2,6‐ジイソプロピルフェノキシ酢酸(DIPA)と植物成長調節剤が多年生水田雑草ウリカワ(Saggitaria pygmaea)の成長と開花に与える相互作用

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Original Article

Interaction between 2,6-diisopropylphenoxyacetic acid (DIPA) and plant growth regulators on shoot growth and flowering of a perennial aquatic plant, *Sagittaria pygmaea*

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2,6-Diisopropylphenoxyacetic acid (DIPA) as well as gibberellins (GAs) was found to promote shoot growth and flowering in a perennial paddy weed *Saggitaria pygmaea*. In this paper, plant growth regulators, which have phytohormonal activities including 2,4-D, *S*-abscisic acid (*S*-ABA), ethephon and benzyladenine (BA) or inhibitors of GA biosynthesis (paclobutrazol and prohexadione), were tested alone or in combination with DIPA to examine their effects on shoot growth and flowering in *S. pygmaea*. 2,4-D inhibited the shoot growth of *S. pygmaea*, increased the number of leaves, and reduced shoot elongation induced by DIPA. Ethephon increased shoot growth of the weed. On flowering of *S. pygmaea*, 2,4-D reduced the promotive effect induced by DIPA. In contrast, ethephon slightly enhanced the promotive effect. These results indicate that in addition to GAs, auxin and ethylene play roles in the shoot growth and flowering of *S. pygmaea*.

Keywords: 2,6-diisopropylphenoxyacetic acid (DIPA), plant growth regulators, shoot growth, flowering, *Sagittaria pygmaea*.

Introduction

Sagittaria pygmaea Miq., a perennial aquatic plant, is one of the most problematic paddy weeds in Japan. The weed emerges from corms and grows as rosettes. It produces corms at the tip of rhizomes, which are considered to be the major reproductive structure. Under normal paddy conditions, the weed begins to produce flowers and tubers 50–60 days after planting, irrespective of day length.[1,2] Gibberellic acid (GA_3) has been reported to promote flowering and tuberization in *S. pygmaea*, suggesting that endgenous GAs play an important role in floral induction in this weed.[3] In addition, ethylene was shown to promote growth of the weed.[4] By contrast, synthetic auxins including 2,4-D and naproanilide strongly inhibit the growth of *S. pygmaea* and are used as herbicides for this weed.[5]

In our previous study, we confirmed that some GAs (A_1, A_3, A_4 and A_5), AC-94377, which shows GA-like effects in various bioassays, and 2,6-diisoproplyphenoxyacetic acid (DIPA) promoted both shoot growth and flowering of *S. pygmaea*.[6] Although DIPA exhibited an antagonistic effect against auxins, neither 2,6-dichlorophenoxyacetic acid, a typical anti-auxin, nor 2,3,5-triiodobenzoic acid (TIBA), an inhibitor of auxin transport, promoted flowering.[7] By contrast, inhibitors of GA biosynthesis, uniconazole and prohexadione, which block different steps in GA biosynthesis, inhibited flowering.[8] These results clearly indicate that flowering in this weed is controlled by endogenous GAs. Structural requirements of GAs for the promotion of flowering seem to be different from those for shoot growth. In addition, since the course of flowering induced by DIPA was clearly different from that observed in plots treated with GA_3 or AC-94377, different mechanisms may be involved in the promotion of flowering.[6]

In this paper, plant growth regulators (PGRs) including 2,4-D, *S*-abscisic acid (*S*-ABA), 2-chloroethylphosphonic acid (ethephon), benzyladenine (BA), and two GA biosynthetic inhibitors, paclobutrazol and prohexadione, were tested alone or in combination with DIPA for their effects on the shoot

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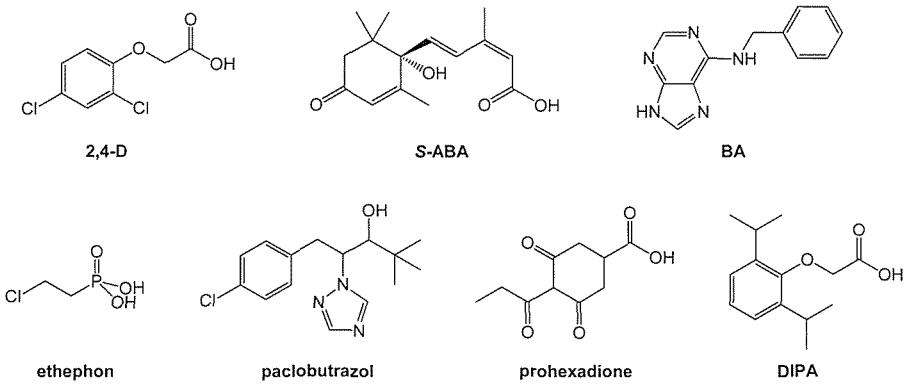

Fig. 1. Chemical structures of 2,4-D, *S*-ABA, BA, ethephon, paclobutrazol, prohexadione and DIPA.

growth and flowering of *S. pygmaea*.

Materials and Methods

1. Chemicals

DIPA was prepared in our laboratory according to the reported method[7] and the other chemicals were purchased from Wako Pure Chemical Industries, Ltd. The structures of these chemicals are shown in Fig. 1.

2. Plant materials and assay conditions

Plant materials and assay methods were essentially the same as in our previous reports.[6,7] Tubers of *Sagittaria pygmaea* were collected locally from paddy fields in winter and stored in moist vermiculite at 4°C until use. Tubers of uniform size (0.07–0.08 g) were germinated in an incubator at 30°C in the dark for 24 hr. All test compounds were examined for their effects on shoot growth, plant height and flowering of *S. pygmaea* using ceramic pots and plastic containers in a greenhouse.

Ceramic pots (15 cm i.d.) were filled with paddy soil and excessively watered to create paddy conditions. Six germinated tubers were transplanted to each pot to a depth of 2 cm. Aqueous solutions of the chemicals were added to irrigation water the following day. The dosages were: DIPA, 1000 g/ha; paclobutrazol, 250, 500 g/ha; prohexadione, 2000, 4000 g/ha; 2,4-D, 125, 250 g/ha; *S*-ABA, 2000, 4000 g/ha; ethephon, 2000, 4000 g/ha; BA, 1000, 2000 g/ha. The pots were placed in a greenhouse maintained at 25–30°C under natural daylight conditions. The level of flooding water was maintained at 3 cm throughout the experiments. The number of leaves was counted and plant heights were measured 30 days after treatment.

In the experiment using plastic containers (20 cm×30 cm×15 cm), 28 germinated tubers were planted in each container. The application rates of the chemicals were: DIPA, 1000 g/ha; paclobutrazol, 500 g/ha; prohexadione, 2000 g/ha; 2,4-D, 62.5 g/ha; *S*-ABA, 2000 g/ha; ethephon, 2000 g/ha; BA, 1000 g/ha. The number of flowering plants was counted 30, 35, 40, 45 and 50 days after treatment. The results were averaged and expressed as percentages of the number of flowering plants in the treated containers. All experiments were performed using three replications.

Results

1. Effects of PGRs on the number of leaves and shoot growth of S. pygmaea

Table 1 shows the number of leaves and the plant height of *S. pygmaea* treated with PGRs. Among the compounds tested, only 2,4-D inhibited the shoot growth of *S. pygmaea*, the shoot length in the treated plots being 61% that of the control at 250 g/ha, while about a 2-fold increase of the number of leaves was observed at 125 g/ha. Although DIPA is also a phenoxyacetic acid, it strongly promoted shoot growth. The mean plant height in plots treated with DIPA at 1000 g/ha was 189% that of the control. In addition, DIPA increased the number of leaves by 12% compared with the control. Furthermore, only DIPA induced the formation of spoon-like leaves, which were produced as 5th and subrequest leaves. After two or three spoon-like leaves had expanded, racemes were produced and flowering began. In the previous study, these leaves were observed in plots treated with GA_3 or AC-94377. On the other hand, ethephon treatment resulted in shoot growth of the weed. *S*-ABA and BA showed no effects even at the highest application rate (4000 g/ha).

Two GA biosynthesis inhibitors with different sites of action, paclobutrazol and prohexadione, did not retard shoot growth even at the highest rates tested. In contrast, prohexadione induced slight shoot growth, the plant height in the treated plots being about 120% that of the control. In addition, prohexadione increased the number of leaves by 18% compared with that of the control at 4000 g/ha.

2. Effects of PGRs applied with DIPA on the number of leaves and shoot growth of S. pygmaea

Table 2 shows the effects of PGRs, each applied with DIPA, on the number of leaves and shoot growth of *S. pygmaea*. All compounds, except *S*-ABA, significantly increased the number of leaves and promoted shoot growth as compared with

Table 1. Effects of PGRs on shoot growth of *S. pygmaea*

Chemical	Dosage (g/ha)	Number of leaves		Plant height	
		Mean±SE	% control	Mean±SE (cm)	% contol
Paclobutrazol	250	9.33±0.33	103.2	4.90±0.30	114.2*
	500	8.83±0.31	97.7	4.23±0.17	98.7
Prohexadione	2000	9.83±0.40	108.7	5.13±0.20	119.7**
	4000	10.67±0.57	118.0**	5.43±0.18	126.7**
2,4-D	125	15.67±1.82	173.3**	3.38±0.31	78.9**
	250	11.17±1.17	123.5**	2.62±0.14	61.0**
ABA	2000	9.17±0.17	101.4	4.70±0.16	109.6
	4000	9.00±0.00	99.6	4.90±0.26	114.2*
Ethephon	2000	9.50±0.43	103.2	5.40±0.22	125.9**
	4000	9.67±0.33	106.9	5.22±0.59	121.6**
BA	1000	9.50±0.22	105.1	4.22±0.17	98.3
	2000	9.67±0.21	106.9	3.97±0.10	92.5
DIPA	1000	10.08±0.26	111.5*	8.10±0.13	188.8**
Control		9.04±0.15	100.0	4.29±0.11	100.0

Significantly different from the control according to LSD Test, *: $p=0.05$, **: $p=0.01$

Table 2. Effects of PGRs applied with DIPA on shoot growth of *S. pygmaea*

Chemical	Dosage (g/ha)	Number of leaves		Plant height	
		Mean±SE	% DIPA	Mean±SE (cm)	% contol
Paclobutrazol	250	12.17±0.54	120.7**b	8.05±0.23	99.4**
+DIPA	500	12.20±0.73	121.0**a	8.02±0.15	99.0**
Prohexadione	2000	10.83±0.70	107.4*	8.83±0.21	109.1**b
+DIPA	4000	11.50±0.56	114.1**	9.20±0.27	113.6**b
2,4-D	125	15.83±1.89	157.0**b	6.50±0.43	80.3**b
+DIPA	250	14.67±1.63	145.5**b	6.20±0.31	76.5**b
ABA	2000	9.17±0.17	90.9	8.43±0.37	104.1**
+DIPA	4000	9.17±0.31	90.9	7.60±0.32	93.8**
Ethephon	2000	10.00±0.68	99.2	8.37±0.23	103.3**
+DIPA	4000	10.83±0.48	107.4*	8.58±0.12	106.0**
BA	1000	10.50±0.34	104.1*	8.27±0.29	102.1**
+DIPA	2000	10.33±0.21	102.5	8.28±0.14	102.3**
DIPA	1000	10.08±0.15	100.0*	8.10±0.13	100.0**
Control		9.04±0.73	89.7	4.29±0.11	53.0

Significantly different from the control according to LSD Test, *: $p=0.05$, **: $p=0.01$. Significantly different from DIPA according to LSD Test, a: $p=0.05$, b: $p=0.01$

the control.

Among the PGRs, only 2,4-D reduced the shoot growth induced by DIPA and increased the number of leaves. The other PGRs showed little effect on the number of leaves and shoot growth as compared with a single application of DIPA at the high rate tested (4000 g/ha).

The two inhibitors of GA biosynthesis showed different effects. Paclobutrazol increased the number of leaves but did not affect shoot growth. By contrast, prohexadione increased the shoot growth induced by DIPA. Spoon-like leaves were

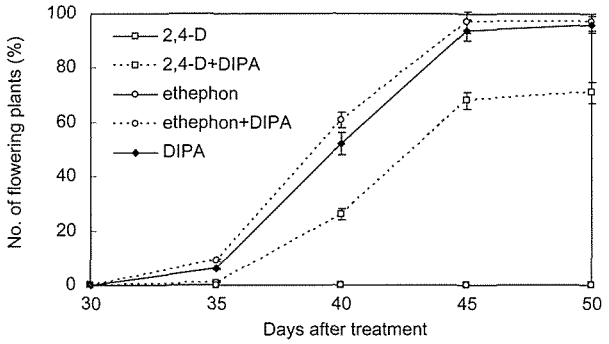

Fig. 2. Effects of 2,4-D, ethephon, and DIPA on flowering of *S. pygmaea*.

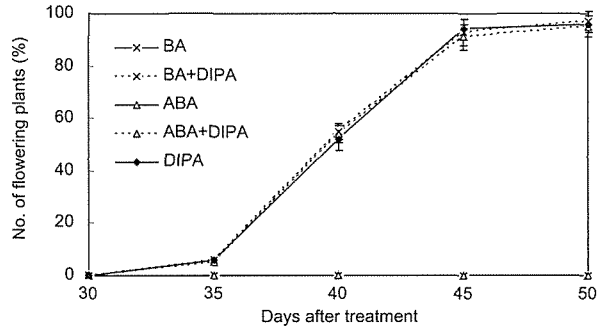

Fig. 3. Effects of BA, *S*-ABA, and DIPA on flowering of *S. pygmaea*.

observed in all DIPA-treated plots.

3. *Effects of PGRs on flowering of* S. pygmaea

Under the experimental conditions, no flowers were observed in the untreated plots even 50 days after treatment. Figs. 2 and 3 show the effects of 2,4-D, ethephon, *S*-ABA, BA, and DIPA on the flowering of the weed. Among the compounds tested, only DIPA promoted the flowering of *S. pygmaea*. In the plots treated with DIPA alone, the weeds began to flower about 30 days after treatment. At 50 days after treatment, more than 90% of the weeds produced inflorescence. By contrast, in the plots treated with the other compounds, no flowers were observed even 50 days after treatment.

Different effects on the flowering of *S. pygmaea* were observed when each PGR was applied with DIPA. 2,4-D reduced the promotive effect induced by DIPA. In contrast, ethephon slightly increased the promotive effect. The other PGRs did not affect the number of flowering plants.

Discussion

Plant growth regulators and plant hormones are expected to play important roles in the growth and development of *S. pygmaea*. In our previous study, we confirmed that GAs and DIPA promoted the shoot growth and flowering of *S. pygmaea*.[8] Furthermore, in the weed, the structural requirements for the promotion of shoot growth by GAs are different from those for flowering, suggesting that these two events depend on separate processes.[6] In this study, not only did DIPA promote shoot growth and flowering, but also slightly increased the number of leaves of *S. pygmaea*.

2,4-D, a typical synthetic auxin, inhibited the shoot growth and flowering of *S. pygmaea*. Since auxin-type herbicides are used to control *S. pygmaea* in paddy fields, this weed would be sensitive to disturbance of endogenous auxin levels.

Ethephon slightly promoted the shoot growth of *S. pygmaea*. Suge *et al.* reported that the growth of *S. pygmaea* was strongly promoted by ethylene and CO_2.[2] In some aquatic plants, it is known that ethylene induces elongation of the coleoptile, mesocotyl, stem and leaf petiole.[10,13] Ethephon did not affect the flowering of *S. pygmaea* when applied alone, but slightly enhanced flowering induced by DIPA. It was shown that ethylene promoted flowering in several plant species forming tulip-like bulbs when their requirements for temperature were satisfied.[14] In *S. pygmaea*, ethylene functioned in a similar manner and enhanced flowering induced by DIPA.

In our previous study, two inhibitors of GAs biosynthesis, uniconazole and prohexadione, slightly reduced the shoot growth of *S. pygamea* at 4000 g/ha, 25 days after treatment; however in the present study, two inhibitors of GA biosynthesis, paclobutrazol and prohexadione, did not retard the shoot growth of the weed at 4000 g/ha, 30 days after treatment. In contrast, prohexadione, which blocks the 2β- and 3β-hydroxylation of GAs,[15,16] slightly promoted shoot elongation. The presence of two biotypes of *S. pygmaea* was reported.[17] The biotype used in our study may be the dwarf type with low levels of endogenous GAs, and consequently GA biosynthetic inhibitors affected its shoot growth, but to a lesser extent. Hisamatsu *et al.* suggested that prohexadione promoted stem elongation and flowering in stock (*Matthiola incana*) by inhibiting 2β-hydroxylation of GAs.[18] Prohexadione is a stronger inhibitor of 2β-hydroxylation than 3β-hydroxylation in *S. pygmaea*, and thus biologically active 3β-hydroxy-GAs would persist longer, resulting in the promotion of shoot growth. In addition, rapid degradation of prohexadione in soil[19] would allow rapid restoration of GA biosynthesis.

The shoot growth and flowering of *S. pygmaea* appeared to be influenced by auxin and ethylene as well as GAs. Complex cross-talk among these plant hormones occurs in plants and thus the observed effects of auxin and ethylene may be the consequence of interactions among them and with other plant hormones, including GAs.

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英文編掲載報文・短報等の要旨

報　文

2,6-ジイソプロピルフェノキシ酢酸（DIPA）と植物成長調節剤が多年生水田雑草ウリカワ（*Saggitaria pygmaea*）の成長と開花に与える相互作用

吉村　巧，倉持仁志，米山弘一，桑原重文

2,6-ジイソプロピルフェノキシ酢酸（DIPA）がジベレリン（GAs）と同様に多年生水田雑草ウリカワ（*Saggitaria pygmaea*）の成長と開花を促進することを見出した．本論文では，2,4-D, S-アブシジン酸（S-ABA），エテホン，ベンジルアデニン（BA），GA 生合成阻害剤（パクロブトラゾールとプロヘキサジオン）などの植物ホルモン様活性を有する植物成長調節剤が，単独または DIPA と混合して処理することで，ウリカワの実生の成長と開花に対してどのような影響を及ぼすかを調べた．2,4-D は，ウリカワの生育を阻害するのと同時に葉数を増加させ，DIPA によって誘導される実生の伸長を減少させた．エテホンは，雑草の生育を促進させた．ウリカワの開花に対しても，2,4-D は DIPA による促進効果を抑制した．対照的にエテホンはわずかに促進効果を高めた．これらの結果は，GAs に加えてオーキシンとエチレンも，ウリカワの実生成長と開花に何らかの役割を果たしていることを示している．