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Changes in propagule formation and plant growth in *Potamogeton crispus* induced by exogenous application of gibberellic acid (GA_3) and 6-benzyladenine (6-BA)

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ABSTRACT: Submerged aquatic macrophytes such as *Potamogeton crispus* can absorb large quantities of nutrients from sediment and water, and therefore mitigate pollution and improve water quality of polluted lakes. Turions are the main propagules of *P. crispus*; their formation is an important process in the life cycle of the plant and relates closely to the population dynamics of the species in lakes. The clarification of the physiological mechanisms specific to plant hormone regulation in propagule formation versus plant growth will help to better understand the population decline in eutrophic waters and to develop management strategies for the species. In the present study, different treatment concentrations and application frequencies of gibberellic acid (GA_3) and 6-benzyladenine (6-BA), a cytokinin substances, were used to investigate their effects on propagule formation and plant growth in this plant. The results showed that, when a cultured plant was treated with GA_3 or 6-BA before propagule morphogenesis, propagule production was inhibited or delayed. Under repeat applications of GA_3 or 6-BA, propagule formation was arrested completely except in the treatment with 2.5 mg l^{-1} GA_3, which led to some phylloclade turions. Plants treated with GA_3 had more phylloclade turions and longer turions compared to the control, whereas less difference was observed under 6-BA treatments. This suggests that cytokinin is a more effective substance in regulating propagule differentiation in *P. crispus*. Morphological and physiological analysis showed that the propagule formation was more sensitive than plant growth to the plant growth regulators. Both plant growth regulators promoted photosynthetic pigments in plants but inhibited starch accumulation in propagules, which may be related closely to the changes in propagule growth and development.

KEY WORDS: Turion · Cytokinin · Gibberellin · Carbohydrate accumulation · Physiological characteristics

INTRODUCTION

Degradation in shallow lakes and other open wetlands begins with the loss of submerged aquatic vegetation due to turbidity and shading, in which the excessive nutrient loads may lead to high algal biomass and loss of biodiversity (Chow-Fraser 1998). Submerged macrophytes can absorb large quantities of pollutants, such as nitrogen, phosphorus and metals from water and sediment (Ali et al. 1999). Friedrich et al. (2003) found that, on average, >76% of nitrogen and phosphorus discharged into the

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Danube Delta lakes was taken up by submerged plants and phytoplankton during their growing seasons, and they proposed harvesting submerged and floating biomass to alleviate water pollution. Planting of submerged macrophytes can be an effective way to reduce nutrient content and re-suspension of sediment in water bodies (Hansson et al. 1998) and to repair eutrophication (Hupfer & Dollan 2003).

Potamogeton crispus is a widespread submerged aquatic plant that grows over winter and generates turions as vegetative propagules in summer in temperate and subtropical zones (Catling & Dobson 1985). Because of its strong adaptability to polluted water (Tobiessen & Snow 1984), *P. crispus* has been widely used as a pioneer species in re-vegetating degraded lakes in China (Wu et al. 2009). Although the plant can occasionally produce seeds (Chen et al. 2006), seed germination is extremely low (0.001%) (Rogers & Breen 1980). Therefore, turion formation is a major channel for the species to propagate and spread and has a key impact on its population dynamics.

The *Potamogeton crispus* turion is an abnormal shoot apex with a compressed stem and leaf scales and differentiates from a shoot apical meristem (SAM). Leaf scale, which originates from leaf but has a smaller area and harder texture, is highly enriched with a nonstructural carbohydrate reserve (mainly starch) (Sastroutomo et al. 1979). There are 2 morphological types of turions in *P. crispus*: standard turion and phylloclade turion. The standard turion usually grows from the apex of a stem or a branch, and is considerably larger than the phylloclade turion, which usually grows from leaf axils. A larger propagule is hypothesized to support the higher competitive ability of young seedling(s) because of a larger starch reserve during propagule germination in aquatic plants (Grace 1985, Spencer & Rejmanek 1989).

The carbohydrate reserve in turions is derived from photosynthetic assimilation in the plant. Therefore, plant physiological characteristics such as photosynthetic strength, chlorophyll content, and carbon and nitrogen metabolisms, particularly at the stem apices where turions form, may have impacts on the reserve accumulation in turions. Spencer & Anderson (1987) observed degradations in chlorophyll *a* (chl *a*) and carotenoids in leaves when propagule formation was induced and plant senescence initiated under short photoperiods in *Potamogeton nodosus*. Woolf & Madsen (2003) found that thick and robust plants that contain high levels of total nonstructural carbohydrates tend to produce larger turions in *P. crispus* in lakes. Huang et al. (2010) reported that an increase in turion weight coincided with increases in leaf photosynthetic efficiency and total biomass in *P. crispus* under higher nitrogen and phosphorus availability in sediment within a certain range. James (2007) found that plant growth, turion development, and carbohydrate accumulation in turions were simultaneously suppressed by the application of lime to *P. crispus*, due to lower photosynthesis in leaves resulting from the lime-induced dissolved inorganic carbon limitation in the water.

Many aquatic plants produce vegetative propagules such as turions and tubers in order to survive unfavorable conditions and to ensure vegetative reproduction (Sculthorpe 1967). Under natural conditions, turions of *Potamogeton crispus* start to form when light, temperature, and day length increase in summer (Kunii 1989). Chambers et al. (1985) showed that turion formation was triggered by long photoperiods ($\geq$16 h d^{-1}) and a certain level of light intensity, when the ratio of red light to far-red light was >1. Sastroutomo (1980) found a combined effect of temperature and photoperiod in regulating turion formation in *P. crispus*. Turion initiation was induced under a daily mean air temperature range of 13–24°C on both 12 and 16 h days, and 24–35°C on 8 h days.

Propagule production has been found to be influenced by plant regulation substances in species other than *Potamogeton crispus*. Abscisic acid (ABA) has been reported to induce propagule formation in *Hydrilla verticillata* (Van et al. 1978, Klaine & Ward 1984) and *Myriophyllum verticillatum* (Weber & Nooden 1976), 2 thermophilic submerged species. Klaine & Ward (1984) reported that ethylene (applied as ethephon at 2 d intervals) reduced turion production in *H. verticillata* by 80%. However, there is little information on the function of plant hormones in propagule production in *P. crispus*.

Different nitrogen and phosphorus supplies can induce rapid changes in plant hormone metabolisms and subsequently result in morphological alterations in various organs of a plant (Franco-Zorrilla et al. 2005, Sakakibara et al. 2006). Water eutraphication, mainly due to high nitrogen and phosphorus discharge from poorly managed catchments to freshwater systems such as lakes or reservoirs, has become a big problem in China in the past decades. Furthermore, plant growth regulators such as gibberellin- and cytokinin-type substances have been extensively applied in fruit, melon, and other horticultural crops. These regulators may discharge into water bodies when they are used inappropriately. Jana & Choudhuri (1983) found that kinetin, a cytokinin-type substance, could delay leaf senescence and promote plants to take up more phosphorus from water

and sediment when applied at a concentration of 50 mg l^{-1} in *Potamogeton pectinatus*, *Vallisneria spiralis*, and *Hydrilla verticillata*. Sastroutomo (1981) reported that gibberellic acid (GA_3) could fully break turion dormancy and promote shoot elongation in light when applied both at 21.5 and 215 mg l^{-1} in *P. crispus*. However, very little is known about the roles of these substances, if any, on propagule formation of aquatic plants.

In the present study, we compared the effects of GA_3, a gibberellin, and 6-benzyladenine (6-BA), a cytokinin, on turion formation and plant growth in *Potamogeton crispus*. The objectives were to understand the regulation of plant hormones on plant growth and development and to develop management strategies for the species, which plays a significant role in pollution control in lakes.

MATERIALS AND METHODS

Sampling and incubation

A local ecotype of *Potamogeton crispus* was collected from a pond near Lake Nanhu (30° 28′ 20″ N, 114° 21′ 49″ E) in Wuhan, Central China on 22 March 2008. Plant materials were kept in buckets filled with water, and immediately transported to a controlled culture experimental field with a movable transparent rain-proof shelter installed about 2.5 m above ground. After the materials were gently cleaned from epiphytes with tap water, apical stems of about 7 cm in length without root were cut from individual plants, and 10 apical stems were then transplanted into each bucket (16 cm in diameter and 22 cm in depth), using acid-rinsed quartz sand as matrix. The incubators were filled with 10% nitrogen- and phosphorus-free Hoagland solution, and the nitrogen concentration was subsequently adjusted to 0.25 mg l^{-1} with NH_4NO_3, and phosphorus concentration to 0.025 mg l^{-1} with NaH_2PO_4. The materials were cultured for 6 d before the treatments were conducted.

Treatment

A multi-factorial (2 regulators × 2 concentrations × 2 applications) design with 3 replicates was used. Cultured plants were treated with a solution containing a low (2.5 mg l^{-1}) or high (25 mg l^{-1}) concentration of GA_3 or 6-BA on 28 March 2008. The 2 application methods were either a single treatment for 4 h on the first day only, or repeat treatments every 3 d for 4 h each time (10 times in total until trial termination on 30 April 2008). The treatment combinations were: (1) GA_3 + low concentration + single application (GALS); (2) GA_3 + high concentration + single application (GAHS); (3) GA_3 + low concentration + repeat applications (GALR); (4) GA_3 + high concentration + repeat applications (GAHR); (5) 6-BA + low concentration + single application (BALS); (6) 6-BA + high concentration + single application (BAHS); (7) 6-BA + low concentration + repeat applications (BALR); and (8) 6-BA + high concentration + repeat applications (BAHR). After the regulator treatments, plants were rinsed immediately and then cultured in regulator-free incubation solution. In addition, plants growing in regulator-free solution were used as a control. Incubation solutions were replaced every 3 d for maintaining the nutrient concentrations and suppressing algal growth throughout the experiment. For repeat applications, the treatments were re-applied prior to the incubation-solution change each time.

The 27 buckets in total were arranged randomly in a completely randomized design. A rain-proof shelter was used to cover the buckets and prevent contamination and dilution of the solutions when rainfall occurred. During the course of the experiment, day length ranged from 12.29 to 13.52 h d^{-1}, midday photosynthetically active radiation ranged from 200 to 1000 µmol m^{-2} s^{-1}, and average daily mean air temperature was 17.1°C (ranging from 14.1 to 21.5°C).

Morphology

Turion formation on individual plants was closely observed daily during the trials. On 30 April 2008, when turions of the control plants were close to maturity (their color began to turn brown from green and the structure became hardened; Huang et al. 2010), plants of all treatments were harvested and initially rinsed with tap water, then cleaned with distilled water. Propagule production and plant growth were then measured, including the number of propagules, number of leaf scales, length and fresh weight of standard and phylloclade turions, plant height, number of nodes on the caulis (stem), length of branches (both primary and secondary branches), and fresh biomass of individual plants. Fresh samples were oven-dried at 105°C for 30 min initially and maintained at 80°C until constant dry weights were achieved to determine the dry biomass of the turions and plants (Yang et al. 2010).

Physiology

Chl *a*, chlorophyll *b* (chl *b*), and carotenoid (car) contents were analyzed in harvested fresh plants. The photosynthetic pigment was extracted from 0.2 g fresh sample from the top 6 nodes of stems in a test tube with 5 ml of 95% ethanol at 70°C in dark until it was totally discolored. Light absorbance of the extract was measured at 470, 649, and 665 nm. The contents (in mg g^{-1} fresh weight [FW]) of chl *a*, chl *b*, and car were calculated by the following formulae (Hu et al. 2007):

$$chl\ a = (13.95A_{665} - 6.88A_{649}) \times 10^{-3} \times V_t \times N/FW \quad (1)$$

$$chl\ b = (24.96A_{649} - 7.32A_{665}) \times 10^{-3} \times V_t \times N/FW \quad (2)$$

$$car = (4.08A_{470} - 3.31A_{665} - 11.64A_{649}) \times 10^{-3} \times V_t \times N/FW \quad (3)$$

where V_t and N represent total volume (ml) and dilution times of the extract, respectively, and A is the absorbance at a specific wavelength (470, 649, or 665 nm).

Soluble protein was extracted from 0.2 g dried powder of harvested plants in 25 ml distilled water, and followed by centrifugation at 1400 × *g* for 10 min. One ml of supernatant and 5 ml Folin-phenol A solution were added to a test tube and blended, and placed in 30°C for 10 min. Then 0.5 ml of Folin-phenol B solution was added and mixed immediately. This was kept at 30°C for 30 min, and then the soluble protein content was determined by colorimetric assay at 650 nm, using bovine serum albumin (BSA) as a standard (Lowry et al. 1951).

Soluble sugar, sucrose and starch contents were analyzed in harvested plants and turions using the anthrone method (Dreywood 1946, Yemm & Willis 1954). Dried powder (0.2 g) of samples was homogenized in 80% ethanol and then centrifuged at 800 × *g* for 10 min following incubation in an 80°C water bath for 30 min. The same extraction procedure was repeated 3 times and the supernatant amalgamated to make up to 100 ml. A volume of 0.2 ml supernatant and 1.8 ml distilled water were put into a test tube to determine soluble sugar. After reacting with 0.5 ml anthrone reagent and 5 ml sulfuric acid in boiling water for 1 min, light absorbance was measured at 630 nm. For sucrose analysis, 0.5 ml supernatant was put into a test tube together with 0.2 ml 30% KOH and 1.5 ml distilled water. After placement in boiling water for 10 min and then adding 0.5 ml anthrone reagent and 5 ml sulfuric acid, the same reaction procedure and colorimetric assay were conducted to measure soluble sugar. The reducing sugar content was calculated by subtracting the sum of sucrose from soluble sugar.

After sugar extraction, the supernatant was decanted and the sediment dried at 80°C for determining starch. Twenty ml of 1.12% HCl was added to hydrolyze the starch thoroughly, in a boiling water bath. The soluble sugar content was determined in the hydrolysate and starch content was calculated from the soluble sugar content (Dreywood 1946, Yemm & Willis 1954).

Statistical analyses

Data on propagule production and plant growth metrics of all treatments were analyzed using 3-way ANOVA, with plant growth regulator, treatment concentration, and frequency as the main factors. Prior to running the ANOVA, the data with non-normal distributions were transformed as $x' = \sqrt{x + 1}$ for the turion number and biomass, and the numbers of primary and secondary branches. Duncan's multiple comparison was used to test the significance of differences at $\alpha = 0.05$. For the morphological and physiological metrics of propagules, differences were compared only between the treatments that produced propagules and the control. All data analyses were performed using SPSS 11.5.0.

RESULTS

Propagule production

Observations showed that turion formation in *Potamogeton crispus* was inhibited or delayed under GA_3 and 6-BA treatments compared to the control. Plants in the control began to produce turions on Day 13 of the experiment, 12 d earlier than those in the treatments GALS and BALS. Measurement at harvest indicated significant differences in turion production between treatments and control (Table 1). Turion formation was arrested completely in BALR, BAHR and GAHR. The average number of standard turions per plant was significantly ($p < 0.05$) lower for GALS and BAHS, and the average number of phylloclade turions per plant for GALS and the dry biomass of individual phylloclade turions for GALR were significantly ($p < 0.05$) higher than those for the control. But no significant differences ($p > 0.05$) were found between the remaining treatments that produced propagules and the control, in the average number of both types of turions per plant and/or dry biomass of individual turions (Table 1). The total number of propagules (standard and phylloclade turions) per

Table 1. *Potamogeton crispus*. Propagule growth under treatment with gibberellic acid (GA_3) or 6-benzyladenine (6-BA). Data are mean ± SE. Different superscripted letters indicate significant differences ($p < 0.05$) within a column. GALS: GA_3 + low concentration + single application; GALR: GA_3 + low concentration + repeat applications; GAHS: GA_3 + high concentration + single application; GAHR: GA_3 + high concentration + repeat applications; BALS: 6-BA + low concentration + single application; BALR: 6-BA + low concentration + repeat applications; BAHS: 6-BA + high concentration + single application; BAHR: 6-BA + high concentration + repeat applications. na: not applicable

Treatment	Standard turion				Phylloclade turion			
	Average no. per plant	No. of leaf scales	Length (cm)	Dry matter (mg turion^{-1})	Average no. per plant	No. of leaf scales	Length (cm)	Dry matter (mg turion^{-1})
GALS	0.3 ± 0.1^{b}	5.8 ± 0.2^{a}	3.1 ± 0.2^{a}	18.6 ± 3.8^{a}	3.4 ± 0.7^{a}	4.7 ± 0.1^{ab}	4.4 ± 0.1^{b}	17.0 ± 2.3^{ab}
GALR	0	na	na	na	1.0 ± 0.3^{b}	5.2 ± 0.2^{a}	5.8 ± 0.2^{a}	22.7 ± 2.7^{a}
GAHS	0	na	na	na	0.9 ± 0.6^{b}	3.5 ± 0.2^{d}	4.5 ± 0.3^{b}	12.0 ± 0.8^{b}
GAHR	0	na	na	na	0	na	na	na
BALS	3.2 ± 0.3^{a}	4.3 ± 0.2^{b}	2.5 ± 0.2^{b}	15.6 ± 2.7^{a}	0.7 ± 0.1^{b}	4.0 ± 0.3^{cd}	3.0 ± 0.4^{c}	17.7 ± 5.0^{ab}
BALR	0	na	na	na	0	na	na	na
BAHS	0.9 ± 0.3^{b}	4.1 ± 0.3^{b}	2.7 ± 0.2^{b}	14.9 ± 5.0^{a}	0	na	na	na
BAHR	0	na	na	na	0	na	na	na
Control	4.0 ± 0.5^{a}	4.4 ± 0.2^{b}	2.6 ± 0.1^{b}	22.4 ± 1.4^{a}	0.5 ± 0.3^{b}	4.8 ± 0.6^{abc}	3.2 ± 0.2^{c}	11.5 ± 3.8^{b}

plant was significantly ($p < 0.05$) lower in the regulator treatments than in the control except in treatments GALS and BALS.

Most of the turions that formed in the control treatment were standard turions and only 11.1% were phylloclade turions. In the GA_3 treatments that produced turions, however, >90% were phylloclade turions (Table 1). In the 6-BA treatment which produced both types of turions (BALS), there was no significant difference ($p > 0.05$) in the ratio of the 2 types of turions in comparison with the control.

Compared to the control, the lengths of both types of turions were significantly ($p < 0.05$) greater for GA_3 treatments; the number of leaf scales of standard turions was higher for GALS, but that of phylloclade turions was lower for GAHS (Table1). No significant difference was detected in the length or number of leaf scales of propagules between the 6-BA treatments and the control.

Dry biomass of standard turions and total propagules per plant was significantly ($p < 0.05$) lower for the regulator treatments that produced propagules than the control (Fig. 1A,C). However, dry biomass of phylloclade turions per plant was higher ($p < 0.05$) in GALS than in the control (Fig. 1B).

Plant growth

There was an overall trend of increase in plant height, the number and length of primary branches, and dry biomass of stems and leaves under GA_3 treatments, compared to the control (Table 2). The differences in all these parameters became significant ($p < 0.05$) between GALS and the control and between GAHR and the control. In GALR and GAHS, the length of primary branches and the dry biomass of stems and leaves (GALR only) were significantly ($p < 0.05$) greater than the control. There were significant ($p < 0.05$) decreases in the number of nodes on a caulis in GALR and GAHR but not in GALS and GAHS, in comparison with the control (Table 2). The number of nodes on a caulis and dry biomass of stems and leaves in GALS were significantly ($p < 0.05$) higher than those in GAHS. However, there were no significant ($p > 0.05$) differences in the metrics of plant growth between GALR and GAHR (Table 2).

There were greater variations in plant growth among 6-BA treatments. Compared to the control, significant ($p < 0.05$) decreases were observed in plant height, number of nodes on a caulis, and number of primary branches in BALR, BAHS, and BAHR, and in the length of primary branches and the dry biomass of stems and leaves in BAHR. However, there was a significant ($p < 0.05$) increase in the dry biomass of stems and leaves in BALS (Table 2). There was general decrease in the number of nodes on a caulis, the number and length of primary branches, and the dry biomass of stems and leaves with increasing concentration and frequency of application of 6-BA (Table 2). Some secondary branches were found in BAHS only.

Photosynthetic pigment content in plants

The contents of photosynthetic pigments in plants varied among treatments (Fig. 2). GA_3 and 6-BA treatments significantly increased ($p < 0.05$) the total chlorophyll and chl *a* in plants, except in the BALS

treatment (Fig. 2A). GA_3 promoted chl *b* content regardless of concentration and frequency of treatment. However, 6-BA promoted chl *b* only in BALR and BAHS treatments (Fig. 2A). Carotenoid content was significantly ($p < 0.05$) higher in repeat applications of both regulators, with the exception of GALR compared to the control (Fig. 2B).

Soluble protein content in plants

Soluble protein content in plants did not change significantly ($p > 0.05$) under both plant growth regulator treatments in comparison with the control, except for a significantly ($p < 0.05$) lower value detected in GAHR and higher values in BALR and BAHS (Fig. 3). For a single application of the same regulator, high-concentration treatment increased the soluble protein content significantly ($p < 0.05$), compared to the low concentration. For repeat applications, however, a significant ($p < 0.05$) difference was detected only in the 6-BA treatments, with BAHR plants showing lower soluble protein content than BALR plants (Fig. 3).

Carbohydrate content in plants and propagules

The contents of soluble sugar and sucrose in plants increased in BALR but decreased to various degrees in other treatments, in comparison with the control (Fig. 4A). The content of reducing sugars was significantly ($p < 0.05$) higher in the single application of both regulators and BALR than in the control (Fig. 4A). Starch content increased in GALR and GAHS, but

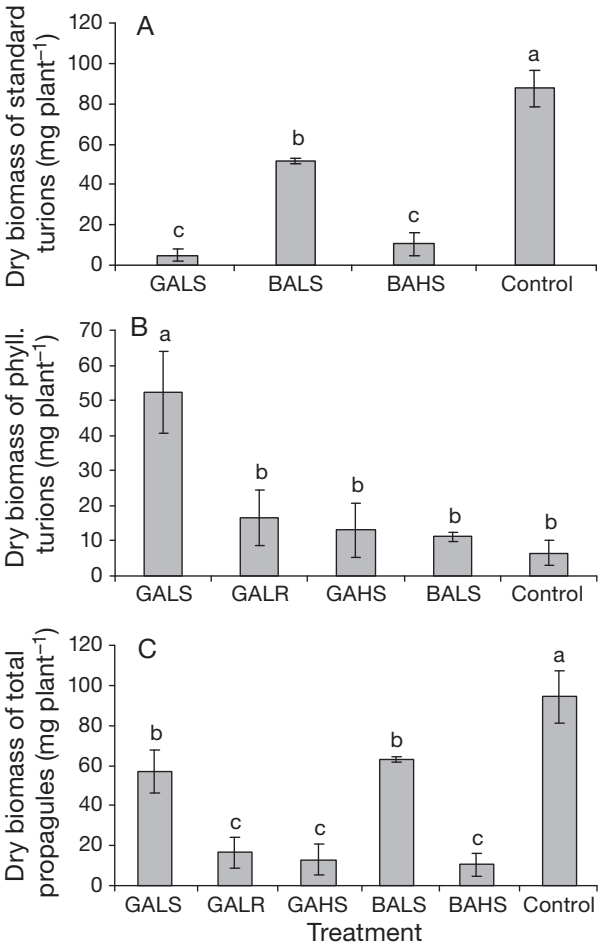

Fig. 1. *Potamogeton crispus*. Dry biomass per plant of (A) standard turions, (B) phylloclade turions, and (C) total propagules under treatment with gibberellic acid (GA_3) or 6-benzyladenine (6-BA). Different letters above SE bars indicate significant differences ($p < 0.05$) between treatment groups. See Table 1 caption for treatment abbreviations

Table 2. *Potamogeton crispus*. Morphological characteristics of plants under treatment with gibberellic acid (GA_3) or 6-benzyladenine (6-BA). Data are mean ± SE. Different superscripted letters indicate significant differences ($p < 0.05$) within a column. na: not applicable. See Table 1 caption for treatment abbreviations

Treatment	Plant height (cm)	No. of nodes on caulis	Primary branches		Secondary branches		Dry matter of stems and leaves (g plant^{-1})
			Average no. per plant	Average length (cm)	Average no. per plant	Average length (cm)	
GALS	41.0 ± 0.8^{a}	28.6 ± 1.6^{a}	10.6 ± 1.4^{a}	6.8 ± 0.8^{bc}	0	na	0.28 ± 0.05^{a}
GALR	36.8 ± 2.4^{ab}	20.3 ± 1.7^{de}	9.5 ± 0.9^{ab}	8.9 ± 1.7^{ab}	0	na	0.27 ± 0.02^{ab}
GAHS	36.8 ± 2.0^{ab}	24.5 ± 1.0^{bc}	8.8 ± 0.4^{ab}	6.5 ± 0.7^{bc}	0	na	0.21 ± 0.00^{bc}
GAHR	41.8 ± 2.9^{a}	22.4 ± 1.8^{cd}	10.1 ± 0.3^{a}	10.7 ± 0.3^{a}	0	na	0.29 ± 0.02^{a}
BALS	33.8 ± 1.2^{b}	29.6 ± 1.3^{a}	9.3 ± 1.2^{ab}	5.7 ± 1.1^{cd}	0	na	0.25 ± 0.02^{ab}
BALR	21.2 ± 1.3^{c}	15.7 ± 0.3^{f}	0.9 ± 0.2^{d}	3.2 ± 0.5^{e}	0	na	0.17 ± 0.01^{de}
BAHS	15.7 ± 1.0^{d}	18.3 ± 1.0^{ef}	4.2 ± 1.3^{c}	4.7 ± 0.3^{cde}	1.5 ± 0.5^{a}	3.0 ± 0.2^{a}	0.17 ± 0.02^{de}
BAHR	17.5 ± 0.8^{cd}	12.8 ± 0.3^{g}	0.3 ± 0.3^{d}	0.8 ± 0.8^{f}	0	na	0.11 ± 0.01^{e}
Control	33.1 ± 1.0^{b}	26.3 ± 0.7^{ab}	6.9 ± 0.6^{b}	4.2 ± 0.1^{de}	0	na	0.18 ± 0.00^{cd}

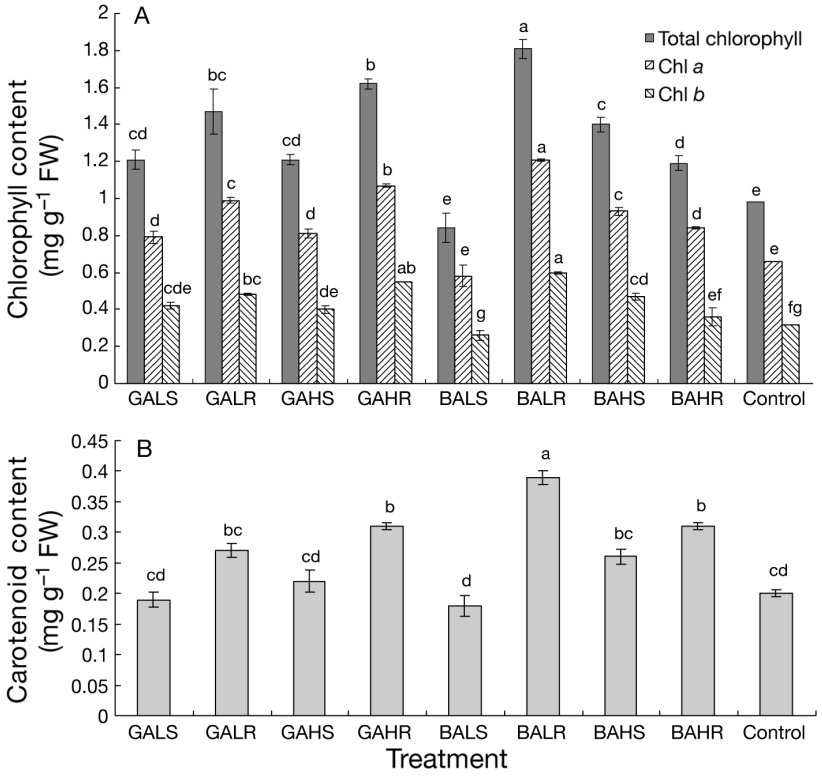

Fig. 2. *Potamogeton crispus*. Content of (A) total chlorophyll, chl *a*, and chl *b*, and (B) carotenoids in plants under treatment with gibberellic acid (GA_3) or 6-benzyladenine (6-BA). Different letters above SE bars of the same parameter indicate significant differences ($p < 0.05$) between treatment groups. FW: fresh weight. See Table 1 caption for treatment abbreviations

decreased for 6-BA applications except in BALR in comparison with the control (Fig. 4B). The contents of soluble sugar, sucrose, and starch increased significantly ($p < 0.05$) with the single-application treatments, but decreased significantly ($p < 0.05$) in repeat applications in the treatments with high concentration of the 2 regulators, compared to the low concentration. This also applied to the content of reducing sugars in repeat applications of 6-BA (Fig. 4).

In propagules, there were significant ($p < 0.05$) increases in the soluble sugar content for all treatments except for GALS, the sucrose content except for GALS and GALR, and the reducing sugar content except for GALS and BAHS (Fig. 5A), compared to control. The starch content in propagules under GAHS and BAHS decreased significantly, compared with the control (Fig. 5B).

Regarding carbohydrate accumulations in propagules per plant, the soluble sugar, sucrose, and reducing sugars decreased in all treatments except for BALS, which had significantly ($p < 0.05$) higher accumulation of sugars, and GALS, which had a similar level of reducing sugars, compared to the control (Fig. 6A). Starch accumulation in propagules per plant was significantly ($p < 0.05$) reduced in all regulator treatments (Fig. 6B). In the single-application treatments with the 2 regulators, carbohydrate accumulation in propagules per plant was significantly ($p < 0.05$) lower at high concentrations compared with low concentrations (Fig. 6).

DISCUSSION

Regulation of propagule production by plant hormones

Propagule production is a critical stage in the life cycle of submerged aquatic macrophytes, which depend on vegetative propagules for population dispersal, but the physiological mechanism in the regulation by plant hormones has not yet been clarified. In the present study, we found that propagule formation was inhibited by gibberellin- and cytokinin-type substances in *Potamogeton crispus*, suggesting the involvement of both substances in the regulation of propagule formation. Klaine (1986) reported that propagule formation was inhibited by the application of thidiazuron (N-phenyl-N′-1,2,3-thidiazol-5-ylurea), an ethylene stimulator and cotton defoliant with cytokinin-like activity (Mok et al. 1982, Genkov & Ivanova 1995), in *Hydrilla verticillata*. Our results revealed that cyto-

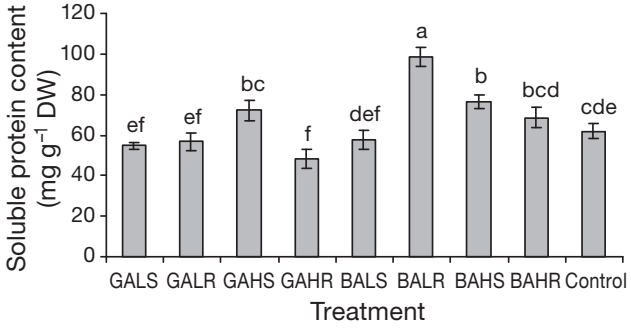

Fig. 3. *Potamogeton crispus*. Soluble protein content in plants under treatment with gibberellic acid (GA_3) or 6-benzyladenine (6-BA). Different letters above SE bars indicate significant differences ($p < 0.05$) between treatment groups. DW: dry weight. See Table 1 caption for treatment abbreviations

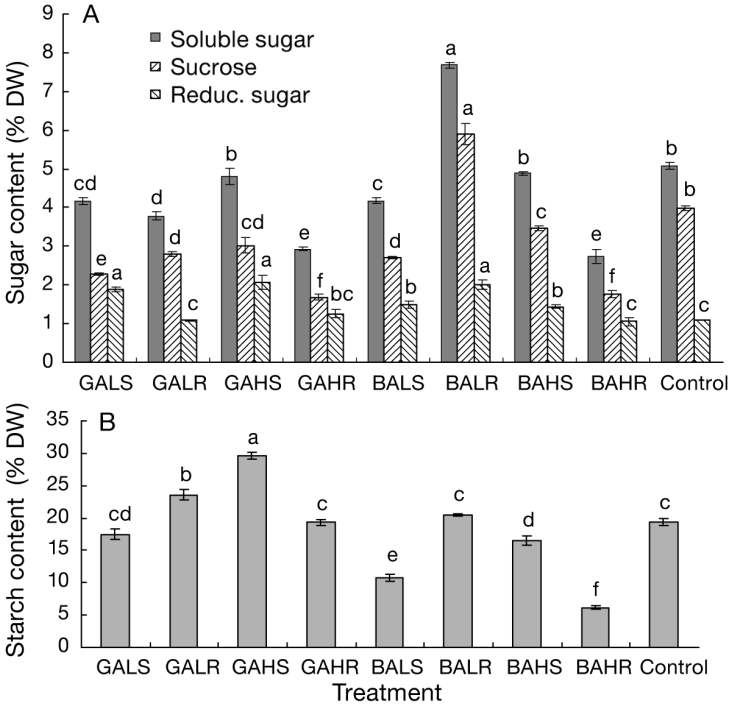

Fig. 4. *Potamogeton crispus*. Contents of (A) soluble sugar, sucrose, and reducing sugars, and (B) starch in plants under treatment with gibberellic acid (GA_3) or 6-benzyladenine (6-BA). Different letters above SE bars of the same parameter indicate significant differences ($p < 0.05$) between treatment groups. DW: dry weight. See Table 1 caption for treatment abbreviations

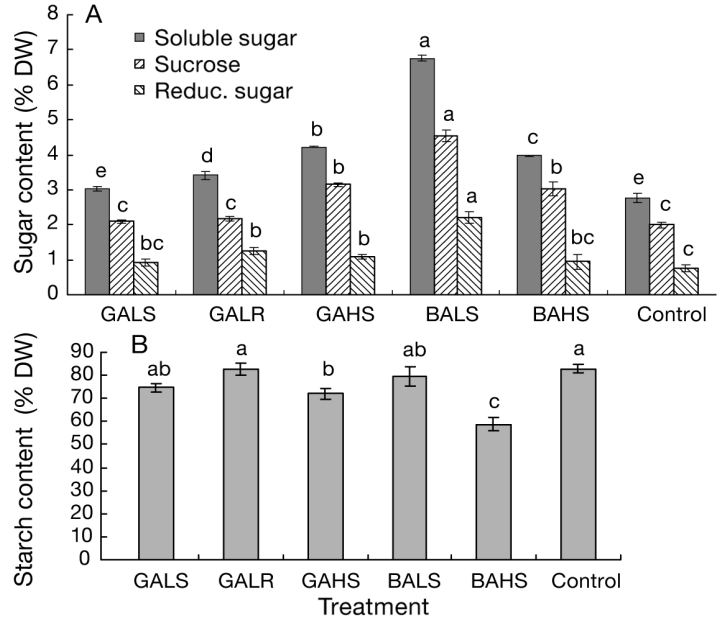

Fig. 5. *Potamogeton crispus*. Contents of (A) soluble sugar, sucrose, and reducing sugars, and (B) starch in propagules under treatment with gibberellic acid (GA_3) or 6-benzyladenine (6-BA). Different letters above SE bars of the same parameter indicate significant differences ($p < 0.05$) between treatment groups. DW: dry weight. See Table 1 caption for treatment abbreviations

kinin might be more effective than gibberellin in the regulation of propagule formation, because propagule formation was arrested completely by repeat applications of 6-BA at low concentrations, but not by GA_3 in *P. crispus*.

We also found that there was less morphological variation in propagules under 6-BA treatments than under GA_3 treatments compared to the control, probably due to differences in the physiological functions of the 2 regulators. Cytokinin may inhibit differentiation of propagules, but not affect their growth, while gibberellin inhibits both processes and induces some standard turions to develop into smaller phylloclade turions. Because larger turions in *Potamogeton crispus* show a stronger ability to spread (Shen et al. 2008), a higher rate of phylloclade turion production is unfavorable to this species' propagation in water.

Development and growth of plant organs are largely determined by the activities of SAM. Recent evidence has shown that plant hormones are involved in sensing and signaling extrinsic factors and controlling meristematic activity to mediate the morphological response of a plant in adapting to an environment (Werner & Schmülling 2009). It is known that nitrate rapidly induces cytokinin and gibberellin biosynthesis in plants (cited in Takei et al. 2001, Ohkama-Ohtsu & Wasaki 2010), indicating a link between nitrogen nutrition and these hormones. Based on our results that cytokinin and gibberellin altered the differentiation of SAM to propagules in *Potamogeton crispus*, it is important to establish the links between the metabolisms of endogenous cytokinin and gibberellin and the ambient nutrient status that coincides with decreased propagation in future studies. This would provide a deeper insight into its decline in eutrophic waters, and would be conducive to developing appropriate management methods.

Effects of plant growth regulators on plant growth

The response of propagule production and plant growth in aquatic plants to plant growth regulators may vary considerably (Smart & Trewavas 1983). In the present study, propa-

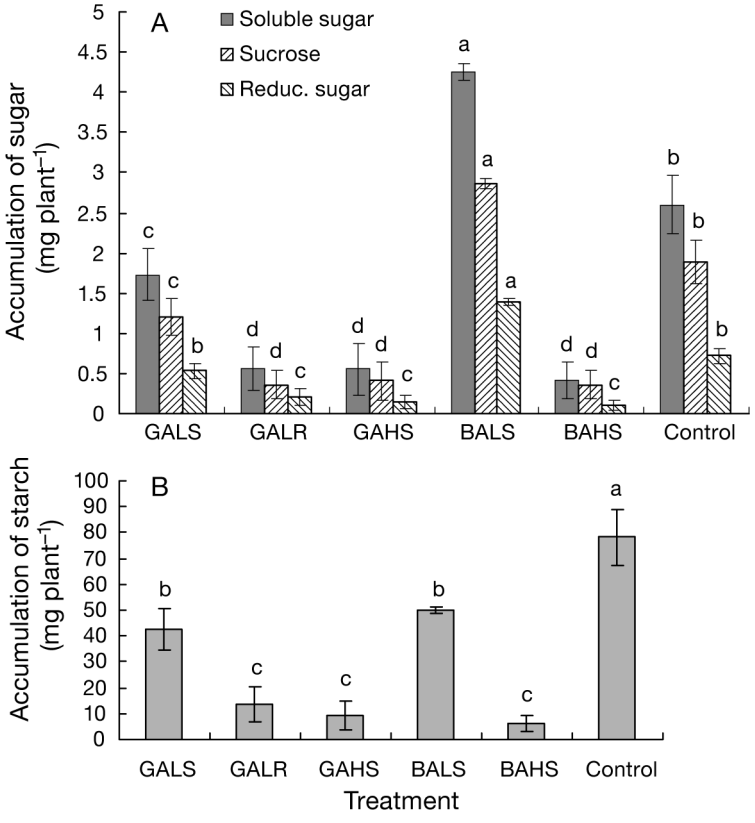

Fig. 6. *Potamogeton crispus*. Accumulation per plant of (A) soluble sugar, sucrose, and reducing sugars, and (B) starch in propagules under treatment with gibberellic acid (GA_3) or 6-benzyladenine (6-BA). Different letters above SE bars of the same parameter indicate significant differences ($p < 0.05$) between treatment groups. See Table 1 caption for treatment abbreviations

gule production was inhibited by GA_3 and 6-BA, but plant growth was generally not affected or in some cases it was even promoted, though inhibition was detected in terms of biomass of stems and leaves under the repeat applications of 6-BA at high concentrations. These results suggest that propagule formation is more sensitive to the 2 plant growth regulators than the plant itself.

Jana & Choudhuri (1983) found that plant senescence was delayed by cytokinin. In the present study, the plant growth period in *Potamogeton crispus* was found to be prolonged by GA_3 and 6-BA, due to the inhibition of propagule formation. This may contribute to the use of the plant to improve water quality because of a longer growing period. However, as propagule production can be substantially inhibited when the plant is exposed to these substances either for a short term at high concentrations or repeatedly at low concentrations during turion morphogenesis, this could be detrimental to the plant's regeneration and propagation over the course of years. In this regard, the runoff of cytokinins and gibberellins from farming or horticultural systems into bodies of water harbouring *P. crispus* should be controlled.

Effects of physiological characteristics on propagule formation

Plants rely on a wide variety of metabolic and physiological responses to adapt their growth and development to variations in the environment. When a storage organ is forming, metabolism in source stems and leaves may decrease and assimilation availability transferred to sink tissue (McCormick et al. 2006), which consequently affects plant growth. Spencer et al. (1994) noted that once *Hydrilla verticillata* is initiated to produce propagules under short photoperiods (11 h d^{-1}), nitrogen and carbon are directed from shoots and roots into newly formed tubers and turions. Spencer & Anderson (1987) also found decreases in chl *a* and carotenoids in leaves coinciding with propagule formation in *Potamogeton nodosus*. In the present study, the contents of photosynthetic pigments increased in plants in both GA_3 and 6-BA treatments except for in BALS compared to the control. This implies that the inhibition of propagule production by gibberellin and cytokinin is also associated with increases in photosynthetic pigments in the plant.

The regulatory functions of hormones on the sink–source relationship have well been established in some terrestrial plants, although the roles of endogenous hormones in these processes remain unknown (Baker 2000). In the present study, we found that the accumulation of starch reserve in propagules declined significantly under all applications of GA_3 and 6-BA in *Potamogeton crispus*. Starch content decreased to different degrees, but soluble sugar, sucrose, and reducing sugar contents increased in the propagules, demonstrating that the starch synthesis from sugars was retarded. Furthermore, the changes in the carbohydrate content in propagules were dose-sensitive under GA_3 single-application: the contents of soluble sugar, sucrose, and reducing sugars increased and starch content decreased with increasing GA_3 concentration. But under 6-BA single-application, the contents of all carbohydrates were lower at high concentration than at low concentration, implying that

the inhibition of starch synthesis in propagules might be partially attributed to lower influx of assimilates into the propagules in the high-concentration 6-BA treatment, in comparison with low concentration. However, analysis showed that there were no corresponding changes in carbohydrate contents of plants in contrast to the propagules under the 2 regulator treatments. The regulation of gibberellin and cytokinin on the sink–source relationship in propagule formation of *P. crispus* remains largely unknown and needs further investigation.

CONCLUSIONS

The results of the present study are conducive towards explaining the regulation on propagule formation by plant hormones, which may be involved in the population decline of some submerged aquatic plants such as *Potamogeton crispus* in eutrophic waters. Propagule production was inhibited or delayed by applying GA_3 or 6-BA to *P. crispus*. The effect became greater with increasing concentrations and number of applications. 6-BA inhibited the differentiation of the propagule, but had little effect on its growth. GA_3 not only inhibited propagule formation, but also induced some standard turions to develop into phylloclade turions. The effects of the 2 regulators on propagule formation were coincident with promoting photosynthetic pigments in the plants and inhibiting starch accumulation in the propagules. Both plant growth regulators had stronger effects on propagule formation than on plant growth. In this regard, reduced discharge of cytokinin- and gibberellin-type regulators from farming or horticultural systems into waterways should be enforced to protect natural regeneration and propagation of *P. crispus*.

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