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Comparative morphometric, physiological and chemical studies of wild and cultivated plant types of *Withania somnifera* (Solanaceae)

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Ashwagandha (*Withania somnifera* Dunal.) is an important medicinal plant cultivated in about 4000 ha in India. It is mainly distributed in the northwestern region of Madhya Pradesh and also in limited areas of different states. However, wild collection of the species continues to be a source of raw drug in Ayurvedic preparations. In the present communication, results of a comparative study of the wild type distributed locally in Anand, Gujarat and the superior variety, Jawahar Ashwagandha (JA-20), are presented. Results revealed conspicuous differences between the cultivated and wild-type plants in most of the characters studied. One of the major differences between cultivated and wild-type plants is that the former are annual, whereas the latter are perennial. Photosynthetic rate was higher in the wild type, which was reflected in its higher biomass production. Another distinguishing character was the floral structure which favours self-pollination in the cultivated plants because of short stigma covered with anther lobes, which is in contrast to the wild type having long, projected stigma inviting cross-pollination. The cultivated plants were in full bloom during December–February; however, in the wild type flowering was a continuous process throughout its lifespan. Flow cytometer study revealed the same ploidy level for both the plant types. However, chemical profile showed variation between the two plant types, even though targeted chemical constituents tested in the study were common to both. However, HPLC quantification of these constituents showed superiority of the wild type compared to JA-20.

Keywords: Chemical profile, floral structure, flow cytometer, *Withania somnifera*.

ASHWAGANDHA (*Withania somnifera* Dunal.) is an important medicinal plant cultivated in the northwestern region of Madhya Pradesh and also in different parts of India in about 4000 ha (ref. 1). It is also found growing wild throughout India. Even now, wild collection of the species continues to be a source of raw drug in Ayurvedic preparations. Ashwagandha roots and occasionally its leaf and seeds are used in Ayurveda and Unani medicines. Roots

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are prescribed as medicines for hiccups, several female disorders, bronchitis, rheumatism, dropsy, stomach and lung inflammation, and skin diseases. It is also an ingredient in medicines prescribed for curing disability and sexual weakness in males. The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), and saponins containing an additional acyl group (sitoindoside VII and VIII)[2].

The species has been domesticated for a long time in Central India. The cultivated plants are distinct from the wild-type plants not only in their therapeutic properties but also in a variety of morphological characters, including those of root, stems, leaves, flower, pollen, etc. though the alkaloids present are the same in both[3]. Some botanists, therefore, described the cultivated plant type as a distinct form and have coined a new name, *Withania ashwagandha*[3]. However, it was not widely accepted and challenged by a group of taxonomists because these two types freely cross and produce intermediary forms[4]. There is a belief among some local practitioners that the wild types are superior in therapeutic action; however, this has not yet been confirmed authentically.

The cultivars available at present are the selections made by the farmers and researchers from time to time. Jawahar Ashwagandha-20 (JA-20) developed by All India Co-ordinated Research Project on Medicinal and Aromatic Plants, Jawahar Lal Nehru Krishi Viswa Vidyalaya, Mandsaur, Madhya Pradesh, under the aegis of ICAR is one of the popular varieties of the species[5]. A comparative study of the wild type distributed locally in Anand, Gujarat and JA-20 was attempted and results are presented in this communication.

The study was carried out at the Directorate of Medicinal and Aromatic Plants Research (DMAPR, formerly National Research Centre for Medicinal and Aromatic Plants), Anand. The Institute is located between 22.5°N lat. and 73.0°E long., having about 800 mm average annual rainfall. Minimum and maximum temperatures range between 12.7°C and 42°C.

Wild type used was locally available in the fields and JA-20 was used as cultivated type (Figure 1 *a*). Morphometric data were collected from plants of the same age. Plants were randomly selected from the populations for observation of various traits. Root yield was recorded on five-month-old plants when the cultivated types were ready for harvesting.

Plant height; number of primary, secondary and tertiary branches; number of leaves per plant; leaf margin; leaf area; leaf-hair types; leaf stomatal index; fresh and dry weights of leaf, stem and root; root morphology; root and root cortex diameter and root bark thickness were studied in selected plants of each type ($n = 10$). Floral measurements were recorded ($n = 25$) in freshly opened flowers from the population of both the plant types. Measurements of various parts of the flower such as calyx, corolla and gynoecium were also made. Fruit size was compared along with persistent calyx. Fruit morphology and colour of berry were also noted and photographed. Test seed weight was recorded by weighing 100 dried seeds from three lots.

Anthesis was studied by observing fully matured bud to flower opening and data were recorded for three days. The experiment was repeated twice in January and February. Pollen measurement was made in freshly collected pollen grains with an ocular micrometer under a microscope[6]. The measurements were recorded from ten fields per micro-slide and each such slide was considered as one replication. Data of three replications were analysed.

Physiological parameters like photosynthetic rate, respiratory rate and leaf conductance were studied using a portable photosynthesis system (Licor-make) during December and February (two-month-old and four-month-old plants). Randomly selected plants ($n = 10$) of both the types were used in the study. Leaf area was measured using a leaf area meter (Licor-make) from freshly excised leaves ($n = 50$) of both the plant types.

Ploidy comparison of the two plant types was made by flow cytometer (Partec PA II). About 0.5 cm^2 of fresh leaf was chopped with a sharp razor blade in nuclei extraction buffer (Partec Cystain UV Precise P). The suspension nuclei was filtered through a CellTrics disposable filter of mesh size 30 μm directly into the sample tube and DAPI staining buffer (Partec Cystain UV Precise P) was added. The sample was analysed with UV excitation by flow cytometry (Partec PA II system). Three runs were made, viz. both the plant types individually and the mixed sample of both the plant types in replications. In the first run wild-type plant sample was run and the flow cytometer was adjusted so that the peak representing nuclei of G_1 stage was at channel 200, and the position was kept constant for other sample runs of G_1 nuclei.

For chemical profile analysis dried root powder (1.0 g each) was mixed with 2 ml of ammonia solution and kept for 1 h and thereafter refluxed in methanol for 1 h, filtered and the solvent was removed under reduced pressure of 330 psi in a rotary vacuum evaporator (HEIDOLPH-Laborota 4010-Digital). Residue was dissolved in 50 ml of water and partitioned in chloroform. The chloroform extract was concentrated to 10 ml and used for sample loading for HPLC analysis (Camag system equipped with a sample applicator – Linomat-5, twin development chamber, TLC scanner-3 and integration software (Wincat), documentation system Reprostar-3 with camera – Canon G2). HPTLC aluminium sheet pre-coated with silica gel 60 (1.05547 E Merck) was used as the adsorbent. Toluene : ethyl acetate : formic acid (5.0 : 1.5 : 0.5) were used as the mobile phase. Reference standards used for the HPTLC study were withaferin-A ($R_f = 0.10$), withanolide-A ($R_f = 0.14$) and 12-deoxywithastramonolide ($R_f = 0.08$; Chromadex, USA-make). The chromatographic development chamber was saturated with mobile phase for 10 min prior to placement of the plates. The plates

were run up to 8 cm height and derivatized (10% H_2SO_4 in methanol). The derivatized plates were heated at 100°C for 4 min, bands were observed and scanned at 366 nm and photographs taken for record. Pooled root samples collected from plants (n = 10) of both types were used in the study.

Withaferin-A, 12-deoxy withastramonolide and withanolide-A were targeted for quality analysis by HPLC. Since JA-20 is a released variety of Ashwagandha for cultivation, all plants in the population of JA-20 are expected to be uniform. Hence roots randomly collected from 10 plants of JA-20 were pooled and analysed. However, in case of wild type, roots were separately collected from 10 different plants and analysed for the targeted chemicals. One gram dried root powder was extracted thrice in 50 ml methanol. Extracts were evaporated to dryness and reconstituted in methanol of HPLC-grade according to the calibration curves. HPLC analysis was performed on a Shimadzu system consisting of LC-20AD binary pump, SPD-20A UV/Vis detector, SIL-20AC HT auto-sampler along with DGU $20A_3$ degasser and with ClassVP software. Separations were achieved using column Li Chro CART® 250-4.6, 5 μm particle size and mobile phase of acetonitrile, and 1% acetic acid in water in the ratio of 60 : 40 at a flow rate of 1 ml/min. Standards as well as the sample spectra of withaferin-A, 12-deoxywithastramonolide and withanolide-A were recorded at λ_{max} = 230 nm, at a retention time of about 9.90, 10.88 and 14.08 min respectively. The calibration curve for each compound was drawn with five different concentrations of the reference standards and compared with the samples peaks for quantification. Each analysis was repeated twice.

One of the major differences observed between the two plant types was their plant habit. The cultivated plants were annual, whereas the wild-type plants were perennial. Morphologically, the wild-type plants were bigger in size than the cultivated plants, and this was reflected in all the morphometric measurements (Table 1). Leaf margin was wavy in the cultivated plants, whereas it was straight in the wild-type plants (Figure 1 *b*). Roots of the cultivated plants were brittle, whereas those of wild-type plants were fibrous and not brittle (Figure 1 *c*).

Flower size was smaller in the cultivated plants. Length of corolla was 4.6 ± 0.5 mm in the cultivated plants and 6.4 ± 0.5 mm in the wild-type plants. In the cultivated plants, lengths of ovary, style and stigma were 1.04 ± 0.04, 1.70 ± 0.04 and 0.18 ± 0.02 mm respectively. In case of wild-type plants, these were 1.80 ± 0.038, 2.44 ± 0.10 and 0.31 ± 0.015 mm respectively.

Berry was yellow in cultivated and red in wild-type plants (Figure 1 *d* and *e*). The seed size was comparatively bigger in the cultivated type (Figure 2 *a*). Average test seed weight recorded was 224.0 ± 3.14 mg in cultivated plants compared to 135.1 ± 3.40 mg in the wild type.

There was little variation in leaf stomata index in both plant types. In the lower surface of leaves of cultivated

Table 1. Morphological comparison of cultivated and wild-type plants at harvesting stage

Character	Cultivated*	Wild-type
Plant height (cm)	23.25 ± 4.03	70.6 ± 13.23
Number of primary branches	2.5 ± 1.00	2.9 ± 0.56
Number of secondary branches	3.75 ± 0.50	3.3 ± 2.11
Number of tertiary branches	1.25 ± 0.95	2.1 ± 1.59
Number of leaves per plant	128–208	346–711
Leaf area (cm^2)	11.95 ± 3.95	40.34 ± 5.71
Leaf margin	Wavy	Straight
Number of flowers per plant	0.00	13.0 ± 2.91
Number of fruits per plant	29–109	56–261
Root diameter (mm)	8.52 ± 0.74	29.37 ± 1.51
Root bark thickness (mm)	0.85 ± 0.11	2.49 ± 0.14
Root cortex thickness (mm)	6.4 ± 0.88	23.51 ± 0.89
Fresh root weight (g $plant^{-1}$)	6.52 ± 2.38	152.3 ± 65.18
Fresh weight of aerial part (g $plant^{-1}$)	20.63 ± 6.67	213.80 ± 92.44
Fresh weight of leaf (g $plant^{-1}$)	9.36 ± 6.79	65.60 ± 18.10
Fresh weight of stem (g $plant^{-1}$)	7.23 ± 2.53	112.46 ± 43.01
Fresh weight of fruit (g $plant^{-1}$)	5.06 ± 2.33	23.43 ± 9.68
Fresh weight of bark (g $plant^{-1}$)	3.43 ± 1.69	50.95 ± 9.36
Fresh weight of cortex (g $plant^{-1}$)	2.74 ± 0.74	65.57 ± 9.19
Dry weight of leaf (g $plant^{-1}$)	2.05 ± 0.52	16.85 ± 5.31
Dry weight of stem (g $plant^{-1}$)	2.47 ± 0.94	32.25 ± 18.04
Dry weight of fruit (g $plant^{-1}$)	3.29 ± 2.21	10.03 ± 4.34
Dry weight of root bark (g $plant^{-1}$)	0.92 ± 0.29	20.99 ± 3.62
Dry weight of root cortex (g $plant^{-1}$)	1.22 ± 0.58	30.44 ± 3.00

*There was no flower in the cultivated plants at harvesting stage.

plants, stomatal index was 0.21 ± 0.01, whereas in the wild type it was 0.22 ± 0.01. In the upper surface of leaves of cultivated plants, stomatal index was 0.18 ± 0.01 and in the wild type it was 0.17 ± 0.01. Stomata were anisocytic in both the plant types. Leaf hairs were shorter in the cultivated than in the wild type, i.e. 0.46 ± 0.05 cm in cultivated and 0.64 ± 0.05 cm in the wild-type plants.

Photosynthetic rate measured in the cultivated plants during December was 12.44 ± 1.51 μmol $CO_2/m^2/s$, respiratory rate was 2.39 ± 1.84 μmol $CO_2/m^2/s$ and conductance was 0.128 ± 0.04 mol $H_2O/m^2/s$. During February, these values were 12.09 ± 2.05 μmol $CO_2/m^2/s$, 1.43 ± 0.99 μmol $CO_2/m^2/s$ and 0.31 ± 0.10 mol $H_2O/m^2/s$ respectively. In the wild type, photosynthetic rate during December was 16.02 ± 5.91 μmol $CO_2/m^2/s$, respiratory rate was 2.06 ± 1.09 μmol $CO_2/m^2/s$ and conductance was 0.18 ± 0.03 mol $H_2O/m^2/s$. During February, these values were 20.34 ± 3.03 μmol $CO_2/m^2/s$, 2.16 ± 1.08 μmol $CO_2/m^2/s$ and 0.34 ± 0.06 mol $H_2O/m^2/s$ respectively. It was also found that photosynthetic rate was comparatively higher in the wild type in both the months (Table 2).

The cultivated plants were in full bloom during December–February. Thereafter, the flowering phase declined and flowering stopped at the end of March. However, in the wild-type plants, flowering was a continuous process. The number of flowers opened per day during December in cultivated plants was 19 ± 5 per plant and in wild type 7 ± 1 per plant. However, during February, number of flowers opened per day increased considerably in cultivated plants (27 ± 9 per plant), whereas the increase was marginal in the wild type (10 ± 2 per plant). The number of flowers recorded in the wild type at the harvesting time (March/April) was 13 ± 3, whereas there was no flowers in the cultivated type.

Maximum number of flowers opened (anthesis) during the daytime (8 am–6.00 pm) in both cultivated and wild-type plants. However, negligible number of flowers opened at night (6.00 pm–8.00 am). Anthesis was about 97.13% in cultivated plants during the daytime and 2.87% at night. In the wild type, it was 93.57% during the daytime and 6.43% at night. Maximum percentage of anthesis was recorded between 10.00 am and 12.00 noon in both the plant types (Figure 3).

Time taken from bud opening to full flower opening stage was between 45 and 60 min in the cultivated plants, in contrast to 15 and 30 min in the wild type. Corolla withered faster in cultivated plants than in the wild type. Corolla tube was shed within 2–3 h in cultivated plants and 5–7 h in the wild-type plants. The flowers remained open for prolonged duration in wild-type plants, which is an indicator of cross-breeding.

Pollen grain was circular in both plant types. In the pollen of cultivated plants, axial plane measurement was 24.4 ± 4.4 μm and equatorial plane measurement was 24.8 ± 3.9 μm. In the case of wild-type plants, these were

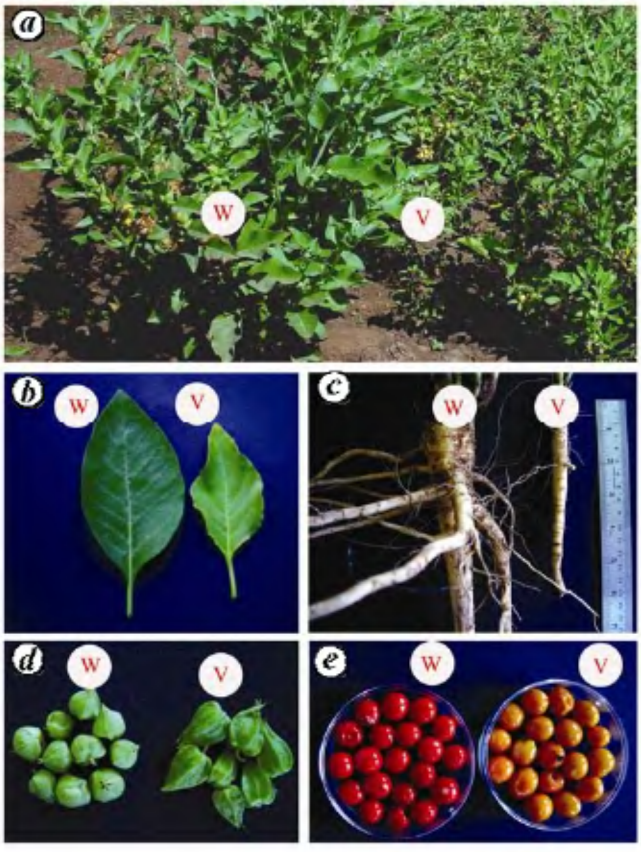

Figure 1. Ashwagandha (*Withania somnifera* Dunal). W, Wild-type plant; V, Cultivated variety JA-20. ***a***, Plant habit; ***b***, Leaf; ***c***, Root; ***d***, Fruit with persistent calyx; ***e***, Berry.

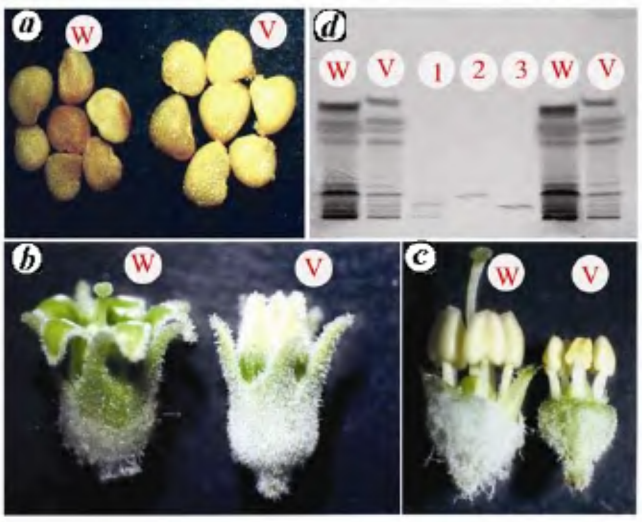

Figure 2. ***a***, Seed; ***b***, Flowers showing exposed stigma in the wild-type and concealed stigma in the cultivated plants; ***c***, Reproductive floral parts showing stigmatic position above the staminal level in the wild type and concealed stigma within the staminal cone in the cultivated plants; ***d***, HPTLC chemical profile along with reference chemicals – (1) Withaferin-A (R_f = 0.10), (2) Withanolide-A (R_f = 0.14) and (3) 12-Deoxywithastramonolide (R_f = 0.08).

Table 2. Physiological comparison of the plant types

Month of observation	Plant type	Photosynthetic rate (μmol $CO_2/m^2/s$)	Respiration rate (μmol $CO_2/m^2/s$)	Conductance (mol $H_2O/m^2/s$)
December	Wild	16.03 ± 5.91	2.06 ± 1.09	0.18 ± 0.03
	Cultivated	12.44 ± 1.51	2.39 ± 1.84	0.13 ± 0.04
February	Wild	20.34 ± 3.03	2.16 ± 1.08	0.34 ± 0.06
	Cultivated	12.09 ± 2.05	1.43 ± 0.97	0.31 ± 0.10

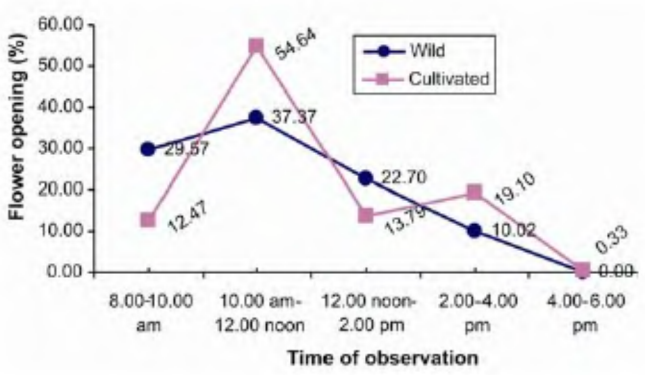

Figure 3. Flowers open (%) during the daytime in both plant types.

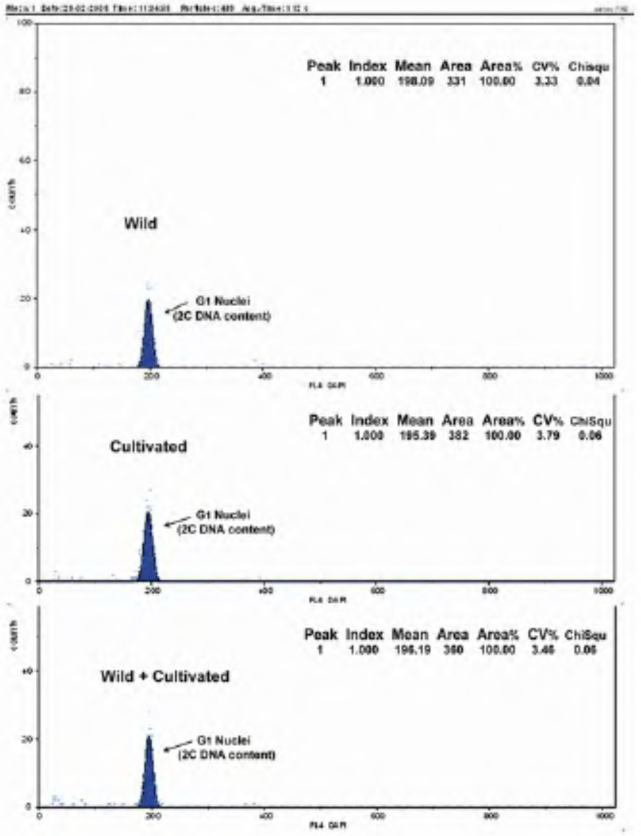

Figure 4. Histograms of relative nuclear DNA content showing similar ploidy level in the wild-type and cultivated plants of *W. somnifera* by flow cytometry.

25.0 ± 4.1 μm and 26.8 ± 3.8 μm respectively. The category of pollen size in both plant types was 'very small', according to the classification of Erdtman[7].

In a fully opened flower, apical portion of the corolla tube was almost straight or converged a little towards the central axis, the stigma was at the level of the anthers and almost concealed within the corolla tube in the cultivated plants. However, in the wild-type plants corolla tube apex was curved outwards and the stigma placed above the level of the anthers in such a way that it is exposed and accessible to pollen grains from the outside (Figure 2 *b* and *c*).

Analysis of DAPI-stained nuclei of wild and cultivated plant types separately by flow cytometry showed a single dominant peak representing nuclei in G_1 phase of the cell cycle. This result indicated that there were almost no dividing cells in the leaf tissue used for sample preparation. In the mixed sample run where both cultivated and wild-type plants were used together, only a single peak appeared (Figure 4). The results were consistent over replicated sample analysis also.

HPTLC chemical profile of the two plant types showed the presence of different chemicals (Figure 2 *d*). The three reference chemical standards used, viz. withaferin-A, withanolide-A and 12-deoxywithastramonolide were present in both the plant types with quantitative differences.

In the cultivated variety, withanolide-A content in the root was 0.433 μg/mg, withaniferin A was 0.094 μg/mg and 12-deoxywithastramanolide was 0.005 μg/mg. In the case of wild-type plants, withanolide-A in the root varied from 0.045 to 2.557 μg/mg, withaniferin A from 0.058 to 0.554 μg/mg and 12-deoxywithastramanolide from 0.003 to 0.023 μg/mg (Table 3). In general, the tested chemical constituents were several times greater in some plants of the wild type.

The present study has revealed the differences between the two plant types in a majority of the characters studied. Comparatively higher rate of photosynthesis in the wild-type plants could be correlated to their higher biomass production. According to the present market standards, quality of raw drug and price fixation are determined by the physical quality parameters of the root, where root brittleness is an important criterion for fetching higher price. In the wild type, roots are mostly fibrous and thus are not acceptable according to the present market standards.

Table 3. Contents of targeted chemical constituents in the wild and cultivated plants of Ashwagandha

Plant type	Withanolide-A (μg/mg)	Withaferin-A (μg/mg)	12-Deoxywithastramonolide (μg/mg)
Wild 1	1.733	0.314	0.014
Wild 2	1.499	0.427	0.012
Wild 3	1.935	0.386	0.023
Wild 4	2.557	0.546	0.019
Wild 5	2.484	0.554	0.011
Wild 6	0.662	0.221	0.007
Wild 7	1.756	0.422	0.013
Wild 8	0.302	0.142	0.003
Wild 9	0.063	0.058	0.003
Wild 10	0.045	0.105	0.005
Cultivated type (JA-20)	0.482 ± 0.105	0.088 ± 0.028	0.026 ± 0.005

Photosynthetic value showed increasing trend in the wild type in the two months studied, whereas in the cultivated plants, it was almost the same. This indicated that the wild-type plants were in active growth stage and the cultivated plants were stabilized in growth during these months.

Chromosome number reported in *W. somnifera* is $2n = 4x = 48$ (refs 8–10). Appearance of a single peak at channel number 200 in the mixed sample run using flow cytometry confirmed that the ploidy levels of both plant types were the same. Hence it could be inferred that higher biomass production in the wild type is not due to the difference in ploidy level.

Lattoo *et al.*[10] reported the mixed mating system in *W. somnifera* as a consequence of partial temporal dichogamy under which the stigma remains exserted beyond the undehisced staminal cone. Under the condition of non-receipt of pollen grains from the outside, self-pollination is guaranteed by the upward prolongation of the staminal cone. In the present study, it was found that the observation made by Lattoo *et al.*[10] was for wild-type flowers. In cultivated plants, self-pollination is the major mode of pollination since the stigma is placed at the level of the staminal cone and the corolla also restricts stigma accessibility to foreign pollen grains. However, gene flow from the cultivated to the wild type plants is possible.

It could be also inferred that the variability reported in the species[11] is due to the mixed mating system reported in the wild-type plants[10], and the cultivated plant type which is developed by selection from the wild type is pollinated predominantly by self-pollination due to its floral structure. This would lead to reproductive isolation within the cultivated group and may emerge as a new species over a period of time. The differences in the breeding system within the two plant types also could be correlated to the plant habits, i.e. the wild-type plants are perennial and the cultivated plants are short-lived and seasonal (4–5 months). Richards[12] reported that reproductive constraints are much heavier in short-lived plants since their opportunity to contribute to the next generation is limited due to the unreliable agents of cross-pollination and there will be selection pressure for reproductive input to be maximized by adopting selfing. In *W. somnifera*, the cultivated plants are seasonal in habit and favour to self-pollination.

It was also found that the chemical profile of the two plant types was dissimilar, as reported by Kaul[3]. Quantitative estimation of the selected chemical constituents showed greater variability among the wild-type plants. This indicates the scope for selection of elite genotypes in terms of therapeutically important chemical yield as well as root yield from the wild-type plant populations. Detailed pharmacological and clinical studies are required for conclusive remarks on the superiority of the two plant types.

The present study showed that cultivated and wild-type plants differ in most of the characters studied. Similar observations were made by Kaul[3], and Singh and Kumar[11]. Atal and Schwarting[4] found smaller calyx size in cultivated plants than in the wild-type plants, which was a deviation from the other reports. In the present study, however, the cultivated plants had an enlarged calyx similar to that reported by Kaul[3], who suggested a new species name for the cultivated plant type, i.e. *W. ashwagandha*. However, giving a new species status to the cultivated plant type is not convincing, because the morphological characters of the cultivated and wild plant types do not differ fundamentally. On the contrary, cultivated plant type is a miniature form of the wild type. Also, there is no barrier of gene flow between these two types. Due to the presence of exposed stigma, wild-type plants invite greater cross-pollination resulting in a high degree of variation in natural populations. The present study also suggests that cultivation of the wild-type Ashwagandha plants for quality drug production would be a logical proposition. Currently, cultivated Ashwagandha roots are fetching higher price due to less fibre content, which makes it easier for powder-making and have fine powder quality. The advantage of easy powdering and fine powder quality of cultivated varieties can be

achieved, in wild types by advanced machinery available for pulverization of such material. In addition, wild-type plants are more easily available in the market due to their several fold higher productivity of roots compared to cultivated plants. At present DMAPR has a large collection of wild-type Ashwagandha and utilization of these for selecting superior chemo-types is in progress.

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Bamboo resources mapping using satellite technology

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Identification of bamboo-growing areas is important for proper planning and management of the resources. Space technology has been playing a vital role in the mapping and identification of natural resources. Not much work has been done on the estimation of bamboo-growing areas using remote sensing and geographic information system due to the mixing up of reflectance value with other forest classes. The present study explores the possibility of developing an index specific to bamboo (bamboo index) using red, NIR and SWIR bands of IRS P6 LISS-III imageries to identify the bamboo-growing areas of the NE region.

Keywords: Bamboo index, remote sensing, resources mapping, satellite technology.

MAPPING and monitoring of the spatial extent of bamboos growing in the NE region is a high-priority requirement for the planners and resources managers. The conventional method of surveying and estimating the growing stock is time-consuming and also involves high cost. However, the development in space technology, particularly the repetitive satellite remote sensing (RS) across various spatial and temporal scales, offers the most economic means of assessing, planning, managing and monitoring the forestry resources, including bamboo. The multi-faceted bamboo-based livelihood development plan can be effectively monitored using RS and Geographic Information System (GIS) techniques due to its inherent advantages and scientific knowledge-based analysis. The role of RS and GIS in the field of resources mapping has already been established as a scientific and cost/time-effective means[1]. There have been few attempts to identify bamboo-growing areas using RS and GIS techniques. One such attempt was made to prepare a map showing open bamboo brakes and forest areas for Meghalaya[2]. In the study, hybrid knowledge-based approach was attempted because unsupervised and supervised classification has limitations to be used in the regional-level classification covering large areas due to intermixing of various land-cover types, radiometry of each scene, mapping of spectrally non-discriminable objects, etc. RS and GIS have been used extensively to identify bamboo-growing areas using spectral analysis of forest-cover types and a knowledge-based classification technique for different bamboo species[3]. GPS, training set data and Normalized Differ-

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