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A Review on Clonal Propagation of Medicinal and Aromatic Plants through Stem Cuttings for Promoting their Cultivation and Conservation

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Abstract

Plant based drugs are being popularized in recent past owing to their long-term benefits without causing side effects. Medicinal and aromatic plants are major sources of these drugs, whose quality is largely dependent on active ingredients present in them. Today a large number of species are on the verge of extinction due to unscientific management practices, over-exploitation, destructive harvesting, poor seed set, low seed viability, pest and disease incidence *etc.* Hence, there is a great need for their conservation through cultivation for which standardization of propagation methodologies is of prime importance. Multiplication of these species clonally through cuttings can make their cultivation economic by providing true to type plants that are known to have optimum levels of active ingredients. Rooting of stem cuttings is a crucial step in plant propagation and there is great variability in the rooting ability of different species. Even though propagation through cuttings appears to be the simplest of all methods, its success depends on a number of factors and the present review concerned highlighting their importance in the context of medicinal and aromatic plants multiplication.

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Introduction

Medicinal and aromatic plants (MAPs) form the basis for plant based therapies including the Indian/ Chinese/ Tibetan Systems of Medicines, which are being popularized in many parts of the world. Quality of these plants largely depends on active ingredients

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present in them. Though most of the raw drugs are sold on fresh/dry weight basis in the domestic market,[1] during the extraction process it is observed that superior the chemotype, better is the quality of the extract, and maximum the returns obtained per unit of charge. From time immemorial, crude drugs have been collected from wild and thus, population of most of the important MAP species is dwindling in nature.[2] A number of species are on the verge of extinction due to problems such as destructive harvesting, poor seed set, low seed viability, pest and disease incidence *etc.*[3-4] Though the existing populations of these species are being regenerated in the nature by means of sexual reproduction, the rate of multiplication is slow and thus insufficient to meet the requirements in most of the cases.[5]The ever-increasing demand for these products in domestic and international markets also calls for a faster multiplication strategy.[6] Good number of improved varieties of MAPs is now available[7] that are being popularized amongst farmers due to their higher net returns and ease of cultivation, thereby reducing extra burden on the forests. Propagation by vegetative means is not only relevant in case of improved varieties/hybrids of commercially cultivated species but also for conservation of those, which are endemic, threatened and are on the verge of extinction.[3] Multiplication of these species by clonal means such as cuttings can make cultivation much economic, providing more uniform population and active ingredient yield per unit area.[8] In some cases such as *Centella asiatica*, plants raised from cuttings have been proven to perform better for biomass production than those raised from seeds.[9]

Understanding the skills involved in vegetative propagation techniques ensures faster multiplication to meet the growing demand from consumers, and thereby keeping collectors away from forests for collecting such resources. Adventitious root formation has been regarded as a complex process as it is highly influenced by different internal and external factors[10]. Poor adventitious root development is considered as a major hindrance in propagation through cuttings[11]. Some species may produce roots even without any treatments as observed in case of *Cinnamomum asomicum and C. impressinervium*[12]*,* whereas in other species propagation by cutting might not be feasible as in *Aconitum heterophyllum*[13]. For example, hard wood cuttings of a threatened species *Saraca asoca* exhibited rooting in mere 16.67% cuttings, when treated with 500 mg/l Indole-3 butyric acid (IBA) and hence, propagation *via* air layering was advocated for propagation with 90% success[14]. Nevertheless, considering the practicality of the technique, ease of operation, cost involved *etc.*, propagation by cuttings has remained a technique of choice in the past and will continue to be in future as well. Even though propagation through cuttings appears to be the simplest among all the methods of vegetative propagation, some aspects need to be understood

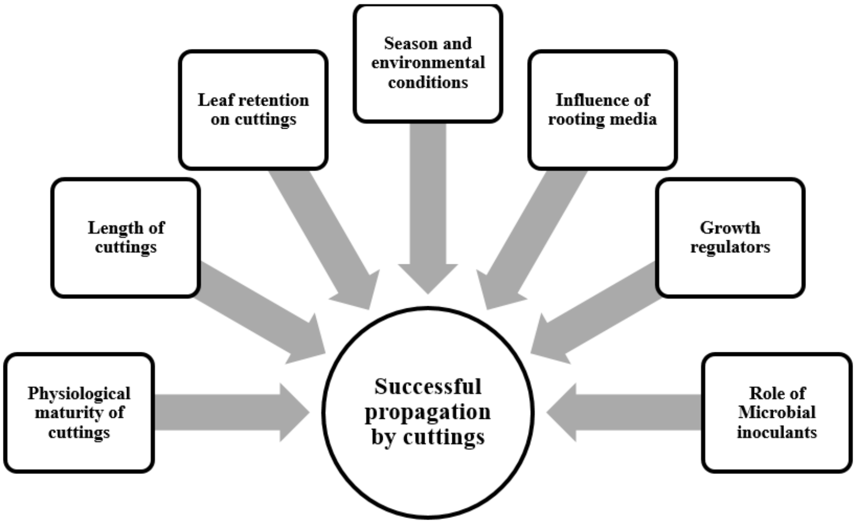

Fig. 1: Major factors governing success of propagation by cuttings in MAPs

to obtain better results. Considering these, factors governing the success of vegetative propagation through cuttings in MAPs (Figure 1) have been reviewed hereunder.

Factors Governing Success of Propagation Through Cuttings in MAPs

Physiological Maturity of Cuttings

Stem cuttings are classified as hardwood, semi-hardwood, softwood and herbaceous cuttings based on physiological age of the wood from which they are excised.[15-16] Hardwood cuttings are taken from dormant, mature stems of more than one year and are commonly adopted in MAPs such as Indian Myrrh, *Rosa* spp., Henna *etc.* Semi-hardwood cuttings are usually prepared from partially mature wood of the current season's growth,which is practiced in species such as *Embelia* spp., *Salacia* spp. *etc*. Softwood and herbaceous cuttings are prepared from soft, succulent new growth of plants[16] and are commonly employed for the propagation of species of mints (*Mentha spp*.), brahmi (*Bacopa monnieri*) and other herbs.

Dick and Dewar[17] opined that variation in carbohydrate pools could be the main factor determining rooting ability. Classically, internal factors such as auxins, rooting co- factors, C:N ratio *etc*. have shown to influence root initiation process.[18] Hardwood stem cuttings of *Gongronema latifolia*, an important African medicinal species, produced significantly higher number of roots compared to when propagated by semi-hardwood and softwood cuttings.[19] Similarly, in case of *J. grandiflorum,* hardwood cuttings exhibited highest regeneration capacity compared to semi-hardwood and softwood cuttings.[20] In case of *Pogostemon heyneanus,* two nodal hardwood cuttings performed better in rooting parameters when compared to semi-hardwood and softwood cuttings.[21] Positive relationship between diameter of cutting and rooting capacity in *Azadirachta indica* has been previously reported,[22] which could be explained by higher carbohydrate reserves in thicker cuttings.

On the contrary, in *Salacia reticulata* and *Embelia ribes*, use of semi- hardwood cuttings gave superior rooting response than that by hardwood cuttings.[4,23-24]

Table 1: Some examples of different types of cuttings based on their physiological maturity in MAPs

Type of cutting	Species	Reference
Herbaceous cutting	*Bacopa monnieri, Centella asiatica, Pogostemon patchouli*	7, 9, 90
Softwood cutting	*Nothapodytes nimmoniana*, *Pelargonium graveolens, Tylophora indica*	83, 91, 92
Semihard wood cutting	*Adhatoda vasica, Bixa orellana, Boswellia serrata*, *Clerodendrum indicum, Crataegus oxyacantha, Embelia ribes, Ginkgo biloba; Jasminum sambac, Leptadenia reticulata, Nothapodytes nimmoniana, Piper longum, Plumbago rosea, Plumabgo zeylanica, Ruta graveolens; Salacia fruticosa*	4, 7, 23, 33, 82, 83, 84, 94, 95, 96, 97
Hard wood cutting	*Celastrus paniculata, Jasminum grandiflorum, Lawsonia inermis¸ Nothapodytes nimmoniana, Premna integrifolia, Rauwolfia serpentina, Streblus asper, Rosa damascena, Taxus baccata, Vitex negundo, Wrightia tinctoria*	20, 83, 97, 93 99, 100, 101, 102
Terminal cutting	*Baliospermum montanum, Bursera delpichiana, Coleus forskohlii, Lippia javanica*	32, 94, 95, 103
Root cuttings	*Hemidesmus indicus, Chlorophytum boriviliamum, Rauwolfia serpentina, Rubia cordifolia*	94

Based on diameter, thin (0.43 cm) and medium (0.52 cm) cuttings were found to be superior for increasing sprouting and survival in *Embelia tsjeriam-cottam* and *Caesalpinia bonduc* than thicker cuttings.[3] Secondly, portion of the stem used for excising cutting has been found to contribute in root induction. Rooting ability of cuttings taken from different parts of the stem of *Ficus* species indicated that higher rooting rates (>52%) were obtained with pole and nodal cuttings as against 5% in case of terminal cuttings.[25] Low rooting ability of apical cuttings could be attributed to their herbaceous character, which made them sensitive to moisture stress, resulting in desiccation.[26] Middle or apical cuttings of about 25 cm length were adjudged as the best planting material for regeneration of *Commiphora wightii*, the Indian myrrh.[27] Similarly, in Damask rose (*Rosa damascena)*, basal woody cuttings were found to give highest rooting percentage with maximum number of roots.[28] Nevertheless, the response is species dependent as Khosla and Pushpangadan[29] observed that the current year, young lateral shoots were the best planting material for rooting of clocimum (*Ocimum gratissimum*). Most suitable physiological maturity of the cuttings in some important MAPs have been presented in Table 1.

Length of Cuttings

Went[30] observed that functional buds present on the cutting produced root promoting chemicals, which signifies the importance of length of cutting in inducing rooting. The length that could produce sufficient root and shoot system for the plant to grow however varies with the species. For example, about 15 cm long cuttings were rated superior in *Plumbago zeylanica* under mist,[31] while longer cuttings of 25 cm were required in case of *Bursera delpichiana*[32] and *Ruta graveolens* to obtain maximum rooting percentage, fresh root weight and higher field survival.[33] Superior rooting percentage with better survival has been reported using 12-15 cm long cuttings in *Rosa damascena*[34] and *Drymis brasiliensis*[35] under polyhouse conditions.

Availability of mother stock is a limiting factor in a number of species and efforts are being made to increase the multiplication ratio to make it cost efficient as well. For instance, in noni (*Morinda citrifolia*), earlier reports[36] recommended use of longer (20 to 40 cm) hardwood cuttings; however, subsequent reports suggested that 4 node[37] or even 3 node[38] cuttings could suffice development of healthy plantlets. Similarly, in case

Table 2: Influence of leaf retention on success of propagation through stem cuttings

Species	Remarks	Reference
Barleria prionitis	Better survival of 81.48% was noticed in leaved cuttings than leafless cuttings (70.37%)	104
Commiphora wightii	Leafless stem cuttings are preferred for early and better rooting	94
Dillenia suffructicosa	Juvenile cuttings with leaves gave 100% survival as against 100% mortality in leafless cuttings.	45
Enantia chlorantha	Full-leaf cuttings were superior (100% rooting) to half-leaf cuttings even without application of auxins	50
Lavandula dentata	Maintaining higher number of leaves (2/3 leaf retention) was beneficial for obtaining better rooting percentage	51
Marsdenia tenacissima	Fresh and healthy leafy cutting having two or three nodes of axillary buds gives better rooting	94
Pogostemon cablin	Removal of all the leaves was not recommended due to higher mortality percentage	54
Piper sarmentosum	Retention of leaves on the cuttings did not enhance. rooting percentage but improved plant growth	43
Plectranthus barbatus	Removal of all leaves except apical bud gives better rooting	94
Salacia oblonga	Highest regeneration percentage (72.3%) was observed, when leafless cuttings were used as against cuttings with leaves (55.5%) using 300 ppm IBA	6

of black pepper (*Piper nigrum*), Singh and Singh[39] obtained maximum rooting and subsequent plant development using two node cuttings than that from three and four node cuttings. However, recently, single node cuttings have been found to increase the multiplication ratio by manifolds.[40] In a related

Table 3: Influence of season and growing environment on success of propagation in MAPs

Species	Remarks	Reference
Caralluma edulis, Leptadenia reticulata and *Tylophora indica*	Aeroponics system was found to be superior in terms of rooting percentage, plant growth and survival than the conventional soil planting	68
Commiphora wightii	Four to five days seasoning of cuttings in the month of August supported better sprouting and field survival (50%)	105
Clerodendrum serratum	Stem cuttings treated with IBA 400 ppm for early sprouting	106
Embelia ribes	Semi-hardwood cuttings collected during January-April were most suitable	23
Ginkgo biloba	Cuttings maintained in polyhouse (25 °C and 70% RH) gave 56.7% rooting after 6 months as against two years under open condition	84
Ginkgo biloba	Cuttings taken during July rooted better (90%) than those taken during April (40%) with the same dose of IAA	107
Jasminum sambac, J. auriculatum and *J. grandiflorum*	Better rooting under mist than in open condition	59, 60
Magnolia fuscata	Propagation under mist reported highest rooting (%) and survival of rooted cuttings	58
Operculina turpethum	Stem cuttings with two nodes may either be planted directly in the field during monsoon (July) or may be rooted in mist chamber during March-June	94
Pelargonium graveolens	Shade of *Putranjeeva roxburghii* was equally suitable as shadenet house for rooting	108
Piper longum	Growth and field establishment was improved in greenhouse than those in natural shade	66
Plumbago zeylanica	15 cm long cuttings were rated superior under mist conditions	31
	Basal stem cuttings up to seventh to ninth nodes under mist chamber gives 80-100% rooting success	94
Salacia fruticosa	Semi-hardwood cuttings collected during January-April were most suitable	4
Stevia rebaudiana	Growth and field establishment was improved in greenhouse than those in natural shade	65
Stevia rebaudiana	Better rooting in autumn season with 50 ppm NAA + 500 ppm IBA, while 2000 ppm NAA + 2000 ppm IBA is required during kharif season	109, 110
Tinospora cordifolia	Obtaining stem cuttings from mother plant during June-July gives better rooting and field survival	94
Vanilla planifolia	Growing cuttings under 50% shade was found to be cost effective	61
Vanilla planifolia	Greenhouse was most suitable for producing vigorous plants, early rooting with highest rooting percentage	64
Zanthoxylum aramatum	Stem cuttings planted in nursery during monsoon (July – August) gives good rooting	94

species, long pepper (*Piper longum*), Kempe Gowda *et al.,*[41]. reported that triple node cuttings treated with IBA 1000 mg/l produced better shoots and roots. Efforts could be made to propagate long pepper using single node cuttings as in black pepper. However, reduction in length of cuttings is not always useful[42] as significantly higher sprouting percentage with better shoot and root growth were observed, when 15 cm cuttings were used over 7.5 cm in stevia (*Stevia rebaudiana*). Similarly, four nodal cuttings were found to be superior to two nodal cuttings for increasing sprouting and survival in *Embelia tsjeriam- cottam* and *Caesalpinia bonduc*[3]. In *Piper sarmentosum*, use of double node cuttings was rated superior to single and triple node cuttings.[43]

Leaf Retention on Cuttings

Retention of leaves on the stem cutting is known to have an impact on the rooting success in some species, mainly because of the presence of auxins in leaves, which are translocated to the base of cuttings for promotion of rhizogenesis.[44] Leaf is also known to provide energy to the cuttings especially those with limited food reserves.[45] However, if excessive leaves are present on the cuttings, moisture may get lost from cuttings due to evaporation resulting into dehydration of cuttings.[46] The requirement for retention of leaves on a cutting is variable, as success varies greatly amongst the species. In some species, cuttings with lesser leaves root better than more leafy ones,[47] while rooting cannot occur in other species in the absence of leaves.[48] According to Okoro and Grace,[49] the initial carbohydrate reserve in leafless hardwood cuttings may not act as a limiting factor for root induction but may govern the subsequent growth and development of leaves.

In case of *Piper sarmentosum*, retention of leaves had no positive influence on rooting percentage but improved plant growth.[43] Limited efforts have been made in MAPs to study the effect of this factor

Table 4: Effect of substrate on rooting, survival and plant growth

Species	Substrate	Remarks	Reference
Barleria prionitis	Sand+ soil+ vermicompost (1:1:1)	Better (97%) survival and plant growth	104
Cinnamomum verum var. Navashree and *Nityashree*	Sand and coir dust (1:1)	Superior rooting parameters	78
Leptadenia reticulata	Sand : FYM : Red soil	Good rooting and field establishment	94
Morinda citrifolia	Forest soil + sand, volcanic cinder + compost	Superior rooting parameters	36
Piper longum	Soil + sand + vermicompost (1:1:1)	Better shoot and root growth parameters	66
Piper nigrum	Soil + Bio-rigi + sawdust (1:1:1) and soil +sand (1:1); while soil was not recommended	Lowest mortality with better root growth	72
Piper nigrum	Vermicompost	Better plant growth	73
Piper nigrum var. Panniyur-1	Topsoil, sand and vermicompost (1:1:1)	Better multiplication ratio (23.5 cuttings per vine) under rapid multiplication technique	74
Pogostemon heyneanus	Top soil: sand: compost (1:1:1)	Better root growth	21
Premna integrifolia	Sand : soil : FYM (1:1:1)	Better rooting	94
Salacia reticulata	Top soil and compost (1:1)	Highly congenial for establishment of cuttings	24
Stevia rebaudiana	Sand: perlite (1:3)	Better rooting and growth	75
Stevia rebaudiana	Soil + sand + vermicompost (1:1:1)	Better shoot and root growth parameters	65
Vanilla planifolia	Vermicompost and coir pith compost	Most efficient and cost effective	76

on success of propagation (Table 2). A comparison was made between half-leaf and full-leaf stem cuttings in *Enantia chlorantha* using different auxins. Results revealed that full-leaf cuttings were superior to half-leaf cuttings as 100% rooting was noticed in those cuttings even without application of auxins.[50] In another study on *Lavandula dentata*, cuttings were planted with 1/3, 1/2 or 2/3 of their leaves. It was noticed that maintaining higher number of leaves was beneficial and 2/3 leaf retention was found to be most suitable for obtaining better rooting percentage.[51] In *Salacia oblonga*, highest regeneration percentage (72.3%) was observed, when leafless cuttings were used as against cuttings with leaves (55.5%) at 300 ppm IBA.[6]

In one case, rooting and successful establishment in patchouli (*Pogostemon cablin*) was found to be superior with 6 leaves + IBA (2000 mg/l),[52] while other report suggested use of 2-4 leaves + IBA (150 mg/l) combination.[53] These variations could be attributed to the differences in the growing environments and the varieties used in these studies. Removal of all the leaves in patchouli cuttings was not recommended due to higher mortality percentage.[54]

Season and Environmental Conditions

Season plays an important role on success of different methods of vegetative propagation including cuttings (Table 3). Day temperature, cloud cover and relative humidity have great influence on the success of rooting, sprouting and growth of propagules.[25,55] These variations largely govern the physiological activities in a plant system, including the sugar levels (due to retention of leaves on the cuttings, which is common in evergreen species) and temperature of the substrate.[55] It has been reported that wet season with high relative humidity is congenial for rapid callus production and early rooting.[56] In *Salacia fruticosa* and *Embelia ribes*, plant propagation by semi-hardwood cuttings was most successful when cuttings were collected during January-April.[4,23]

Under open conditions, ideal conditions for propagation cannot be maintained throughout the year; however, manipulation of environment is possible under protected conditions. Kind of structures used for raising nursery also governs the success as light penetration, temperature, relative humidity and gaseous composition inside the structure varies with the material used. Several workers have reported good results with cuttings under mist. Raines[57] was first to report the use of mist chamber for induction of rooting in stem cuttings. Balakrishna and Bhattacharjee[58] reported in *Magnolia fuscata* that shoot tip cuttings with two leaves treated with IBA (6000 ppm) gave the highest rooting percentage and survival of rooted cuttings under mist. Stem cuttings of *Jasminum sambac*, *J. auriculatum* and *J. grandiflorum* were rooted better under mist than in open condition.[59-60] Under cost effective growing conditions, Konedenedo[61] obtained best results with one meter long vanilla (*Vanilla planifolia*) cuttings with 50% shade. Under Indian conditions, use of low cost polyhouse with ambient condition was recommended for propagation of MAPs through cuttings as against shade net conditions.[62] Medium cost greenhouse was also found to be ideal due to maintenance of relatively high temperature and humidity,[63] wherein the extent of rooting of MAPs was better (76.3%) than that in shade net (25.0%).

Propagating structures *viz.* greenhouse, shade net and natural shade of *Muntingia calabura* were evaluated for propagation of vanilla (*Vanilla planifolia*), which revealed that greenhouse was most suitable for producing vigorous plants, early rooting and maximum rooting percentage[64]. Similarly, rooting, shoot growth and field establishment of stevia (*Stevia rebaudiana*) and long pepper cuttings were improved when experiments were conducted in greenhouse than those in natural shade[65,66]. Higher temperature and relative humidity prevailing in such structures compared to outside condition could result in enhanced photosynthetic efficiency due to early sprouting and leaf production, better root growth and establishment of cuttings [67]. Recently, aeroponics system has become popular in the multiplication of planting material. In three medicinal species viz. *Caralluma edulis, Leptadenia reticulata* and *Tylophora indica* the aeroponics system was found to be superior in terms of rooting percentage, plant growth and survival than the conventional soil planting.[68] Major advantage of this system is production of completely disease free planting material, and hence could be exploited on commercial scale.

Table 5: Interactions between type of cuttings and different growth regulators in propagation of MAPs

Species	Type of cutting	Growth regulators	Remarks	Reference
Adhatoda vasica	SHWC	1500 ppm IBA	Higher (67.5%) rooting than control (30.8%)	96
Azadirachta indica	SC	1000 ppm IBA	Superior (51%) rooting than control (35%)	112
Baliospermum montanum	SHWC	IBA, NAA, Boric acid	Improved sprouting percentage to nearly 90 to 100 %	94
Berberis aristata	TC	5000 ppm IBA	Significantly higher (50%) rooting as against no rooting in control	98
Bursera delpichiana	HWC	2000 ppm IBA	Better rooting (67%) than control (15.3%)	32
Caesalpinia bonduc	SC	1000 ppm IBA	Superior sprouting and field survival	3
Celastrus paniculata	HWC	2000 ppm IBA	Improved (72.6%) rooting percentage as against 54% in control	102
Caralluma edulis	SC	2000 ppm IBA	100 % root induction in hydroponics system	68
Coleus forskohlii	TC	500 ppm IAA	Superior rooting response	103
Commiphora wightii	SC	100 ppm IBA	Hasten rooting and better survival	94
Embelia ribes	SHWC	3000 ppm IBA	100 percent rooting	23
Embelia tsjerium-kottam	SC	1000 ppm NAA	Superior sprouting and Survival	3
Glycyrrhiza glabra	SC	500 ppm IBA	90% rooting as compared to 67% in control	113
Gymnema sylvestre	BC	500 ppm IAA 100 PPM IBA	Superior rooting, root length and survival	93, 95
Jasminum auriculatum	SC	4000 ppm IBA	Better rooting percentage (13.3%) than control (6.7%)	60
Jasminum grandiflorum	SC	2000 ppm IBA	Better rooting percentage (50%)	60
Jasminum sambac	SC	2000 ppm IBA	Better rooting and survival	114
Lavendula angustifolia	BC	2000 ppm IBA	Superior rooting percentage	111
Leptadenia reticulata	SC	3000 ppm IBA	Better root induction (97.7 %) in hydroponics system	68 94
Lippia javanica	BC and TC	3000 ppm IBA	Better rooting percentage	103
Magnolia fuscata	SC	6000 ppm IBA	Superior rooting and survival under mist	58
Marsdenia tenacissima	SC	100 ppm IBA	Better rooting	94
Nothapodytes nimmoniana	1. SWC 2. SHWC 3. HWC	1. 2000 ppm IBA 2. 3000 ppm IBA 3. 4000 ppm IBA	Superior rooting, survival and growth parameters	83
Pelargonium graveolens	SC	2000 ppm IBA	Better rooting percentage and field survival	92
Picrorhiza kurroa	Runners	50 µM IBA for 12 h	Superior rooting (87%) than control (53%)	115
Piper longum	BC	500 ppm IAA	Superior rooting percentage, root length and survival	95
Piper longum	SC	500 ppm IBA	Better rooting and field survival	116
Piper nigrum	NC	2000 ppm IBA	Superior root growth parameters	117

Piper sarmentosum	SWC	1000 mg/l	Superior rooting (92.5%)	43
Plumbago zeylanica	SC	500 ppm NAA	Promote quick rooting	94
Pogostemon patchouli	LHC	1500 ppm IBA	Superior rooting percentage and survival	90
Pogostemon patchouli	SC	IBA and NAA	4-6 folds increased rooting over control	118
Premna integrifolia	HWC	1000 ppm IBA	Superior rooting percentage, growth and field survival	101
Rauwolfia serpentina	HWC	30 ppm IAA	75-95% success with better rooting	119
Rosa damascena	SC	50 ppm NAA	Maximum rooting and shoot growth	99
Ruta graveolens	SHWC	300 ppm IBA	Maximum rooting and field survival	33
Salacia fruticosa	SHWC	3000 ppm IBA	Superior rooting percentage and root length	4
Taxus baccata,	HWC	500 ppm IBA	Higher callusing percentage, rooting percentage, number of roots	93
Terminalia arjuna	SC	2000 ppm IBA	Superior rooting parameters, shoot proliferation, maximum shoot and root biomass	120
Terminalia chebula	Juvenile SC	4000 ppm IBA	Better (55%) rooting than control (25%)	121
Tinospora cordifolia	SC	100 ppm IBA	Better rooting and plant growth	122
Thymus vulgaris	SC	150 ppm IBA	Early and better rooting	123
Tylophora indica	SWC	1000 ppm IBA	Maximum rooting and survival	124
Tylophora indica	SC	3000 ppm IBA	Better RP (93.3 %) in hydroponics	68
Vitex negundo	SC	3000 ppm IBA	100% sprouting	125
Wrightia tinctoria	HWC	4000 ppm IBA	Higher root induction	100

(BC: basal cuttings; HWC: hard wood cuttings; LHC: leafy herbaceous cuttings; NC: nodal cuttings; SC: stem cuttings; SHWC: semi hard wood cuttings; SWC: soft wood cuttings; TC: terminal cuttings)

Influence of Rooting Media/ Substrate

Various characteristics of rooting medium *viz.* structure, texture, porosity, chemical composition, water holding ability of the media and pH have pronounced effect on rooting ability as well as the quality of root system formed.[18,69-70] Hence, adequate attention should be paid, while selecting a medium for the concerned species. In a number of medicinal plant species, such studies are lacking, however spice species having numerous medicinal properties such as black pepper are adequately studied (Table 4). In black pepper variety Panniyur-1 highest rooting (30.9%) and plant growth were obtained in the medium containing sand + farmyard manure (FYM), followed by sand alone.[71] Bogantes- Arias[72] tested six substrates as rooting media for black pepper cuttings, wherein lowest mortality with better root growth was observed in soil + Bio-rigi + sawdust (1:1:1) and soil + sand (1:1) combinations, while soil was not recommended. Thankamani *et al.,*[73] reported that use of vermicompost as a substrate supported better plant growth over standard potting mixture comprising of soil, sand and farmyard manure (3:1:1). Mixture containing topsoil, sand and vermicompost (1:1:1) was found to be the best for root induction and recovery percentage in black pepper var. Panniyur-1 under rapid multiplication technique that could produce 23.5 rooted cuttings per vine within three and a half months.[74]

Better rooting and growth response have been reported in stevia (*Stevia rebaudiana*), when stem cuttings were planted in sand: perlite (1:3) media under polythene film.[75] In another study on stevia, use of soil + sand + vermicompost media (1:1:1) was found to produce longer and thicker sprouts, better root girth, dry weight of shoot and roots.[65] Same medium combination was also found to be promising in long pepper (*Piper longum*) also[66]. In *Pogostemon heyneanus*, a combination of top soil, sand and compost (1:1:1) was found to be the most suitable potting medium.[21] In *Salacia reticulata*, use of top soil and compost (1:1) was found to be highly congenial for establishment of cuttings.[24] *Siddagangaiah et al.,*[76] evaluated various rooting substrates and found that vermicompost and decomposed coir pith were most efficient, cost effective and hence ideal for the production of vanilla stem cuttings. Vermicompost is commonly incorporated in the rooting medium due to various properties such as its richness in nutrients and growth stimulants, higher water holding capacity and improved soil texture that facilitate the root growth.[27,77] Krishnamoorthy *et al.,*[78] reported that sand and coir dust (1:1) was the most appropriate rooting medium for two cinnamon (*Cinnamomum verum*) varieties *viz*. Navashree and Nityashree as highest rooting percentage, length of primary roots and secondary roots per cutting were obtained in this combination. Nelson[36] recommended weed and nematode free forest soil mixed with sand, volcanic cinder and composted organic matter for propagation in noni (*Morinda citrifolia)*.

Growth Regulators

Inherent capability of a cutting to induce rooting could largely be altered by chemical treatments. Many synthetic growth substances have been used to aid the rooting of cuttings since long time.[79] of those, auxins are the most commonly used as they are known to help in accumulation of metabolites, synthesis of new proteins, cell enlargement and increase nitrogen in roots.[80] They also regulate different aspects of plant growth and development by affecting physiological processes including cell division, cell elongation and differentiation.[81]

Indole-3-butyric acid (IBA), naphthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) are the most commonly employed auxins due to their ability to initiate roots, stability and low mobility in plants. Although the induction of rooting by auxin application has been reported in different plant species, reports on the mechanisms of this response are contradictory.[81]

In *Bixa orellana,* use of 4000 mg/l concentration of IAA supported root induction in 22% cuttings, whereas none of the IBA concentrations could induce rooting in these cuttings.[82] It is noticed that the concentration of growth regulators required for rooting varies according to nature of the plant and as a thumb rule, woody plants require higher concentration than the herbaceous ones. The

Table 6: Examples of use of microbial inoculants in propagation of MAPs

Species	Microbial inoculant	Remarks	Reference
Coleus aromaticus	*G. fasciculatum*, *T. harzianum*	Maximum growth, total biomass and nutrients in plants	126
Piper longum	*T. viride*	Improved plant growth and yield	66
Piper nigrum	*Trichoderma viride* (1 g/kg)	Superior growth, survival percentage and lower disease incidence	127
Piper nigrum	*Azospirillum sp*. and Phosphobacteria	Superior plant growth and biomass	128
Piper nigrum	*Trichoderma spp*. and VAM	Robust, disease free cuttings	129
Piper nigrum	*T. harzianum* and *Pseudomonas fluorescens*	Improved growth, reduced incidence of soil borne diseases	130
Piper nigrum	*P. fluorescence* or *T. harzianum* (1g/kg)	Superior plant growth and biomass	131
Stevia rebaudiana	*T. viride*	Superior growth and field establishment compared to growth regulator treatments	65
Vanilla planifolia	*T. harzianum* (2 g/polybag)	Better rooting and plant growth than in growth regulators treated cuttings	67

optimum kind and concentration of auxin are species-specific e.g. a dose of 2000 mg/l of IBA is recommended for *Jasminum grandiflorum*, whereas double dose of 4000 mg/l was required in case of *Jasminum auriculatum.*[60] Hormonal requirement also differs with the type of cutting used for propagation e.g. in *Nothapodytes nimmoniana*, IBA concentrations of 2000 mg/l, 3000 mg/l and 4000 mg/l were optimum for soft wood, semi hardwood and hardwood cuttings, respectively.[83] Hence, a number of other factors need to be studied together with growth regulators to get comprehensive results.

Unlike other aspects discussed in this article, role of plant growth regulators have been studied in multiplication of a number of MAPs (Table 5). In a number of instances, rooting could not be obtained in the absence of external application of growth hormones, thereby indicating their pivotal role in the propagation of these species. Apart from auxins, compounds such as catechin 5 mg/L and gallic acid 10 mg/L have been reported to induce rooting in semi hardwood cuttings of *Ginkgo biloba* with 53 and 57% success.[84]

Role of Microbial Inoculants

Microbial inoculation could help in getting healthy and vigorous transplants with well-developed root system. This in turn, helps in reducing the transplant injury and improving field establishment. Further, a number of microbial species are known to offer protection against soil borne nursery diseases. Microorganisms such as *Trichoderma, Azotobacter, Azospirillum, Bacillus, Rhizobacteria, Pseudomonas, Phosphate Solubilising* Bacteria (PSB), Vesicular Arbuscular Mycorrhiza (VAM) *etc*. have been used to induce rooting in several species.

The mechanism of action of these species is diverse. *Trichoderma* strains are known to assist the process of decomposition of plant residues in the soil[85] and also act as bio-control agent.[86] Root colonization by *Trichoderma* spp. enhances root growth and development, crop productivity and the uptake and use of nutrients.[87] Incorporation of VAM fungus, *Glomus fasciculatus* into the rooting medium enhanced rooting and increased the plant biomass mainly by increasing concentration of endogenous hormone level in black pepper cuttings.[88] Similarly, increased feeder root production and absorptive surface area due to colonization of *Pseudomonas fluorescen*s has been reported.[89] Table 2 represents the applications of different microbial strains on nursery propagation of MAPs. It is evident that a good number of reports are available on black pepper; however, studies on other species are limited (Table 6).

Conclusion

In recent years, there has been an ever increasing demand for MAPs from national and international markets leading to over-exploitation of their wild sources, resulting in their dwindling population in the wild. Hence, there is an urgent need to bring these plants under cultivation for which standardization of propagation techniques is of prime importance. Though sexual propagation through seeds helps in maintaining the diversity in nature, commercial scale exploitation of these species demands uniformity, thereby necessitating the standardization of an alternative vegetative propagation method. Considering the work done in various species as described in the article, there is tremendous scope for studying various factors influencing rooting of stem cuttings in MAPs. Once standardized, it could increase the efficiency of this method for mass multiplication in the MAPs for their cultivation and conservation.

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Conflict of interest

Authors declare that there is no conflict of interest.

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