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The role of gibberellins and ethylene interaction on gibberellins biosynthesis and plant growth

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Abstract

Gibberellins (GAs) and ethylene are involved in many aspects of plant growth and development. Bioactive GAs are associated with many processes in higher plants, such as seed germination, leaf expansion, stem elongation and flower development. Like gibberellins, ethylene are involved in the regulation of diverse developmental processes, including seed germination, seedling growth, leaf and petal abscission, fruit ripening, cell elongation, flower senescence sex determination and pathogen responses. The physiological role of plant hormones has been investigated for many years, but the interaction between plant hormones is not well understood. Recently, we found that activated ethylene signaling reduces bioactive GA levels, thus enhancing the accumulation of DELLAs and ethylene acts on DELLAs via the CTR1-dependent ethylene response pathway (Achard et al., 2007) and double mutants gaixeto2-1 had enhanced content of active GA(4) at both the seedling and the rosette stages (De Grauwe et al. 2008). In present study we have analyzed the effect of ethylene-gibberellins interaction on the content of gibberellins by using different ethylene and GA signal transduction mutants, as well as double mutants. Our results show that ethylene can promote plant growth and development through stimulation gibberellins biosynthesis.

Key words: Gibberellin, ethylene, cross-talk, hypocotyl, growth parameter

Introduction

Gibberellins (GAs) are a family of hormones, some of which are bioactive hormones that are essential for development and growth of plants. Bioactive GAs are associated with many growth and developmental processes in higher plants, such as seed germination, leaf expansion, stem elongation and flower development (Achard *et al.*, 2007; De Grauwe *et al.*, 2008).

Like gibberellin, ethylene are involved in the regulation of diverse developmental processes, including seed germination, seedling growth, leaf and petal abscission, fruit ripening, cell elongation, flower senescence sex determination and pathogen responses (Abeles *et al.*, 1992).

Gibberellins promote the growth of plants by opposing the effects of nuclear DELLA protein growth suppressors. *Arabidopsis* contains genes encoding five DELLA proteins (*GAI, RGA, RGL1, RGL2* and *RGL3*). The gibberellin mutants used in this study, are gibberellin insensitive lines (*gai*). In *Arabidopsis*, *gai* confers a dark green, dwarfed phenotype (Koornneef *et al.*,

1985), and gai plants contain high levels of endogenous GAs (Talon *et al.*, 1990).

Ethylene inhibits the elongation growth of dark-grown seedlings, induces swelling of the stem, and mediates tight closure of the apical hook (triple response). In *Arabidopsis thaliana ETR1* gene codes for members of the ethylene-receptor gene family (Sakai *et al.*, 1998).

Recently, Achard *et al*. (2003) have found that auxin and ethylene regulates root growth by modulating the effect of GA on the levels of a DELLA growth-repressing protein.

In present study we have studied the interaction between ethylene and gibberellins by using different ethylene and GA signal transduction mutants, as well as crosses between ethylene and gibberellin mutants. Here we mainly focused on analyzing the effect of both hormones and their interaction on some growth parameters and GAs levels.

Materials and methods

Hypocotyls measurement

Arabidopsis seeds were sterilized for 2 min in 70% ethanol and 0.1% (v/v) Triton and rinsed with 95% ethanol. Sterilized seeds were sown on Petri dishes containing 25 ml of rich medium (1/2MS) supplemented with 1% sucrose and 0,7% agar, with a pH of 5.6 after cold treatments the plates were put in chamber at 23°C in long day conditions (16 h light/ 8 h dark). The hypocotyl length was measured after 12 days. For hormonal and their inhibitor treatments, gibberellin, ACC, AgNO3 and PAC were directly added to the growth medium

Growth condition

For experiments on soil, seeds were stratified for 3 to 4 days at 4°C before sowing. Plants were grown in plastic pots containing a mixture of substrate and vermiculite (3:2), under an 18-h long-day photoperiod of 130 µE m-2 s-1 consisting of white fluorescent light with a temperature of 23°C.

Plant Growth Analysis

The flowering time was defined as the number of days from the time the seeds were placed in the growth chamber until the flower buds become visible. For the flowering time, the total number of leaves was recorded as an adequate measurement. The days and total leaves were scored from germinating until the opening of first flower. Rosette diameter was measured as the distance between the most distant leaves edge.

Determination of endogenous GA content

The rosette leaves of plants were harvested and immediately frozen in liquid nitrogen. Samples (0.1-0.2 g fresh weight) were homogenized and extracted for 3 min with Mixer mill in 500 µl of 80% methanol. [$^2H_2$]GAs (17, 17-[$^2H_2$] GA; purchased from Prof. L. Mander, Canberra, Australia) was added as internal standards before extraction.

After extraction and purification the different fractions of gibberellins were analyzed by GC GC/MS (Moritz and Olsen, 1995).

Statistical analysis

The data shown are mean values ± standard deviation. Differences between genotypes were compared using LSD at the 0.05 probability level. Levels of significance are represented by * at $P < 0.05$, ** at $P<0.01$, *** at $P<0.001$ and ns: not significant.

Results

For many authors the *Arabidopsis* hypocotyl is convenient

system in studying the interaction of plant hormones and cell expansion (Collett *et al.*, 2000). In our experiment the hypocotyls of wild type (Table 1), grown in the presence of 100 µM GA4, were significantly longer than that of untreated plants. The Paclobutrazol which is an inhibitor of gibberellin biosynthesis strongly decreased the hypocotyl of wild type seedlings. The wild type seedlings showed a decrease in response to $AgNO_3$ (inhibitor of ethylene binding). In ethylene mutants (*etr1-3*), GA4 strongly increased the hypocotyl length. The treatment with PAC significantly decreased the hypocotyls growth of *etr1-3*. However the ACC and AgNO3 had almost no effect on hypocotyl growth of ethylene mutants. Finally, the hypocotyl length of *gai* mutants, was unaffected by ACC and GA4, however the PAC and AgNO3 significantly decreased the hypocotyl of *gai* mutants.

The plant hormone is one of the most important endogenous factors that control growth and development. In *Arabidopsis*, both GAs and ethylene are known to be involved in stem growth and bolting and flowering induction. Table 2 shows the number of leaves, rosette diameter and flowering time of gibberellin and ethylene mutants grown under long day conditions. In Table 2, it can be seen that the *etr1-3* mutant flowers earlier and increases the rosette diameter, but these results were statistically insignificant ($P>0.05$). The *gai* plants (Table 2), flowered later than wild type and had a smaller rosette diameter ($P<0.001$).

The double mutant *gai-etr1-3* had a dwarf phenotype as *gai* mutants. *gai-etr1-3* flowered very late with high number of leaves than wild type ($P<0.001$; Table 2). The rosette of *gai-etr1-3* was significantly smaller than that of *gai* plants ($P<0.05$; Table 2). These results suggest that *etr1-3* mutation was responsible for delay to flower and for stem shortening in *gai-etr1-3* mutants.

The endogenous GA levels in rosette of wild type plants, *gai*, *etr1-3*, *gai-etr1-3*, were analyzed by GC/MS (Tables 2). The analyses of endogenous GA levels were performed in rosette leaves (Table 2). Comparing the GA levels of wild type and ethylene mutants, the results show that *etr1-3*

Table 1. Effect of exogenous GA4, PAC, ACC and $AgNO_3$ on hypocotyl length of wild type, *etr1-3* and *gai* plants. Hypocotyl length was expressed as a percentage relative to untreated seedlings.

	wild type	*etr1-3*	*gai*
Control	1.1±0.07	1.11±0.03	1.04±0.10
100 µM GA4	1.69±0.16	1.97±0.25	0.99±0.11
100 µM PAC	0.66±0.05	0.68±0.06	0.62±0.04
100 Mm ACC	1.20±0.13	1.12±0.04	0.99±0.10
100 Mm AgNO3	0.82±0.12	1.15±0.05	0.80±0.08

Table 2. Comparison of the Phenotype and GAs concentration (GA1+GA4) of wild-type with the *etr1-3*, *gai, gai-etr1-3* mutants grown under long day conditions.

	Number of leaves	Rosette diameter (cm)	Flowering time (days)	GAs levels (pg g-1 fresh weight)
wild type	13.0±1.41	9.73±1.08	23.6±0.70	304.4±38.6
etr1-3	11.9±1.29	10.2±0.47	23.1±1.52	488±95.5
gai	16.4±1.71	5.38±0.22	32.6±1.26	2993±497
gaixetr1-3	29.5±3.50	5.04±0.43	43.4±3.64	7872±1549

mutants contain significantly higher endogenous levels GA1 and GA4 than wild type. The *gai* mutants accumulate 10-fold more GAs than the wild type plants. The double mutants *gai-etr1-3* contain 2-fold more GAs than *gai* plants.

Discussion

The possible interaction between ethylene and gibberellin was studied by adding to the growth medium gibberellins, ethylene precursor ACC, and their inhibitors (PAC and $AgNO_3^-$). Our results (Table 1) show that gibberellins promoted strongly the hypocotyl length of *etr1-3*. Nevertheless, the elongation of hypocotyl was inhibited when ethylene mutants are grown on media containing PAC. These results indicate that gibberellin probably does not induce the hypocotyl growth via ethylene signal. On the other hand, when wild-type seedlings are grown on media supplemented with 100 μM ACC, the hypocotyl length was slightly increased, but the hypocotyl of *gai* was unaffected by this treatment. These results indicate that ethylene may exert its effect on hypocotyl growth through gibberellins.

In relation to growth parameters (Table 2), *etr1-3* mutation decreased the flowering time and the number of leaves and increased the rosette diameter, these results are consistent with previously reported data (Chang *et al.*, 1993).The dwarf phenotype of the *gai* mutant can be explained by the repressor character of GAI, which increased protein stability and leads to a stronger inhibition of GA responses in the *gai* mutant (De Grauwe *et al.*, 2008)

The double mutant *gai-etr1-3* leaves are smaller than *gai* ones (Table 2), whereas the flowering time is significantly higher than wild type and the stem length was very short. These results suggest that the *etr1-3* mutation affected negatively the growth of *gai* mutants, leading to dramatic effects on the phenotype of *gaixetr1-3*. Moreover these data demonstrate that the *etr1-3* mutation is acting through gibberellin.

The results of GA levels (Table 2) suggest that *etr1-3* mutation increased significantly the content of bioactive GA_1 in rosette leaves. These results are consistent with the phenotype of *etr1-3* mutant (big rosette flowered with lower number of leaves than wild type).

Talon *et al.* (1990) suggested that *gai* mutant was presumably a GA-receptor mutant or blocked in the transduction pathway between the receptor and stem elongation. The latter is responsible for the dwarf phenotype observed in *gai*, *gaixetr1-3* and high GA accumulation in this mutant. Comparing the GAs levels in *gai* and *gaixetr1-3* mutants, we found that *etr1-3* significantly increased the levels of all GAs (Table 2). Probably, these date suggest that *etr1-3* mutation increases the GA biosynthesis or decrease the GA catabolism. Indeed, several studies have suggested that GA biosynthesis was controlled by various negative feedback mechanisms (De Grauwe *et al.* 2008).

Conclusions

The results obtained from hypocotyl experiments suggested that gibberellins did not induce the hypocotyl growth via ethylene signal, whereas the ethylene might exert its effects through gibberellins. In double mutants, the higher concentration of bioactive GAs was due to stimulation of GAs biosynthesis by the *etr1-3* mutation.

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