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A Complete Review on Tissue Culture of *Terminalia arjuna*: A Medicinally and Economically Important Tree

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ABSTRACT: *Terminalia arjuna* is an important multipurpose tree species. It is one of the major trees of sericulture industry to rear cocoon of tasar silkworm. The bioactive component present in this tree make it highly valuable tree in pharma industries. But, due to unscientific method of harvesting, poor seed germination and limits of conventional propagation method, its population is facing the threat of being endangered species. To replenish its loss, fulfill the need of therapeutic agents in short time and conserved such plant species, some alternate propagation methods for mass production of *T. arjuna* have been developed. The present review was done with the aim to highlight such non-conventional propagation methods. The literature described the different methods of propagation with different types of explants and potential of plant growth regulators in development of *in vitro* callus, somatic embryo, shoot and root system. The collective information on tissue culture methods of *T. arjuna* will be helpful in conservation of such tree species.

Keywords: *Terminalia arjuna*, medicinal plant, *in vitro*, callus and somatic embryo.

INTRODUCTION

Trees represent the majority of terrestrial biomass production and the forestry. India, Russian Federation, Brazil, Canada, United States of America, China, Democratic Republic of the Congo, Australia, Indonesia and Sudan are ten most forest-rich countries of the world which account for 67 percent of total forest area of the world. We get a large number of important ecological, economic, medicinal and social benefits from forest. The social benefits lie in the communities that live off the forest and in their intrinsic aesthetic value. The economic importance lies in the usefulness of the wood for fuel, construction and communication. The ecological benefits of forests lie in their biodiversity, erosion control, soil improvement, carbon sequestration and improved air, which have long been recognized. The role of forest in socio- economic and rural development is of paramount importance, particularly in a developing country like India where a considerable part of population lives in abject poverty. The medicinal benefits of forest lie in usefulness of plant secondary metabolites in therapeutic use. Out of many tree species used in drug preparation, *Terminalia arjuna* is one of the trees with multipurpose uses.

A. Terminalia arjuna common name and distribution in India

Terminalia arjuna belongs to flowering plant family combretaceae. The genus *Terminalia* is derived from Latin word *terminus* which means the tip of shoot occupied with leaves. It is a valuable multipurpose tree commonly known as Arjuna, Koha, Kahu, Arjan, White Marudah, White Murdh, Arjuna Myrobalan, Orjun, Yerramaddi, Sadada, Sadaru and many more (Ahmad *et al*., 1983). This tree has different Sanskrit Names due to its characteristic feature. These names of Arjuna are Dhaval (whitish or grey bark), Kakubha (a large tree), Indradu (covering a large area) and Viravriksha (hard woody nature).

The tropical region of the world has around 250 species of *Terminalia* (Chouhan *et al*., 2008) and approximately 12 species of *Terminalia* are native to India. Some major related species of *T. arjuna* are *T. bellerica*, *T. chebula*, and *T. ciliate*. In Indian subcontinent, it is most commonly found in indo sub-Himalayan tracts, Bihar, Jharkhand, Delhi, Orissa, Assam, Gujarat, Maharashtra, Madhya Pradesh, Uttar Pradesh, Tamil Nadu, West Bengal and Deccan region. It is found in plenty along riverside, riverlets and ponds. It is a cultivated tree in Punjab and main species of Gir forests of Gujarat.

B. Botanical description

Arjuna is a large, evergreen and perennial tree of tropical and sub-tropical forests. Generally, it has 20-26 m height and about 3 m girth. The stem of the tree is mostly long, buttressed and straight. The wood is brown, hard and do not have any characteristic odor and taste. The wood of Arjuna is heavier, harder and stronger in shock resistance than teak.

Fresh bark is light, spongy and green, grey or pinkish grey in colour. The bark started exfoliating in large irregular sheet when it attains thickness up to 9 cm.

Growth of bark occurs in rainy season and each Arjuna tree yields 9 to 45 kgs bark.

Leaves are long (10-15 cm) and sub opposite. Leaflets are 10-15 cm long, 8-10 cm wide and tongue shaped. Small nodes are present at the top of the petioles immediately below the leaves (Ali, 1994).

The flowers are borne on pendulous terminal and axillary spikes. Each spike consists of 45±3.5 flowers. Flowers are small and pale yellow in colour, bracteates, hermaphrodite, actinomorphic, epigynous and has one whorl of gamopetalous perianth (Chouhan *et al*., 2008). Flowers appear from April to July.

Tree starts fruiting at the age of 6-7 years. Fruit is 2.5 - 3.5 cm long, fibrous woody and glabrous with 5-7 hard wings (Gupta *et al.,* 2018). Unripen fruits are green turn brownish- black at maturity. Fruiting starts in the first week of June and the fruits mature within three months. These fruits do not detach from the tree and remain attached until the next fruiting season reach. Dispersion take place by wind and birds (Sedgley and Griffin, 1989).

Arjun seeds are photoblastic in nature. Seed germination starts in rainy season. Partially buried seeds germinate successfully than the seeds directly exposed to sun light. Seed germination is epigeous and germination capacity is 50-60%.

Arjun tree grow luxuriantly in its natural habitat, mostly in moist cool areas on a variety of soil but prefers fertile alluvial loam and deep sandy well drained soil (Handa and Kaul, 1996). The tree is a moderate shade bearer and does not tolerate dense overhead shade. The growth of arjun is quite well in sunlight on favourable conditions and adequate availability of moisture on the ground. The absolute maximum shade temperature varies from 35°C to 45°C and absolute minimum temperature ranges between 0° to 15°C. The tree pollards well and also produce root suckers. Tree can be coppiced and coppicing power is satisfactory upto 76 cm girth.

C. Medicinal and Economic importance

According to WHO approximately 80% of the developing world's populations are using traditional medicine as a primary healthcare due to lesser side effects (Kamboj, 2000). In India, Ayurvedic, Unani and Siddhi medicine systems are existed. The ancient Indian science of Ayurveda is plant based and significant as a preventive & curative medicine system. Himalayan region of India is rich in medicinal flora and traditional medicine knowledge. Medicinal plants are drawing attention due to its contribution in large scale therapeutic phytochemicals which are used in development of novel drugs. Therefore, the evaluation of rich heritage of traditional medicine is essential (Padmaa *et al*., 2008).

T. arjuna is one of the such plant with greater prospective in medicine industry. It is an important tree of both Ayurvedic and Yunani Systems of medicine and widely used to prepare important ayurvedic formulations like Kashaya, ksheerpaka, arjunaishtam, Cintamanirasam, Laksagugula (Chatha *et al*., 2014; Bishnoi and Ahlawat, 2015).

The leaves, bark, gum, flowers and fruits of *T. arjuna* are mainly used in herbal product formation (Kumar and prabhakar, 1987). It has hypolipidemic, anti-carcinogenic (Nagpal *et al*.,2000), gastro-protective effects (Verma and Singh, 2013), antibacterial (Singh *et al.,* 2008), antioxidant (Gupta *et al*., 2001), anti-inflammatory effects, anti-hypotensive effects (Maulik, *et al.,* 2016; Meghwani, *et al.,* 2017) and protects against ischemia reperfusion injury. The bark of this tree is acrid, sweet and rich in specific medicinally active constituents like tannins, cardenolide, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin, arjun glycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proantho cyanidins (OPCs), phytosterols, calcium, magnesium, zinc, and copper (Akhter *et al*., 2012). These natural ingredients are useful in heart disease (Gauthaman and Mishra, 2004). Clinical studies on of its natural ingredients suggested that it can be of benefit in the treatment of coronary artery disease, heart failure and possibly hypercholesterolemia. It has also been found to be antiviral and antimutagenic. Bark is also useful in fracture, ulcers, blood disease, intoxications, urinary discharge, excessive perspiration, asthma, tumours, leucoderma and false presentation of foetus. The ash of the plant is prescribed for snake bites and bark for scorpion sting. Fruit is used as tonic and deobstruent (Paarakh, 2010). The juice of fresh leaves is used in earache and healing the wounds. A brownish clean golden coloured, transparent gum is obtained from bark of the tree. The edible gum is very nutritive and energetic for heart. The leaves contain 10.10% crude protein, 7.78% crude fibre, 4.30% reducing sugar, 5.75% total sugar, 11.09% starch and 7.00% minerals.

Apart from being the source of medicinal constituents, Arjuna plays an important role in the sericulture industry (Orwa *et al*., 2009) and wood industry. The leaves are used as a food for the Tasar silkworm *Antheraea mylitta* (Dutta *et al*., 1995; Jolly *et. al*., 1968). India ranks second in tasar silk production, which provide employment for many Indian villagers. Hence, it is necessary to propagate *T. arjuna* to improve the feeding material for the silk worm and thus improve the quality of the silk product. Timber of Arjuna is locally used for making carts, agricultural implements, water troughs, traps, boat building, house building, electric poles, tool-handles, jetty-piles and plywood. Arjuna wood is used as firewood and in the production of charcoal. These trees are recommended for reclamation of saline, alkaline soils and deep ravines. It is also used for agro forestry, social forestry programmes, soil conservation and wasteland afforestations.

Arjuna is one of the sacred trees of India. It has acquired the social and religious sanctity with the passage of time. It is said that Arjuna has been born of the two sons of Kubair after saint Narada cursed him. The leaves and flowers of this tree are offered to the Lord Vishnu and Lord Ganpati on the several religious occasions.

Table 1: Detail of tissue culture work done on *T. arjuna*.

Sr. No.	Authors and year	Mode of propagation	Type of explant used	PGR used	Observations and results
1.	Rajan, (1996)	Direct and indirect organogenesis, somatic embryogenesis	Nodal explant and leaves	***In vitro* shoot proliferation:** BAP, Kn, GA_3, 2,4-D, IBA **Shoot multiplication:** BAP, Kn, NAA **callus initiation:** BAP, Kn, NAA, 2, 4-D **Shoot differentiation from Callus:** lAA, NAA, 2,4-D, BAP, GA3 **Rooting:** BAP, NAA, IBA **Direct somatic embryo**: (globular stage): 2,4-D, Kn, BAP	**Shoot initiation:** 63.1% on MS + 4.44 µM BAP + 0.04 µM Kn. **Shoot multiplication:** 4.6 shoots per explant on MS medium + 8.86 µM BAP + 4.65 µM Kn. **callus initiation:** 73.6% on MS+ 13.57 µM 2,4-D+ 0.44 µM BAP + 9.29 µM Kn. **Shoot differentiation from Callus:** MS medium supplemented with 4.44 µM BAP +0.05 µM NAA produced 13.3% shoots. **Rooting:** 33.6% rooting obtained on half strength MS + 0.004 µM BAP + 5.37 µM NAA and 9.84 µM IBA. **Direct somatic embryo:** 2,4-D (13.57 µM), Kn (13.57 µM), BAP (0.44 µM) and L- glutamine promoted embryo induction. **Embryo maturation:** Occurred on lower concentration of 2,4-D (0.045 µM) and BAP (4.44 µM) with 1.5% sucrose in liquid MS medium.
2.	Kumari *et al.* (1998)	Somatic Embryogenesis	leaf	2,4-D, Kn, BAP, NAA, IBA, IAA	**Callus induction:** Maximum 58.57% callus were initiated on MS + 22.62 µM 2, 4-D + 0.05 µM Kn. **Somatic embryo formation:** Globular embryo formation was observed on MS basal medium containing 2% sucrose. **Somatic embryo germination:** Normal cotyledonary embryo germination was maximum (100%) on MS basal medium supplemented with 3% sucrose. Maximum 66.7% germination of fused cotyledonary embryos occurred on MS + 8.66 µM GA_3 medium.
3.	Pandey and Jaiswal (2002)	Direct organogenesis	Cotyledonary nodes	***In vitro* shoot proliferation:** BAP **Shoot multiplication:** BAP ***In vitro* rooting:** IBA	***In vitro* shoot proliferation:** MMS medium enriched with 2.22 µM BAP. **Shoot multiplication:** Total 45-55 shoots obtained from single cotyledonary node on MMS medium + 2.22 µM BAP. ***In vitro* rooting:** Pulse treatment of shoots with 4.92 µM IBA for 15 hrs in liquid MS medium followed by transferred on MMS medium without hormone supported 88% rooting.
4.	Pandey *et al*. (2006)	Direct organogenesis	Nodal segment from mature tree	***In vitro* shoot proliferation multiplication:** BAP, Kn, TDZ, NAA, IBA ***In vitro* rooting:** IBA, NAA	***In vitro* shoot proliferation and multiplication:** Maximum bud break (86%) and multiplication was observed on MMS medium fortified with 4.44 µM BAP +0.54 µM NAA. **In vitro rooting:** Half strength MS medium supplemented with 4.92 µM IBA induced 44.5% rooting.
5.	Kumar *et al.* (2010)	Direct organogenesis, callus culture	shoot, apical bud and leaf from 10-12 days old seedling	***In vitro* shoot and callus initiation**: BAP, Kn, NAA, IBA, GA_3, TDZ	***In vitro* shoot proliferation:** MS medium containing 13.32 µM BAP + 2.69 µM NAA+ coconut water found effective for shoot proliferation from seedling nodal and apical bud explant. **Callus induction:** Maximum Callus induction obtained on 22.62 µM 2,4-D. Upper hypocotyl with shoot apex was best explant for plant regeneration.
6.	Yadav and Jaiswal, (2011)	Somatic Embryogenesis	leaf	2,4-D, BAP, Kn	**Embryogenic callus and Somatic embryo induction:** Somatic embryo formed indirectly. 2,4-D is essential PGR for embryogenic callus induction. 23.0 µM 2,4-D + 0.23 µM Kn developed maximum number of somatic embryos along with embryogenic callus. **Embryo development and germination:** Further embryo development and germination observed on 0.11 µM and 0.22 µM BAP containing medium. Germination frequency was 30%.
7.	Arumugam and Gopinath, (2011)	Indirect organogenesis	Cotyledon (C), hypocotyls (H), epicotyl (E), leaves (L) from 10-12 days old seedling	2,4-D, NAA, IAA, BAP	**Callus induction:** Maximum Callus induction: L > E > H > C MS medium containing 13.57 µM 2,4-D induced maximum callus regeneration. **Organogenesis:** Shoot and root development initiated in MS medium enriched with 22.62 µM 2,4-D +0.05 µM Kinetin+ 2.89 µM GA_3.
8.	Mushke *et al.* (2014)	Direct organogenesis and somatic embryogenesis	Nodal segments and mature leaf	***In vitro* shoot proliferation:** BAP, Kn, TDZ ***In vitro* rooting:** IBA, NAA, IAA **Somatic embryogenesis:** 2,4-D, Kn	***In vitro* shoot proliferation:** Lower concentration of TDZ (0.22 µM) was effective in *in vitro* shoot proliferation. ***In vitro* rooting:** Total 80 % regenerated shoots produced rooting on modified B5 medium (1/3 of the nitrogen concentration) with 4.92 µM IBA. **Somatic embryogenesis:** Combination of 2,4-D and Kn induced 68% somatic embryogenesis.
9.	Gupta *et al.* (2014)	Direct organogenesis	Nodal segments from lopped branches of mature tree	***In vitro* shoot proliferation:** BAP, Kn, NAA **Shoot multiplication:** BAP, NAA ***Ex vitro* rooting:** IBA, NOA	***In vitro* shoot proliferation:** 85% explants responded on MMS medium +13.32 µM BAP+ 0.54 µM NAA **Shoot multiplication:** MMS medium enriched with 2.22 µM BAP +2.69 µM NAA produced maximum 11.2 shoots. ***Ex vitro* rooting:** 1230.1 µM IBA and 1236.3 µM NOA induced 73.3% rooting.
10.	Ravi *et al.* (2014)	Direct organogenesis	Seedling nodal segments	***In vitro* shoot proliferation:** BAP, Kn, NAA, IAA **Shoot multiplication:** BAP, Kn, NAA, IAA ***In vitro* rooting:** IAA, NAA, IBA	***In vitro* shoot proliferation:** MS medium enriched with 6.65 µM BAP supported 9.0 shoots with 1.70 cm shoot length. **Shoot multiplication:** maximum on 6.65 µM BAP. ***In vitro* rooting:** 60% rooting on 0.49 µM IBA.
11.	Choudhary *et al.* (2015)	Direct organogenesis	Nodal segments from lopped branches of mature tree	***In vitro* shoot proliferation:** BAP, NAA, IAA **Shoot multiplication:** BAP, NAA, IAA ***In vitro* rooting:** NAA, IBA	***In vitro* shoot proliferation:** 100% bud break response on MMS medium + 8.86 µM BAP. **Shoot multiplication:** 11.38 shoots obtained on MMS medium + 4.44 µM BAP +0.54 µM NAA. ***In vitro* rooting:** Maximum 80.0 % rooting recorded after pulse treatment with 984 µM IBA for 10 min and then transferred on half strength MS medium containing 0.1% activated charcoal.
12.	Salim, (2018)	Callus culture	young leaves, leaf petioles and internodes	2, 4-D	Best explant for callus induction and proliferation on 13.57 µM 2, 4-D was internodes.

ROLE OF TISSUE CULTURE IN *T. arjuna*

Presently, medicinal plants play a very important role in modern economy. Today, this rich biodiversity of medicinal plants is facing a serious threat due to the increasing demand of some specific plant-based drugs resulted in overexploitation of such plant species from wild (Srivastava, 2018). To protect these plants, several policy measures have been taken but despite of all these measures, the existing plants are decline rapidly due to unsustainable harvesting and extensive illegal collection of medicinal plants to gain financial profit (Sharma *et al.*, 2012). Since the beginning of this century, more than half of the world's tropical forest area has been destroyed.

Thus, it is important to conserve such plant species by applying suitable conventional as well as non-conventional propagation techniques. In *T. arjuna*, sexual propagation through seeds or vegetative propagation by cuttings and air layering are the main conventional propagation techniques. But again, these methods have some drawback which limit their application for conservation programme. Conventional propagation methods of *T. arjuna* are not satisfactory due to poor seed germination and seedling viability as well as inefficiency in rooting by cuttings and air-layering methods (Pandey *et al.*, 2006). Also, *T. arjuna* is liable to get infected and damaged by many casual organisms, pathogens and insect pests at various stages of growth i.e. seedling, nursery and plantation. Nearly 18 insect pests are damaging Arjuna in different parts of India. Major insect pests of Arjuna are *Trioza fletchari, Antheraea paphia* and *Lymantria Mathura.*

So, other non-conventional propagation method should be adopted to conserve the germplasm and for propagation of *T. arjuna* for sustainable utilization. Plant biotechnology, having tissue culture, is an important tool for mass production of any plant species from a single individual in a relatively short time. Present review highlighted the tissue culture work done by different researcher on *T. arjuna*.

A. Direct organogenesis

Development of adventitious shoots direct from Somatic tissue of plants under *in vitro* conditions is known as direct organogenesis.

(i) Culture media for direct organogenesis. The media and its constituents on which explants grow and multiplied played crucial role in plant growth and development. The explants were cultured on MS (Murashige and Skoog, 1962), B_5 (Gamborg, *et al.*, 1968) and WPM (Lloyd and McCown, 1981) medium. In most of the *T. arjuna* studies, MS medium showed superiority over other media (Ravi *et al.*, 2014). Slight modification in MS medium (MMS) salt concentration (half strength of NH_4NO_3 and KNO_3 salts) improved regeneration capacity and the health of newly regenerated shoots (Pandey and Jaiswal, 2002; Pandey *et al*., 2006; Gupta *et al*., 2014; Choudhary *et al*., 2015).

(ii) Explant collection and preparation. Direct organogenesis of *T. arjuna* were reported with cotyledonary nodes (Pandey and Jaiswal, 2002), seedling nodal explants (Ravi *et al*., 2014) and nodal explants from tree (Pandey *et al*., 2006; Gupta *et al*., 2014; Choudhary *et al*., 2015). It is well known that older shoots may contain inhibitory substances and hidden microbes that can inhibit the plant growth and later deteriorate the explants. The explants were also collected from lopped and unlopped branches (Gupta *et al*., 2014; Choudhary *et al*., 2015; Choudhary *et al*., 2020b) of *Terminalia arjuna*. The month of explant collection also affect the *in vitro* response. Explants collected in April- May months was highly responsive in terms of shoot proliferation. Explants were initially treated with 2% (v/v) cetrimide or tween -20 to remove dust particles. Surface sterilization was done with Sodium hypochlorite [4% (w/v) available chlorine] or 0.05-0.1% $HgCl_2$. Sometimes dipping the nodal explants in 70% alcohol for 30 second also helped in removal of contaminants. The browning of culture medium was main problem in *T. arjuna* which caused additional risk of microbial contamination and necrosis in explants. The problem of phenolic exudation during culture establishment was overcome by agitating the explants in pre chilled solution of Ascorbic acid, citric acid and PVPP (Gupta *et al*., 2014; Choudhary *et al*., 2015; Choudhary *et al*., 2020b) or addition of Ascorbic acid and PVP into the medium with activated charcoal (Rajan, 1996).

(iii) *In vitro* shoot proliferation. The hormonal requirement of any plant species depends on the endogenous level of hormone. Different plant growth regulators like cytokinin [BAP (6- Benzylaminopurine), Kinetin (Kn), Thidiazuron (TDZ)] and auxins [Naphthaleneacetic Acid (NAA), Indole-3-butyric acid (IBA), Indole acetic acid (IAA)] affected the *in vitro* shoot proliferation. Among all these PGRs, 8.86 µM BAP was found to be a best plant growth regulator for bud break response (Ravi *et al*., 2014; Choudhary *et al*., 2015). The cytokinin and auxin interaction also stimulate bud break in some plants. In Arjuna, BAP with little concentration of NAA supported 85-86% of shoot bud induction (Pandey *et al*., 2006; Gupta *et al*., 2014). Combination of BAP with Kn also had synergistic effect on shoot initiation (Rajan, 1996). Mushke (2014) reported the effectiveness of TDZ in early bud break. In case of cotyledonary explant, low concentration of BAP (2.22 µM) was found to be sufficient for optimum 86.11% *in vitro* shoot proliferation (Pandey and Jaiswal, 2002).

(iv) *In vitro* shoot multiplication. Many different methods were applied to achieve improved *in vitro* multiplication. A repeated transfer of mother explant having newly emerged shoot clump or shoot clump without mother explant were done to minimize or remove apical dominance and promote lateral bud growth (Gupta *et al*., 2014). Shoot proliferated from nursery nodal explant showed better multiplication on 6.65 µM BAP (Ravi *et al*., 2014). Pandey *et al*. (2006), Gupta *et al*. (2014) and Choudhary *et al*. (2015) reported the synergetic effect of cytokinin and auxin on *in vitro* shoot multiplication and confirmed that low concentration of auxin was the prerequisite for multiplication in this plant species. Choudhary *et al*.

(2015) reported 11.38 shoots from propagule of three shoots on 4.44 µM BAP with 0.54 µM NAA. Rajan (1996) noticed the synergistic effect of BAP and Kinetin on shoot multiplication and observed maximum shoot elongation on half strength MS medium. Pandey and Jaiswal (2002) obtained 45-55 shoots from single cotyledonary node after 60 days of culture initiation on MMS medium supplemented with 2.22 µM BAP.

(v) *In vitro* and *ex vitro* rooting. Rooting is an important step in micropropagation as the complete plant formation depends on successful transfer of plant from lab to land conditions. Plants with good root system survived better during hardening, acclimatization and ultimately in field conditions. Both *in vitro* and *ex vitro* rooting reported in *T. arjuna*. *In vitro* rooting can be done by either incorporate the best rooting hormone into the MS medium (Pandey *et al.*, 2006) or pulse treated the shoots with optimum concentration of hormone and then transferred these shoots on half strength MS medium containing 0.1% activated charcoal (Choudhary *et al.*, 2015). Incorporation of 4.92 µM IBA into the medium induced only 44.5 % root initiation (Pandey *et al.*, 2006) whereas pulse treated shoot with 984 µM IBA for 10 min produced 80% rooting (Choudhary *et al*., 2015). But, according to Mushke *et al.*, (2014) modified B5 medium (one third of the nitrogen concentration) is better than MS medium which developed roots in 80 % *in vitro* regenerated shoots.

The *ex vitro* rooting has many advantages over *in vitro* rooting as both rooting and acclimatization step performed simultaneously which shorten the propagation protocol and plants can rapidly adapt the field conditions with less casualty. Gupta *et al.* (2014) achieved 73.3% rooting when pulse treated the shoot in solution of 1230.1 µM IBA and 1236.3 µM NOA. In addition to auxins, other factors like rooting mixture and incubation conditions also affect the *ex vitro* rooting in *T. arjuna* (Choudhary *et al*., 2018; 2020a).

(vi) Hardening and acclimatization and field transfer. The *in vitro* and *ex vitro* rooted plantlets were transferred in autoclaved bottle having soilrite (Gupta *et al*., 2014) or vermiculite (Choudhary *et al*., 2015) for hardening and acclimatization under greenhouse conditions. During this period a strong root and shoot system developed. The 90% plants were successfully hardened which showed 100% survival rate after field transfer (Choudhary *et al*., 2015). Pandey and Jaiswal (2002) reported 70% survival of field transferred plants.

B. Indirect organogenesis

Plant organ can also regenerate from the somatic tissue after intervening callus phase *i.e.* through indirect organogenesis.

(i) Explant collection and preparation. A variety of explants i.e. cotyledon, hypocotyl, epicotyl and leaves were used in indirect organogenesis. These explants were washed with liquid soap and surface sterilized with 70% ethanol for 1 min. followed by 0.1% $HgCl_2$ for 5 min. The explants were cut into pieces and cultured on MS medium having nicotinic acid, pyridoxine HCl, thymine HCl, glycine, myoinositol, 3% sucrose and different concentration of 2, 4-D. Medium was solidified with 0.7% agar- agar (Salim, 2018).

(ii) Callus induction. Callus induction efficiency depends on the type of explants used. Callus can be produced at wounded site from variety of explants but mitotically active cells are good for callus induction. In *T. arjuna*, cotyledon, hypocotyl, epicotyl and leaves were used as explants. Among all these explants, leaves were found to be most appropriated explant for callus induction followed by epicotyl. Cotyledon explant was least responsive for callus induction (Arumugam and Gopinath, 2011; Rajan, 1996). Salim (2018) used young leaves, leaf petiole and internodes as explants for callus induction and found the internodes as best explant. Incorporation of different hormones like 2, 4-D, NAA, IAA, BAP, Kn into the medium suggested that 2, 4 -D has maximum potential to de-differentiate the differentiated tissue for callus induction (Arumugam and Gopinath, 2011). The quality of these callus improved by supplementing the other PGRs with 2,4-D (Kumar *et al.*, 2010). In *T. arjuna*, incorporation of 0.44 µM BAP and 9.29 µM Kn along with 13.57 µM 2,4-D proved better for callus induction (Rajan, 1996).

(iii) Shoot differentiation. Similar to de differentiation, re differentiation is also regulated by PGRs. Rajan (1996) reported the withdrawal of 2,4-D and Kn from callus induction medium and addition of NAA with BAP promoted the shoot differentiation from callus. GA_3 had inhibitory effect on shoot differentiation. In contrast, Arumugam and Gopinath (2011) reported the shoot and root differentiation in the presence of 2,4-D and Kn along with GA_3 in the medium.

(iv) Root differentiation. Development of root mainly depends on endogenous and externally supplied auxins. IBA, NAA and IAA are key regulatory factors for root differentiation from callus. Generally, IBA is considered best for root induction. But, in *T. arjuna* interaction of 9.84 µM IBA and 5.37 µM NAA with lower concentration of BAP was found best for root differentiation (Rajan, 1996).

C. Somatic embryogenesis

Somatic embryogenesis from non-gametic cells is an alternative method for mass production, germplasm conservation and genetic transformation of tree species. Somatic embryos are developmentally analogue to zygotic embryos. It can develop directly from the explants or indirectly from callus. Somatic embryogenesis has four important stages i.e. induction, maintenance, embryo development or maturation and germination. Somatic embryo maturation occurs through four developmental stages i.e. globular, heart, torpedo and cotyledonary stage.

(i) Explants for somatic embryogenesis. Somatic embryo can be developed from hypocotyl, endosperm, nucellus and leaf explants. In *T. arjuna* both direct (Rajan, 1996; Mushke *et al*., 2014) and indirect Somatic embryogenesis (Kumari *et al*., 1998; Yadav and Jaiswal, 2011) was reported from leaf explant. Leaf explants were surface sterilized with 70% ethanol for 30 seconds and with 0.05% $HgCl_2$ for 5 min. leaf disc were cut out from leaf and cultured on induction medium (Kumari *et al*., 1998).

(ii) Induction of embryogenic callus and somatic embryo. In all studies, it was found that 2,4-D is essential PGR for direct and indirect somatic embryogenesis. The addition of low concentration of Kn (0.047-2.32 µM) along with 2,4-D (13.57 -22.62 µM) maximized the number of somatic embryo and embryogenic callus formation (Kumari *et al*., 1998; Yadav and Jaiswal, 2011; Mushke *et al*., 2014). Rajan (1996) reported direct differentiation of globular shaped embryo from leaf disc on MS medium enriched with 13.57 µM 2,4-D, 9.29 µM Kn, 0.44 µM BAP and 1.5% sucrose. The addition of L-glutamine in the medium promoted the direct embryo formation and inhibited embryogenic callus formation.

(iii) Embryo maturation. The development of globular shaped embryo into heart shaped occurred on liquid MS medium supplemented with 0.045 µM 2,4-D, 4.44 µM BAP and 1.5% sucrose. Deletion of 2,4-D from the medium promoted the conversion of heart shaped embryo into torpedo shaped (Rajan, 1996). Yadav and Jaiswal (2011) observed that instead of conversion of globular shaped embryo into heart shaped embryo, globular shaped embryo again formed callus which was prevented by addition of activated charcoal into the medium.

(iv) Germination of somatic embryo. Germination of somatic embryo to form complete plantlet is critical stage for tree species. In *T. arjuna* only 30% somatic embryo germinated on 0.11 µM and 0.22 µM BAP (Yadav and Jaiswal, 2011). Kumari *et al.* (1998) obtained two types of somatic embryo i.e. normal cotyledonary embryo and fused cotyledonary embryo. The normal cotyledonary embryo showed 100% germination on MS basal medium containing 3% sucrose and addition of GA_3 lower down the germination. Whereas fused cotyledonary embryo showed maximum 66.7% germination on 8.66 µM GA_3 containing medium.

CONCLUSION AND FUTUTRE SCOPE

Medicinal plants like *Terminalia arjuna* have high impact on medicine industry due to presence of many natural therapeutic compounds. Due to certain limitation of conventional propagation methods, today, conservation of *T. arjuna* by rapid multiplication is only possible through plant tissue culture method. The mass production of *T. arjuna* through direct organogenesis, indirect organogenesis and somatic embryogenesis can help in conservation of Arjuna tree and fulfil the demand of raw material of therapeutic medicine. The finding of present study suggests that there is need of further refinement of *in vitro* propagation protocol through indirect organogenesis and somatic embryogenesis methods to make it more reproducible.

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Abbreviations. MMS: Modified Murashige and Skoog medium; WPM: Woody Plant Medium; B5: Gamborg medium; PGRs: Plant Growth Regulators; BAP: 6-Benzylaminopurine; Kn: Kinetin; TDZ: Thidiazuron; 2,4-D: 2,4-Dichlorophenoxyacetic acid; NAA: Naphthaleneacetic Acid; IBA: Indole-3-butyric acid; IAA: Indole acetic acid; GA_3: Gibberellic acid; NOA: 2- Naphthoxyacetic acid.

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