

Effects of Salicylic and Acetylsalicylic Acid on Direct Somatic Embryogenesis in *Oncidium*

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The effects of salicylic and acetylsalicylic acid on direct somatic embryogenesis were investigated using leaf explants of two cultivars of *Oncidium* on 1/2 MS medium with or without thidiazuron. In cv Gower Ramsey, salicylic acid (1, 5, 10, 15, 20, 50 μM) either alone or in combination with thidiazuron (4.54 μM) retarded and delayed embryogenesis. In contrast, in the presence of 4.54 μM of thidiazuron, acetylsalicylic acid at 0.1 μM concentration promoted embryogenesis. In cv Sweet Sugar, all concentrations of salicylic acid with or without thidiazuron proved inhibitory on embryo induction. However, in the presence of 0.45 μM thidiazuron, 0.1 and 1 μM acetylsalicylic acid promoted embryogenesis. In addition, in the presence of 4.54 μM thidiazuron, 0.01, 0.1 and 1 μM acetylsalicylic acid promoted embryogenesis.

Key words: acetylsalicylic acid, embryogenic response, leaf explant, orchid, salicylic acid, thidiazuron.

Salicylic acid is a signal factor involved in hormone regulation and systemic resistance of plants. It has been used in tissue culture media for promotion of somatic embryogenesis of carrot (1), *Coffea arabica* (2), *Astragalus adsurgens* (3) and *Avena nuda* (4). However, salicylic acid combined with thidiazuron had no positive effect on somatic embryogenesis from hypocotyl of geranium (5). Besides, 5 – 20 mM of salicylic acid inhibited somatic embryogenesis of *Medicago sativa* (6). Another related chemical, acetylsalicylic acid (aspirin), a drug often used as an analgesic, antipyretic and as an anti-inflammatory, has also been used in plant tissue culture. It was found to be effective in promotion and synchronization of thidiazuron-induced somatic embryogenesis in geranium (5). It has been suggested that salicylic acid through the inhibition of ethylene biosynthesis, may promote the expression of somatic embryogenesis related proteins (3, 7, 8). *Oncidium* orchids, commonly known as 'dancing ladies', have high economic value in flower markets in the world. A series of studies have reported on the requirements of *Oncidium* leaf cultures for direct embryo induction and optimization of culture conditions for embryogenesis (9-14). In these early studies, cytokinins, ancymidol, PP-333 and ACC (1-aminocyclopropane-1-carboxylic acid) were found to be effective on direct embryo

induction of *Oncidium* cv Gower Ramsey and the best embryo production was found at 20 μM ACC (12). This paper attempts to further improve direct embryogenesis from leaf explants of two *Oncidium* cultivars, Gower Ramsey and Sweet Sugar by adding suitable concentrations of salicylic and acetylsalicylic acid in the culture medium.

Materials and Methods

In vitro-grown plantlets derived from leaf somatic embryos of *Oncidium* cvs Gower Ramsey and Sweet Sugar were maintained on hormone-free 1/2 MS (15) medium with a 2-month-interval of subculture as described by Chen *et al* (9). These plantlets were used as mother plants. About 1 cm long leaf tips taken from young leaves of donor plants, were used as explants to induce direct somatic embryogenesis (9). Plant growth regulators were sterilized by filtration using 0.2 μm filters (Product No. 4612, Gelman Sciences), and added to 1/2 MS medium. Explants were placed adaxial-side-up on the surface of culture media and incubated in 80 mm \times 15 mm Petri dishes under a 16/8-h photoperiod at 28 – 36 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (daylight fluorescent tubes FL-30D/29, 40 W, China Electric Co., Taipei, Taiwan) and at 26 \pm 1 $^{\circ}\text{C}$. Five independent experiments were performed to evaluate the effects of salicylic acid and acetylsalicylic acid (1, 5, 10, 15, 20 and 50 μM) either alone or in combination with 1-phenyl-3-(1,2,3-thiadiazol-

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5-yl)-urea [thidiazuron; 0.45 and 4.54 μM] on direct embryo formation from leaf explants of cvs Gower Ramsey and Sweet Sugar. The frequency of embryo-forming explants and the mean number of embryos per explant were determined for each trial. Four explants were placed in each dish as one replicate. Five replicates were established for each treatment. The percentage of explants forming somatic embryos was recorded. The number of embryos formed from each responding explant was counted under a stereomicroscope (SZH, Olympus, Tokyo, Japan) at the protocorm stage. Data expressed as percentage were transformed using arc sine prior to ANOVA and converted back to the original scale (16). Treatment means were compared by following Duncan's Multiple Range Test (17).

Results and Discussion

In *Oncidium* orchids, the effects of growth regulators, explant length, explant orientation and medium additives (NaH_2PO_4 and nitrogen sources) had been tested to optimize the production of somatic embryos from leaf cultures (9-14). This communication mainly deals with the effects of salicylic and acetylsalicylic acid on direct somatic embryogenesis on leaf explants.

Effect of salicylic acid— In cv Gower Ramsey, salicylic

acid induced significant high browning rate than the control treatment and embryogenesis was retarded at 5-50 μM concentrations (Table 1). In cv Sweet Sugar, salicylic acid also had negative effect on direct embryo formation; the embryo numbers decreased at all the concentrations tested (Table 1). In the presence of 4.54 μM of thidiazuron, 5-50 μM of salicylic acid inhibited the percentage of embryo-bearing explants and decreased the number of embryos in both cultivars (Table 2). In cv Gower Ramsey, 1 μM of salicylic acid slightly increased the percentage of embryo-bearing explants, but decreased the number of embryos (Table 2). In cv Sweet Sugar, 1 and 5 μM of salicylic acid slightly increased the percentage of embryogenesis, but decreased the number of embryos (Table 2). Altogether, in the presence of thidiazuron, the browning rate was lower (Tables 1 & 2). Roustan *et al* (1) reported that 10-100 μM of salicylic acid promoted somatic embryogenesis from suspension culture of *Daucus carota*. In *Astragalus adsurgens*, less than 200 μM of salicylic acid was suitable for induction of somatic embryogenesis (3). In addition, very high concentration (0.5 mM) of acetylsalicylic acid improved somatic embryogenesis from young leaf bases of naked oat (*Avena nuda*) (4). However, very low concentration (10^{-6} μM) of salicylic acid was found to be effective in somatic embryo formation from callus of *Coffea*

Table 1. Effect of salicylic acid on direct somatic embryogenesis from leaf explants of *Oncidium* cvs Gower Ramsey and Sweet Sugar

Salicylic acid (μM)	Percentage of browning	Percentage of embryogenesis on different explant positions*		Percentage of embryogenesis	No. of embryo per explant
		Leaf tip	Adaxial side		
Gower Ramsey					
0	25 b	30 a	35 ab	45 a	18.0
1	55 ab	40 a	50 a	55 a	17.0
5	75 a	5 b	5 c	5 b	9.0
10	75 a	0 b	10 bc	10 b	2.0
15	75 a	0 b	0 c	0 b	0
20	65 ab	0 b	0 c	0 b	0
50	95 a	0 b	0 c	0 b	0
Sweet Sugar					
0	85 a	10 a	0 a	15 a	17.0
1	95 a	0 a	0 a	0 a	0
5	90 a	0 a	5 a	5 a	1.0
10	100 a	0 a	5 a	5 a	2.0
15	95 a	0 a	5 a	5 a	3.0
20	95 a	0 a	0 a	0 a	0
50	85 a	0 a	0 a	0 a	0

Data were scored after 3 months of culture. Formation of direct embryo was counted at entire explants, leaf tips and adaxial sides. Five replicates (dishes) each with four leaf explants were performed for each treatment. Means with the same letters within columns are not significantly different at $p < 0.05$.

*None were recorded on abaxial sides or cut ends.

Table 2. Effect of salicylic acid on direct somatic embryogenesis from leaf explants of *Oncidium* cultured on 4.54 μM thidiazuron-containing medium

Salicylic acid (μM)	Percentage of browning	Percentage of embryogenesis on different explant positions*		Percentage of embryogenesis	No. of embryo per explant
		Leaf tip	Adaxial side		
Gower Ramsey					
0	10 ab	30 ab	75 a	75 a	14.1
1	5 ab	50 a	60 a	85 a	11.3
5	0 b	30 ab	70 a	50 ab	5.7
10	20 ab	40 ab	45 ab	50 ab	12.8
15	0 b	25 ab	45 ab	45 ab	5.0
20	15 ab	20 ab	10 bc	20 bc	3.5
50	35 a	0 b	0 c	0 c	0
Sweet Sugar					
0	10 ab	70 a	65 ab	70 a	23.2
1	10 ab	40 ab	80 a	85 a	15.3
5	5 b	60 ab	50 ab	80 a	6.5
10	15 ab	55 ab	45 ab	55 ab	13.4
15	35 a	45 ab	50 ab	55 ab	1.7
20	35 a	55 ab	25 b	55 ab	10.3
50	35 a	25 b	15 b	25 b	4.4

Data were scored after 2 months of culture. Formation of direct embryo was counted at entire explants, leaf tips and adaxial sides. Five replicates (dishes) each with four leaf explants were performed for each treatment. Means with the same letters within columns are not significantly different at $p < 0.05$.

*None were recorded on abaxial sides or cut ends.

arabica (2). Besides, 5-20 mM of salicylic acid inhibited somatic embryogenesis of *Medicago sativa* (6). Obviously, the previous studies show that the performance of salicylic acid on somatic embryogenesis was affected by several factors such as species, genotype and explant type, etc. In this study, salicylic acid could promote or inhibit direct embryo formation depending on the concentration and the genotypic effect.

Effect of acetylsalicylic acid— In *cv* Gower Ramsey, even a very low concentration (0.01 μM) of acetylsalicylic acid significantly increased the number of embryos, but the percentage of embryo-bearing explants was significantly lower (Table 3). However, at 0.1 μM of acetylsalicylic acid, the percentage of embryo-bearing explants and the number of embryos were promoted slightly (Table 3). Except for 1 μM of acetylsalicylic acid, other concentrations (5, 10, 15, 20 and 30 μM) proved inhibitory on direct embryogenesis (Table 3). In *cv* Sweet Sugar, all concentrations of acetylsalicylic acid retarded direct embryogenesis (Table 3). In *Daucus carota*, higher concentrations (50-100 μM) of acetylsalicylic acid were reported to be suitable for embryo induction from suspension culture (1). However, in this study, higher concentrations of acetylsalicylic acid retarded direct embryogenesis. When higher concentrations were used,

more inhibitions were obtained (Table 3).

Effect of acetylsalicylic acid in the presence of thidiazuron—

In *cv* Gower Ramsey, except for 4.54 μM of thidiazuron combined with 0.1 μM of acetylsalicylic acid, other combinations decreased the percentage of embryogenesis (Table 4). In *cv* Sweet Sugar, five combinations (0.45 μM thidiazuron + 0.1 μM or 1 μM acetylsalicylic acid, 4.54 μM thidiazuron + 0.01, 0.1 or 1 μM acetylsalicylic acid) resulted in promotion of embryogenesis (Table 5). It means that in the presence of 0.45 or 4.54 μM thidiazuron, lower concentrations of acetylsalicylic acid were suitable for direct embryo induction. In contrast, in the presence of thidiazuron at 4.54 μM , higher concentrations (5–50 μM) of acetylsalicylic acid inhibited embryogenesis (Table 5). Hutchinson and Saxena (5) reported that 1–20 μM of acetylsalicylic acid combined with 10 μM of thidiazuron was effective in embryo induction of *Geranium*. They also found that acetylsalicylic acid synchronizes thidiazuron-induced somatic embryogenesis. In this study, although lower concentrations of acetylsalicylic acid promoted embryogenesis, but no obvious promotion of synchronization was observed. Somatic embryos of both cultivars grew normally (Fig. 1a, b), and were successfully converted into plantlets following the standard culture procedure established by the previous

Table 3. Effect of acetylsalicylic acid on direct somatic embryogenesis from leaf explants of *Oncidium*

Acetylsalicylic acid (μM)	Percentage of browning	Percentage of embryogenesis on different explant positions*		Percentage of embryogenesis	No. of embryo per explant
		Leaf tip	Adaxial side		
Gower Ramsey					
0	15 c	35 ab	60 a	60 ab	11.4
0.01	25 bc	15 abc	20 abc	20 bcd	36.8
0.1	20 bc	25 abc	60 a	65 a	14.7
1	30 bc	40 a	40 ab	50 abc	21.3
5	55 ab	0 c	30 abc	30 abcd	6.7
10	35 bc	0 c	10 bc	15 cd	3.7
15	55 ab	5 bc	10 bc	10 d	8.5
20	60 ab	5 bc	0 c	5 d	1.0
50	75 a	0 c	0 c	0 d	0
Sweet Sugar					
0	85 a	10 a	0 a	15 a	17.0
0.01	95 a	0 a	0 a	0 a	0
0.1	85 a	5 a	5 a	10 a	9.0
1	60 b	10 a	0 a	10 a	2.5
5	50 b	5 a	0 a	5 a	1.0
10	90 a	0 a	0 a	0 a	0
15	90 a	0 a	0 a	0 a	0
20	90 a	0 a	0 a	0 a	0
50	85 a	0 a	0 a	0 a	0

Data were scored after 3 months of culture. Formation of direct embryo was counted at entire explants, leaf tips and adaxial sides. Five replicates (dishes) each with four leaf explants were performed for each treatment. Means with the same letters within columns are not significantly different at $p < 0.05$.

*None were recorded on abaxial sides or cut ends.

Table 4. Effect of salicylic acid and TDZ on direct somatic embryogenesis from leaf explants of *cv* Gower Ramsey

Acetylsalicylic acid (μM)	TDZ (μM)	Percentage of browning	Percentage of embryogenesis on different explant positions				Percentage of embryogenesis	No. of embryo per explant
			LT	Ad	Ab	CE		
0	4.54	5 ab	50 a	85 abc	0 a	0 a	85 ab	30.0
0.01	0.45	0 b	70 a	90 a	5 a	5 a	90 a	21.6
0.1	0.45	5 ab	70 a	85 abc	0 a	0 a	85 ab	16.4
1	0.45	30 a	40 a	65 abcd	0 a	0 a	70 ab	16.6
0.01	4.54	10 ab	40 a	85 ab	0 a	0 a	85 ab	14.9
0.1	4.54	0 b	45 a	90 ab	0 a	0 a	90 a	32.2
1	4.54	0 b	60 a	90 ab	0 a	0 a	90 a	26.1
5	4.54	5 ab	45 a	55 bcd	0 a	0 a	65 ab	17.2
10	4.54	5 ab	40 a	70 abcd	0 a	0 a	75 ab	9.9
15	4.54	5 ab	30 a	45 cd	0 a	0 a	50 b	9.1
20	4.54	5 ab	55 a	70 abcd	0 a	0 a	80 ab	14.9
50	4.54	10 ab	40 a	45 d	0 a	0 a	55 ab	13.2

Data were scored after 2 months of culture. Formation of direct embryo was counted at entire explants, leaf tips (LT), adaxial sides (Ad), abaxial sides (Ab) and cut ends (CE). Five replicates (dishes) each with four leaf explants were performed for each treatment. Means with the same letters within columns are not significantly different at $p < 0.05$.

Table 5. Effect of acetylsalicylic acid and TDZ on direct somatic embryogenesis from leaf explants of *cv* Sweet Sugar

Acetylsalicylic acid (μM)	TDZ (μM)	Percentage of browning	Percentage of embryogenesis on different explant positions				Percentage of embryogenesis	No. of embryo per explant
			LT	Ad	Ab	CE		
0	4.54	0 a	70 a	70 abc	5 a	25 ab	85 abc	16.3
0.01	0.45	40 a	45 a	60 bcd	0 a	5 cd	60 abcd	19.0
0.1	0.45	15 a	45 a	85 ab	0 a	0 d	85 ab	23.4
1	0.45	0 a	55 a	100 a	5 a	0 d	100 a	22.7
0.01	4.54	5 a	75 a	95 ab	0 a	45 a	95 a	25.5
0.1	4.54	30 a	50 a	70 abc	0 a	10 bcd	70 abcd	27.0
1	4.54	20 a	65 a	80 ab	10 a	25 ab	80 abc	32.5
5	4.54	20 a	55 a	35 cd	0 a	0 d	60 abcd	8.4
10	4.54	15 a	45 a	55 bcd	0 a	0 d	60 abcd	9.3
15	4.54	35 a	30 a	15 d	0 a	0 d	35 cd	8.3
20	4.54	15 a	45 a	30 cd	0 a	0 d	45 bcd	9.1
50	4.54	35 a	25 a	20 d	0 a	0 d	25 d	9.0

Data were scored after 2 months of culture. Formation of direct embryo was counted at entire explants, leaf tips (LT), adaxial sides (Ad), abaxial sides (Ab) and cut ends (CE). Five replicates (dishes) each with four leaf explants were performed for each treatment. Means with the same letters within columns are not significantly different at $p < 0.05$.

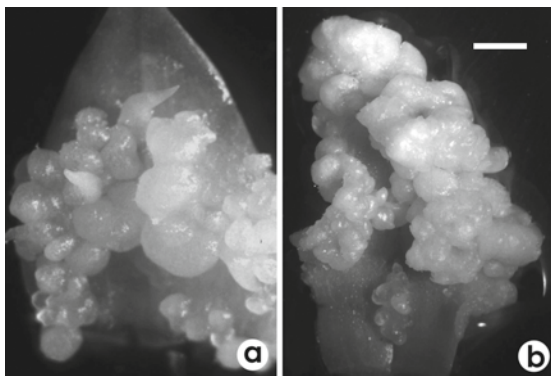


Fig. 1. Direct somatic embryogenesis from leaf explants of *Oncidium* cvs Gower Ramsey and Sweet Sugar. (a) Somatic embryos formed on a leaf explant of *cv* Gower Ramsey after two months of culture in the presence of 4.54 μM of thidiazuron and 0.1 μM acetylsalicylic acid (bar = 2 mm), and (b) Somatic embryos formed on a leaf explant of *Oncidium cv* Sweet Sugar after two months of culture in the presence of 4.54 μM thidiazuron plus 0.01 μM acetylsalicylic acid (bar = 2 mm).

study (10).

In conclusion, salicylic acid or acetylsalicylic acid when used alone retarded or delayed embryogenesis in both cultivars of *Oncidium*. But, in the presence of thidiazuron, lower concentrations of acetylsalicylic acid promoted embryogenesis. However, further studies are required for using acetylsalicylic acid in combination with thidiazuron and ACC to test their effect on optimization of direct somatic embryogenesis in *Oncidium*.

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