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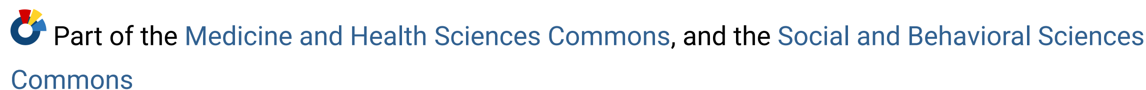
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Effects of chitosan and salicylic acid on Stemona alkaloid production in hydroponic culture of Stemona curtisii Hook. f.

Abstract

The objective of this study was to investigate the effects of the elicitors, salicylic acid (SA) and chitosan, on the improvement of *Stemona* alkaloid production in hydroponic cultures of *S. curtisii.* In vitro plantlets were used as plant materials. The elicitors were added into the culture medium and samples of the roots and medium were collected on week 2 and 4 after the elicitor addition and then analyzed for *Stemona* alkaloid production by high performance liquid chromatography (HPLC). This study revealed that both SA and chitosan increased production of three Stemona alkaloids and that chitosan is better than SA for the enhancement of the production of these alkaloids. The elicitation by 20 mg L^{-1} of chitosan for 4 weeks induced highest amount of oxyprotostemonine (274.31 µg g^{-1} DW) stemocurtisine (35.46 µg g^{-1} DW) and stemocurtisinol (99.48 µg g^{-1} DW), which were 1.9, 2.0 and 1.5 fold higher than that of the control, respectively.

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Contributed Paper

Effects of Chitosan and Salicylic Acid on *Stemona* Alkaloid Production in Hydroponic Culture of *Stemona curtisii* Hook. f.

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ABSTRACT

The objective of this study was to investigate the effects of the elicitors, salicylic acid (SA) and chitosan, on the improvement of *Stemona* alkaloid production in hydroponic cultures of *S. curtisii*. *In vitro* plantlets were used as plant materials. The elicitors were added into the culture medium and samples of the roots and medium were collected on week 2 and 4 after the elicitor addition and then analyzed for *Stemona* alkaloid production by high performance liquid chromatography (HPLC). This study revealed that both SA and chitosan increased production of three *Stemona* alkaloids and that chitosan is better than SA for the enhancement of the production of these alkaloids. The elicitation by 20 mg L^{-1} of chitosan for 4 weeks induced highest amount of oxyprotostemonine (274.31 μg g^{-1} DW) stemocurtisine (35.46 μg g^{-1} DW) and stemocurtisinol (99.48 μg g^{-1} DW), which were 1.9, 2.0 and 1.5 fold higher than that of the control, respectively.

Keywords: chitosan, hydroponic culture, salicylic acid, *Stemona* alkaloid, *Stemona curtisii*

1. INTRODUCTION

Stemona curtisii is an insecticidal plant belonging to the monocotyledon family Stemonaceae. This family is the only source of *Stemona* alkaloids [1]. Three kinds of *Stemona* alkaloids (stemocurtisine oxyprotostemonine and stemocurtisinol) have been isolated from the root extracts of *S. curtisii* [2-3]. These extracts had insecticidal activity against *Spodoptera littoralis* [4] and larvicidal activity against *Anopheles minimus* [3]. In addition, the use of *S. curtisii* extracts as a biopesticide were reported [5], which revealed that this pesticide was effective against *Phyllotreta chontanica*, *Plutella xylostella*, *Lipaphis erysimi*, *Trichoplusia ni* and *Spodoptera littoralis* in agricultural field trials.

Hydroponics have become an important technique to enhance the production of

secondary metabolites in several plants, for example, genistein in *Lupinus luteus* [6] and isoflavones in *Pueraria Montana* [7]. Moreover, the treatment of plants with various elicitors has been an effective strategy for improving the production of secondary metabolites in plants. Many elicitors such as salicylic acid (SA), chitosan or yeast extract were confirmed as effective for the enhancement of *Stemona* alkaloid production from *in vitro* cultured *Stemona* plants [8-9]. Although past studies have examined the effect of elicitors in tissue cultures, no previous investigations have examined their effects in hydroponic cultures. Hence, the purpose of this study was to assess the effects of the elicitors SA and chitosan on the improvement of *Stemona* alkaloid production especially oxyprotostemonine stemocurtisine and stemocurtisinol in hydroponic cultures of *S. curtisii*.

2. MATERIALS AND METHODS

2.1 Plant Materials and Hydroponic Culture

The intact plants of *S. curtisii* (a voucher specimen No. 17581) were collected from Trang Province in Thailand. Micropropagation of *S. curtisii* was used to produce plantlets following the method described by Palee *et al.* [10]. The *in vitro* shoots of *S. curtisii* were cultured on Murashige and Skoog (MS, 1962) [11] solid media containing 1 mg L^{-1} naphthalene acetic acid (NAA) for 8 weeks to induce roots. After that, eight week–old plantlets were used as plant materials, which were transferred into the modified hydroponic culture (Figure 1). These plantlets were first washed with running tap water to remove agar from the roots, then soaked with 0.2% solution of fungicide (Carbendazim) for 30 minutes, and then finally transplanted to hydroponic trays having a mixture of perlite and vermiculite in the ratio of 1:1 (w/w). The hydroponic systems were aerated by bubbling compressed air through the nutrient solution.

2.2 Feeding of Elicitors

The elicitors, consisting of chitosan at concentrations of 10, 20 and 50 mg L^{-1} or SA at concentrations of 100, 200 and 500 µM, were added into the culture medium. Non elicitor treatment was the control conditions. The fifteen plantlets in each treatment were kept in the greenhouse for 4 weeks at a temperature range 25-30 °C and 11-13 h photoperiods. Samples of the roots and the culture medium for each treatment were collected two times on weeks 2 and weeks 4 after adding the elicitors and were then analyzed for the presence of the *Stemona* alkaloids, oxyprotostemonine stemocurtisine and stemocurtisinol by high performance liquid chromatography (HPLC).

2.3 Root and Medium Extraction

Fresh roots were cut into small pieces and dried in the oven at 35-40 °C. Then, the dried roots were powdered and extracted 3 times with methanol (MeOH). For medium extraction, the culture medium for each treatment was extracted 3 times with dichloromethane (DCM). The solutions of the roots and medium extracts were filtered and evaporated to give a crude extract which was dissolved in 1 mL MeOH and 1 mL water before extraction with DCM, and then the organic phase was evaporated to give a partially purified extract (DCM crude extract). Finally, the DCM crude extracts of the roots and the culture medium were analyzed by HPLC.

2.4 Quantification of *Stemoma* Alkaloids by HPLC

Analytical HPLC was performed using an Agilent 1200 series. Chromatographic separation was achieved with a C_{18} column

(Inertsil ODS-3, 5 μm, 4.6 I.D. × 150 mm, GL sciences Inc., Japan). The mobile phase was Milli-Q water and MeOH (HPLC grade) (30:70, v/v), at a flow rate of 1 mL min^{-1} with an injection volume of 20 μL and UV detection at 297 nm [12]. Standards of oxyprotostemonine, stemocurtisine and stemocurtisinol were separated and purified by column chromatography from *S. curtisii* root extract, with purities of approximately 97.3, 92.1 and 96.3% (based on peak area), respectively. Quantification was based on the external standard method using calibration curves. Standard solutions of oxyprotostemonine, stemocurtisine and stemocurtisinol were prepared in methanol. Each concentration of standard solution was injected onto the HPLC column in triplicate. The retention times of oxyprotostemonine, stemocurtisine and stemocurtisinol were 2.9, 3.4 and 4.8 min, respectively**.** The mean peak areas for each concentration were calculated and the standard calibration curves were constructed by plotting concentrations against the peak areas.

2.5 Statistical Analysis

The experiment was laid out in completely randomized design(CRD). All experiments were conducted in three replicates with 15 plantlets per treatment. The values are expressed as the Mean±SD. The data were analyzed using one-way analysis of variance (ANOVA) and then followed by the Turkey test. All statistical tests were considered significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Influence of Chitosan on *Stemona* Alkaloid Production

The accumulation of the *Stemona* alkaloids namely oxyprotostemonine stemocurtisine and stemocurtisinol in the roots and medium are shown in Table 1. It was found that the elicitation by chitosan enhanced the production of these three *Stemona* alkaloids in the roots and also stimulated the secretion of these alkaloids into the medium. The highest contents of total oxyprotostemonine (274.31 μg g^{-1} DW), stemocurtisine (35.46 μg g^{-1} DW) and stemocurtisinol (99.48 μg g^{-1} DW) were observed from treatment with 20 mg L^{-1} chitosan for 4 weeks, which were 1.9, 2.0 and 1.5 fold significantly higher than the control, respectively. The positive effects of chitosan on *Stemona* alkaloid production were also reported by Chaichana *et al.* [9] and Jadwadtanakul [13] in tissue cultures of *Stemona* sp., where the highest contents of 1′, 2′-didehydrostemofoline and stemofoline were found from treatment with 25 mg L^{-1} chitosan for one week. Chitosan has been used as a strong elicitor to improve the production of secondary metabolites in tissue culture and hydroponic culture of numerous plants, for example, *Lupinus luteus* [6], *Plumbago rosea* [14], *Ocimum basilicum* [15] and *Withania somnifera* [16]. Fan *et al.* [17] demonstrated that chitosan not only enhanced the production of triterpenoids in cell suspension cultures of *Betula platyphylla* but also increased the activity of enzymes involved in triterpenoid biosynthesis, *i.e.* phenylalanine ammonia lyase, superoxide dismutase, endochitinases and exochitinases, which were 1.6 - 3.0 fold when compared to unelicited cells. Likewise in hairy root cultures of *Armoracia lapathifolia*, Flocco and Giulietti [18] reported that total peroxidase activity increased about 170% after 48 h of elicitation with chitosan at 100 mg L^{-1}. Thus, in the present study, chitosan may be stimulating the activity of enzymes involved in *Stemona* alkaloid biosynthesis, resulting to the enhancement of oxyprotostemonine, stemocurtisine and stemocurtisinol production in *S. curtisii*.

Table 1. Effects of chitosan concentration on *Stemona* alkaloids accumulation in *S. curtisii* roots and the culture medium.

week	Concentration of chitosan	Oxyprotostemonine content ($\mu g\ g^{-1}$ DW)			Stemocurtisine content ($\mu g\ g^{-1}$ DW)			Stemocurtisinol content ($\mu g\ g^{-1}$ DW)		
		Root	Medium	Total	Root	Medium	Total	Root	Medium	Total
2	Control	158.05± 3.28^{c}	5.17± 0.03^{e}	163.22± 3.26^{c}	13.34± 0.35^{d}	ND*	13.34± 0.35^{d}	57.66± 1.19^{e}	1.45± 0.03^{c}	59.11± 1.19^{e}
	10 mg L^{-1}	134.33± 2.90^{d}	4.78± 0.09^{f}	139.11± 2.93^{de}	13.20± 0.51^{d}	ND	13.20± 0.51^{d}	49.96± 0.60^{f}	0.77± 0.03^{e}	50.73± 0.57^{f}
	20 mg L^{-1}	115.53± 1.80^{e}	6.14± 0.06^{d}	121.67± 1.75^{f}	9.52± 0.56^{e}	ND	9.52± 0.56^{e}	39.40± 0.98^{g}	1.55± 0.02^{c}	40.95± 0.96^{g}
	50 mg L^{-1}	126.27± 2.16^{de}	6.19± 0.09^{d}	132.46± 2.19^{def}	8.93± 0.05^{e}	2.11± 0.02^{a}	11.04± 0.07^{de}	72.63± 0.68^{c}	1.22± 0.04^{d}	73.85± 0.63^{c}
4	Control	135.44± 0.73^{d}	5.50± 0.05^{e}	140.94± 0.78^{d}	17.59± 0.59^{c}	ND	17.59± 0.59^{c}	64.20± 1.21^{d}	1.21± 0.04^{d}	65.41± 1.25^{d}
	10 mg L^{-1}	120.56± 1.84^{e}	8.17± 0.07^{c}	128.73± 1.86^{ef}	17.55± 0.26^{c}	ND	17.55± 0.26^{c}	80.36± 1.44^{b}	1.54± 0.02^{c}	81.90± 1.42^{b}
	20 mg L^{-1}	260.51± 1.71^{a}	13.80± 0.08^{a}	274.31± 1.66^{a}	35.46± 0.65^{a}	ND	35.46± 0.65^{a}	96.69± 1.21^{a}	2.79± 0.02^{a}	99.48± 1.22^{a}
	50 mg L^{-1}	182.28± 2.91^{b}	8.93± 0.06^{b}	191.21± 2.94^{b}	22.95± 0.78^{b}	1.34± 0.01^{b}	24.29± 0.79^{b}	92.23± 1.21^{a}	2.25± 0.04^{b}	94.48± 1.23^{a}

The experiments were conducted in three replicates. Each replicate had 15 plantlets per treatment. * ND = Not-detected.

* Values expressing the Mean ± SD followed by similar letters in a column indicates as non-significantly difference at $P < 0.05$ (ANOVA; Turkey test).

3.2 Influence of Salicylic Acid (SA) on *Stemona* Alkaloid Production

The effects of SA on *Stemona* alkaloids production in the modified hydroponic culture of *S. curtisii* were also examined. Data in Table 2 revealed that the elicitation by SA for 4 weeks had a positive effect on the enhancement of the production of the *Stemona* alkaloids in the roots. This also stimulated the secretion of oxyprotostemonine and stemocurtisinol into the medium, however SA did not affect stemocurtisine secretion that may be cause from the amount of stemocurtisine in the roots is much less. The highest amounts of total oxyprotostemonine (213.37 $\mu g\ g^{-1}$ DW) and stemocurtisine (33.30 $\mu g\ g^{-1}$ DW) were observed from the culture treated with 100 μM SA for 4 weeks, which were 1.5 and 1.9 fold significantly higher than that of the control, respectively. The elicitation by 500 μM SA for 4 weeks showed the highest amount of total stemocurtisinol (96.20 $\mu g\ g^{-1}$ DW), which was 1.5 fold significantly higher than that of the control. The stimulating effect of SA on *Stemona* alkaloid production has been reported in *S. curtisii* root cultures, whereas the highest amount of oxyprotostemonine, stemocurtisine and stemocurtisinol were found in the treatment with 500 μM SA for 16 weeks [8]. For *in vitro* cultures of *Stemona* sp., the elicitation by 100 μM SA for one week revealed the highest amount of Stemofoline and 1′, 2′-didehydrostemofoline, which were 1.6 and 1.7 fold higher than the control, respectively [19].

Salicylic acid is an important stress-signaling molecule in plant resistance to pathogens and other stress factors [20-21]. SA and its chemical derivatives, acetylsalicylic acid and trifluoroethyl salicylate have been reported to enhance the production of secondary metabolites in various plants [22-24]. The stimulatory effects of SA on secondary metabolite production may be due to the stimulation of biosynthetic enzymes [25-26]. In *Capsicum frutescens*, 200 μM SA

enhanced capsaicin production in the cell and stimulated the release of capsaicin into the medium. The total capsaicin content was the highest on the 12th day of culture, which correlated with higher activity of capsaicin synthase in the capsaicin biosynthetic pathway [27]. Kang *et al.* [28] reported that the highest amount of scopolamine in the adventitious roots of *Scopolia parviflora* was found in the roots treated with 1.0 mM SA for 24 h, which was associated with the over expression level of the key enzymes, putrescine *N*-methyltransferase and hyoscyamine 6β-hydroxylase in the biosynthesis of tropane alkaloids.

Table 2. Effects of SA concentration on *Stemona* alkaloids accumulation in *S. curtisii* roots and the culture medium.

week	Concentration of chitosan	Oxyprotostemonine content (µg g^{-1} DW)			Stemocurtisine content (µg g^{-1} DW)			Stemocurtisinol content (µg g^{-1} DW)		
		Root	Medium	Total	Root	Medium	Total	Root	Medium	Total
2	Control	158.05± 3.28^{d}	5.17± 0.03^{e}	163.22± 3.26^{d}	13.34± 0.35^{f}	ND*	13.34± 0.35^{f}	57.66± 1.19^{de}	1.45± 0.03^{c}	59.11± 1.19^{de}
	100 µM	189.60± 1.87^{b}	3.75± 0.01^{f}	193.35± 1.87^{b}	13.83± 0.28^{ef}	ND	13.83± 0.28^{ef}	62.13± 0.60^{cd}	0.39± 0.03^{f}	62.52± 0.63^{cd}
	200 µM	140.89± 1.43^{e}	3.40± 0.01^{g}	144.29± 1.43^{e}	16.32± 0.54^{de}	ND	16.32± 0.54^{de}	54.28± 0.76^{e}	0.71± 0.02^{e}	54.99± 0.79^{e}
	500 µM	138.85± 3.13^{e}	2.18± 0.01^{h}	141.03± 3.12^{e}	17.93± 0.20^{d}	ND	17.93± 0.20^{d}	56.79± 0.91^{e}	0.36± 0.02^{f}	57.15± 0.93^{e}
4	Control	135.44± 0.73^{e}	5.50± 0.05^{d}	140.94± 0.78^{e}	17.59± 0.59^{d}	ND	17.59± 0.59^{d}	64.20± 1.21^{c}	1.21± 0.04^{d}	65.41± 1.25^{c}
	100 µM	202.61± 1.15^{a}	10.76± 0.05^{a}	213.37± 1.19^{a}	33.30± 0.58^{a}	ND	33.30± 0.58^{a}	72.93± 1.03^{b}	2.63± 0.04^{a}	75.56± 0.99^{b}
	200 µM	172.09± 3.46^{c}	6.39± 0.07^{c}	178.48± 3.45^{c}	24.72± 0.58^{c}	ND	24.72± 0.58^{c}	77.12± 1.48^{b}	1.84 ± 0.02^{b}	78.96± 1.48^{b}
	500 µM	176.17± 1.82^{c}	6.67± 0.07^{b}	182.84± 1.88^{bc}	28.64± 0.95^{b}	ND	28.64± 0.95^{b}	95.07± 0.80^{a}	1.13± 0.03^{d}	96.20± 0.83^{a}

The experiments were conducted in three replicates. Each replicate had 15 plantlets per treatment. * ND = Not-detected.

* Values expressing the Mean ± SD followed by similar letters in a column indicates as non-significantly difference at $P < 0.05$ (ANOVA; Turkey test).

Figure 1. The modified hydroponic culture.

4. CONCLUSIONS

Elicitation by chitosan and SA had a positive effect on the enhancement of the production of the three *Stemona* alkaloids, oxyprotostemonine, stemocurtisine and stemocurtisinol in the modified hydroponic cultures of *S. curtisii*. Treatment with 20 mg L^{-1} chitosan for 4 weeks showed the highest amount of total oxyprotostemonine, stemocurtisine and stemocurtisinol, which were 1.9, 2.0 and 1.5 fold higher than the control, respectively.

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REFERENCES

[1] Zhou Y., Jiang R.W., Hon P.M., Xu Y.T., Chan Y.M., Chan T.W.D., Xu H.X., Ding L.S., But P.P.H. and Shaw P.C., *Rapid Commun. Mass Sp.*, 2006; **20**: 1030-1038. DOI 10.1002/rcm.2409.

[2] Mungkornasawakul P., Pyne S.G., Jatisatienr A., Supyen D., Lie W., Ung A.T., Skelton B.W. and White A.H., *J. Nat. Prod.*, 2003; **66**: 980-982. DOI 10.1021/np020612s.

[3] Mungkornasawakul P., Pyne S.G., Jatisatienr A., Supyen D., Jatisatienr C., Lie W., Ung A.T., Skelton B.W. and White A.H., *J. Nat. Prod.*, 2004; **67**: 675-677. DOI 10.1021/np034066u.

[4] Kaltenegger E., Brem B., Mereiter K., Kalchhauser H., Kahlig H., Hofer O., Vajrodaya S. and Greger H., *Phytochemistry*, 2003; **63**: 803-816. DOI 10.1016/S0031-9422(03)00332-7.

[5] Sastraruji T., *Bioinsecticide Production from Stemona Extract and its Application in Agricultural Use*, PhD Thesis, Chiang Mai University, Thailand, 2006.

[6] Kneer R., Poulev A.A., Olesinski A. and Raskin I., *J. Exp. Bot.*, 1999; **50(339)**: 1553-1559. DOI 10.1093/jxb/50.339.1553.

[7] Kirakosyan A., Kaufman P.B., Chang S.C., Warber S., Bolling S. and Vardapetyan H., *Plant Cell Rep.*, 2006; **25**: 1387-1391. DOI 10.1007/s00299-006-0198-2.

[8] ChotikadachanarongK., Dheeranupattana S., Jatisatienr A., Wangkarn S., Mungkornasawakul P., Pyne S.G., Ung A.T. and Sastraruji T., *Curr. Res. J. Biol. Sci.*, 2011; **3(4)**: 322-325.

[9] Chaichana N., Dheeranupattana S., Jatisatienr A., Wangkarn S., Pyne S.G., Mungkornasawakul P., Sangthong P. and Sastraruji T., *Curr. Res. J. Biol. Sci.*, 2012; **4(4)**: 449-454. DOI 10.3923/ajps.2011.338.341.

[10] Palee J., Dheeranupattana S., Jatisatienr A., Wangkarn S., Mungkornasawakul P., Pyne S.G., Ung A.T. and Sastraruji T., *Asian J. Plant Sci.*, 2012; **11(6)**: 294-299. DOI 10.3923/ajps.2012.294.299.

[11] Murashige T. and Skoog F., *Physiol. Plant*, 1962; **15**: 473-497. DOI 10.1111/j.1399-3054.1962.tb08052.x.

[12] Palee J., Dheeranupattana S., Jatisatienr A. and Wangkarn S., *Chiang Mai J. Sci.,* 2013; **40(3)**: 356-363.

[13] Jadwadtanakul G., *Effects of Chitosan and Sodium Acetate on 1′, 2′-Didehydrostemofoline*

Production of In Vitro Stemona sp. PhD Thesis, Chiang Mai University, Thailand, 2012.

[14] Komaraiah P., Ramakrishna S.V., Reddanna P. and Kavi Kishor P.B., *J. Biotechnol.*, 2003; **101**: 181-187. DOI 10.1016/S0168-1656(02)00338-3.

[15] Deschamps C. and Simon J.E., *J. Essent. Oil Res.*, 2006; **18**: 618-621. DOI 10.1080/10412905.2006.9699183.

[16] Sivanandhan G., Arun M., Mayavan S., Rajesh M., Mariashibu T.S., Manickavasagam M., Selvaraj N. and Ganapathi A., *Ind. Crops Prod.*, 2012; **37(1)**: 124-129. DOI 10.1016/j.indcrop.2011.11.022.

[17] Fan G., Li X., Wang X., Zhai Q. and Zhan Y., *Afr. J. Biotechnol.*, 2010; **9(19)**: 2816-2820. DOI 10.5897/AJB09.1975.

[18] Flocco C.G. and Giulietti M., *Appl. Biochem. Biotechnol.*, 2003; **110(3)**: 175-183. DOI 10.1385/ABAB:110:3:175.

[19] Chaichana N. and Dheeranupattana S., *Int. J. Biosci. Biochem. Bioinform.*, 2012; **2(3)**: 146-150. DOI 10.7763/IJBBB.2012.V2.89.

[20] Draper J., *Trends Plant Sci.*, 1997; **2**: 162-165. DOI 10.1016/S1360-1385(97)01030-3.

[21] Shah J., *Curr. Opin. Plant Biol.*, 2003; **6**: 365-371. DOI 10.1016/S1369-5266(03)00058-X.

[22] Bulgakov V.P., Tchernoded G.K., Mischenko N.P., Khodakovskaya M.V., Glazunov V.P., Radchenko S.V., Zvereva E.V., Fedoreyev S.A. and Zhuravlev Y.N., *J. Biotechnol.*, 2002; **97**: 213-221. DOI 10.1016/S0168-1656(02)00067-6.

[23] Wang Y.D., Yuan Y.J. and Wu J.C., *Biochem. Eng. J.*, 2004; **19**: 259-265. DOI 10.1016/j.bej.2004.02.006.

[24] Jeong G.T., Park D.H., Ryu H.W., Hwang B., Woo J.C., Kim D. and Kim S.W., *Appl. Biochem. Biotechnol.,* 2005; **121-124**: 1147-1157. DOI 10.1007/978-1-59259-991-2_96.

[25] Ali M.B., Hahn E.J. and Paek K.Y., *Molecules*, 2007; **12**: 607-621. DOI 10.3390/12030607.

[26] Yu Z.Z., Fu C.X., Han Y.S., Li Y.X. and Zhao D.X., *Biotechnol. Lett.*, 2006; **28(13)**: 1027-1031. DOI 10.1007/s10529-006-9035-5.

[27] Sunda G. and Ravishankar G.A., *Curr. Sci.*, 2003; **85(8)**: 1212-1217.

[28] Kang S.M., Jung H.Y., Kang Y.M., Yun D.J., Bahk J.D., Yang J.K. and Choi M.S., *Plant Sci.*, 2004; **166(3)**: 745-751. DOI 10.1016/j.plantsci.2003.11.022.