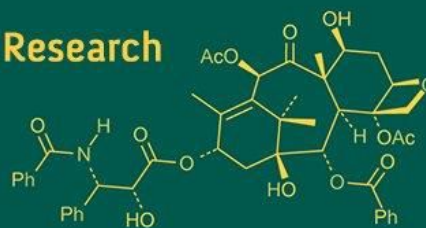


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Gaurav Aggarwal
School of Agricultural Studies,
Quantum University Roorkee,
Haridwar, Uttarakhand, India

Sakshi Raj
School of Agricultural Studies,
Quantum University Roorkee,
Haridwar, Uttarakhand, India

Jagatpal
School of Agricultural Studies,
Quantum University Roorkee,
Haridwar, Uttarakhand, India

Corresponding Author:
Gaurav Aggarwal
School of Agricultural Studies,
Quantum University Roorkee,
Haridwar, Uttarakhand, India

Optimizing micro propagation systems for productive *in-vitro* regeneration of the medicinal plant Ashwagandha (*Withania somnifera* L.)

Gaurav Aggarwal, Sakshi Raj and Jagatpal

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Abstract

Ashwagandha, scientifically referred to as *Withania somnifera* L. is one of the most economically significant medicinal plants in India. Its adaptogenic, immunomodulatory, and medicinal properties are utilized extensively in Ayurveda and the world's herbal industries. Micro-propagation provides a workable alternative for the large-scale production of genetically homogeneous and disease-free planting materials due to the increasing demand and the drawbacks of traditional seed-based propagation, such as poor germination, genetic variability, and disease susceptibility. Using leaf and petiole explants grown on Murashige and Skoog (MS) media supplemented with different quantities of cytokinins (BAP, Kinetin) and the auxin Indole-3-acetic acid (IAA), this study sought to optimize micro-propagation methods. In accordance with the results, TP9 caused the maximum shoot induction in petiole explants (4.10 shoots), while TL9 treatment (2.0 mg/L Kn + 0.2 mg/L IAA) produced the highest shoot regeneration (4.20 shoots per leaf explant). On MS media supplemented with 0.20 mg/L IAA, root induction was optimized. In order to create an effective micro-propagation system for *Withania somnifera* L., the study emphasizes the importance of exact growth regulator balance, explant type, and aseptic culture conditions. This valuable medicinal species' commercial cultivation, conservation, and mass propagation are all made possible by the improved method being used.

Keywords: Ashwagandha, *Withania somnifera* L., micro propagation, MS medium, cytokinins, auxins, tissue culture, regeneration

Introduction

Traditional medical systems throughout the world are built on medicinal herbs. *Withania somnifera* L. (Ashwagandha), often known as Indian ginseng or the winter cherry, is one of the most widely recognized. Known for its restorative and adaptogenic attributes, it is one of the most valued herbs in Ayurveda. Its anti-inflammatory, anti-oxidant, anti-stress, anti-anxiety, and immunomodulatory properties are mainly related to bioactive substances called withanolides.

The requirement for reliable, superior planting materials has increased because to the increasing demand for ashwagandha-based goods worldwide, including capsules, powders, herbal tablets, nutraceutical supplements, and cosmetic formulations. However, limited viability, erratic germination, seasonal limitations, and genetic heterogeneity plague traditional seed production, resulting in variable phytochemical content. *in vitro* plant tissue culture, or micro-propagation, represents a viable substitute for producing consistent plantlets in large quantities. It depends upon plant cells' totipotency, which allows a single cell to regenerate into a whole organism under specific circumstances. Success in micro propagation depends on optimizing growth media, plant growth regulators (PGRs), sterilizing processes, incubation conditions, and explant type.

The purpose of this study was to use leaf and petiole explants to optimize a repeatable and effective strategy for ashwagandha shoot and root regeneration. Commercial farmers, the herbal industry, academic research groups, and conservation efforts are anticipated to get benefits from the findings.

Although they provide vital therapeutic ingredients that are used to cure various kinds of illnesses, medicinal plants have been considered an essential component of traditional healthcare systems throughout civilizations.

Among these, *Withania somnifera* L. Dunal, also referred to as winter cherry, Indian ginseng, or ashwagandha, stands out as one of the most significant Ayurvedic therapeutic plants. Its broad range of pharmacological actions, which include adaptogenic, anti-inflammatory, immunomodulatory, antioxidant, neuroprotective, anti-stress, and rejuvenating effects, account for its prevalence in traditional formulations. Ashwagandha is a valuable crop in the pharmaceutical, nutraceutical, and herbal sectors because of its medicinal qualities, which are mostly due to a class of steroidal lactones called withanolides. The requirement for ashwagandha has increased dramatically in recent years due to the increasing worldwide interest in natural medicines and herbal supplements, particularly during the post-pandemic era when products that enhance immunity and relieve stress have grown increasingly significant.

Ashwagandha continues to be mostly produced, consumed, and exported in India, with Madhya Pradesh, Rajasthan, Maharashtra, and regions of Uttar Pradesh and Gujarat becoming the main cultivation regions. The plant is frequently grown under rain fed conditions and does well in subtropical and semi-arid regions. Although its vital position in the supply chain, traditional horticulture faces numerous challenges. Whereas seeds are the primary means of propagation, their viability, germination rates, and genetic stability vary. Due to stiff seed coverings, environmental stressors, and inconsistent maturity, seeds frequently exhibit poor germination. Additionally, there is significant genetic heterogeneity in seed-propagated plants, which has an impact on growth, yield, and withanolide content consistency. Industries that use standardized raw materials with uniform phytochemical profiles find this diversity difficult. In addition, crops grown within conventional farms are highly susceptible to soil-borne illnesses, weather changes, and diseases, which could reduce yields.

Modern techniques in biotechnology provide workable solutions to these constraints. A highly effective method for the rapid replication of superior, disease-free, and genetically homogeneous plant material is plant tissue culture, especially micro-propagation. The totipotent feature of plant cells the capacity of just one cell to regenerate into an entire plant under carefully regulated *in vitro* conditions is exploited by micro-propagation. Regardless of seasonal or environmental limitations, a large number of comparable plantlets can be generated all year round using sterile culture settings, nutritional mix, and plant growth regulators. This method guarantees the bulk generation and preservation of highly productive genotypes with higher phytochemical content for medicinal plants like ashwagandha.

A number of critical elements, especially explant selection, medium substance, balance of hormones, sterilization, and incubation conditions, are essential to the success of micro-propagation. Among these, the morphogenic pathway is largely determined by the selection of plant growth regulators (PGRs). While auxins like indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and the naphthalene acetic acid (NAA) encourage root initiation, callus formation, and tissues differentiation, cytokinins like the 6-benzylaminopurine (BAP) and kinetin (Kn) are frequently used to promote cell division and shoot proliferation. Optimizing organogenesis requires achieving the right ratio and concentration of these hormones. The most widely used nutrient foundation for tissue culture is still Murashige and

Skoog (MS) medium, which was created in 1962 and has a balanced composition of macronutrients, micronutrients, essential vitamins, and carbon sources.

Several scientists have tried to recreate *Withania somnifera* L. using a variety of explants, such as nodal segments, stem margins, leaves, and cotyledons. Reproducibility frequently varies because to variations in the genotype, explant age, physiological circumstances, and culture environment, despite reports of encouraging outcomes. Furthermore, a number of studies highlight the necessity of optimizing for both species and explants in order to enhance root induction and shoot regeneration. Developing a consistent, effective, and repeatable micro-propagation procedure is crucial from both a commercial and scientific standpoint, given the increasing requirement for ashwagandha across several industries.

In regard to this, the current study uses leaf and petiole extracts cultivated on MS medium enriched with different amounts of BAP, Kinetin and IAA to optimize micro-propagation techniques for *Withania somnifera* L. The study's objectives are to determine the best hormone concentrations for inducing shoots, assess each explant's capacity for regeneration, and create an effective rooting system for full plantlet development. This research supports sustainable production methods, the preservation of elite genotypes, and the expanding worldwide market for herbal remedies by producing high-quality, consistent, and disease-free Ashwagandha plantlets.

Methodology

The purpose of this research was to optimize an effective micro-propagation procedure for *Withania somnifera* L. at Quantum University's Plant Tissue Culture Research Facility in Roorkee, Uttarakhand. Explants came from robust, healthy plants kept in a greenhouse. Because of their strong capacity for regeneration and their sensitivity to growth regulators, newly formed leaves and petiole were selected.

Explant Collection and Surface Sterilization

In order to achieve eliminate of contaminants and surface impurities, freshly obtained explants were first cleaned under running tap water. After that, they were submerged in a moderate Teepol detergent solution and gently shaken for 20 to 30 minutes. After cleaning, distilled water was used to give the explants a thorough rinse. After one minute and thirty seconds of final sterilization in a laminar airflow chamber with 0.1% mercuric chlorides (Hg Cl₂), the tissue specimens were washed between three and four times with sterilized distilled water to remove any remaining chemical residue. Prior to inoculation, sterilized explants were aseptically cut to eliminate any oxidized or damaged tissue.

Preparation of Culture Medium

For every experiment, the nutritional foundation was the Murashige and the Skoog (MS) basal medium. 3% sucrose was added to the medium as a carbon source, and 0.8% agar was used to solidify it. After adjusting the medium's pH to 5.8, it was autoclaved for 20 minutes at 121 degrees Celsius and 15 pressure. The medium was transferred through sterilized test tubes and flasks once it had cooled. To find the best combinations for root formation and shoot induction, different concentrations of the plant growth regulators BAP, Kinetin (Kn), and IAA were added.

Experimental Treatments

Ten hormonal treatments were developed for leaf explants (TL1-TL10) and petiole explants (TP1-TP10) in order to promote shoot regeneration. These treatments included a fixed concentration of IAA (0.2 mg/L) in Kinetin-based combinations, as well as varying quantities of BAP (0.5-2.5 mg/L) and Kinetin (0.5-2.5 mg/L). In order to maximize surface contact, the explants were injected vertically on the medium.

Three IAA concentrations 0.05, 0.10, and 0.20 mg/L were used to assess root induction.

Culture Conditions and Observations

Every culture was incubated at 26 ± 2 °C with a 16-hour photoperiod and 2000-2500 lux of light. The relative humidity was kept at about 60%. To avoid contamination, sterilized cotton caps or aluminum foil were placed over the flasks. Every week, the callus formation, shoot emergence, and the growth of roots of the explants were observed. Parameters including rooting efficiency, the number of shoots per explant, and percentage response were all recorded.

Results and Discussion

Ashwagandha leaf and petiole explants cultivated on MS media supplemented with different amounts of auxin (IAA) and cytokinins (BAP, Kinetin) were used in the current work to design an optimum micro-propagation strategy. In order to understand the biological reactions and underlying elements controlling tissue behaviour, a thorough explanation of the findings obtained through the regeneration of shoots and induction of roots experiments is provided.

1. Shoot Regeneration from Leaf Explants

Based on the hormonal treatments, leaf explants displayed a wide range of morphogenic responses. The majority of

Kinetin-and BAP-based media showed callus induction, but only treatments with higher Kinetin and IAA concentrations showed shoot regeneration. The treatments TL1, TL2, and TL6 demonstrated no shoot response, suggesting that organogenesis could not be triggered by reduced cytokinin levels.

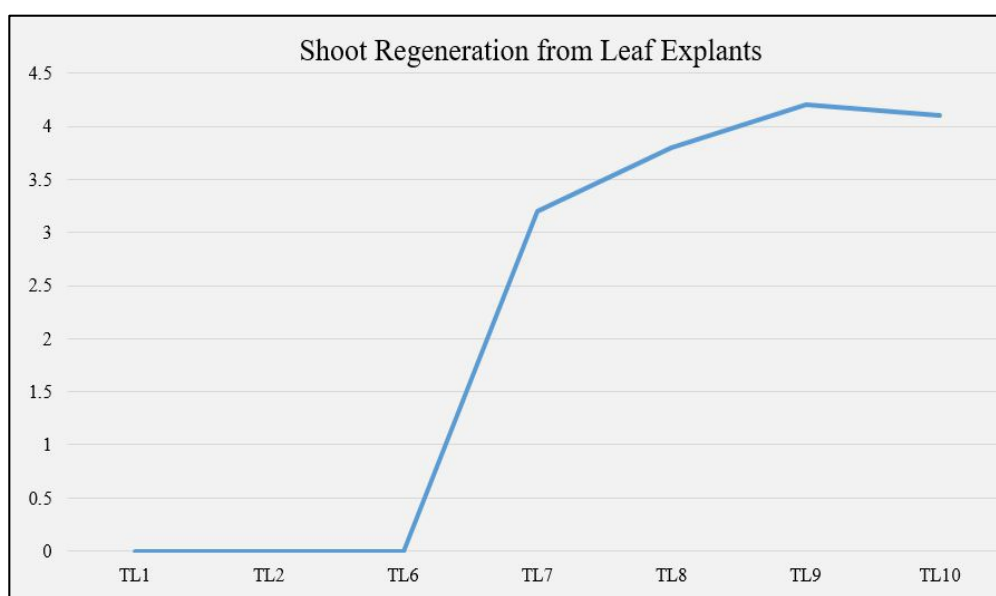
Table 1: Shoot Regeneration Response from Leaf Explants

S. No.	Treatment	Average Shoots
1	TL1	0.0
2	TL2	0.0
3	TL6	0.0
4	TL7	3.2
5	TL8	3.8
6	TL9	4.2
7	TL10	4.1

The data clearly indicates in the table 1, that shoot reaction rose steadily as kinetin concentration increased. With an average of 4.2 shoots per explant, TL9 (2.0 mg/L Kn + 0.2 mg/L IAA) had the highest regeneration rate, closely followed by TL10. This suggests that the ideal hormonal concentration of kinetin is 2.0 mg/L, after which the cytokinin action starts to plateau.

The lack of shoot regeneration in TL1, TL2, and TL6 is probably caused by insufficient cytokinin levels, which are required to promote meristematic activity. Leaf cells need a larger hormonal stimulus to re-enter the cell cycle since they are more differentiated and photo-synthetically active. By promoting vascular differentiation and bud primordia development, the addition of IAA improved shoot induction, underscoring the significance of cytokinin auxin synergy.

The occurrence of favourable hormonal circumstances for direct organogenesis is suggested by the increase in shoot numbers from TL7 to TL9. TL10, on the other hand, showed a small decrease that could be linked to cellular stress or vitrification caused by cytokinin excess.



Graph 1: Shoot Regeneration from Leaf Explants

2. Shoot Regeneration from Petiole Explants

When compared to leaf explants, petiole explants responded more effectively and reliably. Higher quantities of kinetin in

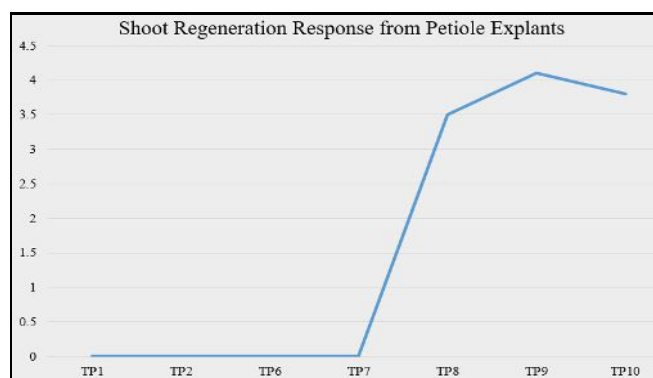
the treatments resulted in homogeneous shoot buds with faster initiation timings.

Table 2: Shoot Regeneration Response from Petiole Explants

S. No.	Treatment	Average Shoots
1	TP1	0.0
2	TP2	0.0
3	TP6	0.0
4	TP7	0.0
5	TP8	3.5
6	TP9	4.1
7	TP10	3.8

Decreased cytokinin levels were adequate to activate shoot primordia, as evidenced by the petiole explants' lack of responsiveness in TP1-TP7. Regeneration started with TP8 (3.5 shoots) and reached its peak in TP9 (4.1 shoots per explant). The hormonal saturated threshold seen in leaf cultures was once again reflected in the small drop in TP10. Because they have more rapidly dividing cells close to the circulatory region, which react quickly to exogenous cytokinin, petiole explants typically fared better. This benefit, along with effective nutrient uptake and less phenolic exudation, accounts for the earlier and superior shoot responses that petiole explants displayed in comparison to leaves.

By minimizing callus development and decreasing somaclonal variance, the initial organogenic pathway seen in petiole explants improves clonal fidelity. Petiole explants are preferred for large-scale micro-propagation because of these qualities.

**Graph 2:** Shoot Regeneration from Petiole Explants

3. Root Induction

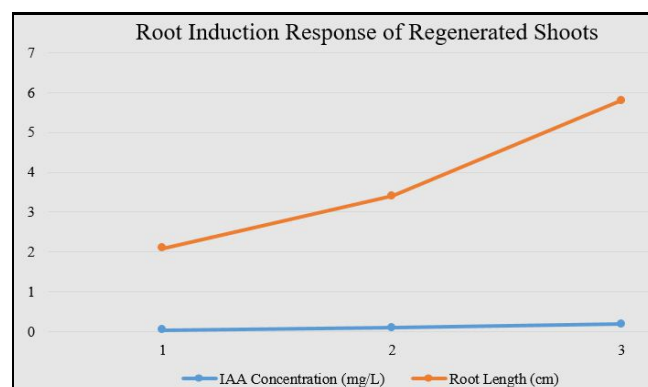
Three IAA concentrations were added to MS media in order to test rooting. The main metric for assessing auxin effectiveness was root length.

Table 3: Root Induction Response of Regenerated Shoots

S. No.	IAA Concentration (mg/L)	Root Length (cm)
1	0.05	2.1
2	0.10	3.4
3	0.20	5.8

As IAA concentrations rose, root development steadily increased; 0.20 mg/L IAA produced much larger and lengthier fibrous roots. (5.8 cm). This suggests that *Withania somnifera* L. reacts substantially to low-to-moderate auxin levels, which is in line with earlier research showing that IAA accelerates the development of root primordia and cell elongation.

The explants may have maintained endogenous auxin reserves that complemented exogenous IAA, particularly at higher concentrations, based on the linear rise in rooting. Better acclimation and survival were supported by the morphologically superior roots generated at 0.20 mg/L, which were thicker, longer, and more branching.

**Graph 3:** Root Induction Response

4. Comparative Interpretation across Explants and Treatments

Assessing the responses from different explant types and hormonal treatments revealed clear patterns that showed which combinations were better at enhancing *Ashwagandha* micro-propagation. Petiole explants generally demonstrated faster and more consistent regeneration of shoots than leaf explants due to their close connection to vascular tissue and greater amount of naturally produced growth regulators that enhance reactivity to exogenous cytokinins. Surprisingly, in leaf explants, callus formation frequently occurred before shoot emergence, delaying regeneration and necessitating higher kinetin concentrations to initiate organogenesis.

Among the hormonal combination tested, treatments consisting of 2.0 mg/L Kinetin using 0.2 mg/L IAA, specifically TL9 during leaf explants and TP9 during petiole explants, produced the most shoots, underscoring the importance of appropriate cytokinin and auxin relationship in promoting ideal morphogenic responses. Excessive cytokinin levels resulted in a slight decrease in regeneration efficiency, suggesting that the saturation point is the maximum level exceeding and that this issue hormonal stress may occur, even though smaller cytokinin concentrations that were unsuitable to induce shoots. Rooting responses also showed a clear pattern as IAA concentrations increased, with deeper and healthier roots growing to the highest point at 0.20 mg/L IAA. Overall, all of these results illustrate that choosing of explants, precise hormonal balance, and specific culture condition optimization are critical to the success of micro-propagation; petiole explants along with Kinetin IAA configurations are the most efficient methods to achieve accurate and stable plant regeneration.

Conclusion

The result of this effort reveals that proper explant choice, together with optimization in the use of MS hormone combinations, can result in efficient micro-propagation in *Withania somnifera* L. The synergistic effect of CK & auxins in leaf, as well as petiole, explants contributed largely towards their efficient regeneration, while petiole explants had been more reliable as well as faster in initiating

direct shoots for regeneration, without any added hormone supplement in the MS medium, till 10 days, with 2.0 mg/L Kinetin + 0.2 mg/L IAA (TL9 & TP9) exhibiting the highest frequency of normal shoots in most experiments conducted. In MS media containing MS treated with 0.20 mg/L IAA, root initiation was also quite successful since it resulted in healthy and long roots that were ideal for acclimation. Overall, it can be said that the improved method developed by this study offers an economical and feasible approach for mass-producing Ashwagandha plantlets that can serve as disease-free and genetically uniform planting material for use by the pharmaceutical and nutraceutical industries that have been expressing an increasing demand for high-quality Ashwagandha planting stocks for commercial cultivation.

References

1. Autade RH, Dalvi SM. Effect of BAP on direct organogenesis in *Withania somnifera*. Bioscience Biotechnology Research Communications. 2016;9(1):88-93.
2. Chavan JJ, Nimbalkar MS. *in vitro* propagation and callus induction studies in *Withania somnifera*. Indian Journal of Biotechnology. 2009;8(2):236-240.
3. Choudhary RS, Sharma KC. Micropropagation efficiency of various explants of Ashwagandha. International Journal of Medicinal Plant Studies. 2016;4(1):130-134.
4. Hossain MF, Anisuzzaman M. Influence of auxin concentrations on rooting efficiency in Ashwagandha. International Journal of Biosciences. 2019;14(4):292-304.
5. Lata H, Chandra S. Evaluation of clonal fidelity in micropropagated plants of *Withania somnifera* using ISSR markers. Plant Cell Reports. 2013;32(7):1347-1354.
6. Rahman MA, Karim MR. Comparative study of explant response in micropropagation of medicinal species. African Journal of Biotechnology. 2014;13(8):857-864.
7. Sabir F, Qureshi R. Shoot regeneration performance under various cytokinin combinations in *Withania somnifera*. Journal of Herbs, Spices & Medicinal Plants. 2008;13(4):123-133.
8. Sangwan RS, Khan S. Enhanced withanolide production in tissue cultured plants of *Withania somnifera*. Physiologia Plantarum. 2008;133(2):278-287.
9. Sinha P, Roy SK. Role of cytokinins in shoot proliferation of medicinal plants grown *in vitro*. Journal of Plant Biochemistry and Biotechnology. 2002;11(1):45-49.
10. Sivanesan I, Jeong BR. Somatic embryogenesis and plant regeneration in Ashwagandha. HortScience. 2007;42(6):1345-1348.
11. Thomas TD, Shah MM. Plant growth regulators and their role in tissue culture of medicinal plants. Journal of Plant Sciences. 2010;5(4):222-235.
12. Waman AA, Bohra P. Standardization of micropropagation protocol in Ashwagandha. Journal of Applied Horticulture. 2011;13(2):150-153.