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Influence of type of cuttings and growth regulators on sprouting and rooting of Madhunashini [*Gymnema sylvestre* (Retz.) R. Br. ex Schult.] cuttings

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Abstract

Madhunashini (*Gymnema sylvestre* (Retz.) R. Br. ex Schult.) is a highly valued medicinal crop for its anti-diabetic and immense therapeutic properties. The current investigation was carried out at the ICAR-Indian Institute of Horticultural Research, Bengaluru, to study the influence of types of cuttings and growth regulators on sprouting and rooting in Madhunashini. Among the three types of cuttings, basal cuttings recorded significantly higher values for sprouting per cent (40.60%), fresh (1.14 g) and dry weight (0.62 g) of sprouts, per cent rooting (25.14%), fresh and dry weight of roots (0.84 and 0.48 g). Among growth regulators and their combination recorded significantly higher values for percentage sprouting (45.00%) and rooting (33.33%) as also for other root parameters. whereas, maximum fresh weight (1.89 g) and dry weight of sprouts (1.09 g) was observed in IBA @750 ppm + NAA @250 ppm treatment. The interaction effect of type of cutting, growth regulators and their combination on all sprouting and rooting parameters was found superior in basal cuttings treated with IBA @750 ppm + NAA @250 ppm are the best for propagation through cuttings.

Keywords: Basal cuttings, growth regulators, *Gymnema sylvestre*, Madhunashini, propagation, rooting percentage and sprouting percentage

Introduction

Gymnema sylvestre (Retz.) R. Br. ex Schult. is a native medicinal plant, which occurs abundantly in the monsoon forests of India and belongs to the family Apocynaceae. It is generally found in the tropical and subtropical regions of the world and thus the origin of this medicinal herb is tropical or sub-tropical Asia, South Africa, Oceania, China and Australia. Generally, it is a slow-growing, large perennial woody climber. The active ingredient in its leaves is gymnemagenin. Chewing of Gymnema leaves destroys the ability to discriminate sweet taste, giving it its common name 'Madhunashini' in Sanskrit, meaning 'sugar destroyer' (Saneja et al., 2010)^[16]. It is a highly valued medicinal crop for its anti-diabetic and immense therapeutic properties. Madhunashini is being collected from the wild habitat to meet the demand, thus posing a threat to its genetic diversity. It has been designated endangered on the IUCN Red List (Shailasree et al., 2012)^[18]. The only way to meet increase in demand and reduce pressure on wild collection of this species is its large scale propagation but low fruit set, short span seed viability and a delay in rooting due to latex exudation are the three main challenges to Madhunashini cultivation (Madhavan and Manivanan, 2007)^[12]. The estimated annual demand for Madhunashini is over 2700 metric tonnes per year by the Indian herbal industry, but the present availability is only 200 to 500 metric tonnes per year (Goraya and Ved, 2017)^[4]. Therefore, it must be cultivated using the proper agro-techniques and propagation techniques must be developed. As a reason, the current study was initiated to standardise the propagation technique in Madhunashini for the development of high quality planting material production.

Materials and Methods

The present study was carried out at ICAR-Indian Institute of Horticultural Research, Bengaluru. The experiment was laid out in Factorial Completely Randomized Design with three replications. Plant material required for the experiment was collected from experimental plot, Block-VII, Division of flower and Medicinal crops, ICAR-IIHR.

Two main treatments were imposed, i.e., type of cutting with 11 sub-treatments, i.e., growth regulators and their combinations. Each treatment was replicated thrice; in each replication, 10 cuttings were used. The cuttings were prepared by giving a slant cut just below a node and the leaves were removed for basal and median cuttings. The upper part of cuttings with four leaves were retained for terminal cuttings. All types of cuttings, measuring 10-15 cm long with 2-5 nodes, were taken from three different parts of the climber, hence the names basal, median and terminal cuttings. The basal portion of the cuttings (about 4-6cm) was dipped in a different growth regulator solution for 10 minutes and airdried. Treated cuttings were planted in media containing Riverbed sand, red soil and well decomposed farmyard manure in the proportion of 1:2:2. The rooting media was thoroughly mixed and was filled in polythene bags of 15cm long and 8cm width with 200 gauge thickness. Cuttings were planted by inserting one of its basal nodes into the media and carefully pressing the medium around them to remove any air pockets. One set of cuttings prepared as above was kept under the medium cost polyhouse. Observations on various sprouting and rooting parameters were recorded 60 days after planting.

For recording sprouts and roots fresh as well as dry weight, cuttings were uprooted at 60 days after planting. The sprouts were separated from the cuttings and weighed. After recording fresh weight, the samples were dried in a hot air oven at 60°C until constant weight was attained. Similarly, the root system was washed thoroughly in water and roots were separated from the cuttings and air dried. Their fresh weight and dry weights were recorded. For recording sprouting and rooting per cent all cuttings from each treatment were considered and for other parameters, five cuttings were tagged and mean data was recorded.

The data recorded were statistically analysed under Factorial Complete Randomised Design (FCRD) using analysis of variance (ANOVA) at 1% level of significance and the standard error mean was analysed with the help of statistical software KAU-GRAPES, Version 1.1.0 (KAU-GRAPES, 2022).

Results and Discussion

The results are discussed to know the effect of auxins and their combination such as, indole butyric acid (IBA) and naphthalene acetic acid (NAA) on rooting and sprouting of different types of stem cuttings *viz.*, terminal cuttings, median cuttings and basal cuttings in Madhunashini and depicted in Table 1 and 2.

Among different types of cuttings (C), C₃ (basal cuttings) was recorded as the highest sprouting per cent (40.60%) and in terminal cuttings (C₁) minimum of 16.36 per cent was observed. The maximum sprouting per cent was observed in T₁₁ (IBA @750 ppm + NAA @250 ppm) (45.00%) and the minimum sprouting per cent (23.32%) was recorded in T₁ (control) over different growth regulator concentrations (T). The interaction effect between the types of cuttings and growth regulators (C×T) was varying significantly. C₃T₁₁ (basal cuttings with IBA @750 ppm + NAA @250ppm) recorded the highest (53.33%) sprouting per cent. However, C₁T₅ (terminal cuttings with NAA @150 ppm), C₁T₈ (terminal cuttings with IBA @500 ppm + NAA @250 ppm) was recorded the lowest sprouting per cent (10%) (Table 1). All types of cuttings are sprouted initially, but as time goes on, terminal cuttings do not survive. This might be due to the fact that basal cuttings contain higher amount of food reserve and terminal cuttings lack rooting nodes, are poor sources of stored carbohydrates and are susceptible to microbial infection. Similar findings were reported by Subbaraj *et al.* (1997) ^[21], Chandrashekaran (2001) ^[25], Karoshi and Hegde (2001) ^[6], Somashekhar and Sharma (2002) ^[20], Pandey (2012) ^[13], Akshitha *et al.* (2014) ^[1], Kumari *et al.* (2018) ^[9], Ishwarya *et al.* (2021) ^[5] in Madhunashini; Basak *et al.* (2014) ^[2] in Pippali; Rahdari *et al.* (2014) ^[15] in *Cordyline Terminalis*; Ky-Dembele *et al.* (2014) ^[24] in *Commiphora wightii.*

Concerning types of cuttings (C) it was observed that the maximum fresh weight of sprouts was detected in basal cuttings (C₃) (1.41 g) and the minimum (0.89 g) was noticed in median cuttings (C_2) . In the case of different growth regulator treatments (T) the fresh weight of sprouts was highest in T_{11} (IBA @750ppm + NAA @250ppm) (1.89g) and the lowest was observed in T_1 (control) (0.41 g). The interaction effect between the types of cuttings and growth regulators (C×T) showed, C_3T_{11} (basal cuttings with IBA @750ppm + NAA @250ppm) recorded the higher (2.44 g) while the C_3T_1 (basal cuttings with control) showed the lower (0.38 g) fresh weight of sprouts (Table 1). The variation in fresh weight of sprouts might be due to the ample supply of stored food in basal cuttings and the combined effect of cuttings with IBA and NAA combination. These results were in accordance by the findings of Subbaraj et al. (1997)^[21], Subhash (2000) ^[22], Chandrashekaran (2001) ^[25], Karoshi and Hegde (2001) ^[6], Somashekhar and Sharma (2002) ^[20], Pandey (2012) ^[13], Akshitha *et al.* (2014) ^[1], Kumari *et al.* (2018)^[9], Ishwarya et al. (2021)^[5] in Madhunashini; Basak et al. (2014)^[2] in Pippali; Rahdari et al. (2014)^[15] in Cordyline Terminalis; Ky-Dembele et al. (2016) ^[10] in Pterocarpus santalinoides; Tripathi et al. (2014)^[24] in Commiphora wightii.

Among the types of cuttings (C), it was observed that the maximum (0.62 g) dry weight of sprouts was recorded in basal cuttings (C₃) and the minimum was noticed in median cuttings (C₂) (0.44 g). With respect to different growth regulators and their combination (T), T₁₁ (IBA @750 ppm + NAA @250 ppm) recorded the maximum dry weight of sprouts (1.09 g), while the minimum (0.09 g) was recorded in T₁ (control). The interaction effect between the types of cuttings and growth regulators (C×T) on the dry weight of sprouts showed, C_3T_{11} (basal cuttings with IBA @750 ppm + NAA @250 ppm) recorded the higher (1.27 g) dry weight of sprouts whereas, the lower (0.07 g) was noticed in C_2T_{10} (median cuttings with IBA @500 ppm + NAA @250ppm) (Table 1). This could be due to the highest mean fresh weight recorded in the sprouts produced. Similar results were observed by Subbaraj et al. (1997)^[21], Subhash (2000)^[22], Chandrashekaran (2001)^[25], Karoshi and Hegde (2001)^[6], Somashekhar and Sharma (2002) ^[20], Pandey (2012) ^[13], Akshitha et al. (2014)^[1], Kumari et al. (2018)^[9], Ishwarya et al. (2021) ^[5] in Madhunashini; Basak et al. (2014) ^[2] in Pippali; Rahdari et al. (2014)^[15] in Cordyline Terminalis; Ky-Dembele et al. (2016) ^[10] in Pterocarpus santalinoides: Tripathi et al. (2014)^[24] in Commiphora wightii.

Among the different types of cuttings, the rooting percent was highest in basal cuttings (C3) (25.14%) and lowest in median

cuttings (C2). There was no rooting in terminal cuttings (C_1) after 60 days of planting. This could be due to terminal cuttings lacking rooting nodes, lack of stored carbohydrates and susceptibility to microbial infection (Chandrashekaran, 2001) $^{\left[25\right]}$. The higher rooting per cent was found in T_{11} (IBA @750 ppm + NAA @250 ppm) (33.33%), while the least rooting per cent was recorded in T_1 (control) (10.00%), among the different growth regulator treatments. In terms of the interaction effect between the types of cuttings and growth regulators (C×T) on the rooting per cent reported, C_3T_{11} (basal cuttings with IBA @750 ppm + NAA @250 ppm) recorded the maximum (40.00%) rooting percent while, the minimum (3.00%) was noticed in C_2T_{11} (median cuttings with IBA @750 ppm + NAA @250 ppm) (Table 2). The combined effect of cuttings with IBA and NAA combinations, as well as the fact that the basal cuttings had high reserves of carbohydrates and nitrogen, may have contributed to the higher rooting percentage. These results are in accordance with Subbaraj et al. (1997) [21], Subhash (2000) [22], Chandrashekaran (2001)^[25], Karoshi and Hegde (2001)^[6], Saraswathy et al. (2002), Somashekhar and Sharma (2002) ^[20], Chandrasekar et al. (2003) ^[3], Madhavan and Manivannan (2007) ^[12], Sundharaiya et al. (2010) ^[23], Lal and Jha (2012) ^[11], Pandey (2012) ^[13], Akshitha et al. (2014) ^[1], Prasad and Ray (2015) ^[14], Kumari et al. (2018) ^[9], Ishwarya et al. (2021) ^[5] in Madhunashini; Singh et al. (2015) ^[19] in Grewia asiatica; Kouakou et al. (2016) [8] in Garcinia kola. Ky-Dembele et al. (2016) ^[10] in Pterocarpus santalinoides; Basak et al. (2014)^[2] in Pippali; Tripathi et al. (2014)^[24] in Commiphora wightii;

The maximum (0.84 g) fresh weight of root was recorded in basal cuttings (C₃) and the minimum was noticed in median cuttings (C₂) (0.68 g) among the different types of cuttings (C). Influence of different growth regulators and their combination (T) over cuttings showed, the maximum (1.26 g) fresh weight of root was observed in T_{11} (IBA @750ppm +

NAA @250ppm) and the minimum (0.26 g) fresh weight of root was recorded in T₁ (control). Concerning the interaction effect between the types of cuttings and growth regulators (C×T), the maximum (1.68 g) fresh weight of roots was recorded in C_3T_{11} (basal cuttings with IBA @750 ppm + NAA @250 ppm) while the minimum (0.280 g) was recorded in C_3T_1 (basal cuttings with control) (Table 2). This might be due to the fact that basal cutting contained more stored carbohydrates, which was responsible for more root production, coupled with the positive response of IBA and NAA due to their synergistic effect, leading to an increase in fresh weight of roots. these results are in line with the reports of earlier researchers Subhash (2000) [22], Chandrashekaran $(2001)^{[25]}$, Akshitha *et al.* $(2014)^{[1]}$, Ishwarya *et al.* $(2021)^{[5]}$ in Madhunashini; Rahdari *et al.* $(2014)^{[15]}$ in *Cordyline* terminalis; Basak et al. (2014)^[2] in Pippali; Ky-Dembele et al. (2016)^[10] in Pterocarpus santalinoides.

Among different types of cuttings(C), basal $cuttings(C_3)$ recorded the maximum (0.48 g) dry weight of roots and the minimum (0.30 g) was observed in median cuttings (C_3). The maximum dry weight of root was recorded in T₁₁ (IBA @750 ppm + NAA @250 ppm) (0.77 g) while the minimum (0.030 g) was recorded by T_1 (control) for different growth regulators and their combinations (T). The interaction effect between the types of cuttings and growth regulators (C \times T) showed, C₃T₁₁ (basal cuttings with IBA @750 ppm + NAA @250 ppm) recorded the highest (1.14 g) whereas C_2T_1 (median cuttings with control) recorded the lowest (0.01 g) dry weight of roots (Table 2). This could be due to the highest mean fresh weight recorded in the roots produced. These results are in agreement with Subhash (2000) ^[22], Chandrashekaran (2001) ^[25], Sundharaiya et al. (2010) [23], Lal and Jha (2012) [11], Akshitha et al. (2014)^[1], Ishwarya et al. (2021)^[5] in Madhunashini; Rahdari et al. (2014)^[15] in Cordyline terminalis; Basak et al. (2014)^[2] in Pippali; Ky-Dembele et al. (2016)^[10] in Pterocarpus santalinoides.

 Table 1: Influence of type of cuttings and growth regulators on various shoot parameters in Madhunashini [Gymnema sylvestre (Retz.) R. Br. ex

 Schult.]

Treatments	Sprouting per cent				Fresh weight of sprouts (g)				Dry weight of sprouts (g)				
	C ₁	C ₂	C ₃	Mean	C ₁	C ₂	C ₃	Mean	C ₁	C ₂	C ₃	Mean	
G1- Control	30.00	19.99	26.66	23.32	0.00	0.43	0.38	0.41	0.00	0.12	0.07	0.09	
G2- IBA @500 ppm	20.00	23.30	36.66	29.98	0.00	1.02	1.08	1.05	0.00	0.64	0.44	0.54	
G3- IBA @750 ppm	23.33	40.00	50.00	35.00	0.00	1.85	1.94	1.42	0.00	1.22	0.98	0.69	
G4- IBA @1000 ppm	16.66	30.00	40.00	33.33	0.00	1.54	1.17	1.35	0.00	0.92	0.46	0.65	
G5- NAA @150 ppm	10.00	16.66	30.00	23.33	0.00	0.64	0.72	0.68	0.00	0.18	0.09	0.13	
G ₆ - NAA @250 ppm	13.33	33.00	36.66	34.83	0.00	1.88	0.96	1.02	0.00	1.10	0.16	0.60	
G7- NAA @500 ppm	16.66	13.33	36.66	24.99	0.00	0.52	0.84	0.68	0.00	0.19	0.12	0.15	
G ₈ - IBA @500 ppm + NAA @150 ppm	10.00	16.66	40.00	28.33	0.00	0.47	1.81	1.14	0.00	0.08	0.92	0.50	
G9- IBA @750 ppm + NAA @150 ppm	13.33	23.30	50.00	36.65	0.00	0.52	2.36	1.44	0.00	0.08	1.24	0.76	
G10- IBA @500 ppm + NAA @250 ppm	10.00	20.00	46.66	33.33	0.00	0.47	1.91	1.19	0.00	0.07	1.14	0.67	
G11- IBA @750 ppm + NAA @250 ppm	16.66	13.33	53.33	45.00	0.00	0.40	2.44	1.89	0.00	0.06	1.27	1.09	
Mean	16.36	22.68	40.60		0.00	0.89	1.41		0.00	0.44	0.62		
	$S.Em\pm$	CD@1%			$S.Em\pm$	CD@1%			S.Em±	CD@1%			
	(P = 0.01)				(P=0.01)				(P=0.01)				
С	0.15	0.62			0.0069	0.027	_		0.0039	0.014			
G	0.37	1.44			0.016	0.046	_		0.0092	0.026			
C×G	0.52	2.06			0.022	0.09			0.013	0.015			

 $C_1-Terminal\ cuttings,\ C_2-Median\ cuttings,\ C_3-Basal\ cuttings$

S.Em± - Standard error mean, CD - Critical difference

Conclusion

In this study, the effect of different types of cuttings, growth regulators and their combinations on the sprouting and rooting behaviour revealed that the basal cuttings pre-treated with a combination of IBA @750ppm + NAA @250ppm exhibited, maximum sprouting and rooting per cent and maximum fresh and dry weight of both roots and sprouts.

 Table 2: Influence of type of cuttings and growth regulators on various root parameters in Madhunashini [Gymnema sylvestre (Retz.) R. Br. ex

 Schult.]

Treatments	Rooting per cent				Fresh weight of roots (g)				Dry weight of roots (g)			
	C ₁	C ₂	C3	Mean	C ₁	C ₂	C ₃	Mean	C ₁	C ₂	C3	Mean
G1- Control	0.00	10.00	10.00	10.00	0.00	0.24	0.28	0.26	0.00	0.01	0.05	0.03
G2- IBA @500 ppm	0.00	13.33	13.33	13.33	0.00	0.58	0.59	0.58	0.00	0.09	0.36	0.22
G ₃ - IBA @750 ppm	0.00	30.00	36.66	24.99	0.00	1.35	1.10	1.01	0.00	0.81	0.74	0.59
G4- IBA @1000 ppm	0.00	20.00	30.00	21.50	0.00	1.24	0.91	1.00	0.00	0.80	0.42	0.55
G5- NAA @150 ppm	0.00	6.66	16.66	11.66	0.00	0.44	0.36	0.40	0.00	0.12	0.09	0.10
G ₆ - NAA @250 ppm	0.00	16.66	23.33	19.99	0.00	1.33	0.48	0.90	0.00	0.84	0.14	0.49
G7- NAA @500 ppm	0.00	6.00	20.00	13.00	0.00	0.43	0.32	0.37	0.00	0.15	0.15	0.15
G ₈ - IBA @500 ppm + NAA @150 ppm	0.00	10.00	23.33	16.66	0.00	0.52	1.00	0.76	0.00	0.23	0.64	0.43
G9- IBA @750 ppm + NAA @150 ppm	0.00	13.33	36.66	25.00	0.00	0.66	1.42	1.07	0.00	0.21	0.88	0.61
G10- IBA @500 ppm + NAA @250 ppm	0.00	10.00	26.66	18.33	0.00	0.31	1.22	0.76	0.00	0.05	0.72	0.39
G ₁₁ - IBA @750 ppm + NAA @250 ppm	0.00	3.00	40.00	33.33	0.00	0.35	1.68	1.26	0.00	0.04	1.14	0.77
Mean	0.00	12.63	25.14		0.00	0.68	0.84		0.00	0.30	0.48	
	S.Em±	CD@1%			$S.Em\pm$	CD@1%			$S.Em\pm$	CD@1%		
	(P=0.01)				(P=0.01)				(P=0.01)			
С	0.089	0.34			0.0039	0.0156			0.0024	0.0094		
G	0.21	0.60			0.0091	0.026			0.0056	0.016		
C×G	0.29	1.16			0.012	0.048			0.0079	0.0314		

 $C_1-Terminal\ cuttings,\ C_2-Median\ cuttings,\ C_3-Basal\ cuttings$

S.Em± – Standard error mean, CD – Critical difference

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