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INFLUENCE OF TDZ ON THE IN VITRO PROPAGATION OF VALERIANA JATAMANSI

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Abstract

Valeriana Jatamansi is a critically endangered medicinal herb of the genus Valeriana .It is generally exploited for its roots and rhizomes and is useful as a sedative , antispasmodic , carminative, nervine and a stimulating agent .Valerian ,its main compound has been traditionally used in treatment of sleeping problems, obesity, epilepsy and snake poisoning .The aim of this study was to establish a practical methodology for rapid and large scale multiplication of *Valeriana Jatamansi* combining varying concentrations of growth regulators like THIDIAZURON (TDZ) and Kinetin. The results showed that using lower concentrations of THIDIAZURON (TDZ) and Kn (TDZ 1mg/l +Kn 0.5mg/l)after 9-10 days helped in induction of longer shoots while using higher concentrations (TDZ 5mg/l+ Kn 4 mg/l) within 5-6 days resulted in increase of leaf size and number of shoots.

Keywords: *Valeriana Jatamansi,*Thidiazuron(TDZ),Kinetin, Sedative, Sleeping problem, Carminative.

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Introduction

One of these Himalayan critically endangered list of gems is *Valeriana* belonging to family Valerianaceae, the name valerian was probably derived from the Latin, "valere" to be healthy or strong , which is distributed in all the temperate ranges except Australia (Bennet, 1987), growing at an altitude of 1500- 3000m above sea level. *Valeriana wallichii*, the major species of the genus Valeriana is distributed from Afghanistan to Burma (Polunin and Stainton,1987), India, China and central and western hills of Nepal (Manandhar 1976).

It is an important medicinal herb of North western Himalayas being used in the treatment of sleep problems, obesity, nervous disorders and snake poisoning and skin diseases, epilepsy, leprosy, hysteria and asthma. The active principle of this plant besides having antibacterial and antiprozal activity can be taken as a remedy for snake bite as well as scorpion sting (Chopra *et al*., 1956). It can also be used as potential anti-tumor agents (Bounthanh *et al.,* 1981). The crude drugs from roots/ rhizomes and Valerian derived phytomedicines are used as mild sedatives in pharmaceutical industry.

Material and methods:

Immature shoot tips of valeriana jatamansi were collected. The shoots were washed with tap water 3 times(for 15 minutes) followed by distilled water(for 20 minutes) and detergent (for 30 minutes). After this, shoot tips were treated with bavistin(for 30 minutes),followed by washing with detergent for 15 minutes and then 2-3 washings of distilled water were given.MS medium alongwith varying concentrations of THIDIAZURON (TDZ) and Kn(TDZ 1mg/l + Kn 0.5mg/l to TDZ 5mg/l + Kn 4mg/l) was prepared by adding Sucrose 3% (w/v), Nutrient agar 0.75% and the pH of the culture medium was adjusted to 5.8.Culture medium was then sterilised in autoclave at 15 lbps pressure , 121^{o}C temperature for about 15-20 minutes. All the aseptic cultures were then incubated in light in culture room

adjusted at 25 $\pm 1^{o}$C with 16 photoperiod at 32 µE m-2s-light intensity. Surface sterilisation of the shoot tips was done in laminar air flow with 90% ethanol, followed by 0.1% $HgCl_2$with detergent and then for 3 times by autoclave distilled water.After sterilisation, inoculation of shoot tips in jars was then done by employing varying concentrations of THIDIAZURON (TDZ) and Kn (TDZ 1mg/l with Kn0.5mg/l to TDZ5mg/l with Kn4mg/l).The jars were then kept in the growth chamber for 16 hour light/8 hour dark cycles.Frequency of shoot proliferation and number of shoots developed per culture was then recorded after a period of 5 days.

Media Preparation:

The basal salt media (Murashige and Skoog media) was prepared according to the protocol given in the previous slide.

⬇

Sucrose (3% w/v) and growth regulators were added.

⬇

pH was set at a range of 5.6-5.7 .

⬇

Agar was added to the media (0.8% w/v).

⬇

Then the media was allowed to autoclave at 121°C temperature and 15 Psi pressure after properly boiled to get a homogenous mixture.

⬇

After autoclaving, media was dispensed in culture jars and allowed to solidify for 3 days.

Statistical Analysis

Number of days taken for shoot initiation: The different concentrations of THIDIAZURON (TDZ) and Kn exhibited significant differences on days taken for initiation of shoot in *Valeriana jatamansi*. Early shoot initiation was observed in *Valeriana jatamansi* on MS medium containing TDZ 5mg/l + Kn 4mg/l (5-6 days) followed by other concentrations shown below in Table 1.1

S.No.	Treatment	No. of days
1	MS +BAP(1mg/l)+Kn(1mg/l)+TDZ(0.5mg/l)	20
2	MS +TDZ(3mg/l)+ IAA(2mg/l)	15
3	MS +TDZ(1mg/l)+Kn(0.5mg/l)	9-10
4	MS +TDZ(2mg/l)+Kn(1mg/l)	8-10
5	MS +TDZ(3mg/l)+Kn(2mg/l)	8-10
6	MS +TDZ(4mg/l)+Kn(3mg/l)	7-8
7	MS +TDZ(5mg/l)+Kn(4mg/l)	5-6

Table 1.1: Shows the effect of different concentrations of TDZ and Kn on number of days taken for shoot initiation

Number of days taken for side shoots initiation

The different concentrations of THIDIAZURON (TDZ) + Kn showed significant differences on days taken for side shoot initiation in *Valeriana jatamansi*. Early side shoot initiation was observed on MS medium with TDZ 5mg/l + Kn 4mg/l(5 days) and TDZ 3mg/l + Kn 2mg/l(5 days) whereas other concentrations are shown below in Table 1.2.

S.No.	Treatment	No. of days
1	MS +BAP(1mg/l)+ Kn(1mg/l)+TDZ(0.5mg/l)	-
2	MS +TDZ(3mg/l)+ IAA(2mg/l)	10
3	MS +TDZ(1mg/l)+ Kn(0.5mg/l)	10
4	MS +TDZ(2mg/l)+ Kn(1mg/l)	8
5	MS +TDZ(3mg/l)+Kn(2mg/l)	5
6	MS +TDZ(4mg/l)+ Kn(3mg/l)	6
7	MS +TDZ(5mg/l)+ Kn (4mg/l)	5

Table :1.2 Show the effect of different concentrations of TDZ on number of days taken for side shoot initiation.

Result

Fig. 1.1 (f.1 and f.2) shows the shoot proliferation of *Valeriana jatamansi* in MS Medium containing TDZ(5mg/l) + Kn(4mg/l) :

a) A shoot tip was inoculated on MS Medium containing TDZ (5mg/l) + Kn (4mg/l).
b) After 5 days of inoculation, two larger size leaves were observed.
c) After 10 days of inoculation, leaf size was further increased accompanied with growth of two shoots and a lateral bud.
d) After 15 days of inoculation, numbers of shoots as well as leaves were significantly increased and the leaves started showing glassy effect.

So, it is clear from the above data that shoot number, leaf size and leaf number of *Valeriana jatamansi* was significantly increased as the concentration of THIDIAZURON (TDZ) in combination with Kn was increased. But by increasing the concentration of THIDIAZURON (TDZ) and Kn, the dark green colour of the leaf became light green due to this glassy effect shown assuming that the chlorophyll pigment became minimum in the leaf than the other plants which might be a problem while hardening.

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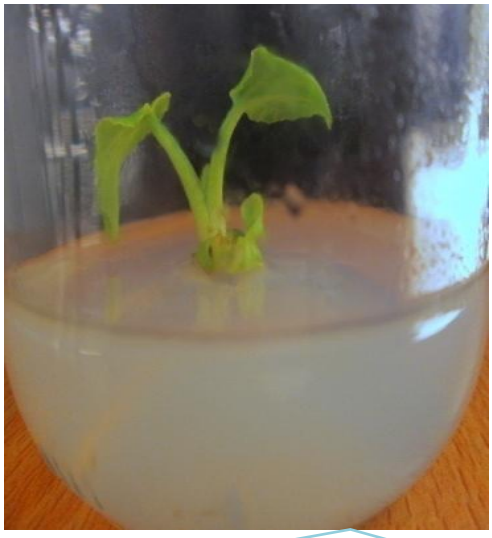
This fig. shows shoot tip after inoculation

This fig. shows that two large leaves were observed after 5 days



This fig. shows after 15 days, two shoots and two large leaves and a lateral bud was observed

This fig. shows maximum number of shoots and leaves after 5-8 days more , but glassy effect (less chlorophyll content) is shown by leaves.

Fig. 1.1 (f.1) MS medium containing TDZ(5mg/l)+Kn(4mg/l)

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This fig. shows shoot tip after inoculation

After 5 days, this fig. shows that two large leaves were observed

This fig. shows two shoots and two large leaves were observed after 10 days and one root was also observed.

This fig. shows two large leaves after15 days, but glassy effect is shown by leaves.

Fig. 1.1(f2)MS medium containing TDZ(5mg/l)+Kn(4mg/l)

Conclusion: The results showed that using lower concentrations of THIDIAZURON (TDZ) and Kn (TDZ 1mg/l +Kn 0.5mg/l)after 9-10 days helped to induce longer shoots while using higher concentrations (TDZ 5mg/l+ Kn 4 mg/l) within 5 days resulted in increase of leave size and number of shoots.

FUTURE ASPECTS: The result showed that higher concentration in combination of kinetin and THIDIAZURON (TDZ) effects the chlorophyll content .In future researcher will use different combination of hormone with THIDIAZURON (TDZ) to check the chlorophyll content.

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