

ORIGINAL PAPER

Synergistic effects of basal media and plant growth regulators on the *in vitro* micropropagation of *Asparagus racemosus*

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Abstract. *Asparagus racemosus* (Shatavari) is a high-value medicinal herb facing genetic depletion due to over-exploitation and climate-induced propagation challenges. This study established an optimized *in vitro* micropropagation protocol by evaluating the synergistic effects of two basal media, Murashige and Skoog (MS) and Woody Plant Medium (WPM), supplemented with varying concentrations (0–4 mg/L) of five plant growth regulators (NAA, IBA, IAA, KN, and BAP). One-way ANOVA confirmed that PGR type and concentration significantly influenced both shoot proliferation and elongation. Cytokinins, particularly kinetin (KN), outperformed auxins for shoot induction. The highest proliferation was achieved in WPM supplemented with 4 mg/L KN, yielding 3.80 ± 0.25 shoots per explant after 30 days. Long-term kinetics monitored over 90 days revealed that WPM supported consistent, aggressive growth, whereas MS medium exhibited irregular kinetics with a growth lag at 60 days. Conversely, MS medium supplemented with 1 mg/L IAA was most effective for elongation. A significant physiological trade-off was observed between shoot number and length. These findings demonstrate that while MS is suitable for specialized elongation, WPM provides a superior, stable environment for sustained, large-scale biomass production of *A. racemosus*.

Keywords: *Asparagus racemosus*, micropropagation, basal media, kinetin, growth kinetics, Woody Plant Medium

1. Introduction

Asparagus racemosus (Family: Asparagaceae), commonly known as Shatavari, is a medicinal herb in both Ayurveda and Thai traditional medicine, traditionally revered as a rejuvenative tonic for female reproductive health, lactation, and immune support (Sharma et al., 2013; Singh et al., 2018). The therapeutic efficacy of its tuberous roots is primarily attributed to a high

concentration of steroidal saponins, particularly shatavarins, alongside a diverse profile of flavonoids, polyphenols, and tannins that exhibit potent antioxidant, anti-inflammatory, and immunomodulatory activities (Hayes et al., 2006; Hasan et al., 2016).

However, the increasing global demand for these bioactive compounds has led to the over-exploitation of wild populations and genetic depletion, a crisis further exacerbated by the unpredictable impacts of climate change (Thakur et al., 2015). Erratic rainfall patterns, increasing frequencies of extreme heatwaves, and shifting soil moisture levels have made traditional outdoor propagation increasingly challenging, often leading to low seed viability and compromised crop yields. To address these vulnerabilities, plant tissue culture and *in vitro* micropropagation serve as critical biotechnological tools for the rapid, large-scale production of uniform, disease-free planting material throughout the year (Salman, 2021; Sharma et al., 2025). These advanced cultivation techniques have demonstrated the potential to far exceed the productivity of traditional farming; for instance, optimized cell cultures have been shown to produce total saponin levels up to 20-fold higher than those found in cultivated plants (Pise et al., 2012). Furthermore, specific *in vitro* protocols utilizing modified media and precise growth regulator combinations can lead to the hyper-production of key marker compounds, such as shatavarin IV, reaching concentrations significantly greater than natural root extracts (Pise et al., 2012). The optimization of culture media and hormonal balance remains a

fundamental prerequisite for maximizing the regeneration and sustained growth of *A. racemosus*. While standard protocols often utilize Murashige and Skoog (MS) and Woody Plant Medium (WPM), the comparative efficacy of these basal media frequently varies depending on the specific growth parameters and the total duration of the culture period (Lloyd and McCown, 1980; Kumar and Sen, 1985; Saad and Elshahed, 2012). Therefore, identifying the effects between media composition and plant growth regulators (PGRs) is essential for developing a highly efficient and scalable micropropagation system for medicinal plants.

2. Materials and Methods

2.1 Plant material and explant preparation

Nodal segments of *A. racemosus* were used as explants for all experimental treatments. The explants were surface-sterilized and prepared according to standard tissue culture protocols before being inoculated onto the treatment media.

2.2 Culture media and treatments

To evaluate the synergistic effects of basal media and plant growth regulators (PGRs), two basal media were tested: Murashige and Skoog (MS) and Woody Plant Medium (WPM). Both media were supplemented with varying concentrations (0, 1, 2, 3, and 4 mg/L) of five different PGRs, including, Auxins: α -Naphthaleneacetic acid (NAA), Indole-3-butyric acid (IBA), and Indole-3-acetic acid (IAA), and Cytokinins: Kinetin (KN) and 6-Benzylaminopurine (BAP).

2.3 Evaluation of shoot multiplication and elongation

The initial morphogenic response, including the number of shoots and shoot length (cm), was recorded after 30 days of culture. Each treatment consisted of ten replicates. The optimal concentration for each PGR was identified based on maximum performance in either shoot number or length.

2.4 Growth kinetics and long-term proliferation

To assess the sustained regenerative capacity of the species, the identified optimal concentrations were monitored over an extended duration. Data for shoot multiplication and elongation were collected at three intervals: 30, 60, and 90 days.

2.5 Statistical analysis

The experiment was conducted using a completely randomized design. Data were subjected to a One-way Analysis of Variance (ANOVA) to determine the significance of the effects of basal media and PGR concentrations. Following ANOVA, Duncan's Multiple Range Test (DMRT) was employed as a post-hoc analysis for pairwise comparisons of treatment means at a significance level of $P \leq 0.05$. Statistical analyses were performed using R program, and results were expressed as mean $\pm$ Standard Error (SE).

3. Results

3.1 Optimization of basal media and plant growth regulators

The optimization of culture media and hormonal balance is a critical prerequisite for maximizing the regeneration potential and subsequent growth of *A. racemosus*. In this study, the synergistic effects of two basal media (MS and WPM) and varying concentrations (0 to 4 mg/L) of five plant growth regulators (NAA, IBA, IAA, KN, and BAP) were evaluated to determine the most effective conditions for shoot proliferation and elongation. One-way ANOVA revealed that the type and concentration of PGRs had a highly significant effect on both the number of shoots and shoot length ($p < 0.01$) (Table 1). Regarding shoot proliferation, the variance between experimental groups was remarkably high ($F(4, 145) = 19.79, p < 0.001$), confirming that certain hormone treatments substantially outperformed others in stimulating axillary bud proliferation.

In contrast, while the impact on shoot length remained statistically significant ($F(4, 145) = 4.07, p = 0.004$), the data exhibited greater within-group variation. This suggests that while exogenous PGRs drive initial elongation, the final shoot length is likely governed by a more nuanced interaction between the basal media composition and the plant's internal endogenous hormonal signaling.

Following the confirmation of significant differences through the one-way ANOVA, Duncan's Multiple Range Test (DMRT) was employed as a post-hoc analysis to perform pairwise comparisons between the treatment means at a 5% significance level ($\alpha = 0.05$) (Table 2).

The data reveals that cytokinins (KN and BAP) were significantly more effective than auxins (NAA, IBA, and IAA) in promoting shoot induction. The highest proliferation was recorded in WPM medium supplemented with 4 mg/L KN, yielding 3.80 ± 0.25 shoots per explant (Table 2). A representative photo of the highest shoot number in this treatment is shown in Figure 1A. This was statistically comparable to WPM + 4 mg/L BAP (3.50 shoots). In MS medium, the maximum performance for shoot number was also observed with 4 mg/L KN (3.00 shoots), suggesting that WPM may be a more suitable basal medium for maximizing shoot density in this plant.

Table 1. One-way analysis of variance (ANOVA) of the effects of basal media and plant growth regulators (PGRs) on shoot number and shoot length of *Asparagus racemosus*.

		Sum of Squares	df	Mean Square	F	Sig.
No. of Shoots	Between Groups	190.81	4	47.70	19.79	0.000
	Within Groups	349.45	145	2.41		
	Total	540.27	149			
Shoot Length	Between Groups	12.74	4	3.19	4.07	0.004
	Within Groups	113.59	145	0.78		
	Total	126.33	149			

A significant physiological trade-off was observed between the number of shoots and their individual lengths. Treatments that produced the highest number of shoots (high KN and BAP) resulted in significantly shorter shoots, with lengths ranging from 0.33 to 0.75 cm. In contrast, the maximal shoot length was achieved in treatments with lower multiplication rates. The longest shoots were observed in MS medium supplemented with 1 mg/L KN (2.10 ± 0.55 cm) and MS + 1 mg/L IAA (1.98 ± 0.48 cm); the vigorous

elongation characteristic of these treatments is clearly visible in the representative cultures of 1 mg/L IAA in MS (Figure 1B). Interestingly, while the lower concentration of 1 mg/L KN in MS promoted elongation, it also triggered early-stage callus initiation at the base of the explant (Figure 1C). These observations suggest that lower concentrations of growth regulators, particularly in MS medium, favor elongation and specialized morphogenic responses rather than dense axillary proliferation.

Table 2. Effect of different types and concentrations of plant growth regulators (PGRs) in WPM and MS media on shoot multiplication and shoot length of *A. racemosus*. Bold values indicate optimal concentrations (Opt. Conc.) selected for each plant growth regulator based on maximum performance. Each value represents the mean of ten replicates. Means followed by the different letter within a column indicate significant differences according to Duncan's Multiple Range Test (DMRT) at a 5% significance level.

Media	PGRs	Opt. Conc. (mg/L)	No. of Shoots	Shoot Length (cm)
			Mean±SE	Mean±SE
WPM	NAA	1	1.70±0.21^{ghijk}	0.13±0.01 ^k
		3	1.30±0.15 ^{ijkl}	0.17±0.02^{jk}
	IBA	3	1.70±0.21^{ghijk}	0.20±0.04 ^{jk}
		1	1.10±0.10 ^{kl}	0.58±0.13^{ghi}
	IAA	4	2.00±0.00^{ghij}	0.97±0.25 ^{de}
		3	1.80±0.25 ^{ghijk}	1.50±0.17^{bc}
	KN	4	3.80±0.25^a	0.47±0.06 ^{bij}
		1	2.40±0.16 ^{defg}	0.76±0.26^{ef}
	BAP	4	3.50±0.22^{ab}	0.33±0.04 ^{ij}
		2	3.20±0.33 ^{abc}	0.48±0.06^{bij}
MS	NAA	1	1.70±0.15^{ghijk}	0.12±0.01 ^k
		3	1.30±0.15 ^{ijkl}	0.18±0.03^{jk}
	IBA	3	2.40±0.34^{defg}	0.21±0.03 ^{jk}
		1	0.80±0.20 ^l	0.28±0.12^j
	IAA	4	2.00±0.00^{ghij}	0.64±0.12 ^{fgh}
		1	1.60±0.16 ^{hijk}	1.98±0.48^{ab}
	KN	4	3.00±0.26^{bcd}	0.75±0.09 ^{efg}
		1	1.30±0.15 ^{ijkl}	2.10±0.55^a
	BAP	2	2.30±0.30^{defgh}	0.78±0.13 ^{ef}
		1	2.00±0.30 ^{fg hij}	1.26±0.27^{cd}

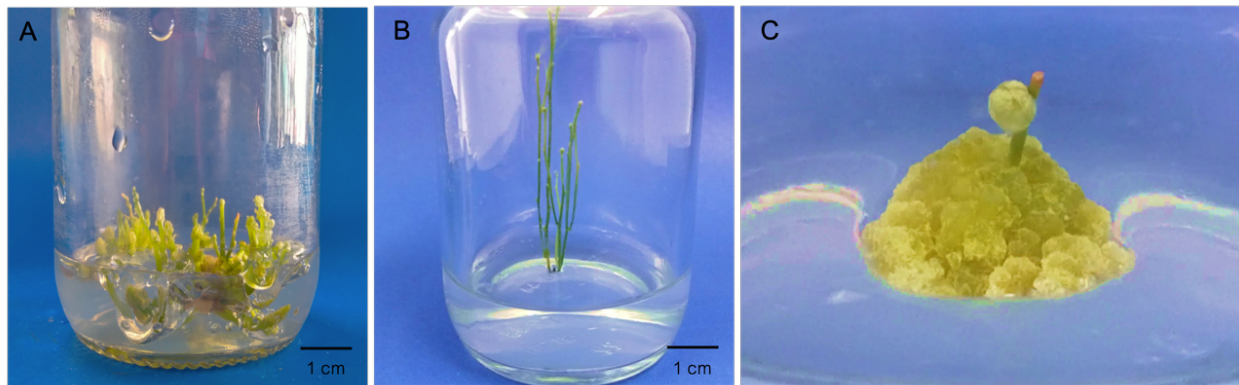


Figure 1. Morphogenic responses of *A. racemosus* at 30 days. (A) Peak shoot multiplication in WPM with 4 mg/L KN; (B) pronounced shoot elongation in MS with 1 mg/L IAA; (C) early-stage callus initiation at the explant base in MS with 1 mg/L KN. (Scale bars = 1 cm)

3.2 Growth kinetics and long-term shoot proliferation and elongation

To evaluate the sustained regenerative capacity of *A. racemosus*, the optimal concentration identified for each PGR was further monitored over an extended culture duration of 30, 60, and 90 days. The resulting growth kinetics, illustrated in two comparative line graphs,

reveal distinct successional patterns between the WPM and MS basal media.

In WPM medium, KN exhibited a consistent and aggressive growth, increasing from 3.80 to 10.8 shoots by day 90, while IAA and NAA also showed steady upward trends (Figure 2A). In contrast, MS medium displayed more irregular kinetics where most regulators including KN and BAP experienced

a significant reduction in shoot numbers at the 60-day mark before recovering by day 90 (Figure 2B). Despite this mid-term lag, KN remained the most effective treatment in MS, suggesting that while WPM supports more stable long-term proliferation, both media benefit from kinetin supplementation for maximal biomass accumulation.

The shoot elongation kinetics over the 90-day period revealed contrasting responses between the two basal media, often showing an inverse relationship with the shoot multiplication rates previously observed. In WPM medium, most treatments showed a significant lag or decline in length at the 60-day mark before recovering by day 90; notably, NAA and KN achieved the greatest final lengths in this medium (Figure 3A). Conversely, IAA in WPM showed a continuous decline in length from 30 to 90 days, likely due to apical dominance suppression as shoot numbers increased. In MS medium, the growth patterns were more specialized. IAA demonstrated a superior and consistent elongation trend, more than doubling its length from 30 days to reach a study-high of 4.08 cm by day 90 (Figure 3B). While KN maintained a steady length of approximately 2.1 cm through the 60-day mark, it experienced a reduction by day 90. These results suggest that while WPM is optimal for maximizing the number of shoots, MS medium supplemented with IAA is significantly more effective for achieving maximal shoot length during long-term culture.

4. Discussion

This study provides key insights into the development of a stable micropropagation protocol for *A. racemosus*, specifically identifying the critical interplay between basal media composition and long-term hormonal kinetics. The results confirm that cytokinins, specifically kinetin (KN) and benzylaminopurine (BAP), are significantly more effective than auxins for promoting shoot induction. WPM supplemented with 4 mg/L KN emerged as the superior treatment for maximizing shoot density. Our results align with the findings of Paudel et al. (2018), which reported that

Kinetin is highly effective for shoot induction. While Paudel et al. demonstrated that a combination of 0.5 mg/L NAA and 1.5 mg/L KN resulted in a peak for shoot development, our study achieved a higher multiplication rate of 10.8 shoots by day 90 using a higher concentration of 4 mg/L KN in WPM. This suggests that *A. racemosus* can respond favorably to higher cytokinin concentrations when the basal medium is optimized to reduce stress. Furthermore, Pant and Joshi (2018) observed that Kinetin concentrations between 1.0–2.0 mg/L significantly induced shoots from nodal explants. With our identification of 4 mg/L KN as the optimal concentration for multiplication extends these findings, demonstrating that higher concentrations may be necessary to maximize biomass accumulation during extended culture periods of up to 90 days.

The higher performance of WPM over MS for shoot multiplication suggests that the lower salt and nitrogen concentrations in WPM may reduce osmotic stress, facilitating more stable long-term proliferation (Lloyd and McCown, 1980; Saad and Elshahed., 2012). In contrast, MS medium exhibited irregular "U-shaped" kinetics, characterized by a growth lag at 60 days. This mid-term reduction in shoot numbers across most regulators in MS suggests a temporary physiological bottleneck, potentially due to nutrient depletion or the accumulation of inhibitory metabolites, before a recovery phase by day 90. The present study also highlighted a significant influence of basal media formulation on the sustained regenerative capacity and overall health of the *A. racemosus* cultures (Bopana and Saxena, S., 2008).

A significant physiological trade-off was observed between the number of shoots and their individual lengths. High concentrations of KN resulted in shorter shoots, while maximal elongation occurred at lower multiplication rates. This is consistent with Pant and Joshi (2018), which reported that individual shoot length increases at lower

concentrations of BAP. Interestingly, our result showed that 1 mg/L KN in MS triggered early-stage callus initiation, which is consistent with a previous study that Kinetin is effective for both callus and shoot induction (Patel and Patel, 2015). The "U-shaped" growth kinetics observed in MS medium treatments (Figure 2B)—characterized by a 60-day growth lag—

suggests a physiological bottleneck that is less pronounced in WPM. These findings suggest that whereas MS is effective for specialized responses such as shoot elongation, WPM provides a more stable environment for sustained long-term proliferation.

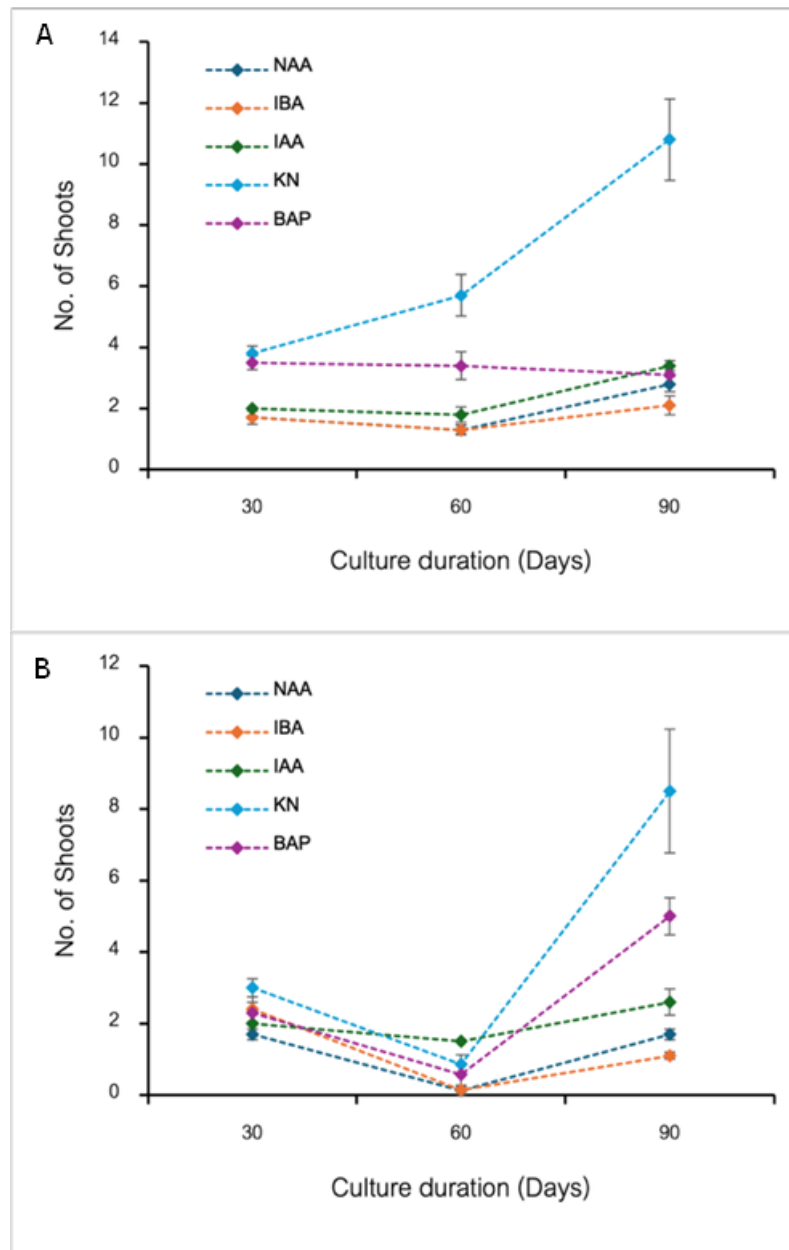


Figure 2. Comparative shoot proliferation kinetics of *A. racemosus* over a 90-day culture period using optimal PGR concentrations. (A) Mean number of shoots in WPM medium; (B) mean number of shoots in MS medium, Error bars represent $\pm$ SE.

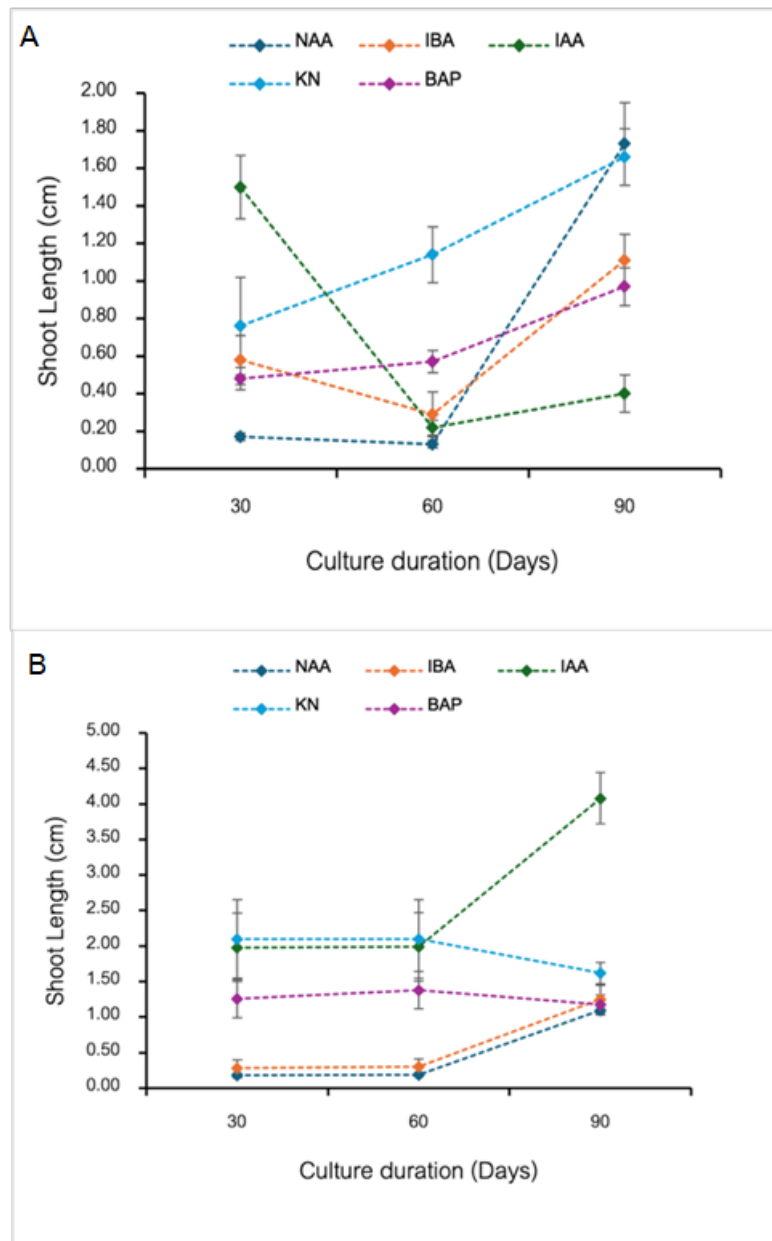


Figure 3. Shoot elongation kinetics of *A. racemosus* over 90 days. (A) Mean shoot length in WPM medium; (B) mean shoot length in MS medium. Error bars represent $\pm$ SE.

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References

- Bopana, N., & Saxena, S. (2008). *In vitro* propagation of a high value medicinal plant: *Asparagus racemosus* Willd. In *Vitro Cellular & Developmental Biology-Plant*, 44(6), 525-532.
- Hasan, N., Ahmad, N., Zohrameena, S., Khalid, M., & Akhtar, J. (2016). *Asparagus racemosus*: for medicinal uses & pharmacological actions. *International Journal of Advanced*

- Research, 4(3), 259-267. ElSohly M, Gul W (2014) Constituents of Cannabis sativa. Handbook of cannabis, 3(1093): 187-188.
- Hayes, P. Y., Jahidin, A. H., Lehmann, R., Penman, K., Kitching, W., & De Voss, J. J. (2006). Asparinins, asparosides, curillins, curillosides and shavatarins: structural clarification with the isolation of shatavarin V, a new steroidal saponin from the root of *Asparagus racemosus*. Tetrahedron letters, 47(49), 8683-8687.
- Kumar Kar, D., & Sen, S. (1985). Propagation of *Asparagus racemosus* through tissue culture. Plant cell, tissue and organ culture, 5(1), 89-95.
- Lloyd, G., & McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture.
- Pant, K. K., & Joshi, S. D. (2018). Comparative study of in vitro root and shoot proliferation from the node explants of *Asparagus racemosus* Willd. Journal of the Institute of Agriculture and Animal Science, 35(1), 121-125.
- Patel, L. S., & Patel, R. S. (2015). Rapid in vitro micro propagation of *Asparagus racemosus* Willd. from nodal explants.
- Paudel, N., Aryal, M. R., & Puri, R. H. (2018). Effect of hormone for in vitro propagation of *Asparagus racemosus* Willd. Current Life Sciences, 4(4), 53-61.
- Pise, M., Rudra, J., Bundale, S., Begde, D., Nashikkar, N., & Upadhyay, A. (2012). *Asparagus racemosus* cell cultures: a source for enhanced production of shatavarins and sarsapogenin. In Vitro Cellular & Developmental Biology-Plant, 48(1), 85-91.
- Saad, A. I., & Elshahed, A. M. (2012). Plant tissue culture media. Recent advances in plant in vitro culture, 25(7), 30-40.
- Salman, S. (2021). In vitro propagation and secondary metabolites production in the wild rare *Asparagus aphyllus* L. plant. Egyptian Journal of Desert Research, 71(2), 149-161.
- Sharma U, Kumar N, Singh B, Munshi RK, Bhalariao S (2013). Immunomodulatory active steroidal saponins from *Asparagus racemosus*. Medicinal Chemistry Research, 22(2), 573-579
- Sharma, A., Tripathi, M. K., Pandey, A., Payasi, D. K., Sharma, M., Mishra, R., ... & Tiwari, S. (2025). Standardization of in vitro propagation protocol for *Asparagus racemosus* employing nodal segments. Plant Cell Biotechnology & Molecular Biology, 26(9-10), 73-90.
- Singh, A. K., Srivastava, A., Kumar, V., & Singh, K. (2018). Phytochemicals, medicinal and food applications of Shatavari (*Asparagus racemosus*): An updated review. The Natural Products Journal, 8(1), 32-44.
- Thakur, S., Tiwari, K. L., & Jadhav, S. K. (2015). In vitro approaches for conservation of *Asparagus racemosus* Willd. In Vitro Cellular & Developmental Biology-Plant, 51(6), 619-625.