藥作誌(Korean J. Medicinal Crop Sci.)2(1):38~43(1994)

Effect of Thidiazuron on Regeneration from Long-Term Cultured Callus of *Solanum spp.*

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ABSTRACT : The effect of thidiazuron on callus growth and shoot regeneration of *Solanum* species was very positive. Increasing concentrations of thidiazuron stimulated callus growth of *Solanum ptycanthum* and *Solanum nigrum*. Shoot regeneration of *S. ptycanthum* and *S. nigrum* was greater on medium with thidiazuron than that with IAA and BA. Thidiazuron at 0.5μM or above increased the number of shoots regenerated from *S. ptycanthum* calli compared to the IAA with BA. High concentrations of thidiazuron (>1μM) increased the number of shoots than BA or low levels of thidiazuron and IAA or BA. The addition of IAA to thidiazuron media reduced *S. ptycanthum* shoot formation.

Plant cell growth and regeneration in tissue culture systems are highly dependent on the use of the appropriate cytokinin. Cytokinin belongs to two different chemical families, derivatives of either 6-aminopurine or the diphenyl ureas (Mok et al. 1987)[5]. Diphenyl urea itself is a rather weak cytokinin compared to adenine-type cytokinins (Mok et al. 1982)[6]. But cytokinin-active phenylureas such as thidiazuron displayed biological activity quantitatively equal to or exceeding that of adenine-type cytokinins.

Thidiazuron (N-phenyl-N'-1, 2, 3-thidiazol-5yl-urea) induces a variety of cytokinin-like responses, including promotion of callus growth and regeneration, induction of organogenesis, and stimulation of ethylene production [6,11,12,14]. For example, thidiazuron levels above 5×10^{-9} or $4\times^{-7}$ molar stimulated soybean callus growth and radish cotyledon expansion, respectively. A wide range of thidiazuron concentrations induced multiple shoot formation from tobacco leaf discs. High concentrations of thidiazuron stimulates callus growth of *Phaseolus lunatus* L. (Mok et al. 1979; Capelle et al. 1983)[2,7]. The activity of

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thidiazuron was slightly higher than that of zeatin in stimulating callus growth of *P. lunatus* cv. Jackson Wonder and about 30 times higher in stimulating callus growth of *P. lunatus* PI 260415. Callus exposed to thidiazuron continued to proliferate in the absence of a cytokinin during subsequent passages[2,7].

Thidiazuron stimulated in *vitro* proliferation of shoots from cultures of apple (Nieuwkerk et al. 1986)[9], and maple (kerns and Myer, 1986)[4]. When shoot tips from a natural hybrid of *Acer* × *Freemanii* were cultured on a modified Linsmaier-Skoog (LS) medium with 0.01−0.05μM thidiazuron, shoot proliferation was initiated (kerns and Myer, 1986)[4]. Shoot proliferation from shoot tip explants of Gala apple (*Malus domestica* Borkh) was stimulated when thidiazuron was incorporated in Linsmaier-Skoog medium at concentrations of 0.1 to 10μM. Shoot numbers with thidiazuron were equivalent to or greater than that produced when using 4.4μM BA (Nieuwkerk et al. 1986)[9]. Thidiazuron has a cytokinin-like effect on a wide range of species, especially those which responded little to conventional cytokinins (Fellman et al. 1987)[3].

Solanum species including *Solanum ptycanthum* and *Solanum nigrum* are common medicinal crops as well as food plants throughout the world. These species contained a steroid alkaloid, solasodine. Solasodine is easily converted to progesterone. Therefore, solasodine is used as starting material for synthesizing various of steroidal hormones. There were many reports that solasodine was produced by using callus culture (Bhatt et al., 1983)[1], suspension culture (Nigra et al., 1990)[10] and hairy root culture (Wei et al., 1986)[15]. Callus maintainance and plant regeneration from long term culture for these purpose is very important. This study was carried out to determine the effect of thidiazuron on callus formation and shoot regeneration of *Solanum* species.

MATERIALS AND METHODS

In Vitro Culture

Callus, originally established from cotyledonary explants, of *Solanum ptycanthum* Dun. and *Solanum nigrum* L. were cultured on medium (Thomas and Pratt, 1982)[13] with 2,4-dichlorophenoxyacetic acid (2 mg/l) and 6-benzyladenine (1 mg/l). The callus had been subcultured every 30 days for over 2 years prior to initiating these experiments. The pH of all media was adjusted to 5.7±0.1 before addition of 7.5 g/l agar. The growth conditions were 16 hr of light and a temperature of 28℃.

Genotype and Thidiazuron Effect on Callus Growth and Shoot Formation

Callus (100mg) of *Solanum ptycanthum* and *Solanum nigrum* was transferred into culture vessels (135ml^3 volume) containing 30ml of medium. The medium consisted of Murashige and Skoog salts (Murashige and Skoog, 1962)[8] along with B5 vitamins (Gamborg et al. 1968). Thidiazuron was included in the medium at 0.01, 1, and 5μM. None of the thidiazuron treatments included an auxin. A treatment consisting of IAA (indoleacetic acid) (1.1μM) and BA (8.9μM) was also included. In preliminary experiments (Yu and Masiunas, 1990)[16], this combination resulted in the greatest regeneration. After culturing on media for 30 days, fresh weight of the callus and number of shoots were recorded.

Effect of Thidiazuron on Solanum ptycanthum Regeneration.

Callus of *S. ptycanthum* was used to further characterize the effect of thidiazuron on re-

generation. *S. ptycanthum* was the most responsive species of those evaluated to thidiazuron. Callus (100mg) was cultured on culture vessels containing 30ml of MSB5 medium. Thidiazuron concentrations used were between 0 and 5.0μM. The IAA or IAA plus BA was added to the thidiazuron media. To determine if thidiazuron increases the rate of regeneration, the number of shoots were determined weekly for 60 days. To determine the effect on growth, the shoot length was determined after 30 days.

The Interaction between Thidiazuron and Other Plant Growth Requlators.

A third experiment characterized the interaction between thidiazuron and IAA or BA on regeneration of *Solanum ptycanthum*. *S. ptycanthum* was the most responsive species to thidiazuron of those species evaluated. Callus of *S. ptycanthum* was transferred to culture vessels containing 30ml of medium consisting of Murashige and Skoog salts (Murashige and Skoog, 1962)[8] along with B5 vitamins (Gamborg et al. 1968). The experiment was a factorial design. The factors were thidiazuron concentrations (0, 0.5, 1.0, and 5μM) and additional plant growth regulators (IAA at 1.1μM or IAA and BA at 1.1 and 8.9μM, respectively). After transferring the callus onto the regeneration media, the number of shoots were counted weekly for 60 days.

RESULTS AND DISCUSSION

Genotype and Thidiazuron Effect on Callus Growth and Shoot Formation.

The effect of thidiazuron differed depending on the genotype and the parameter being measured. Increasing concentrations of thidiazuron stimulated callus growth of *S. ptycanthum* at 1.0uM thidiazuron was similar to growth with the combination of 1.1μM IAA and 8.9μM BA. Other researchers have also reproted thidiazuron to be effective in stimulating callus growth. Capelle et al.(1983)[2] reported thidiazuron was highly active in stimulating callus growth of *Phaseolus lunatus*. Its activity was slightly or 30 times higher than that of zeatin in stimulating callus growth, depending on genotype(Capelle et al. 1983)[2]. The activity of thidiazuron in promoting the growth of *P. lunatus* callus might be associated with an induction of cytokinin autonomy and endogeneous cytokinin biosynthesis (Mok et al. 1979)[7].

Shoot formation in media with thidiazuron in *Solanum ptycanthum* Dun. and *Solanum nigrum* L was very good. The optimum thidiazuron concentration differed depending on the genotype(Table 1). *Solanum ptycanthum* regenerated the highest number of shoots on media with 1.0μM thidiazuron while *Solanum nigrum* with 5μM thidiazuron. Shoot regeneration of *S. ptycanthum* and *S. nigrum* was more effective on medium with thidiazuron than with IAA and BA. At 1.0μM thidiazuron, *Solanum ptycanthum* regenerated 51 shoots per callus while at 5μM thidiazuron *Solanum nigrum* regenerated 66 shoots per callus. Shoot regeneration of these two *Solanum* genotypes was approximately 10 shoots per callus on medium with IAA and BA.

The effect of Thidiazuron on S. ptycanthum.

As in the previous experiment, thidiazuron at 0.5μM or above increased the number of shoots regenerated from *S. ptycanthum* calli compared to the IAA+BA control. Shoot regeneration was the greatest at 5μM thidiazuron(Figure 1). More than 40 shoots per calli were regenerated at 5μM thidiazuron while only 7 shoots per calli were regenerated

Table. 1. Effect of thidiazuron, IAA and BA on the number of shoot from callus of *Solanum ptycanthum* and *Solanum nigrum*.

Genotype	1.1μM IAA +8.9μM BA	Thidiazuron(μM) 0.5	1.0	5.0	LSD 5%
		Shoots / Calli			
S. ptycanthum	9.7	8.0	51	39	16.9
S. nigrum	13.0	11.3	21.3	66	12.8
LSD 5%	6.3	4.3	9.7	9.1	

Table. 2. Effect of thidiazuron, IAA and BA on the shoot length of *Solanum ptycanthum* and *S. nigrum*.

Genotype	1.1μM IAA +8.9μM BA	Thidiazuron(μM) 0.5	1.0	5.0	LSD 5%
		Shoots length(cm)			
S. ptycanthum	2.5	1.6	0.8	0.3	0.5
S. nigrum	0.8	1.5	2.6	2.1	1.3
LSD 5%	0.7	0.5	0.8	0.5	

on medium with IAA and BA.

Mok et al. (1987)[5] also reported that thidiazuron was considerably more active than other cytokinins in multiplying broccoli (*Brassica oleracea* L.) shoots.

In this study, shoots regenerated from media containing thidiazuron were two times more likely to be phenotypic variants than from those from medium with IAA and BA (data not shown). Other researchers have also reported that thidiazuron had induced phenotypic variation (Kerns and Myer, 1986; Nieumkerk et al. 1986; Fellman et al. 1987)[3,4,9]. For example, Nieuwkerk et al.(1986)[9] reported that at 1 and 10μM thidiazuron, leaves of the regenerated apple plants were narrow, pointed laminae, and distorted.

Although the total number of *S. ptycanthum* shoots increased with concentration of thidiazuron at 0.5μM or greater, the number of shoots with a length greater than 5mm

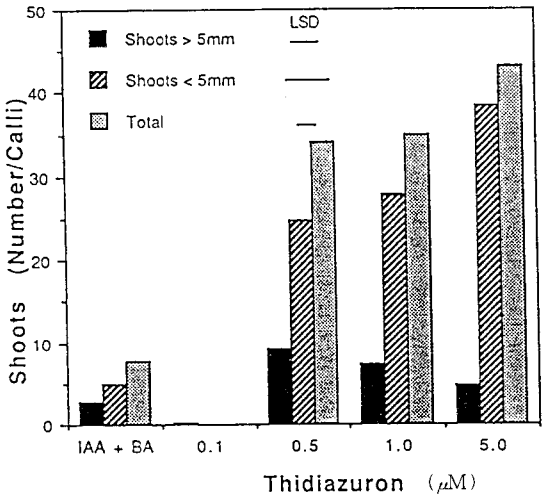

Figure 1. Effect of IAA and BA or thidiazuron on shoot regeneration of *Solanum ptycanthum*. The concentration of IAA was 0.2mg /1 and BA was 2mg /1.

decreased. For example at 0.5μM thidiazuron, 27% of the shoots were larger than 5mm, while at 5μM thidiazuron only 11% of the shoots were. These small shoots generally have poor survival when transferred to rooting media.

Nieuwkerk et al.(1986)[9] also reported that high concentrations of thidiazuron resulted in smaller shoots than BA or low levels of thidiazuron. In tobacco leaf cultures, the greater the concentration of thidiazuron, the more plantlets; however, they were quite small. Larger plantlets that rooted spontaneously were obtained at lower thidiazuron concentrations(i.e.,\t5$\times 10^{-8}$M) (Thoms and katterman, 1986)[14].

Shoot regeneration in *S. ptycanthum* was faster on medium with thidiazuron than on medium with IAA and BA(Figure 2). On thidiazuron media, shoots were initiated within two weeks-culture of subculturing, while shoot initiation required at least 4 weeks when callus

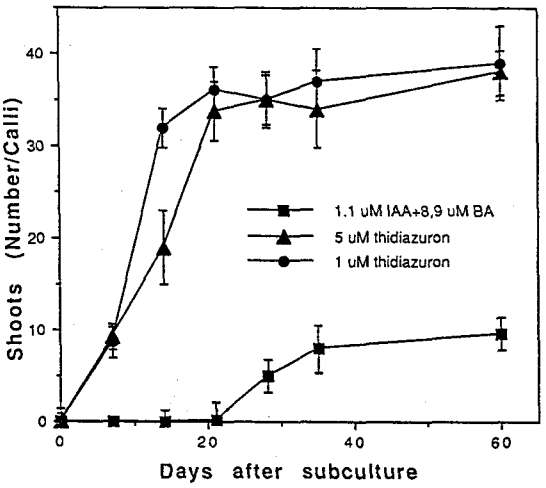

Figure 2. The number of *Solanum ptycanthum* shoots regenerated of weekly after subculturing onto medium with either thidiazuron or 1.1μM IAA and 8.9μM BA. Bars represent the standard error of the mean.

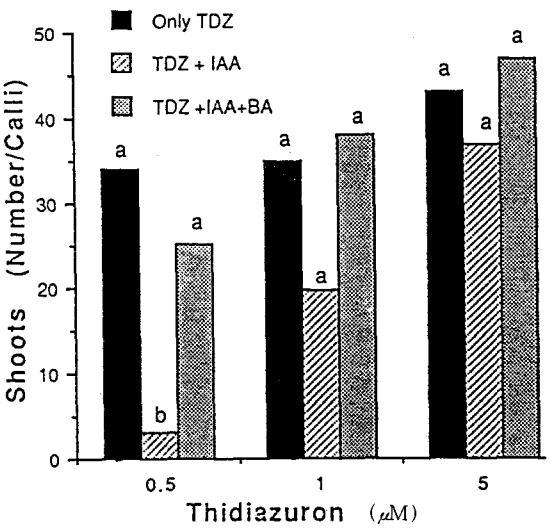

Figure 3. Effect of thidiazuron and interaction of IAA, or IAA and BA on shoot regeneration of *Solanum ptycanthum*. The concentration of IAA is 1.1μM and BA is 8.9μM. The same letters within each treatment are not significantly different at 5% level.

transferred onto medium with IAA and BA.

The Interaction between Thidiazuron and Other Plant Growth Regulators.

There was a significant interaction between thidiazuron and IAA or BA(Figure 3). The addition of 1.1μM IAA to thidiazuron resulted in a significant reduction in the number of shoots formed. This inhibition of shoot formation could be overcome either by increasing the thidiazuron concentration or by adding BA to the media. In an experiment on shoot proliferation of *Acer* × *Freemanii*, Kerns and Myer (1986)[4] found that BA did not significantly enhance the effect of thidiazuron. Thus the interaction we observed may either be specific to overcoming an IAA induced inhibition of regeneration or is a species specific response.

In our study, thidiazuron was more effective than BA and IAA in callus formation, shoot regeneration, and growth of *S. ptycanthum* and *S. nigrum*. Thidiazuron also decreased the length of time required for shoot initiation. Thus, thidiazuron could be useful and economical for tissue culture application in some *Solanum* species. Its biological activity in these species may be important in the studies of the mode of action of cytokinins.

적 요

미국 일리노이에 자생하는 야생 까마중종인 *Solanum ptycanthum*과 *Solanum nigrum*의 어린자엽으로부터 유기된 callus를 2년이상 장기간 계대배양 후 식물체 분화율을 제고하기 위하여 IAA, BA, thidiazuron의 효과를 조사하였다. callus 생장과 식물체분화는 IAA와 BA를 혼합처리시보다 thidiazuron을 단독처리 하였을 때가 4-5배 효과적이었다. Thidiazuron과 IAA와 BA간에는 식물체분화에 상호작용이 있었으며 thidiazuron을 단독처

리시보다 IAA를 첨가시 식물체 분화를 감소하였다.

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