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STRATEGIES FOR CONSERVATION OF ENDANGERED MEDICINAL PLANT *WITHANIA COAGULANS*: A REVIEW

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ABSTRACT

Withania coagulans commonly known as doda paneer or Indian rennet is an important medicinal plant with tremendous medicinal potential. The plant has been reported to possess several biological and pharmaceutical properties. These properties are attributed to presence of several bioactive compounds in the plant. *W. coagulans* also possess traditional and commercial significance as seeds of plant are utilised to convert milk into cheese. Despite having significant medicinal properties the plant remains underutilised. The plant has become critically endangered in the recent past. Reproductive failure, extremely poor germination rate and unrestricted collection from wild stands for various purposes have rendered the plant critically endangered. Hence there is need for development of protocols and strategies for conservation and also mass propagation of the plant. The present work reports various studies conducted for mass propagation and measures which need to be adopted for conservation of the plant.

KEYWORDS: *W. coagulans*, micropropagation, conservation.

INTRODUCTION

Withania coagulans also known as Indian rennet or doda paneer is a well known medicinal plant. *W. coagulans* is naturally found as fragmented population in regions of Iran, Afghanistan, Pakistan, East India and Nepal. **(Jain *et al.*, 2009; Valizadeh and Valizadeh, 2009)**. The plant is a rigid under shrub about 0.3-0.9 m in height. Branches and leaves are covered or clothed with grey or yellowish white tomentum. Plant possesses long and

indistinct petioles and unisexual flowers present in axillary clusters. Similar to branch and leaves, calyx (about 6 mm long, campanulate) are also covered with tomentum. Corolla are 8 mm long. Male flowers possess stamens with 2 mm filaments and anthers are 3-4 mm in length. Ovary is ovoid and glabrous present without style or stigma. Berries of the plant are smooth, globuse, about 6-8 mm in diameter and surrounded by membranous calyx. Seeds are 2.5-3 mm in diameter **(Wealth of India, 1982)**. The plant has been utilized for several traditional and medicinal purposes. Berries of the plant are known to possess milk coagulating property and are used to prepare cheese from milk. Due to this property the plant is known as cheese maker or Indian rennet. Beside this plant is utilized in treatment of insomnia, nervous exhaustion, impotence, disability, wasting diseases, flatulent colic, dyspepsia, diabeties and intestinal infections. *W. coagulans* also exhibits various pharmaceutical activities like anti-inflammatory, anti-microbial, anti-tumor, anti-hyperglycemic, hepatoprotective, free radical scavenging activity, immune suppressive and CNS depressant activities **(Jaiswal *et al.*, 2009; Mathur *et al.*, 2011,2013; Pezeshki *et al.*, 2011; Khodaei *et al.*, 2012, Gaind and Bhudhiraja 1967; Budhiraja *et al* 1977, Chaudhary *et al* 1995)**. Several factors have altogether lead to present endangered status of the plant. The main factor responsible for slow propagation is extremely poor germination rate and reproductive failure owing to dioceious nature of *W. coagulans* **(Jain *et al.*, 2012)**. Over exploitation and unrestricted collection from wild stands for medicinal, pharmaceutical and traditional purposes alongwith destruction of natural habitat have rendered the plant critically endangered. Hence there is requirement of development of laboratory methods, cultural practices for enhancing germination rate and mass propagation of the plant not only for conservation but also for its rehabilitation. In the past *in vitro* micropropagation has emerged as an effective tool for mass propagation of rare and endangered plants. Advent and development of suspension culture have resulted in enhancement of production of bioactive substances of medicinal value. Present work reports different studies conducted pertaining to conservation of *W. coagulans* and probable work which needs to be carried out to conserve the herb.

Fig.1 Mature plant of *W. coagulans.*

Studies conducted for enhancement of germination rate in *W. coagulans*

One of the major problem associated with sow propagation rate of *W. coagulans* is extremely poor germination rate. Few studies have been conducted to enhance germination rate in *W. coagulans*. **Edalatifard L *et al* (2014)** investigated the effect of light and darkness alongwith different germination media (B5, plain agar, filter paper) on germination rate of *W. coagulans*. They selected seeds of 12 ecotypes of *W. coagulans* growing in region of Sistan Baluchistan province, Southern Iran. Seeds from each ecotype were separately inoculated onto each germination media and incubated under the conditions of light and darkness. Irrespective of the germination media utilized enhanced germination rate in all the 12 ecotypes was reported in seeds incubated in darkness as compared to seeds incubated under light. Among the three different media maximum germination rate was reported onto filter paper medium and B5 and plain agar was found to have no effect on germination. Among all the combinations evaluated in the study a maximum of 94% germination rate was reported onto filter paper medium incubated in darkness. In a study conducted by **Sharma *et al* (2015)**, a method to enhance germination rate of *W. coagulans* was reported. In the study seeds were pre treated with different concentration of H_2SO_4 and HCl for varying time interval. Pretreared seeds were allowed to germinate in laboratory (*invitro*) conditions (in sterile petriplates with moistened cotton) and in natural soil. Germination rate was greatly enhanced in both the conditions with a maximum of 78.4% seeds germinating on treatment with 20% H_2SO_4 and 72.6% seeds germinating on treatment with 20% HCl as compared to 1-2% germination obtained in control. Even though the germination rate was enhanced through

this pretreatment, the seedlings failed to grow beyond cotyledonary stage unless transferred aseptically to a culture medium. Only 5-6% germinated seedlings were reported to exhibit growth beyond cotyledonary stage in natural conditions. It was also found in the study that beside poor germination rate plant also suffers from poor root development atleast at initial growth stages.

Micropropagation studies conducted for mass propagation of *W. coagulans*

Plant tissue culture technology is an indispensible tool for conservation of endangered plants. Eminent workers have utilized the technology to for *invitro* mass propagation of the plant **(Table1). Jain *et al* (2009)** reported *invitro* multiplication of axillary buds obtained from nodal segments onto MS medium + Kn (0.5mg/l) and MS + BA (0.5 mg/l). In the same study addition of phloroglucinol (PG) (0.5mg/l) was reported to improve regeneration as well as elongation of shoots buds. In a study carried out by **Valizadeh and Valizadeh (2009)** leaf and internodal segments were utilized as explants and were cultured onto MS medium containing different concentration of 2,4-D, BA and Kn. Callusing was reported in all combinations. Regeneration of shoots were obtained from the callus on medium composition MS + BA (2 mg/l) + IBA (0.5mg/l.). The study by far remains one of the first tissue culture study of *W. coagulans*. In an another study carried out by **Valizadeh and Valizadeh (2011)** protocol for *invitro* propagation of *W. coagulans* was developed. Nodal segments were cultured onto MS medium containing with BA (2-4 mg/l) or IBA either alone or in combination. MS+BA (2 mg/l) + IBA (0.5 mg/l) was reported to be most appropriate medium combination for multiple shooting (7.2 shoots per explant). In another study conducted by **Jain *et al* (2011)** leaves of *W. coagulans* were utilized as explants. In general when leaves of any plants are utilized as explants majority of micropropagation studies reports indirect regeneration through callus formation callus, however **Jain *et al* (2011)** reported direct regeneration of shoot buds from cultured leaf regeneration of shoot buds from culture leaf segments onto MS+ BA(22.2µM)+Kn(23µM). About 80% culture developed shoots onto the mentioned medium combination with an average of 17 shoots. In a study conducted by **Rathore *et al* (2012)** nodal segments of *W. coagulans* were cultured aseptically onto MS medium containing BAP and IAA along with additives (ascorbic acid, L – arginine and adenine sulphate). A maximum of 93.5±0.34% cultures exhibited multiple shooting onto MS +8.88µM BAP+ 0.57µM IAA alongwith 100mg/l ascorbic acid and 25mg/l of citric acid, L-arginine and adenine sulphate. **Nekela (2013)** also utilized nodal segments as explants and reported 100% cultures to develop shoots onto MS + 2mg/l BA with an average of 24 shoots

per culture. **Purushotham *et al* (2015)** in their study germinated seeds of *W. coagulans* in vitro and nodal segments from two month old plant were utilized as explants. Nodal segments were cultured onto MS media fertilised with BAP and Kn alone and also in combinations. BAP was reported to be more appropriate as compared to Kn. About 2.37± 0.3 shoots regenerated onto MS + 1mg/l BAP as compared to 1.96±0.3 shoot obtained onto MS+ 1mg/l Kn . 3.33±0.5 shoots were reported onto medium MS+1mg/l BAP +0.5mg/l Kn. In the same study regeneration of shoots from nodal segments was also achieved onto MS +TDZ.(0.2-1mg/l). **Sharma and Koshy (2017)** in their study cultured cotyledons obtained from seedlings as explants onto MS medium supplemented with 2,4 D and NAA. Development of callus was reported irrespective of type of auxin present in the medium (2,4-D or NAA). Subculture of cotyledonary callus onto BAP (8-10µM) supplemented medium resulted in regeneration of shoots. In the same study TDZ was reported to be extremely effective in inducing shoot bud regeneration from proliferating callus. 100% regeneration was achieved onto MS+ 8-12µM TDZ with an average of 2.5±0.5 shoots and a maximum of 3.5 shoots onto medium combination MS+ 8µM TDZ.

Table 1: Summary of micropropagation studies of *W. coagulans.*

Author(s)	Medium	Explant	Adjuvant (s)	Response			
				Multiple shooting		Callus development	
				% response	Avg. shoot no.	% response	Nature of callus
Valizadeh & Valizadeh, 2009	MS	Internode Leaf	2,4-D (4mg/l) + Kn (0.25mg/l) 2,4-D (2-4mg/l) + BA (0.50-1mg/l)	NR	NR	**36** **100**	
Jain *et al* 2009	MS	Node	BAP (5mg/l) Kn (5mg/l) BAP (0.5mg/l) + Kn (0.5mg/l)	83 73 83	11.2±0.5 3.2±0.3 18.6±0.5	NR	NR
Valizadeh & Valizadeh, 2011	MS	Node	BAP (2mg/l) IBA (0.5mg/l)	100	07	NR	NR
Jain *et al* 2011	MS	Leaf	BA (22.2µM) + Kn (23µM)	80	17	NR	NR
Rathore *et al* (2012)	MS	Node Invitro shoots	BAP(8.88µM) + IAA(0.57µM) BAP(1.11µM) + IAA(0.57µM)	93.5 100	4.1±0.10 19.1±0.2	NR	NR

Nekela 2013	MS	Node	BA (2mg/l)	100	24	NR	NR
Purushothatam *et al* (2015)	MS	Node	BAP (1mg/l) Kn (1mg/l) BAP (1mg/l) + Kn (0.5mg/l) TDZ (0.6mg/l)	100 100 100 100	2.37±0.3 1.96±0.3 3.37±0.6 1.97±0.6	NR	NR
Sharma *et al* 2016	MS	Node	2,4-D (12µM) Kn (12µM) BAP ((12µM	100 100 100	01.4±0.6 11.2±0.6 05.6±0.5	NR	NR
Joshi *et al* 2016	MS	Node	BA (2.5mg/l) Kn (2mg/l) TDZ (2.5mg/l) 2-ip (2mg/l) Zeatin(2.5mg/l) mt (2.5mg/l)	79.17 66.67 75.17 75 83.33 75	4.68±1.2 2.81±1.2 5.16±1.9 4.67±1.0 3.30±1.2 4.50±1.6	NR	NR

NR= no response

***In vitro* rooting of regenerated shoots**

In several studies conducted (Table 2), auxins IBA and IAA have been successfully utilized for *invitro* induction of roots. Both full as well as half strength MS medium (supplemented with PGR) have been reported to be suitable root inducing medium by different workers, however ½ strength medium appears to be more effective as compared to full strength. **Valizadeh and Valizadeh (2009)** utilized full strength MS medium fortified with different concentrations of IBA for *invitro* rooting and 100% rooting was reported onto MS +2mg/l IBA with an average of 23 roots. **Jain *et al* (2009)** and **Rathore *et al* (2012)** utilized ½ strength MS medium as rooting medium. **Rathore *et al* (2012)** in their study achieved rooting in about 67.3% cultures onto MS +29.52µM IBA. A higher rooting percentage was reported when shoots were rooted invivo on soilrite with pulse treatment of IBA. Jain *et al* (2009) achieved rooting in 80% cultures onto MS+ IBA (0.25mg/l) + IBA (0.5 mg/l) + CC (2mg/l). **Joshi *et al* 2016** analysed NAA and IAA as PGR to induce rooting in regenerated shoots. Both full as well as half strength MS media were utilised. In their study ½ strength MS media was far superior as compared full strength for *invitro* rooting. Onto full strength MS media supplemented with NAA no rooting was reported irrespective of concentration of NAA. When full strength MS media was supplemented with IAA rooting was reported only on higher concentration (1.5-2mg/l) of IAA. Rooting was obtained on all concentration of IAA and NAA when strength of medium was reduced to half. Maximum rooting was reported (58.33%) onto half MS+ 2mg/l IAA followed by half MS +0.5 mg NAA onto with 41.67% cultures developed *invitro* roots. In the same study development of callus was

obtained onto full strength medium. No callus formation was obtained onto half strength medium.

In another study conducted by **Purushotham *et al* 2015**, IAA and IBA were utilized as rooting hormones individually as well as combination. When the hormones were utilised individually. IBA was reported to be better root inducing hormone as compared to IAA. A maximum of 98.55% cultured exhibited *invitro* rooting onto 2MS+ 4mg/l IBA. Percentage of hormones were utilised in combination and 100% cultures developed in *invitro* roots onto MS+ 4mg/l IBA +1mg/l. **Sharma and Koshy (2017)** also utilized full and half strength MS medium supplement with difficult concentration of IAA to induce *in vitro* rooting. As reported in other studies half strength medium was found to be superior as compared to full strength. ½ MS +2µM IAA was reported to be the most suitable medium composition. In another study **(Sharma *et al* 2015)** full and half strength MS containing IBA (5- 2.0µM) were utilized. No root induction was reported on full strength medium. All cultures developed roots when strength of medium was reduced to half with a maximum of 52.4% cultures developing invitro roots onto ½MS + 10µM IBA. Beside achieving successful rooting these studies have also reported quite good percentage of survival of *invitro* regenerated plants when transferred to natural conditions through the process of acclimatization (Fig.2).

Table 2: Summary of micropropagation studies of *W. coagulans.*

Author	Media	PGR	% culture developing roots	Avg. number of roots
Valizadeh & Valizadeh (2009)	MS	IBA (2mg/l)	100	23
Jain *et al* (2009)	½ MS	IBA (0.25mg/l) IAA (0.5mg/l) CC (2mg/l)	80	11.5±0.7
Rathore *et al* (2012)	½ MS	IBA (29.52µM)	67.3±1.01	
Purushotham *et al* (2015)	MS	IBA (4mg/l) IAA (4mg/l) IBA+IAA (4mg/l) (1mg/l)	98.55 6.67 100	56.66±1.05 3.83±0.98 57.5±1.12
Sharma *et al* (2016)	MS ½ MS	IAA (20µM) IAA (20µM)	62.6 88.6	6.8±0.8 12.0±0.5
Sharma *et al* (2016)	MS ½ MS	IBA (20µM) IBA (20µM)	42.5 90.0	4.6±0.6 11.2±0.4
Joshi *et al* (2016)	MS MS ½ MS	NAA IAA (2mg/l) NAA (0.5mg/l)	No response 33.33 41.67	- 2±0.82 1.33±0.5

	½ MS	IAA (2mg/l)	58.33	2.43±0.5
Sharma and Koshy (2017)	MS	IAA (20µM)	88.6	14.0±0.6

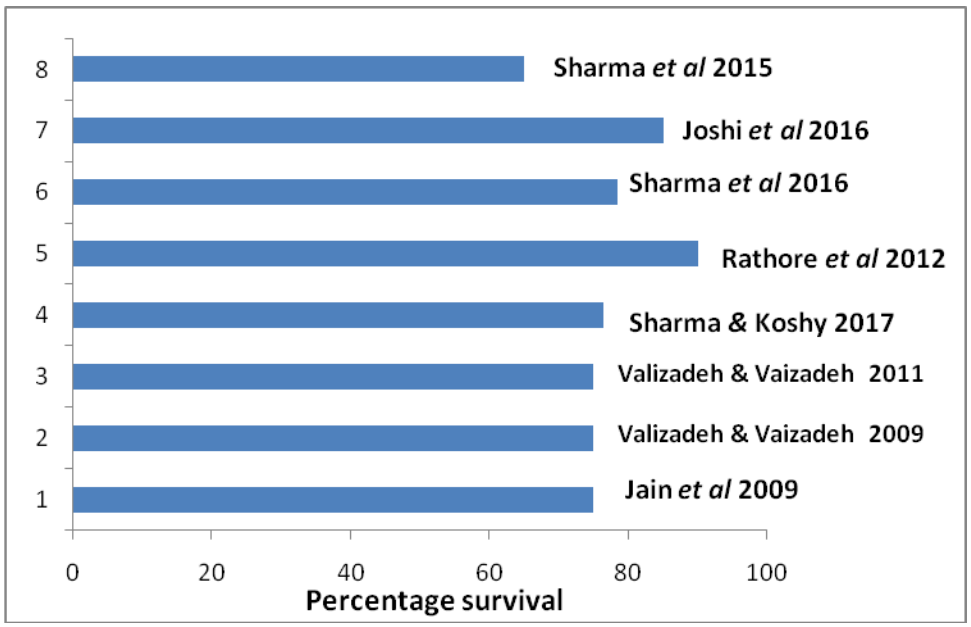

Fig. 2: Percentage survival of *invitro* regenerated plants in different studies during acclmitization.

Epilogue

As evident from the results obtained in above mentioned studies, successful *invitro* regeneration and mass propagation of *W. coagulans* was achieved in all the studies conducted by eminent workers. Moreover in the most of the studies reported pertaining to micro propagation of *W. coagulans* direct regeneration has been reported. Also good percentage of successful acclimatization of *invitro* regeneration plants has been reported in most of the studies conducted so far. Hence at present efforts are required to reproduce the studies to produce large number of plants so as to rehabilitate them in natural stands. Moreover, there is requirement to figure out the causes of poor germination rate exhibited by the species. So as to develop method and technique to further enhance the germination rate.

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