J. Phytol. Res. 24(1) : 15-22, 2011

ECOPHYSIOLOGICAL STUDIES ON SEED GERMINATION AND VEGETATIVE PROPAGATION IN *COMMIPHORA WIGHIIT* (ARNOTT) BHANDARI WITH THE OBJECT OF ENCOURAGING COMMERCIAL CULTIVATION AND *EX-SITU* CONSERVATION

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Commiphora wightii, a highly endangered medicinal plant of Thar desert has been used for the cure of more than 50 diseases by ayurvedic physicians beside being burnt as fire wood in villages. It cannot survive without *ex-situ* conservation. In the present work as a result of protracted sampling year after year from 2001 to 2009, several much cheaper organic chemicals have been discovered which elicited rooting upon stem cutting as good as or even higher than that by hormones. In respect of all the four parameters for which data were recorded, certain concentrations of trypsin elicited the highest values except in respect of the parameter for the average total length of adventitious roots per stem cutting in which Nicotinamide and Ascorbic acid elicited marginally superior results.

Keywords : Hormonal and non-hormonal chemicals; Rooting stem cuttings.

Introduction

When the science of medicinal plants was at the peak of its glory in ancient India, people in the West lived in caves in jungles. Ayurveda, the science of medicinal plants for the cure of human diseases flourished during the Vedic period (2000 BC to 800 BC). The treatise "Vrikshayurveda", written during the Pre-Christian era was the most authentic text-book for pre-medical students. It contained a system of classification[1] of plants which is more modern than the most modern systems among present day classifications. Atharveda is another ancient treatise which contains a detailed account of herbal drugs and the diseases against which they are used. Not with standing the progress in the science of medicinal plants in ancient India, much of the literature was destroyed by the various invaders which plundered India after the 10th century. Non-availability of standard diagnostic description of medicinal plants and their correct identification have been the weakest aspect of Ayurveda. On the other hand, the overexploitation of well known medicinal plants like *Commiphora wightii* (Arnott) Bhandari (valid name of *Balasamodendron mukul* Engl) and many others have made them very highly endangered. Realizing the importance and threat to many medicinal plants of India, in 1969, the government of India constituted the Central Council for Research In Indian Medicine and Homoeopathy to take stock of the present position. The medicinal properties of *Commiphora wightii,* the India bdellium (vern, *guggal)* have been listed in ayurvedic treatises[2].

Its gum is acrid, digestive, astringent, aromatic, anthelmintic, anti-inflammatory, anodyne, antiseptic, aphrodisiac, alterative, antispasmodic, bitter, expectorant, depurative, demulcent, detergent, diuretic, stimulant, emmenagogue, lithontriptic, nervine, liver and general rejuvenating tonic, haematinic, thermogenic and vulnerary. The gum has also been used for the cure of vitiated conditions, gout, osteo-arithritis, asthma, cardiac disorders, dysmenorrhoea, leprosy, pectoral and hepatic disorders, urinary caliculus, scrophula, leucoderma, bronchitis, amenorrhoea, wounds, coronary thrombosis, diabetes and stomatopathy. Beside the above diverse medicinal uses of guggal in Ayurveda, several workers in the field of modern biology and medicine have experimentally proved its medicinal utility. A crystalline steroidal compound isolated from Guggle showed anti-inflammatory activity in experimental animals[3]. The essential oil from Guggle gum has several pharmacological applications, including its utility as sustaining material in making tablet dosage of various medicines[4-5]. A medicinal component Gugglesterol isolated[6] from Guggle yielded a hypolipidemic drug[7]. The hypolipidemic activity of a steroid fraction of Guggle has

been experimentally demonstrated in monkeys[8]. Furanosesquiterpenes have been isolated from the essential oil of Guggle[9]. An extract from Guggle has been shown to be a powerful repellent and toxicant against tics[10]. A detailed review of all the medicinal properties[11-12] of guggal is available whereas its essential oil shows anti-anthelmintic[13] activity. It has been further demonstrated that furanosesqueterpenoids of Guggle[14] are very effective natural insecticides against tics. Likewise, its volatile resin exudates have a potential role in plant defence[15]. Guggle steroids also play a vital role in the inhibition of platelet aggregation[16]. Several steroidal compounds[17] which are antiviral have been isolated from guggal gum beside monocyclic diterpenes[18]. Further the curative properties of ethyl acetate extract of Guggle have been found to be very effective against antherosclerosis[19]. Z-Guggleosterones[20] of Guggle possess strong thyroid stimulating properties and the mechanism of their action has been fully elucidated[21]. Gum Guggle is highly effective hypocholesterolemic and hypolipidemic for patient of coronary heart disease[22]. A compact research team working on female rats experimentally demonstrated the oleo-gum resin of guggal to be potently anti-cholesterolemic, anti-hyperlipidemic drug besides lowering fertility in female rats[23]. Several other researchers[24-26] have found it to be an antioxidant[27] or for curing antherosclerosis[28]. A recent most interesting discovery[29] is that Gram-positive bacterial strains have been found to be most susceptible organisms towards guggal gum, the minimum inhibitory concentration (MIC) being in the concentration range of 0.5- 2.0 mg/ml. That only a single plant species has a cure for so many human disorders naturally gives the impression that *Commiphora wightii* is a grand chemist's store where a curative medicine is available for practically all human ailments, that is a panacea indeed! There are many guggal preparations now-a-days but the pure guggal gum is in extremely short supply and adulteration with coagulated exudates of many other plant species is common which makes these preparations ineffective against many of the ailments which it is expected to cure. The main object of the present study was to find out methods for the production of propagules from seeds and stem cuttings on a massive scale for raising *Commiphora wightii* whose overexploitation over a very long period of time has brought it to a stage where its *in situ* survival has become impossible. Guggal farms need to be established in all the Aravalli districts of Rajasthan to insure *ex-situ* conservation of this unmatchable heritage medicinal plant in this state and to meet the massive need of its gum resin which is used for the manufacture of pure Ayurvedic medicines. Undoubtedly, it would also provide employment to a large number of poor unemployed labourers.

Material and Methods

Seed germination -Several earlier workers[30-32] had reported that seeds do not germinate in nature. Our observation is that guggal shrub in nature inhabit flattened tops and slopes of the Aravalli hills from where the seeds are blown by summer winds down the into the valleys where they usually die due to submersion in water, leaving no chance for the field workers to watch them germinating. It was reported[33] that usually one, sometimes 2-3 seeds develop and germinate from each drupe. The present worker who cultivated guggal in her plot during the years 1991-2003 found that the number of fruits on nine years old shrubs varied from 897 to 1069. The mature fruits are red drupes, each containing usually one, sometime two or even three seeds. On an average 60% of seeds have a dark-brown, rather somewhat blackish seedcoat, are embryonate, the remaining being light brown in colour and are usually ex-exembryonate. The latter usually do not germinate. The dark brown seeds show typical dicot type of epigeal germination and were used in all germination experiments. The embryonate seeds when soaked in water show typical dicotyledonous epigeal type of germination, usually with two cotyledons, rarely with three. After four months, the viability of seed begins to decrease and within 12 months all the seeds become non-viable. It is indeed a matter of great regret that even after the medicinal properties of guggal had been established by Ayurvedic physicians, no efforts were made to find the role of seed in its propagation and thousands of tons of seeds have rotten away during the last 2000 years or so.

Seeds with dark-brown seedcoat, without any treatment do not show more than 40% germination. On account of this, rather low percentage of germination, the seeds were soaked in different concentrations of certain organic chemicals, including growth regulators, vitamins and enzymes for 48 hr prior to spreading them in Petri dishes for studying percentage of germination. The adhering liquid was removed from the seeds by gently pressing them between two filter papers. Seeds soaked in distilled water constituted the control. Another set of seeds were transferred to the soil in pots, directly after the various treatments. Observations in respect of percentage of germination (Table 1) and total average length of primary root from radical per seedling (Table 2) were recorded.

Sprouting of stem cuttings-Uniform sized stem cuttings, 20 cm long with a proximal diameter of about 10 mm were cut out from 9-10 years old shrubs. The proximal

Table1. Showing the effect of soaking of seeds for 48 hrs in different concentrations of certain chemicals on the percentage of germination after a period of 60 days. Values are means of three replicates of 10 seeds each: multiplication each value by 10 gives percentage of germination.

Treatments	Concentrations						
	0	150	300	450	650	800	100
Indol-3-acetic acid	1.33	3.67	7.33	4.67	0.67	0.00	0.00
Naphthalene	1.33	2.67	7.67	7.33	3.00	1.00	0.00
Indole butyric acid	1.33	2.00	3.33	4.00	4.33	7.00	5.67
Gibberellic acid	1.67	2.67	2.67	3.33	1.33	0.33	0.33
Kinetin	2.00	3.67	5.33	4.67	0.67	0.00	0.00
Trypsin	2.00	3.67	4.00	5.00	8.33	5.33	5.67
Nicotinamide	2.33	4.33	4.67	4.67	2.67	1.33	0.00
Folic acid	1.67	3.33	3.33	3.67	6.00	4.67	2.33
Ascorbic acid	1.33	2.67	4.67	5.33	7.00	7.00	4.00

ANOVA TABLE

Variance	DF	SS	MSS	F	SEM ±	CD (5 %)	CD (1%)
Treatment	8	181.43	22.6786	36.950**	0.1710	0.4785	0.6323
Concentration	6	242.25	40.3757	65.784***	0.1508	0.4220	0.5577
T X C	48	496.79	10.3499	16.863*	0.4523	1.2659	1.6730
Error	126	077.33	00.6138				

GM =3.30; CV (%) = 23.73; *Significant; **Highly significant; *** Very highly significant.

seven cm length of stem cuttings was dipped in different concentrations of the various chemicals for 48 hrs constituted the treatments whereas cuttings treated similarly in distilled water constituted the controls. Both the control and the treated cuttings were transferred to the pots filled with garden soil. Medium irrigation was provided to the pots. The cuttings started sprouting within 15 days of the setting of the experiments. The observations were continued for 60 days during which some of the cuttings withered and died. On the 60th day the pot soil was gently washed away and the percentage of cuttings sprouted and the total length of all adventitious roots per cutting in each replicate was recorded.

Results and Discussion

The present work took more than nine years to complete due to a variety of reasons, like paucity of experimental material, selection of appropriate chemicals and their suitable concentrations and most important repeatability of results within reasonable limits during pre-sampling from the year 2001-2009. However, the results presented in this study relate to the year 2008-2009 (Tables 1-4). Data were recorded on average percentage of seed germination (Table 1), average total length of primary root from radical (Table 2), percentage of sprouting of stem cuttings (Table 3) and average total length of all the adventitious roots (Table 4), developed upon one cutting, 60 days after sowing of the pre-treated samples and controls in the soil.

Hormones are exorbitantly costly chemicals. To the best of our search, no serious efforts have ever been made for non-hormonal and cheaper chemicals to achieve results of similar magnitude or even better than those elicited by hormones. The present workers following this idea for the promotion of sprouting of stem cuttings on a massive scale screened some organic chemicals during the period 2001-2009 and could search out a number of such chemicals, other than hormones whose performance in respect of root initiation on stem cuttings was as good

Table 2. Showing the effect of soaking seeds in different concentrations of certain chemicals for 48 hours prior to sowing in polypots on the average total length (cm) of a primary root from radical and lateral branches upon it after a period of 60 days. Values are means of three replicates of three seed.

Treatments	Concentrations						
	0	150	300	450	650	800	100
Indol-3-acetic acid	01.50	04.53	05.67	03.67	07.73	06.97	06.03
Naphthalene	02.23	09.17	12.20	12.90	12.27	11.50	08.63
Indole butyric acid	02.53	05.50	05.63	07.50	08.87	08.10	07.90
Gibberellic acid	01.90	01.07	01.47	01.63	02.47	03.83	03.60
Kinetin	02.43	10.13	15.10	13.00	10.97	10.23	07.53
Trypsin	12.67	27.50	27.83	49.30	44.33	36.50	34.50
Nicotimide	11.83	13.67	13.43	12.67	11.17	09.50	00.00
Folic acid	06.33	11.00	10.63	11.17	16.27	08.13	00.00
Acetic acid	12.00	14.10	15.70	30.67	39.83	34.00	28.17

ANOVA TABLE

Variance	DF	SS	MSS	F	SEM ±	CD (5 %)	CD (1%)
Treatment	8	16961.96	2120.2448	3652.596***	0.1663	0.4653	0.6149
Concentration	6	2297.39	382.8983	659.628**	0.1466	0.4104	0.5423
T X C	48	4094.61	85.3044	146.956**	0.4399	1.2311	1.6270
Error	126	73.14	0.5805				

GM = 12.37; CV (%) = 6.16; **Highly significant; *** Very highly significant

or even better than that elicited by various hormones. A close perusal of data in Tables 1-4 amply brings home this point. The highest average values elicited by the various chemicals and their concentrations are given in the Table 5.

It can be seen from the data in the Tables 1-5 that the effect of certain concentrations of trypsin are superior than all other chemicals in respect of three of the four parameters except the average total length of adventitious roots per stem cutting in which nicotinamide and ascorbic acid elicited marginally superior results.

Ever since the property of auxins to stimulate rooting on stem cuttings was confirmed[34-35], there has been a mad rush for their use wherever rooting needed to be initiated on stem cuttings. The literature on this topic is so massive that only a few references are cited here[36-40]. Further quite many recent workers have observed that the results elicited by Indole-butyric acid are in most cases superior to Indole acetic and Napthyl acetic acids[41-48]. According to a recent report[49] certain high concentrations of auxins, in particular IBA and NAA in combination with one per cent extract of the seaweed *Hypnea muciformis* Lamour and even higher concentrations of seaweed extract alone produced better results.

The present studies show that beside the exhorbitantly costly hormones which have been used in promoting rooting on stem cuttings for the last 70 years or so, there are much less costly organic chemicals which can elicit as good as or even better results than the hormones and this can tremendously reduce the project costs. However, at this stage it cannot be said that each such chemicals like hormones would have universal applicability unless the internal mechanism of their action is revealed at the cellular / molecular level. However, research for the search of such chemicals is most desirable. *Commiphora wightii* itself is an example in which lower concentration of IAA and NAA are not very effective in comparison to their controls but much superior results were elicited at much lower cost with certain non-hormonal chemicals, namely trypsin, nicotinamide, folic and

Table 3. Showing the effect of soaking of stem cuttings for 48 hrs in different concentrations of certain chemicals on the percentage of sprouting of stem cuttings sixty days after insertion in the soil. Values are means of three replicates of 10 cuttings each; multiplication of each value by 10 gives percentage of sprouting.

Treatments	Concentrations						
	0	150	300	450	650	800	100
Indol-3-acetic acid	3.00	3.00	3.00	3.00	5.00	7.00	2.67
Naphthalene	2.00	3.00	3.00	3.00	3.00	3.00	2.33
Indole butyric acid	2.00	3.00	4.00	5.00	4.00	6.00	6.00
Gibberellic acid	2.33	1.67	2.00	2.00	2.67	5.00	5.00
Kinetin	1.00	1.00	2.00	2.00	2.00	4.00	3.00
Trypsin	2.67	5.67	6.00	7.33	8.67	7.67	6.00
Nicotinamide	3.33	4.33	5.33	6.00	7.67	7.33	5.33
Folic acid	2.67	6.33	7.00	7.33	7.67	7.67	4.33
Ascorbic acid	3.33	4.33	4.33	7.00	6.67	7.67	6.33

ANOVA TABLE

Variance	DF	SS	MSS	F	SEM ±	CD (5 %)	CD (1%)
Treatment	8	418.30	52.2870	76.017***	0.1810	0.5065	0.6694
Concentration	6	226.07	37.6790	54.779**	0.1596	0.4467	0.5904
T X C	48	146.59	3.0540	4.440*	0.4788	1.3401	1.7711
Error	126	86.67	0.6878				

GM =4.41; CV (%) = 18.82; *Significant; **Highly significant; *** Very highly significant

Table 4. Showing the effect of different concentrations of certain chemicals on the average total length (cm) of all the adventitious roots developed on the buried portion of a treated stem cuttings 60 days after insertion in the soil. Values are means of three replicate each of three stem cuttings.

Treatments	Concentrations						
	0	150	300	450	650	800	100
Indol-3-acetic acid	1.50	1.50	04.40	05.77	07.63	08.97	07.17
Naphthalene	1.47	9.03	08.13	12.43	11.03	09.40	08.00
Indole butyric acid	1.83	5.13	06.67	06.30	07.07	09.03	07.17
Gibberellic acid	1.40	1.43	01.60	01.57	02.03	03.67	02.00
Kinetin	1.90	7.13	11.53	15.07	13.20	11.23	08.77
Trypsin	1.33	2.77	03.73	13.20	15.90	10.70	07.73
Nicotinamide	1.53	3.47	04.27	13.23	16.17	11.47	09.83
Folic acid	1.50	4.40	05.30	12.23	15.47	11.80	08.47
Ascorbic acid	1.73	4.13	06.43	11.27	16.63	14.40	10.23

ANOVA TABLE

Variance	DF	SS	MSS	F	SEM ±	CD (5 %)	CD (1%)
Treatment	8	1027.33	128.4165	206.243**	0.1722	0.4819	0.6369
Concentration	6	2128.63	354.7709	569.780***	0.1519	0.4250	0.5617
T X C	48	782.51	16.3022	26.182*	0.4556	1.2750	1.6851
Error	126	78.45	0.6226				

GM = 7.33; CV (%) = 10.77; *Significant; **Highly significant; *** Very highly significant.

Table 5. Summary table.

Treating chemicals	Best results and the concentrations of the chemicals eliciting it			
	A	B	C	D
IAA	73.3%	07.73cm	70.00%	08.97cm
	300ppm	650ppm	800ppm	800ppm
NAA	76.7%	12.90cm	30%	12.43cm
	300ppm	450ppm	150ppm	1450ppm
IBA	70.00%	08.80cm	60.00%	09.03cm
	800ppm	650ppm	800ppm	800ppm
GA_3	33.30%	03.83cm	50.00%	03.67cm
	450ppm	800ppm	800ppm	800ppm
Kinetin	53.33%	15.10cm	40.00%	15.07cm
	300ppm	300ppm	800ppm	450ppm
Trypsin	83.33%	49.30cm	86.70%	15.90cm
	650ppm	450ppm	650ppm	650ppm
Nicotinamide	46.70%	13.67cm	76.70%	16.70cm
	300ppm	150ppm	650ppm	650ppm
Ascorbic Acid	70.00%	39.83cm	76.70%	16.67cm
	650ppm	650ppm	800ppm	650ppm

Explanation of lettering A-D : A. Average highest percentage of seed germination and concentration of the chemical eliciting it; B. Average highest length (cm) of primary root and lateral branches upon it and the concentration of the chemical eliciting it; C. Average percentage of sprouting of stem cuttings and concentration of the chemical eliciting it; D. Average highest length (cm) of all the adventitious roots developed upon a stem cutting and the concentration of the chemical eliciting it.

ascorbic acids.

Acknowledgements

The authors are grateful to Prof. N. C. Aery and Prof. Y. D. Tiagi of the Department of Botany , Mohan Lal Sukhadia University, Udaipur.

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