**Short Communication**

**Promotion of Flowering in *Sagittaria pygmaea* Miq. by 2,6-Diisopropylphenoxyacetic Acid**

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The flowering of *Sagittaria pygmaea* Miq. was promoted by 2,6-diisopropylphenoxyacetic acid, as well as by gibberellin (GA$_3$). Uniconazole canceled the promotive effect of the phenoxyacetic acid, while prohexadione shortened the period required for flowering. Endogenous GAs seem to play an important role in the flowering of *S. pygmaea*, and 2,6-diisopropylphenoxyacetic acid might affect GA biosynthesis or metabolism.

**Key words:** flowering; gibberellin; 2,6-diisopropylphenoxyacetic acid; *Sagittaria pygmaea* Miq.

*Sagittaria pygmaea* Miq. is one of the troublesome perennial paddy weeds in Japan. Under paddy conditions, the weed begins to produce flowers and tubers 50-60 days after planting, irrespective of the planting date. Therefore, the flowering of *S. pygmaea* was found to depend not on day-length but on other factors. Gibberellin (GA$_3$) has been reported to promote flowering in *S. pygmaea* and in two cultivars of Chinese arrowhead (*S. trifolia*), suggesting that endogenous GAs played an important role in the floral induction of *Sagittaria* sp.

In the course of our search for herbicidal compounds, some phenoxyalkanoic acid derivatives were found to promote flowering in *S. pygmaea*. Among these compounds, 2,6-diisopropylphenoxyacetic acid was one of the most potent derivatives for promoting flowering of the weed, while this compound was not herbicidally active.

In this paper, we describe the promotive effect of 2,6-diisopropylphenoxyacetic acid on the flowering of *S. pygmaea*, together with the promotive effect of AC-94377 [1-(4-chloro-1,3-dihydro-1,3-dioxo-2H-isooindol-2-yl)cyclohexanecarboxamide, a synthetic compound showing GA-like activity in various biological assays] (Fig. 1). In addition, the interaction on flowering between the phenoxyacetic acid and two types of GA biosynthetic inhibitors, uniconazole [(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol] and prohexadione (4-ethoxycarbonyl-3-hydroxy-5-oxo-3-cyclohexenecarboxylic acid), is reported.

Plastic containers (30 × 35 × 15 cm) were filled with paddy soil and excessively watered to create paddy conditions. Twenty-eight tubers of *S. pygmaea* Miq. of uniform size (0.07–0.08 g) that had been germinated in an incubator at 30°C in the dark for 24 h were transplanted in each plastic container to a depth of 2 cm. An aqueous solution of each compound was added to the irrigation water on the following day, and the containers were placed in a greenhouse maintained at 25–30°C under natural daylight conditions. The depth of the flooding water was maintained at 3 cm throughout the experiments. The number of flowering plants was counted on 30, 35, 40, 45, and 50 days after treatment. The experiments were repeated three times with two replications. In these greenhouse experiments, the weed produced flowers all the year round. However, in particular, the period required for flowering varied slightly with temperature; i.e., higher temperatures shortened the period. Therefore, representative results are shown in the figures.

Figure 2 shows the effects of 2,6-diisopropylphenoxyacetic acid, GA$_3$, and AC-94377, each applied at a rate of 1 kg/ha, on the flowering of *S. pygmaea*. In the treated plots, more than 90% of the plants produced inflorescence at the 6- to 7-leaf stage 50 days after treatment, while this was apparent in less than 10% of the untreated plants. It should be noted that simple phenoxyacetic acid was effective in promoting the flowering of the weed, while the compound...
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Fig. 3. Effects of Uniconazole and Prohexadione on the Flowering of *S. pygmaea*, and on the Flowering Promoted by 2,6-Disopropylphenoxyacetic Acid.

- 2,6-disopropylphenoxyacetic acid (1 kg ha⁻¹)
- uniconazole (0.5 kg ha⁻¹)
- uniconazole (0.5 kg ha⁻¹) + 2,6-disopropylphenoxyacetic acid (1 kg ha⁻¹)
- prohexadione (2 kg ha⁻¹)
- prohexadione (2 kg ha⁻¹) + 2,6-disopropylphenoxyacetic acid (1 kg ha⁻¹)

was essentially inactive in various bioassays typical for GAs; it showed very weak activity in an a-amylase induction test, being 1/10000 as active as GA₃ (S. Yoshida, unpublished results). We also confirmed that AC-94377 had a promotive effect similar to that of GA₃ on the flowering of *S. pygmaea*. Each of these substances also promoted shoot growth in the weed, the means of the sixth leaf length in the plots treated with the phenoxyacetic acid, GA₃, and AC-94377 being 161% (9.03 ± 0.03 cm), 190% (10.63 ± 0.07 cm) and 171% (9.60 ± 0.06 cm) that of the control (5.60 ± 0.06 cm), respectively.

As shown in Fig. 3, the two inhibitors of GA biosynthesis having different sites of action prevented flowering when applied alone. However, they showed different effects on flowering promoted by the phenoxyacetic acid. Uniconazole, an inhibitor of the oxidation of ent-kaurene, ent-kaurenol and ent-kaurenal⁷,⁸ canceled the promotive effect induced by the phenoxyacetic acid. In contrast, prohexadione, which blocks the 2β- and 3β-hydroxylation of GAs,⁹,¹⁰ shortened the period required for flowering.

Although endogenous GAs in *S. pygmaea* and the effects of other GAs on flowering have not yet been examined, these results suggest that 2,6-disopropylphenoxyacetic acid itself may act like GAs in the weed, but not in other plants, or that phenoxyacetic acid may affect GA biosynthesis or metabolism of the weed. In addition, partial recovery from the uniconazole inhibition of flowering by the phenoxyacetic acid may be interpreted as the phenoxyacetic acid acting as an antagonist against uniconazole.

Studies on the structure-activity relationship in the promotion of flowering by substituted phenoxyalkanoic acid derivatives and on the characterization of endogenous GAs in *S. pygmaea* are in progress.

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References