has been used traditionally in medical systems as liver tonic, anti-inflammatory agent, astringent, aphrodisiac and more recently as a treatment for bronchitis, asthma, ulcers, emaciation, insomnia, and senile dementia, among other conditions. The medicinal use of Ashwagandha for neurological diseases, anxiety, inflammation, and Parkinson's disease is supported by clinical trials and animal research. Due to its ability to prevent chemotherapy, Ashwagandha may be a helpful supplement for individuals receiving radiation and chemotherapy. In addition to being used therapeutically as an immune stimulant in patients with low white blood cell counts in the blood, Ashwagandha is also utilized as an adaptogen for patients with nervous weariness, sleeplessness, and debility caused by stress (Verma and Kumar, 2011) [56].

Mechanism of action

Ashwagandha is an adaptogenic plant that enhances the body's resistance to stress by having a favourable impact on the hypothalamic-pituitary-adrenal axis. An exclusive group of plants known as "Adaptogens" aid in the balancing, healing, and defence of the body against the impacts of stress. They are thermostat-like in nature because they are amphoteric. The new discovery of an adaptogens ability to inhibit the stress-activated c-Jun N-terminal protein kinase 1 is one of the most significant aspects of an adaptogen. A stress response is mediated by an adaptogen. An adaptogen promotes both calm and energy. Their special skills aid in reducing stress and enhancing mood, mental clarity, and physical endurance (Wal *et al*., 2018) [57]. The capacity to lower reactive oxygen species, adjust mitochondrial activity, control apoptosis, lower inflammation, and improve endothelial function all contribute to the pharmacologic effects (Ahmad and Dar, 2017) [1]. By retaining larger levels of both enzymatic and non-enzymatic antioxidants, *W. somnifera* was revealed to have a preventive defence mechanism against oxidative damage. Thin layer chromatography analysis of the products revealed that several primary metabolites were integrated into with anolide A, proving that withanolide A is produced de novo inside roots from primary isoprenogenic precursors. As a result, withanolides are produced throughout the plant rather than being imported (by way of the full metabolic pathway operating) (Saxena *et al*., 2008) [48]. Squalene serves as the biosynthetic precursor for withanolides (WTDs), well-known and medicinally significant chemicals of *Withania somnifera*, including the anticancer agent withaferin A (WFA). Squalene synthase (SQS) catalyzes the condensation of farnesyl pyrophosphate (FPP) molecules to generate Squalene (25). Triterpenoids of these beneficial chemical, called C28-steroidal lactones are thought to be synthesized by the sterol pathway using 24-methylene cholesterol as a substrate flow and the mevalonate (MVA) and 2-C-methyl-d-erythritol-4-phosphate (MEP) pathways (42).

Genetic resources and ex situ conservation

For the purpose of conserving biodiversity, the International Union for Conservation of Nature, a membership union made up of both governmental and non-governmental organizations, was founded (Gowthami *et al.*, 2021) [17]. Since the beginning of the 1990s, the Government of India (GoI) has given medicinal plants the attention they deserve. Several initiatives have been made to protect and conserve these species both ex situ and in situ. About 16.5 million ha (5.02%) of India's total geographic area is covered by protected areas, while 70.8 million ha (21.54%) of it is covered by forests. 870 protected areas in all, including 104 national parks, 551 animal sanctuaries, 127 community reserves, and 88 conservation reserves, are designated in India. In addition, India is home to between 100,000 and 150,000 sacred groves, according to (Kandari *et al.*, 2014) [26].

The Government of India has established a number of institutes and organizations for ex situ conservation such as Botanical Survey of India, National Botanical Research Institute (NBRI), Central Council for Research in Ayurvedic Sciences (CCRAS), Central Council for Research in Homoeopathy (CCRH), ICAR-Directorate of Medicinal and Aromatic Plants (ICAR-DMAPR), Central Council for Research in Siddha (CCRS), and Central Council for Research in Unani Medicine (CCRUM), specifically to conduct research on medicinal plants under the Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homoeopathy (AYUSH),

Also active in the preservation and production of these medicinal plants are a number of different non-government and government organizations, businesses and Ayurveda practitioners (Bhattacharyya *et al*., 2006) [5].

The G-15 GEBMAP program established a network of 4 National Gene Banks for Medicinal and Aromatic Plants (GEBMAP) at (1) ICAR-NBPGR, (2) KSCSTE - JNTBGRI, (3) CSIR-CIMAP, and (4) Regional Research Laboratory (RRL) Jammu (added later). The Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, served as the program's nodal agency. This has not only improved focus and impetus, particularly on collection and conservation of medicinally essential vulnerable species, but it has also assisted in coordinating ongoing activities in the nation (Sharma and Pandey, 2013) [51-52].

The Indian government also funded the study of medicinal plants by establishing the NMPB in the Ministry of AYUSH in 2000. Along with NMPB, DBT and the Department of Science and Technology (DST) have offered funding to researchers to study medicinal plants. Ashwagandha was one

of 32 medicinal plants that the NMPB highlighted for conservation recently (in 2019).

The main cultivars are Rakshita and Poshita from CIMAP Lucknow, India, WS-20 (JA-20) and WS-22 from Jawahar Lal Nehru Krishi Vishawavidhalaya, Mandsore, India, and WSR from RRL, Jammu, India. An extensive procedure for the micro propagation of *Withania somnifera* has also been created by the Regional Research Laboratory in Jammu, India. The botanical name for the local variation Nagori, which has starchy roots, is Withania Ashwagandha kaul. For the cultivation of *Withania somnifera*, full Agrotechnology has been established by the Central Institute of Medicinal Aromatic Plants in Lucknow, India. In accordance with accreditation from the worldwide organic certification body ESCOCERT S.A, it has also engaged in the organic cultivation and processing of Ashwagandha root (Shinde *et al*., 2015) [53].

The past and present Ashwagandha breeding

Breeding behavior

The species displayed impressive interpopulation diversity, particularly in terms of the filament, pedicel, and style lengths, which have an impact on its capacity to reproduce. Despite its flowers initially being susceptible to cross pollination, an increase in filament length impairs self-pollination. The flower is protogynous, which means that the anthers mature at full bloom while the stigma becomes receptive at the bud stage. Protogynous flowers and the lower position of the anthers make it possible for first cross pollination. When there is partial xenogamy, an increase in filament length raises the anthers to the same level as the stigma, enabling autogamy. *W. somnifera* uses mixed mating, which involves 66% self-pollination and 34% cross-pollination. This method will be helpful for producing both recombinant and autogamous seeds. As well as showing variation in terms of bud initiation stage, anthesis, maximum flowering stage, fruit initiation, fruit ripening, crop withering stage, etc., different accessions of this species also showed variation in terms of bud ripening stage (Mehta and Raina 2018) [37]. The pollen is fertile in flower buds with a length of 0.6 and 0.7 mm, indicating that the flower was fertilized while still in its bud and that it is of the chasmogamous type (Sahu, 2015) [40].

Breeding objectives

Improved root yield components and quality, high and consistent yield, resistance to biotic and abiotic stresses, safety and high functional value, suitability for technological applications in agriculture, cost-saving and sustainable production, high active compounds and effective protection of plant breeders' rights are some of the major breeding goals for Ashwagandha. (e.g., by hybrid varieties)**,** to prevent decay high antimicrobial properties of the composition, high content of desirable compounds to reduce extraction costs and enable microbiological decontamination procedures, and absence of harmful substances to prevent costs associated with removing them after harvest (De Chandra, 2017) [8].

Genetic architecture of breeding targets

Breeding tactics can be modified by understanding the genetic makeup of key target features. Numerous variables of Ashwagandha have been investigated for their genetic makeup, including data from an experiment used to ascertain component trait variation, the strength of the association between root yield and quality attributes, as well as path coefficient. The phenotypic coefficient of variation for root length, plant height, and seed output per plant was found to be very large. Root yield was positively correlated with plant height and root length, with heritability in the broad sense and genetic progress being determined to be moderate to high. According to a path analysis, plant height directly contributes to root yield, followed by root length (Khatak *et al*., 2013) [28].

The ratio of 9:7 (yellow: red) was found in inheritance studies of yellow versus red berry color, which showed that the trait was under the control of classical double recessive epitasis. According to Deore *et al*. (2014) [9], two genes (Y1 and Y2) with complementing recessive epitasis are responsible for this cross's berry color.

With the exception of plant height and the number of secondary branches, the bulk of the features taken into account were controlled by non-additive gene action, as indicated by the 2gca/2sca. With a little increase in the additive fraction, additive and non-additive gene actions controlled the fresh root production (Balakrishnan *et al*., 2021) [3].

The analysis of variance revealed that the population of genotypes contained a sizable degree of variability. Both the total withanolides content (22.8%) and the total alkaloid content (37.38%) showed large genotypic coefficients of variation (GCV %). Days to maturity (94.0%), total alkaloid content (91.0%), days to flowering (82.0%), and plant height (70.0%) all showed high heritability estimates. The features with high to moderate GCV (%), heritability, and genetic advance (GA) included total alkaloid content, total withanolides content, main root length, and plant height; these variables are therefore controlled by additive gene action (Gami *et al*., 2015) [13].

Biotechnological approaches for enhancement of secondary metabolites

Organ, tissue, and cell culture-based biotechnological techniques provide prospective solutions to the current issues. Elite cultivars may be multiplied quickly using *in vitro* propagation, which also makes it easier to produce high-quality planting materials. For the improvement of secondary metabolites and general crop improvement, genetic engineering and secondary metabolite engineering offer considerable promise. The enormous therapeutic potential of *W. somnifera* encourages investigation of all feasible technologies, including *in vitro* and recombinant DNA techniques, to produce chemotypes with desirable enhanced chemo profiles. Although feasible alternatives, recombinant DNA or cell fusion techniques are limited by the lack of genetic and biochemical understanding of the manufacture of secondary metabolites (Evans and Sharp, 1986) [11]. Therefore, it is urgently needed to make biotechnological advancements in order to increase yield at a faster rate.

Using tissue culture techniques, it is possible to cultivate plant cells on a large scale in order to extract secondary metabolites from them. These approaches provide a continuous, reliable, and renewable source of valuable plant medications. For *W. somnifera*, significant work has been reported utilizing a variety of *in vitro* techniques, with the major focus being on the manipulation of adjuvants for plant growth regulators and the cultural settings for withanolide accumulation (Kaur *et al.*, 2021) [27]. The potential use of *in vitro* techniques to create

cell, organ, and root cultures for increased withanolide production is discussed below (Dhar *et al*., 2015) [10].

Cell suspension culture, *in vitro* shoot culture and root culture

A relatively small portion of the total withanolide content of the native plant is represented by different withanolides. It's crucial to investigate condition-adjusted cultures for resourceful *in vitro* biogeneration of such potent withanolides. Production of withanolide D, WS-1, WS-3, and WS-2 in organogenic cultures has been documented. There are also numerous reports of withanolide D and WS-3 accumulation in cultures of altered roots and shooty teratomas, however WS-1 was purportedly not detected in these cultures. It has been reported and optimized W. somnifera cell suspension cultures have been successfully established for the biogenesis of WS-1.

The main method for producing WS-1 *in vitro* is through root cultures, especially hairy roots, and its bioreactor-based upscaling. However, Agrobacterium rhizogene-transformed hairy roots of *W. somnifera* that have WS-1 present have not been found (Banerjee *et al*., 1994) [4]. According to reports, the aerial sections of the plant are primarily responsible for producing the withanolides seen in these hairy root cultures. Therefore, *W. somnifera* shoot cultures, which are tissue culture complements of the native plant's aerial portions, were used to study WS-1 biogenesis.

Multiple shoot cultures of *W. somnifera* have been observed to produce withanolides in response to hormones (Methyl jasmonate, salicylic acid, benzyladenine) culture conditions, and elicitations. Shoot cultures are a viable medium for withanolide functional genomics research as well.

A. rhizogenes-mediated hairy (transformed) roots provide enormous potential for research on secondary metabolite production because of their rapid development, broad branching, and genetic stability. They can also produce secondary metabolites that are unique to certain types of roots (Giri and Narasu, 2000) [15]. In order to produce important secondary chemicals, hairy roots have been induced in a variety of aromatic and medicinal plants (Santos *et al*., 2005) [43]. Using A. rhizogenes strain R1601, Murthy *et al*. (2008) [39] reported transforming *W. sominifera* and obtaining converted hairy roots from cotyledons and leaf explants.

Somaclonal variants

Both genetic and epigenetic factors can contribute to somaclonal variation (Larkin and Scowcroft, 1981; Lee and Phillips, 1988) [32, 33]. According to (Skirvin *et al*. 1994) [45], activation of transposable elements, point mutations, chromosomal rearrangements, recombination, DNA methylation, and altered sequence copy number are frequently linked to the emergence of somaclonal variation.

Cytological behavior

To learn more about the function and effects of mutagens and to clarify how genotypes react to a given mutagen, cytogenetic investigations are required. There is no premature moment of chromosomes occurring in normal chromosomal pairing. In control plants, mitosis occurred as it should have (Bharathi *et al*., 2014) [6]. However, the chromosomal analysis showed that 2n=48 was the diploid number in all of the examined accessions.

The future of Ashwagandha breeding: integrating advanced breeding methods Omics application

In order to comprehend and examine the available biological data more fully, the omics study has been established in the biological sciences. High-throughput 'omics' technologies, including genomics, transcriptomics, and proteomics, are enabling in-depth analyses of its molecular alterations and elucidating data on many biological pathways. Although the phytochemical profiles and medicinal properties of *W. somnifera* are quite well understood. There are extremely few genes and proteins that are used in the production of these pharmaceutically useful components. Despite the ongoing expansion of *W. somnifera* genetic knowledge, the basic stages in the biosynthesis of withanolide are still unclear. Isoprenoids are the building blocks for anolides; they are produced from traditional cytosolic mevalonate (MVA) and the plastid-specific MEP route that produces IPP. Coding and non-coding RNAs and their expression levels are analyzed using transcriptomics, and two transcriptome sequence data for *W. somnifera* are available in the SRA database of the NCBI. The results were obtained from leaf and root tissues utilizing the 454 sequencing platform and salicylic acid-treated leaf transciptomes. It has been suggested that a small number of potential genes, including cytochrome P450s, glycosyltransferases (GTs), and methyl transferases, are involved in the manufacture of 24-methylene cholesterol. Considered to be a precursor for many withanolide biosynthetic pathways is 24-methylene cholesterol (Siddique, 2014) [49]. The predicted three-dimensional structure of numerous known proteins from *W. somnifera* has been examined in terms of proteomic research. Different enzymes involved in the production of withanolide were investigated in order to determine the structure from the sequence. For their projected structures, HMGR, DXS, DXR, FPPS, SQS, SQE, and CAS have been reported (Sangwan *et al*., 2017) [41-42].

Genome enabled breeding

Molecular gene transfer significantly speeds up the breeding process. The clarification of the biosynthesis pathway of significant secondary metabolites, the identification of associated enzymes, the isolation of encoding genes, and the design of gene transfer techniques are all major areas of ongoing study with medicinal plants. These studies are exceedingly expensive, and it is still mostly unknown how to successfully develop transgenic cultivars with commercial value. An effective method for identifying similarities and evolutionary connections between genotypes of *Withania somnifera* collected from various geographical regions is ISSR-based molecular diversity evaluation. By choosing varied parents for crossing (based on the molecular diversity of the accessions), it is possible to improve the root and other properties of Ashwagandha genotypes and create high-yielding variations (Tiwari and Shrivastava, 2016; Hiremath *et al*., 2021) [54, 21].

Conclusions and future prospects

The successful introduction of Ashwagandha into the Indian herbal market may increase rural jobs, stimulate trade, and improve millions of people's health. One such plant with a consistent market demand, well-established cultivation techniques, and established marketing channels is Ashwagandha. Due to this, Ashwagandha use is now

sustainable despite its high demand and declining natural population.

Ashwagandha is a crop that is ideally adapted to diversify our farming systems and adapt to changing conditions because of its distinctive medicinal profile and biosynthetic route. Ashwagandha is experiencing resurgence as a result of growing public awareness of its medical and pharmacological properties and health advantages. The Ashwagandha gene pool's extensive genetic diversity makes it possible to create and enhance cultivars that are high in secondary metabolites. It is necessary to further evaluate the genetic and phenotypic variety that is available for economic features through genetic research and multi-location examination. In addition, interspecific hybridization between species that are genetically distinct is a promising way to take use of heterosis in Ashwagandha. Recent years have seen significant progress in the Ashwagandha genomic treatments, which has a huge potential to be used in future crop improvement projects. However, Ashwagandha molecular breeding initiatives using various omics methods lag far behind those in other therapeutic crops. Genome sequencing of significant wild and weedy species will be helpful for a number of large-scale genotyping applications, such as cultivar identification, germplasm characterization, and QTL discovery, in addition to cultivated species. The identified markers' inter-species transferability demonstrates their use in deciphering the phylogenetics of this complex genus. Together, these findings will aid in the development of Ashwagandha plants with high medicinal value and high yields for use in future agricultural systems. The current condition of Ashwagandha research and breeding is discussed in this paper, along with recommendations on how to use emerging molecular tools to bring this extinct crop back to life. Ashwagandha research and breeding can catch up to big crops that have been rigorously grown for many years by making full use of the techniques already available.

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