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Research Paper

TDZ Induced Multiple Shoot Regeneration and in Vitro Flowering From Nodal Explant in *Withania Somnifera (L.)*

Botany

KEYWORDS : Thidazuron, invitro flowering, multiple shoot

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ABSTRACT

Withania somnifera better known as Ashwagandha or Indian Ginseng is one of the most privileged medicinal plant of India. The plant is utilized in treatment of various ailments and diseases. Due to excessive harvesting of the plant from natural habitat to fulfill demand of pharmaceuticals and considerably poor germination rate has collaboratively rendered the plant endangered. Hence in vitro micropropagation studies become essential for mass propagation and conservation of the plant. The present report describes the role of TDZ in induction of multiple shoots from nodal segment culture of W. somnifera. Lower concentration of TDZ (2-5µm) induced direct shoot bud regeneration however, higher concentration (8-20µm) initiated callusing followed by shoot bud regeneration. Shoot bud elongated onto MS basal medium. In the same study TDZ also induced in vitro flowering from nodal segment cultures.

INTRODUCTION

Withania somnifera, the Indian Ginseng is one of the most commonly utilized medicinal plant for health management and treatment of various ailments ever since the practice of traditional medicine to modern medicine. Withania somnifera (Linn.) Dunal is a cosmopolitan plant and twenty three known species of Withania are known to be distributed in drier parts of tropical and subtropical zones. Wild species has been reported from Pakistan, Afghanistan, Palestine, Egypt, Jordan, Morocco, Spain, Canary Island, Eastern Africa Congo, Madagascar and South Africa. In India it is found widely distributed in regions of Gujrat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Punjab plain extending to mountains of Himachal Pradesh and Jammu (Sharma et al 2011, Mirjalili et al 2009, Farooqui & Srenarth 2004) The root of Withania somnifera has been specifically utilized in various medicinal formulation, although its seed and leaf also have significant medicinal value. The plant has been utilized in Ayurveda system of medicine for over 3000 years. Withania exhibits medicinal properties such as anti inflammatory, anti stress, anti tumor, antioxidant, antineoplastic effects, rejuvenating tonic, immunomodulatory activity, cardioprotective activity and hypothyroid activity (The biologically active chemical constituents are alkaloids (ashwagandhine, cuscohygrine, anahygrine, tropine etc), steroidal compounds including ergostane type steroidal lactones, withaferin A, withanolides A-Y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone etc (Mishra et al 2000, Verma & Kumar 2011, Sharma et al 2011, Singh et al 2010, Mirjalili et al 2009) Withania somnifera is propagated either vegetatively by cuttings or through seeds. However, vegetative propagation is time consuming. Low germination rate due to presence of inhibitors is main problem in mass propagation of this herb (Karnik 1978). Due to the over exploitation from natural resources for medicinal purposes the plant has been listed as endangered (Govil et al 1993; Manickam et al 2000; Sharma et al 2011) and on verge of extinction (Vitali et al.,1996). Therefore micropropagation and tissue culture studies become significant for mass propagation and conservation of W. somnifera. Thidiazuron (TDZ) is a substituted phenylurea (N-phenyl-1, 2,3 thidiazol-5-ylurea) inducing high rate of regeneration and axillary shoot proliferation in several plant species (Fiola et al 1990, Malik and Saxena 1992). TDZ not only stimulates axillary shoot proliferation but is also known to induce callus formation and shoot organogenesis. Generally low concentration of TDZ has the capacity to induce multiple axillary shoots to proliferate. At higher concentration, TDZ is a powerful agent that may result in generation of callus and formation of adventious shoots and embryo. Therefore Thidiazuron may be the most potent of the diphenyl ureas that have been evaluated for use in plant tissue cultures (Mok et al 1982). Thidiazuron has been reported to induce shoot proliferation in many species such as Mapple hybrid (Kerns & Mayer,1986), Apple (Van Nieuwkerk et al 1986), Azala (Briggs et al 1988). Pear (Singh & Bhatia 1988) and Peach (Zimmerman & Scorza, 1992). In vitro flowering has been proved to be useful for study of physiology of flowering and has been recognized as valuable strategy which can be integrated into breeding programmes for various species. Role of TDZ in induction of in vitro flowering is reported in Dendrobium nobile (Wang ZH et al 2009) and Rose species (Wang GY, 2002).Available reports related to in vitro culture studies in Withania somnifera indicates multiple shoot regeneration or micropropagation (Shukla et al 2010, De Silva and Senarath 2009, Logesh et al 2010,Saritha and Naidu 2007,Sabir et al 2008). Literature on in vitro flowering in Withania is very limited (Saritha and Naidu 2007). Present report signifies role of TDZ in multiple shoot regeneration and in vitro flowering in W. somnifera, an important and endangered medicinal plant.

MATERIAL & METHODS

The plants of Withania somnifera were procured from Botanical Survey of India, Regional station, Allahabad and maintained in the green house of the Department of Tissue Engineering, SHIATS (fig.1). Nodal segments from mature plant were utilized as explants in the study. About 1.0-2.0 cm long nodal segments were taken from plants and these were also initially rinsed with 90% ethyl alcohol followed by washing with tap water. Explant was treated with 0.2% solution of Tween 20 (Commercial Polyoxyethylene sorbitan monolaurate- S.D. fine-chem. Limited , Mumbai.) and kept under running tap water for 30 minutes. The cuttings were sterilized with 0.1% mercuric chloride solution for three minutes and then repeatedly washed with sterile distilled water. Nodal segments were aseptically cultured onto MS (Murashige, T. and Skoog, F.1962) medium containing various concentration of Thiadizuron. At the time of inoculation, both ends of nodal segments were trimmed with help of a scalpel under aseptic conditions.

The cultures were incubated at 26 ± 2^0 C under 16hrs light photoperiod of 15uE/m^2 /s irradiance provided by Philips fluorescent tubes (36W/54, 6500K) in the culture room. For each treatment, a minimum of 20 cultures were raised and each experiment was repeated atleast twice. The cultures were examined periodically and the morphological changes noted on the basis of visual observations. Result are expressed as percent cultures responded and number of shoot and floral buds obtained per culture.

RESULT AND DISCUSSION

When lower concentration of TDZ (2-5 µm) was added onto MS basal medium, explants did not support callus formation. Onto

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higher TDZ concentration (8-20 µm) explants exhibited an initial swelling of node followed by an increase in size to nearly two fold of the original one after two weeks of culture(fig.2A). The size enlargement led to induction of moderate callus in 40-97.8% cultures after 4th week of initial culture (see table I). The callus was compact and whitish green in colour (fig.2B).

The explants exhibited multiple shoot bud initiation directly without callusing or indirectly through callus formation. The lower concentration of TDZ (2-5 µm) showed shoot bud induction after six weeks of culture. The average number (3.7±0.60) and maximum number of shoot buds (8) was highest at 15 µm TDZ supplemented media (fig.3A, see table I). Although TDZ was found effective in shoot bud induction from nodal segments but the regenerated buds did not elongated further after 4-5 weeks of culture and were transferred onto basal medium for further elongation (fig.3B), elongated shoots on further culture led to development of micropropagated plant(Fig. 5). Previous reports also enlight the role of TDZ in invitro induction of multiple shooting and regeneration (Faisal and Anis 2006 & Ahmad and Anis 2007). The role of TDZ in inducing regeneration is attributed to the ability of TDZ in enhancing the synthesis of adenine type cytokinins (Huettman and Preece, 1993). Hence TDZ is known to be an effective plant growth regulation for induction of shoot bud regeneration. Study of Khalafalla and Hatorri (1999) also suggested that TDZ acts as a trigger for initiation of shoot buds which can be further developed on basal MS medium. Work of Neuman et al (1993) showed that the effect of TDZ is persistent even when the regenerated shoots are transferred from medium containing TDZ to TDZ free medium. Although TDZ has been used in a number of medicinal plants for stimulating regeneration, however its use in Withania somnifera is very limited (Fatima and Anis, 2011). They found low concentration initiated shoot bud formation. However, high concentration was reported to have an adverse effect on the same. In the present report, higher concentration of TDZ (15-20 µm) supported callusing as well as shoot bud regeneration.

Nodal segments showed induction of in vitro flowering onto MS media containing higher concentration of TDZ (8-20 µm). Onto medium fortified with 8um TDZ 33.3% cultures exhibited floral bud induction (fig.4A). On increasing the concentration of TDZ (10-20 µm), 35.3% to 66.6% cultures exhibited in vitro flowering. Highest amount of flowering was observed at 20um TDZ concentration (see table II). The flower bud enlarged in size and developed into mature flower within four weeks(fig.4B). The floral buds as well as mature flower were 4-5 times larger in size than the buds and flower obtained in mother plant. In vitro flowering is an important physiological event which can be used for various molecular studies. In vitro flowering has been induced in Dendrobium and Rose using TDZ. In Withania previous reports of flowering induction has been observed using Kinetin and IAA (Sarita and Naidu, 2006). They had observed flowering and fruiting using combination of Kinetin and IAA. In the present report TDZ induced flowering buds together with shoot formation. In vitro flowering and in vitro fruiting can provide inside into the biochemical and molecular events involved in flowering and development of fruit. Further studies can open new frontiers to reveal the physiological changes involved in transformation from vegetative state to reproductive state.

Table I: Response of nodal segments of *Withania somnifera* to PGR

	TDZ (µm)	% culture showing callus	Degree of callusing	% culture showing shoot bud initiation	Avg. No. of shoots	Max. No. of shoots
1	2	-	-	45.4±1.7	2.2±0.73	4
2	5	-	-	68.2±1.1	2.6±0.80	3
3	8	46.0±1.4	++	100±0.0	3.2±0.60	4
4	10	66.1±1.7	++	100±0.0	3.6±1.2	6
5	12	73.3±0.6	+++	100±0.0	3.3±0.51	5
6	15	87.4±1.3	+++	100±0.0	3.7±0.60	8
7	20	98.7±1.5	+++	100±0.0	3.3±0.80	5

Table II: Effect of TDZ on in vitro flowering in Withania somnifera nodal segment culture

TDZ (µm)	% culture showing flower induction	Avg. No. of Floral Buds	Max. No. of Floral Buds
8	39.1±2.1	1.5±0.38	2
10	38.8±2.2	2.0±0.67	3
12	42.8±1.2	2.4±0.54	4
15	58.6±1.6	3.1±0.91	5
20	66.8±0.9	2.8±0.70	4

All values are mean ± standard deviation

Fig. I

Fig. I Mature plant of Withania somnifera

Fig. 2(A)

Fig. 2(A) Swelling of node along with callus induction

Fig. 2(B)

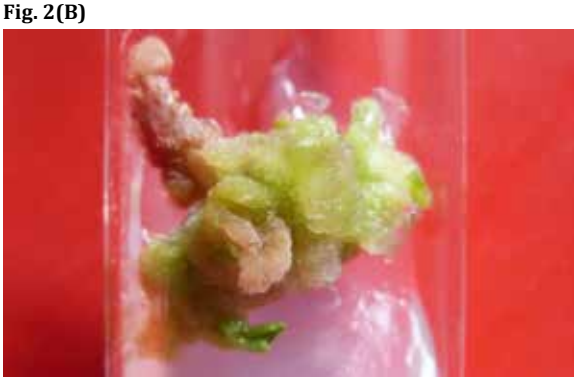

Fig. 2(B) Callus enlargement after 6th week of culture

Fig. 3(A)

Fig. 3(A) Multiple shoot generation

Fig. 3(B)

Fig. 3(B) Elongation of shoot onto basal MS medium

Fig. 4(A)

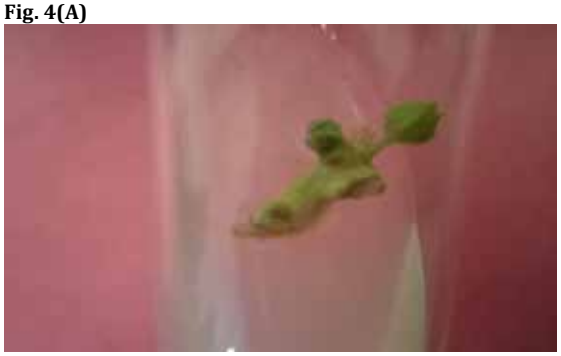

Fig. 4(A) Invitro induction of floral buds

Fig. 4(B)

Fig. 4(B) Development of mature flower

Fig. 5

Fig. 5 Micropropagated plant

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