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The effects of jasmonic acid and ethylenediamine-di--o-hydroxyphenyl-acetic acid on floral induction and induction of turions in *Spirodela polyrhiza* (L.) Schleiden

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We investigated the effects of jasmonic acid (JA) and ethylenediamine-di-o-hydroxyphenyl-acetic acid (EDDHA) on floral induction and induction of turions in the photoperiodically neutral clone Djelekovec of the species *Spirodela polyrhiza* in axenical cultures. JA (0.475–47.5 nmol·L^{-1}) promoted floral induction under long-day (LD) and short-day (SD) conditions, while it had no effect on turion induction. The inhibitory effect of JA on flowering and turion induction was observed at a JA concentration of 237.5 nmol·L^{-1} and 475 nmol·L^{-1}. Under the same conditions, flowering and induction of turions were enhanced by EDDHA (20.5 µmol·L^{-1}). The combination of EDDHA (20.5 µmol·L^{-1}) and JA (47.5 nmol·L^{-1}) had an additive effect on the promotion of floral induction, which was promoted significantly in experiments with LD preculture. The results obtained by quantitative determination of endogenous JA levels in *Spirodela polyrhiza* at two growth stages support our previous findings that JA may regulate floral induction. The levels of endogenous JA decreased from 226±12 ng·g^{-1} fresh weight during the vegetative stage in LD to 38.1±3.5 ng·g^{-1} in the flowering plants. In SD the levels of endogenous JA decreased from 62±9 ng·g^{-1} fresh weight during vegetative stage to 29.2±3.1 ng·g^{-1} in the flowering plants.

Keywords: *Spirodela polyrhiza*, chelating, EDDHA, flowering, turion, jasmonic acid

Introduction

The first report on the effects of JA on flowering was published for *Nicotiana tabacum* cv. Samsun (BARENDSE et al. 1985). JA (10^{-7} to 10^{-5} mol L^{-1}) was shown to inhibit bud initiation. Inhibitory effects of jasmonates on flowering were also described by MACIEJEWSKA et al. (2004) in the SD plant *Pharbitis nil* and by HONDA et al. (2006) who reported that methyl jasmonate (MJA) at a concentration of 10 ppm inhibited anther extrusion in barley ear cultures. On the other hand, it was shown that JA in low concentrations promoted flowering in *S. polyrhiza* (clone Velika Polana) and in *Lemna minor* (KRAJNČIČ and NEMEC 1995, KRAJNČIČ et al. 2006) so it might be expected that the effects of JA are different in different

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plants. JA might also be expected to play a role in the formation of flowers due to the relatively high levels of it in developing plant reproductive tissues (CREELMAN and MULLET 1997).

The chelating agent EDDHA is a strong stimulator of floral induction in several species of *Lemnaceae* (LANDOLT and KANDELER 1987, KRAJNČIČ and SLEKOVEC-GOLOB 1991, KRAJNČIČ et al. 1998). Its stimulatory effects on floral induction occur especially through chelating Zn, Mn and Cu and through eliminating the antagonism between Mn and Zn chelates by EDDHA (KRAJNČIČ and NEMEC 2003). EDDHA enhances the functioning of plant hormones (PIETERSE and MUELLER 1977, KRAJNČIČ 1985, KRAJNČIČ 1994) and so it was included in the present study.

Formation of turions in *S. polyrhiza* is known to be affected by environmental factors such as temperature, phosphate, nitrate, sulphate, sugars and light intensity (LANDOLT 1986, APPENROTH 2002). Moreover, the application of abscisic acid (ABA) at low physiological concentrations (100 nmol L^{-1}) induced turion formation in *S. polyrhiza* (SMART et al. 1995).

The aim of the present paper was to investigate possible clonal variations of the effects of JA on flowering in the day-neutral species *S. polyrhiza*. For this reason the clone Djelekovec (Croatia) was used in the present study and results were compared with those obtained for the clone Velika Polana (Slovenia). In addition the effects of the EDDHA and the combination of JA and EDDHA on flowering and induction of turions in *S. polyrhiza* were investigated. Quantitative determination of endogenous JA levels in the test plants during the vegetative stage and the macroscopically visible flowering stage was also performed.

Materials and methods

Experiments were conducted with axenic cultures of the species *S. polyrhiza* (clone Djelekovec), which is a photoperiodically neutral plant (KRAJNČIČ and DEVIDÉ 1982). The axenic cultures were prepared as described in KRAJNČIČ and DEVIDÉ (1980). The nutrient solution was prepared as previously described (KRAJNČIČ et al. 1995).

We dissolved (±) JA (Apex Organics, UK) and EDDHA (Fluka AG, Buch SG, Switzerland) in 0.1 mol L^{-1} KOH and added to the nutrient solution before or after sterilisation of nutrients. Transplantation was carried out just before or at the onset of the dark period. The experiments were carried out under controlled conditions in growth chambers as described in KRAJNČIČ et al. (1998).

Three groups of experiments were carried out. In the first group, JA concentration ranged between 0 and 475 nmol L^{-1} (0, 0.475, 4.75, 47.5, 237.5 and 475 nmol L^{-1}) under LD and SD conditions with LD (group A experiments) and SD preculture (group B experiments). In the second group of experiments, the combination of EDDHA at the concentration of 20.5 μmol L^{-1} with JA at a concentration 47.5 nmol L^{-1} in group A and 0.475 nmol L^{-1} in group B of experiments also under LD and SD conditions was added to the nutrient solution. EDDHA was added to the nutrient solution at the concentration of 20.5 μmol L^{-1}, because our previous investigations showed that this concentration promoted flowering optimally (KRAJNČIČ and NEMEC 2003).

Each experimental group consisted of three replicates. For each control and for the addition of each substance or combination at a specific photoperiod, three Erlenmayer flasks, each containing 200 mL of the nutrient solution, were used (n=9). Two plants were transferred into the nutrient solution of each Erlenmayer flask. We obtained 2000–3000 *S. polyrhiza* plants from two initial fronds in 1 month. The number of flowering fronds and turions was determined for each replicate under the stereomicroscope. We counted the attached turions and the mature turions that detached from parent plants and sank to the flask bottom.

Statistical analyses of data were performed using one-way analysis of variance available in the statistical package SPSS version 7.5. Comparisons among means were performed using the LSD test. Statistical significance was set at a confidence level of at least 95%.

In the third group of experiments, the endogenous levels of JA in *S. polyrhiza* were determined during the vegetative and macroscopically visible flowering stages. In this case plants were grown in the nutrient solution without JA or EDDHA being added. Plants were harvested and washed with deionised water, frozen in liquid N_2 and ground in a mortar. The powder was stored at –80 °C. The procedure for sample preparation then followed the steps described by KRISTL et al. (2005), except for the Cleanup step 2, which was slightly modified and performed as follows: the eluate was dried under an N_2 stream and the residue was dissolved in 3 mL of diethyl ether: acetic acid (98/2, v/v). The sample was then passed through an anion-exchange aminopropyl SPE cartridge (500 mg/3 mL, Phenomenex, USA) followed by 7 mL of diethyl ether: acetic acid (98/2, v/v). Both eluates were collected and dried under a stream of N_2. The residue was dissolved in 1 mL methanol. An aliquot of this solution was then used for the determination of endogenous JA levels using the method as previously described (KRISTL et al. 2005).

Results

The results (Tab. 1) give the arithmetic means of the percentage of flowering plants and number of turions with standard errors (S.E.) of the means for different day lengths and different concentrations of JA. In lower concentrations, JA promotes floral induction under LD and SD conditions in the studied plants, but different pre-treatments of plants have a significant influence on the level of JA being optimal for the induction of flowering (47.5 nmol L^{-1} in group A of experiments and 0.475 nmol L^{-1} in group B of experiments). JA in a concentration of (237.5 nmol L^{-1}) inhibits floral induction, while a higher concentration of JA (475 nmol L^{-1}) prevents the floral induction in the same plants. The combination of JA (47.5 nmol L^{-1} in group A of the experiment or 0.475 nmol L^{-1} in group B of the experiment) and EDDHA (20.5 µmol L^{-1}) had an additive effect on the promotion of floral induction. Two colours photographs (Fig. 1, Fig. 2) show flowering plants of *S. polyrhiza* grown in the nutrient solution with JA.

From the results in Tab. 1 it is evident that more turions are formed in plants under LD conditions in group A than in group B of the experiments. JA at concentrations of 0.475–47.5 nmol L^{-1} had no effect on turion induction, while at higher JA concentration the induction of turions was inhibited. In group A under SD and LD conditions, the formation of turions was completely prevented at a JA concentration of 475 nmol L^{-1}. Turion forma-

Tab. 1. Flowering percentage and number of turions with standard errors of the mean of *S. polyrhiza* (Djelekovec) plants after treatment with JA and EDDHA under different concentrations and photoperiods.

Group	JA (nmol L^{-1})	EDDHA (μmol L^{-1})	Flowering percentage		Number of turions in	
			LD	SD	LD	SD
A	0.00	0	17.9±0.37	15.1±0.66	320±0.38	220±0.25
	0.47	0	19.7±0.66	15.7±0.66	320±0.38	220±0.25
	4.75	0	26.1±0.58	18.7±0.49	321±0.39	218±0.24
	47.50	0	33.9±0.66	27.5±0.81	325±0.41	226±0.26
	237.50	0	7.1±0.55	6.7±0.75	180±0.25	144±0.20
	475.00	0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
	0.00	20.5	38.1±0.65	25.1±0.71	336±0.58	246±0.59
	47.50	20.5	61.2±0.70	33.2±0.56	386±0.71	375±0.60
B	0.00	0	18.1±0.55	15.7±0.66	290±0.55	182±0.51
	0.47	0	29.7±0.73	32.2±0.55	288±0.54	180±0.52
	4.75	0	25.2±0.55	29.2±0.87	292±0.60	176±0.50
	47.50	0	20.65±0.84	20.7±0.81	296±0.60	184±0.53
	237.50	0	9.5±0.98	8.0±0.71	186±0.46	140±0.41
	475.00	0	0.0±0.00	0.0±0.00	84±0.45	72±0.40
	0.00	20.5	30.2±0.55	18.1±0.61	325±0.55	238±0.56
	0.47	20.5	48.1±0.56	30.2±0.55	356±0.56	364±0.57

Group A of experiments: Preculture of plants under LD (27 d) and culture of plants under LD (26 d) or SD (26 d).
Group B of experiments: Preculture of plants under SD (27 d) and culture of plants under LD (26 d) or SD (26 d).
LD = 16 h light and 8 h darkness,
SD = 8 h light and 16 h darkness
Flowering percentage = arithmetic means of percentage of flowering plants and standard errors of the means.
Number of turions = arithmetic means of turions number and standard errors of the means.

Fig. 1. A group of flowering plants of *Spirodela polyrhiza* (Djelekovec clone) (x2) with visible stamens and brownish coloured turions (above in the middle). The plants were grown in the nutrient solution with JA (47.5 nmol L^{-1}) in LD condition.

Fig. 2. A three-link colony of *Spirodela polyrhiza* (Djelekovec clone), with visible stamens, mother and daughter frond and a brownish coloured turion (in the middle). The plants were grown in the nutrient solution with JA (47.5 nmol L^{-1}) in LD condition.

tion was induced by EDDHA (20.5 μmol L^{-1}) and even more apparently by the combination of JA (47.5 nmol L^{-1} in group A or 0.475 nmol L^{-1} in group B) and EDDHA (20.5 μmol L^{-1}).

In the third group of experiments the endogenous levels of JA in *S. polyrhiza* (Djelekovec clone) were determined (Tab. 2). The endogenous levels of JA decreased from 226±12 ng g^{-1} fresh weight (FW) of *S. polyrhiza* during the vegetative stage in LD (12^{th} day after inoculation of the plant in the nutrient solution) to 38.1±3.5 ng g^{-1} FW in flowering plants (30^{th} day after inoculation). In SD the endogenous levels of JA decreased from 62±9 ng g^{-1} FW during the vegetative stage (12^{th} day after inoculation) to 29.2±3.1 ng g^{-1} FW in the flowering plants (30^{th} day after inoculation). The endogenous level of JA was significantly higher in plants under LD conditions than in plants under SD conditions, especially at the vegetative stage.

Tab. 2. Endogenous levels of JA in *S. polyrhiza* at different growth stages. The results are given in ng g^{-1} FW±standard deviation of duplicate analyses.

Growth stage	Endogenous JA content (ng g^{-1}FW)	
	LD/LD	SD/SD
Vegetative stage (12^{th} day after inoculation in nutrient solution)	226±12	62±9
Flowering plants (30^{th} day after inoculation in nutrient solution)	38.1±3.5	29.2±3.1

LD/LD: preculture of plants under LD and culture of plants under LD conditions.
SD/SD: preculture of plants under SD and culture of plants under SD conditions.

Discussion

The comparison of the results reported by KRAJNČIČ and NEMEC (1995) for the photoperiodically neutral clone Velika Polana (Slovenia) of the species *S. polyrhiza* with those obtained in the present study (Tab. 1) for the Djelekovec clone (North Croatia) shows that there are no clonal differences regarding the effect of JA on flowering. The inhibitory effect of higher concentrations of JA (237.5 and 475 nmol L^{-1}) on the flowering of these two clones corresponds with the results reported by BARENDSE et al. (1985) obtained in thin--layer explants of tobacco (*N. tabacum* cv. Samsun), by ALBRECHTOVÁ and ULLMANN (1994) in plant *Chenopodium rubrum*, by MACIEJEWSKA et al. (2004) in the SD plant *Pharbitis nil* and with the results reported by KRAJNČIČ et al. (2006) obtained in the LD plant *Lemna minor.* In this study the effects of EDDHA and the combination of JA and EDDHA on flowering were studied in this species for the first time. The percentage of flowering plants was significantly higher using the combination of JA (47.5 nmol JA L^{-1} in group A of the experiments or 0.475 nmol JA L^{-1} in group B of the experiments) and EDDHA (20.5 μmol L^{-1})than the percentage of flowering plants using JA and EDDHA separately. An additive effect of those two substances on the promotion of floral induction was observed with LD preculture (group A of experiments under LD and SD conditions), while with SD preculture (group B of experiments) it was observed only under LD conditions. The results obtained from all our investigations show that JA, EDDHA or the combination of JA and EDDHA promote floral induction in other species of *Lemnaceae* from various groups according to their photoperiodic response.

To our knowledge quantitative determinations of endogenous JA levels were performed in photoperiodically neutral plants *S. polyrhiza* in LD and SD conditions in the vegetative stage (12^{th}day after inoculation of the plants in nutrient solution) and in flowering plants (30^{th} day after inoculation in nutrient solution) for the first time. The endogenous levels of JA decreased from 226±12 ng g^{-1} FW during the vegetative stage in LD to 38.1±3.5 ng g^{-1} FW in the flowering plants. In SD the endogenous levels of JA decreased from 62±9 ng g^{-1} FW during vegetative stage to 29.2±3.1 ng g^{-1} FW in the flowering plants. The decreasing levels of endogenous JA in *S. polyrhiza* correspond to a great extent with the results obtained for *Lemna minor* (KRAJNČIČ et al. 2006). For both species JA was measured in whole plants. NAGPAL et al. (2005) measured the quantities of endogenous JA levels in *A. thaliana* in flower closed buds, opening buds and in open flowers. The experimental results for *L. minor* (KRAJNČIČ et al. 2006), *S. polyrhiza* (described in this paper) and for *A. thaliana* (NAGPAL et al. 2005) showed decreasing levels of endogenous JA at the stage of maximum flowering.

The results obtained in this study show that JA at low concentrations (0.475–47.5 nmol L^{-1}) has no effect on turion induction (Tab. 1). At higher JA concentrations (237.5–475 nmol L^{-1}), turion induction was inhibited or prevented. On the other hand turion formation is significantly induced by EDDHA (20.5 μmol L^{-1}) and even more apparently by the combination of JA and EDDHA (Tab. 1). Regarding the mechanism of EDDHA in turion induction, no data were found in the literature. We think that among other possible causes for its stimulating effect, EDDHA possibly decreases the level of endogenous cytokinin (SCHARFETTER et al. 1986), which, when added to the nutrient solution, suppresses turion formation (CHALOUPKOVÁ and SMART 1994). The number of turions is significantly higher in plants growing under LD conditions for all treatments (Tab. 1). As shown

previously, for the clone SJ (APPENROTH et al. 1990) the increase of the number of turions depends almost linearly on the daily light period. There is also no critical day length in the case of turion formation under autotrophic conditions (APPENROTH 2003), which is crucial for the existence of photoperiodic phenomena. A significantly higher number of turions under LD conditions may be ascribed to the extended period of photosynthesis rather than to the photoperiodic LD response.

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