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ORIGINAL RESEARCH

Heterologous Expression of a Gibberellin 2-Oxidase Gene from *Arabidopsis thaliana* Enhanced the Photosynthesis Capacity in *Brassica napus* L.

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Abstract Gibberellins (GAs) are endogenous hormones that play an important role in regulating plant stature by increasing cell division and promoting seed germination. The GA2-oxidase gene from *Arabidopsis thaliana* (*AtGA2ox8*) was introduced into *Brassica napus* L. by *Agrobacterium*-mediated floral-dip transformation with the aim of decreasing the amount of bioactive GA and hence reduced the plant height. As anticipated, the transgenic plant exhibited dwarf phenotype. Importantly, compared with the wild type, the transgenic plants had delayed the seed germination, increased the chlorophyll content (28.7–36.3%) and photosynthesis capacity (14.3–18.7%) in a single leaf. At the same time, the photosynthesis capacity of the whole plants was significantly enhanced (35.7–48.6%) due to the extra leaves and branches.

Keywords *AtGA2ox8* · Dwarf · Seed germination · Chlorophyll content · The photosynthesis capacity

Introduction

In plants, seed is the main mechanism of plant propagation, and the proper regulation of seed dormancy and germination is of critical importance to plant survival (Ariizumi and Steber 2007). It is generally believed that seed germination is tightly controlled by diverse environmental conditions, including moisture, temperature, and light, which can be translated into signals transmitted by plant hormones, such as abscisic acid (ABA), gibberellic acid (GA), brassinosteroid, and ethylene (Finch-Savage and Leubner-Metzger 2006). In addition, several lines of evidence have demonstrated that GAs and ABA are some of the main regulators known (Kucera et al. 2005; Yamaguchi et al. 2007; Rodríguez-Gacio and Matilla 2009; Iglesias-Fernández and Matilla 2010).

As we know, GAs are endogenous plant hormones that play very important roles in plant growth and regulating many aspects of plant development, including seed germination, shoot growth, flower induction, stimulating cell elongation and cell division, hypocotyl elongation, fruit maturation, and leaf expansion (Kende and Zeevaart 1997; Harberd et al. 1998; Hedden and Proebsting 1999). Only a few of the presently known 126 different GAs, such as GA_1, GA_3, GA_4, and GA_7, have been shown to possess biological activity (Hedden and Phillips 2000). Moreover, bioactive GAs is well-known for promoting germination and plant growth (Lo et al. 2008). The past studies have indicated that GA metabolism takes place in three different cellular compartments: plastids, endoplasmic reticulum, and cytosol (Hedden and Phillips 2000; Olszewski et al. 2002; Sun and Gubler 2004). Multiple enzymes are involved in GA metabolism and catabolism, including ent-copalyl diphosphate synthase, ent-kaurene synthase, P450 mono-oxygenases, and dioxygenases. Two dioxygenases, GA20-oxidase and GA 3β-hydroxygenase, catalyze the last few steps in the synthesis of bioactive GA. Another dioxygenase, GA2-oxidase, catalyzes GA catabolism of bioactive GA or their precursors (Thomas et al. 1999; Hedden and

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Phillips 2000; Olszewski et al. 2002). So GA2-oxidases regulate seed germination and plant growth by inactivating endogenous bioactive GAs (Lo et al. 2008).

Decreasing the concentration of endogenous bioactive GA by expression of GA2-oxidase not only can create dwarf plant and elongate the dormancy time of seed but also can alter the concentration of chlorophyll (Biemelt et al. 2004; Dijkstra et al. 2008). The chlorophyll is very closely connected with the photosynthesis. Currently, chlorophyll fluorescence is widely used in analyzing the photosynthetic apparatus and understanding the mechanism of photosynthesis and the mechanism by which a range of environmental factors alter photosynthetic activity under both biotic or abiotic stresses (Sayed 2003).

Overexpression of the genes encoding AtGA2-oxidase can cause a dwarf phenotype, delayed flowering, and reduced bioactive GA levels in *Arabidopsis* (Schomburg et al. 2003; Wang et al. 2004; Zhao et al. 2007). In addition, the heterologous expression of AtGA2-oxidases also results in the production of plants with reduced height and delayed flowering in wheat (Hedden and Phillips 2000), rice (Sakamoto et al. 2001), tobacco (Schomburg et al. 2003; Biemelt et al. 2004), bahiagrass (Agharkar et al. 2007), and *Solanum* species (Dijkstra et al. 2008). But there is any report about heterologous expression of AtGA2-oxidases into *Brassica napus* L. which is a grown worldwide, oilseed-producing crop. In this study, we introduced *AtGA2ox8* gene into *B. napus* L. and described its impacts on the seed germination, the chlorophyll accumulation and photosynthesis capacity.

Materials and Methods

Plant Materials and Genetic Transformation

The cultivar of *B. napus* L. *N529* is used in this study, with the height of more than 2 m, which provided by academy of seed industry of Hunan Yahua. For preparing *AtGA2ox8* transgenic plants, we amplified *AtGA2ox8* (GenBank accession no. AL021960, accession number in Tair Web site is At4g21200) gene from *Arabidopsis thaliana* cDNA using a reverse-transcription polymerase chain reaction (RT-PCR) method and sub-cloned it into the *Sam*I and *Bam*HI sites of pEGAD vector downstream of the CaMV35S promoter. The constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation according to our previous study (Lin et al. 2009). When the *B. napus* L. plants began to bolt and produce flora inflorescences, the grown healthy flora inflorescences at the top of rapeseed plants were selected and immersed into the plastic bag with *Agrobacterium* inoculum for 30 s, then covered with an agricultural parchment bag to maintain high humidity and, after a week, uncovered the agricultural parchment bags and removed the flower buds at the top of dipped inflorescences. When the siliques were brown and dry, the seeds from the dry siliques were collected and stored at −20°C for screening.

Transgenic Lines Screening

Transgenic plants were selected using herbicide Basta (1:1,000, *v*/*v*). Total genomic DNA were extracted from Basta-resistant transgenic lines using hexadecyltrimethylammonium bromide method (Murray and Thompson 1980) for detecting *Basta*-resistant gene (*bar*) by using PCR analysis with primers (*bar*-F: 5′-CTACATCGAGACAAGCACGGT-3′, *bar*-R: 5′-CTGAAGTCCAGCTGCCAGAA-3′). PCR was generally performed with a PCR program: 94°C 5 min (94°C 30 s, 58°C 30 s, 72°C 30 s) 30 cycles, 72°C 5 min. PCR products were analyzed using 1.5% agarose gel electrophoresis.

GA_3 Treatment

To investigate the response of seed germination to exogenous GA_3, about 100 seeds were sown on MS medium saturated with 100 μmol GA_3 (Shanghai Solvent) in a plate. Seeds were placed in the temperature-controlled growth chambers and grown under continuous white light (100 μmol m^{-2} s^{-1}) at 22°C. After 7 days, seed germination was statisticated, the lengths of hypocotyls were measured, and the averages were calculated. Three parallel experiments were carried out.

In order to investigate the transgenic plants whether response to exogenous GA_3, we sprayed GA_3 (10^{-4} M) to the transgenic plants grown in greenhouse. When the seedlings were sown on soil for 6 days, the exogenous GA_3 were started to spray. And then the transgenic seedlings were sprayed once every 6 days to the seeds ripening. At the same time, we sprayed water to wild type (WT) as the control.

Paclobutrazol Treatment

Paclobutrazol (PAC) is a triazole derivative that inhibits GA biosynthesis at the kaurene oxidase reaction (Hedden and Graebe 1985) and usually used to investigate whether the pathway of GA biosynthesis was affected in GA sensitive plants. One hundred seeds were sown on MS medium saturated with 2 μmol L^{-1} PAC (Shanghai Solvent) in a plate. Seeds were placed in the temperature-controlled growth chambers and grown under continuous white light at 22°C. The frequency of seed germination was calculated at 7th day.

GA Preparation and ELISA Analysis

The GA extraction and purification were performed as previously described (Talon and Zeevaart 1990; Zeevaart et

al. 1993). The total contents of GA_1 and GA_4 were then determined by enzyme-linked immunosorbent assay as previously described (Yang et al. 2001). The response assay to the exogenous hormone was performed as previously described (Oikawa et al. 2004).

Agronomic Traits Investigation

For hypocotyl length assay, seeds were sown in clear plastic vials containing MS medium. The clear plastic vials were placed in the incubator exposed to continuous white light (100 μmol m^{-2} s^{-1}). The temperature was controlled at 22°C. After 10 days, for WT and each transgenic line, the hypocotyl lengths of at least 20 seedlings were measured, and the averages were calculated. With a view to investigate the plant height, internode length, branches, and seed yield, WT and transgenic seedlings were sown in an irrigated field in early October 2008. The field was divided into four parts. Each part consisting of three plots was devoted to one of the four lines (WT, *OeAtGA2ox8-6*, *OeAtGA2ox8-7*, and *OeAtGA2ox8-9*). Seedlings were sown at a density of 30 seedlings per plot with 0.24 m between seedlings and 0.5 m between rows. Before harvest, both the straightened plant height and the undisturbed plant height from the ground to the highest point of the plants were measured for all the plants in each of the three plots. Ten individual plants were randomly selected from each plot; the numbers of primary branch and the internode length were recorded. The height of the first branch was measured from ground to the lowest primary branch. The number of primary branches was counted along the main stem.

Germination Analysis

WT and three T_2 independent transgenic lines (*OeAtGA2ox8-6*, *OeAtGA2ox8-7*, *OeAtGA2ox8-9*) seeds were surface-sterilized by soaking in 70% ethanol for 1 min and in 10% NaClO for 20 min after which they were rinsed four times (5 min each) with sterile deionized water. Seeds were germinated and grown on MS (Murashige and Skoog 1962) plates solidified with 0.8% agarose, supplemented with 3% sucrose, pH 5.8. The MS plates were grown under greenhouse conditions, 22°C day and night (photoperiod of 16:8 h light/dark; 100 μmol m^{-2} s^{-1}; Nykiforuk and Johnson-Flanagan 1998; Dunfield et al. 2007).

Chlorophyll Content Estimation

Chlorophyll content of leaf disks (0.5 cm^2 diameter) was determined in 96% ethanol, as described by Lichtenthaler (1987).

Photosynthetic Capacity Measurements and Calculation

The portable photosynthesis system LI-6400 (LI-COR, Lincoln, NE, USA) was used to measure the CO_2 uptake rates of single leaves. The temperature of the leaf was maintained at 20°C, and the CO_2 concentration of the air entering the leaf chamber was adjusted to 400 mmol mol^{-1}. During the measurements of light response curves of the CO_2 gas exchange, the photosynthetic photon flux density varied between 0 and 2,000 mmol photons m^{-2} s^{-1}. Gas exchange of at least four to five different leaves of each plant was recorded for calculation of the photosynthetic capacity of the whole plant on the basis of single leaf measurements. Light intensity was 200 mmol photons m^{-2} s^{-1} in these cases. The total photosynthetic capacity of each plant was estimated in relation to the leaf area by these data.

Gas exchange of whole plants was measured by the canopy chamber PMK-1 (Biemelt et al. 2004; see also Fig. 5a). These measurements were performed under daylight conditions in the greenhouse. During the experiments, the intensity of the illumination was in the range of 100 to 280 mmol photons m^{-2} s^{-1}, and the CO_2 concentration of the inlet gas varied between 380 and 400 μmol^{-1}. Light intensity, volume flow of the inlet gas, temperature, and the molar fraction of CO_2 and water of the inlet and the outlet gas were recorded continuously by the LI-6400 photosynthesis system, and the CO_2 gas exchange rate of the whole plant was calculated. To compare the photosynthetic rate, the transgenic plants and WT were determined under the equal illumination with 200 mmol photons m^{-2} s^{-1}. In order to achieve that, the gas exchange of the whole plants was recorded before the light curves were measured for three different representative leaves of each plant directly. The mean was fitted by the function of the non-rectangular hyperbola. The photosynthetic capacity of the whole plant was calculated by the resulting value when the light intensity was 200 mmol photons m^{-2} s^{-1}. All measurements were replicated at least six times. Statistical analysis of net CO_2 fixation data was made using SPSS statistical software (Chicago, IL, USA; Norusis 1993).

Gene Expression Analysis

Total RNA was purified from the 100-mg seedlings of 10 days or 100-mg leaves of 60 days old of *B. napus* L. using Trizol reagent (Invitrogen Corporation, USA) and treated with RNase-free DNase I (Promega Biotech Corporation, USA). The DNase-digested RNA sample was used for reverse transcription by Superscript III reverse transcriptase (Invitrogen Corporation, USA). Samples, which served as cDNA stocks for PCR analysis, were stored at −80°. Semi-quantitative RT-PCR was used for

gene expression analysis. RT-PCR was performed in a 20-μL solution containing 1-μL cDNA stock using rTaq DNA polymerase (TaKaRa, Dalian, China), using a programmable thermal cycler (Biometra T-gradient 96050-801, Germany). The PCR amplification profile consisted of an initial denaturation step of 5 min at 94°, followed by 22 cycles of 20 s at 94°C, 30 s at 55°C, and 20 s at 72°C. The sense primer of *AtGA2ox8* is 5′-CGGAATCAGAGGCATTAG CAT-3′ and antisense primer 5′-CTCCACCTTTGGG TTCGTCAT-3′. *BnACTIN7* sense primer is (5′-TGGTTGGGATGGG-TAAAAAGA-3′) and antisense primer is (5′-CGGAGGA-TAGCGTGAGG AAGAG-3′). At the same time, we confirm the fusion protein of GFP and *AtGA2ox8* expressed in transgenic plants by PCR. The sense primer of *GFP*+ *AtGA2ox8* is 5′-GTGGATTGATGTGATATCTCCAC and the antisense primer is 5′-GAAACTTTATTGCCAAATGTTTG.

Results

Transformation and Molecular Characterization

The *AtGA2ox8* gene was introduced into *B. napus* L. by *Agrobacterium*-mediated floral-dip transformation (see "Materials and Methods"). The expression cassette contained green fluorescent protein (*GFP*) gene, target gene (*AtGA2ox8*), and herbicide Basta-resistant gene (*bar*) which was used as the select marker gene. *AtGA2ox8* and *GFP* were driven by the CaMV35S promoter and would express fusion protein in the transgenic plants (Fig. 1a). Twenty-four Basta-resistant independent lines were obtained after the first screening with herbicide Basta (Fig. 1b). The *bar* gene were also detected by PCR analysis in all Basta-resistant plants, but nothing was amplified from the WT plants (results showed partly in Fig. 1c), indicating that the transgene integrated into the genomic DNA and the transgenic plants were obtained.

To investigate whether the target gene expressed in *B. napus* L., we identified the green fluorescence of GFP protein in Basta-resistant independent lines using laser scanning confocal microscope. Among of 24 independent transgenic lines, three lines, *OeAtGA2ox8-6*, *OeAtGA2ox8-7*, and *OeAtGA2ox8-9*, were detected strong green fluorescence in roots, hypocotyls, and leaves, whereas the signal was hardly detected in WT (data not shown). At the same time, the segregation ratio of the three Basta-resistant independent lines conformed to the law of inheritance of Mendel was selected to raise homozygous T_2 lines. Furthermore, we detected the target gene expression of the three T_2 lines by semi-quantitative RT-PCR analysis. Semi-quantification RT-PCR revealed strong *AtGA2ox8* signal in the three independent transgenic lines, whereas hardly detectable

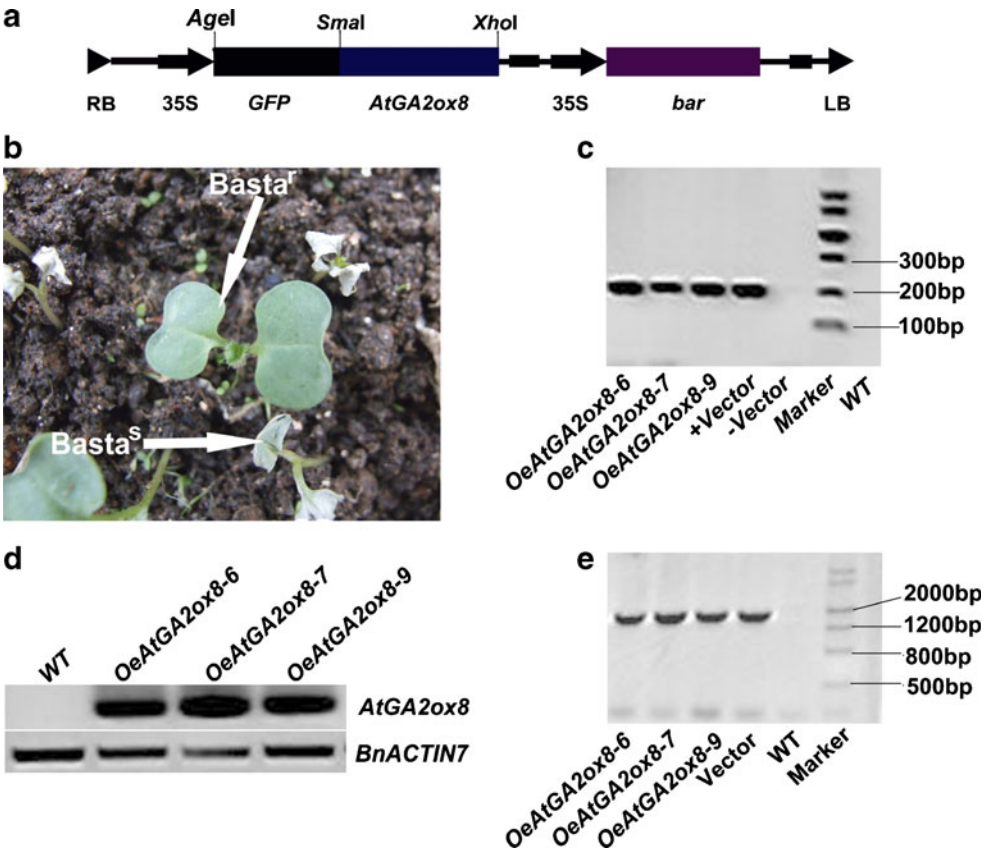

Fig. 1 Molecular characterization of transgenic seedlings. **a** The construct of expression vector. *RB* right border, *35S* cauliflower mosaic virus 35S promoter, *GFP* green fluorescent protein gene, *AtGA2ox8* the *Arabidopsis* gene *AtGA2ox8*, *SmaI SmaI* site, *BamHI BamHI* site, *bar* Basta-resistant gene, *LB* left border. **b** Represent Basta-resistant seedlings on field screening. **c** Investigated the *bar* in 7-day-old transgenic seedlings. **d** Investigated the expression level of *AtGA2ox8* in 7-day-old transgenic seedling by RT-PCR. **e** Amplified the fusion gene of *GFP* and *AtGA2ox8* in transgenic plants by cDNA

signal in the WT (Fig. 1d). In order to identify the fusion protein GFP and *AtGA2ox8* whether expressed in transgenic plants, the fusion gene amplified in three lines, *OeAtGA2ox8-6*, *OeAtGA2ox8-7*, *OeAtGA2ox8-9*, and expression vector 35S::*GFP-AtGA2ox8* by PCR analysis, but not detectable signal in the WT (Fig. 1e). The amplified product was sub-cloned into pGEM®-T easy vector (Promega, USA), and the sequencing result indicated the fusion gene GFP and *AtGA2ox8* between the digest sites *Age*I and *Xho*I in transgenic plants. And also there had a *Sma*I digest site between the GFP and *AtGA2o8*. Those results confirmed that the fusion protein GFP and *AtGA2ox8* was overexpressed in the three independent transgenic lines.

Introduced *AtGA2ox8* Delayed Seed Germination of Transgenic Plants by Altering the Levels of Bioactive GA

Compared to WT, the seed germination was delayed (Fig. 2a, c). To test whether the declined seed germination of transgenic plants was due to reduced levels of bioactive GA, we investigated the transgenic seedling response to exogenous GA_3. It was found that germination rate of

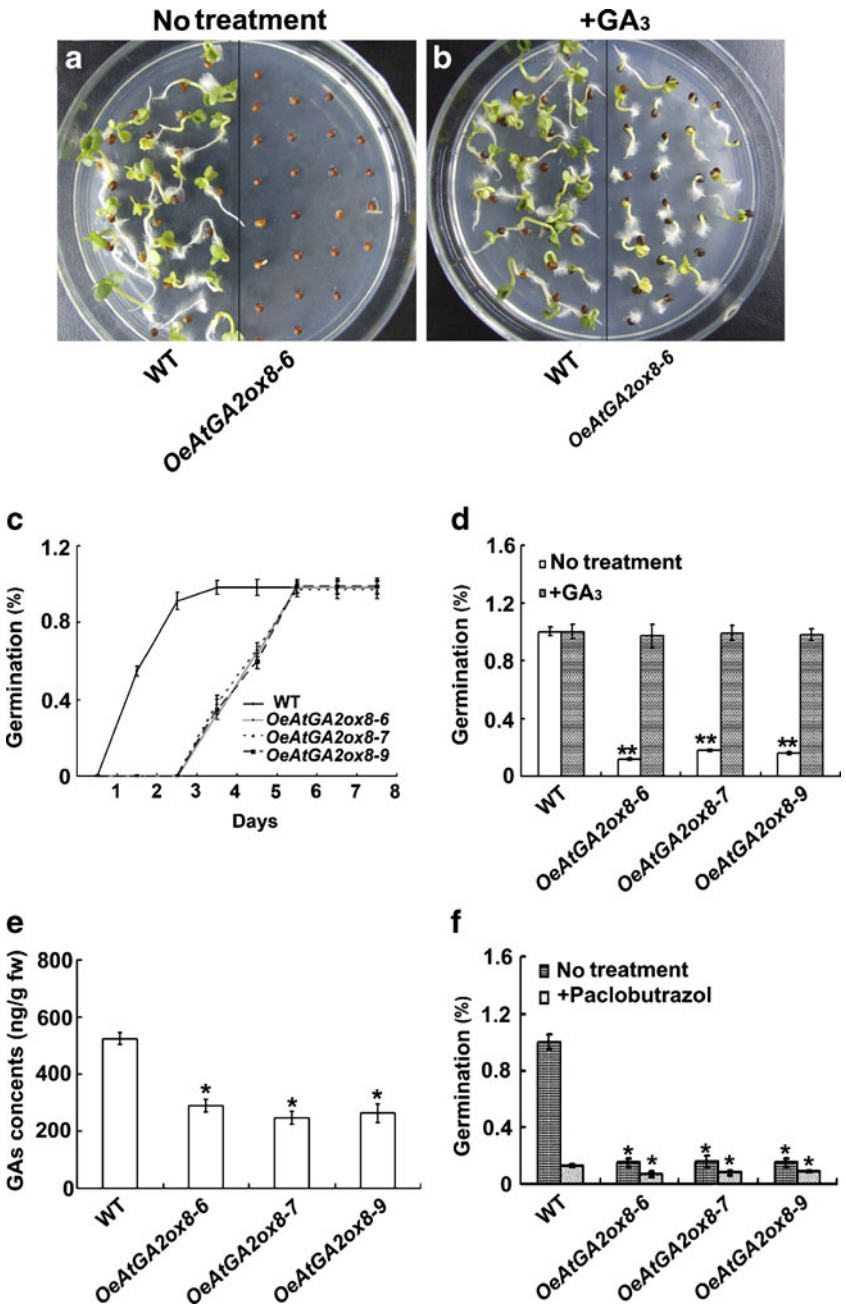

Fig. 2 The GA_3 rescued the phenotypes of transgenic seedlings. **P*<0.05; ***P*<0.01. **a** Transgenic seedlings and WT germinated on MS medium without GA_3 for 4 days. **b** Transgenic seedlings and WT germinated on MS medium contains 10^{-4} mol L^{-1} GA_3 for 4 days. **c** The germination frequency of transgenic seedlings and WT on MS medium. **d** The germination frequency of transgenic seedlings and WT on MS medium treated or untreated with 10^{-4} mol L^{-1} GA_3 for 4 days. **e** The concentration of GAs in 7-day-old of transgenic seedlings and WT. **f** The germination frequency of WT and transgenic seedling treated or untreated with 2 μmol L^{-1} PAC for 4 days

transgenic seedlings was restored to the normal germination frequency by treatment with 10^{-4} M GA_3 (Fig. 2b, d) for 4 days. As we know, GA_1 is the primary bioactive GAs which control shoot growth and floral development in *Brassica* (Rood et al. 1989), and GA_4 is a precursor of GA_1 in *Brassica* and several crop species (Rood and Hedden 1994; Durley and Pharis 1973; Koshioka et al. 1983; Rood et al. 1983; Takahashi et al. 1986; Yamane et al. 1977). So we investigated the active GA levels in the transgenic plants and WT. Indeed, the contents of active GA_1 and GA_4 extracted from the young leaves of the *AtGA2ox8* lines were markedly reduced compared to those of WT in young leaves (Fig. 2e). In addition, we investigated whether the pathway of GA biosynthesis was affected in transgenic

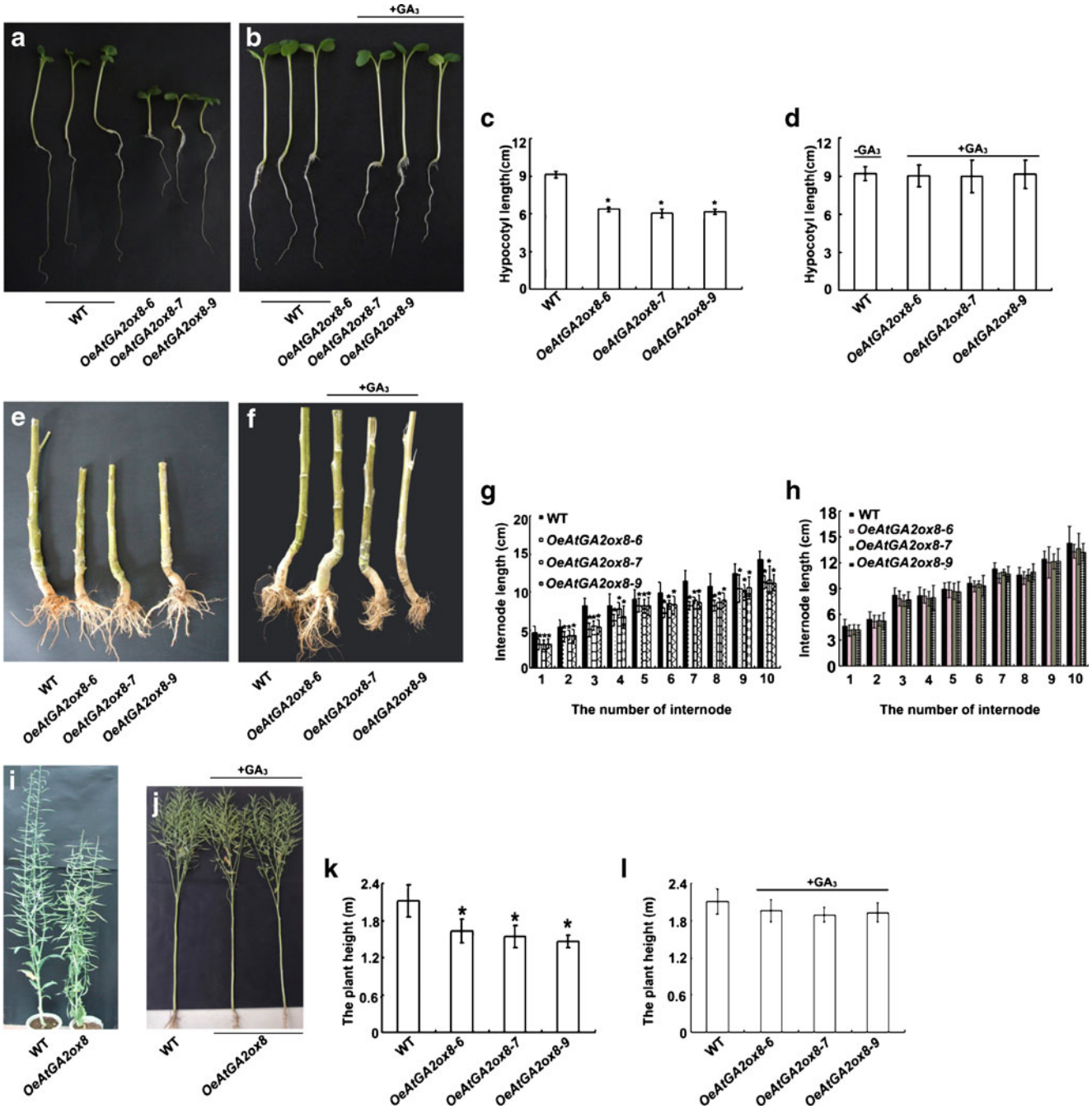

Fig. 3 Phenotypic characteristics of transgenic *B. napus* L. plants expressing *AtGA2ox8*. **a**, **c** The hypocotyl length of 10-day-old seedling growth on MS medium. **b**, **d** The hypocotyl length of 10-day-old seedling growth on MS medium which contains GA_3 10^{-4} mol L^{-1}. **e**, **g** The internode length of old stage of soil-grown plants. **f**, **h** The internode length of old stage of soil-grown plants which treated by GA_3 10^{-4} mol L^{-1} in all life cycle. **i**, **j** The plant height of WT and transgenic plants. **k**, **l** The plant height of WT and transgenic plants which treated by GA_3 10^{-4} mol L^{-1} in all life cycle

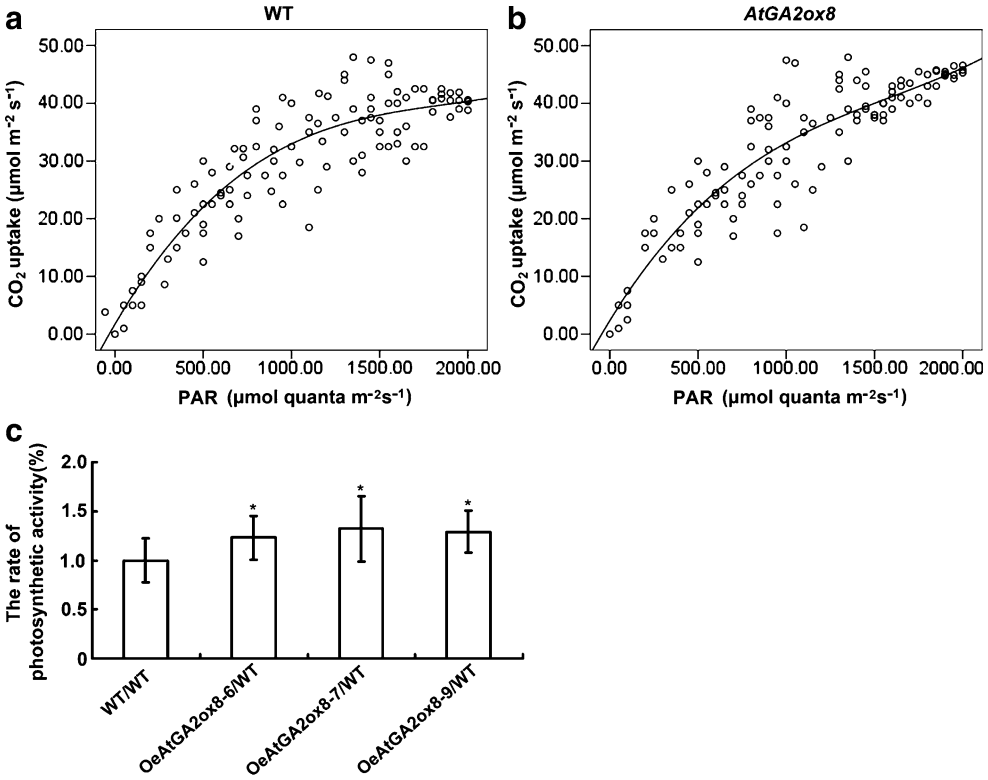

Fig. 4 Single leaf measurements of photosynthesis. **a**, **b** Light response curves of net photosynthetic rate were monitored in fully mature leaves of WT and *AtGA2ox8* transgenic plants with altered GA biosynthesis. Each data point is the mean of 25 independent measurements. **c** Photosynthetic activity of whole plants was calculated from four to five leaves at growth stage 3 (leaves) at 200 μmol quanta m^{-2} s^{-1} light intensity. The mean gas exchange rate of wild-type plants was 0.893±0.069 μmol CO_2 s^{-1}/plant. Data shown are the mean ± SE of 25 independent values

plants by PAC. The transgenic seedlings of the WT and the *AtGA2ox8* transgenic plants grown in continued white light on MS medium, after treating with 2 μmol L^{-1} PAC for 4 days, showed a pronounced inhibition of seed germination, which was similar to the WT seedlings (Fig. 2f). These results indicated that the pathway of GA biosynthesis was affected and the phenotypes of transgenic seedlings were indeed due to the reduced of bioactive GA levels which were caused by the introduced *AtGA2ox8*.

The Effects the Hypocotyl Growth of the *AtGA2ox8* Transgenics

The hypocotyl growth of both WT and transgenic seedlings was investigated under white light condition. The elongation of hypocotyls of transgenic seedling was inhibited, compared to WT (Fig. 3a, c). It was found that the short hypocotyls of transgenic plants could restore to the normal length after being treated with 10^{-4} M GA_3 (Fig. 3b, d). To investigate whether the effect of inhibited hypocotyl elongation would sustain in the subsequent growth stages, the T_2 plants were grown in an irrigated field in winter under a short photoperiod (October to May). Compared with WT, the plant height of transgenic plants at the ripening phase (Fig. 3i, k) was distinctly shorter (decreased by 18.8–23.9%). After harvesting the seed, we measured the length of ten bottom internodes in adult plants and found that all the internodes of transgenic plants were about 17.8–33.6% shorter than the corresponding ones of WT. Moreover, the closer the internode is to the top of the plant, the shorter the internode length (Fig. 3c, f). At the same time, not only the internode length (Fig. 3g, j) but also the plant height (Fig. 3h, l) recovered to normal when treated with the GA_3 in all life cycle of the transgenic plants. Therefore, the reduced height of these lines was primarily due to shorter internodes.

Table 1 Phenotypic characteristics of soil-grown transgenic *B. napus* L. plants expressing *AtGA2ox8*

	WT	*OeAtGA2ox8-6*	*OeAtGA2ox8-7*	*OeAtGA2ox8-9*
Stem height (cm)	163.3±6.70	128.1±6.21	125.4±5.78	122.6±4.31
Stem diameter (mm)	34±0.6	25±0.6	23±0.6	22±0.6
No. of primary branches	11.0±0.14	11.9±0.18	12.0±0.12	11.7±0.13
Leaf thickness (mm)	0.46±0.01	0.63±0.01	0.71±0.02	0.69±0.01
No. of leaves	21±0.23	34.3±0.46	36.1±0.78	33.8±0.84
Total chlorophyll (mg m^{-2})	618±24	776±18	788±22	803±29

All data investigated at the flowing stage

Increased the Photosynthesis Capacity of the Unit Area of a Single Leaf by Increasing the Thick and the Chlorophyll Content of Leaves

With the method of altering the levels of bioactive GA, we evaluated whether the photosynthetic capacity was affected. CO_2 uptake was measured in leaves of *AtGA2ox8* and WT plants. Figure 4a, b illustrated the effect of actinic light intensity on CO_2 uptake of a fully mature leaf of the different lines. In contrast, photosynthetic activity was increased in *AtGA2ox8* plants under nearly saturating illumination. The enhanced photosynthetic activity of *AtGA2ox8* leaves was not only due to the increased total chlorophyll contents (about 28.7–36.3%) in the unit area of a single leaf but also due to the increased thickness of a leaf in transgenic plants, compared with WT (Table 1). Moreover, the maximum quantum yield for the CO_2 assimilation of leaves can be calculated from the initial slope of the light response curves. The comparison of these parameters revealed a significant increase in the maximum quantum yield in leaves of *AtGA2ox8* plants (14.3–18.7%). Those results indicated that overexpression of *AtGA2ox8* could enhance the photosynthesis capacity of a single leaf by increasing the chlorophyll content and thickening the leaf.

Increased the Photosynthesis Capacity of the Whole Plant by Increasing the Leaves and Branches

Except for a single leaf, we also investigated the photosynthesis capacity of the whole plant using the design of Biemelt et al. (2004). As shown in Fig. 5, the photosynthesis capacity of the whole plant enhanced 35.7–48.6%. The increased photosynthesis capacity was major caused by the growing in number of leaves and branches (Table 1). Those results demonstrated that overexpression of *AtGA2ox8* could enhance the photosynthesis capacity of the whole plant by increasing the leaves and branches.

Discussion

In this study, we expressed a heterologous *AtGA2ox8* gene in *B. napus* L. from *Arabidopsis*. The transgenic plants displayed on dwarf, delayed seed germination, increased

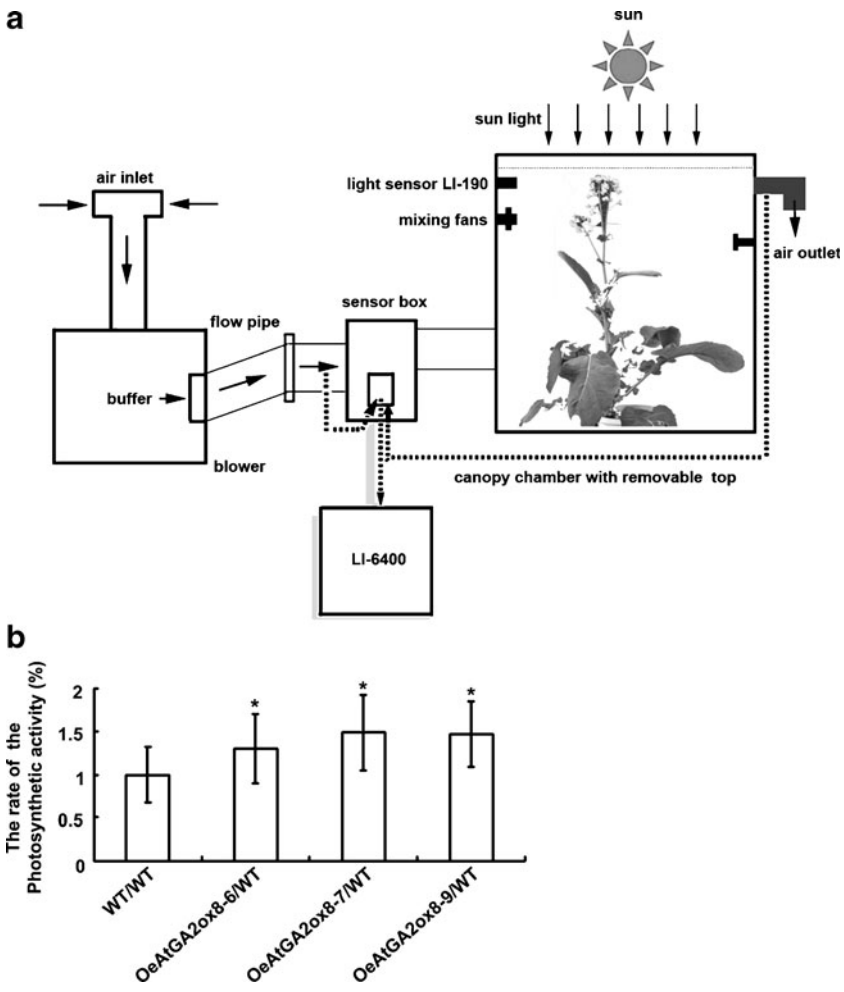

Fig. 5 CO_2 uptake rates of *AtGA2ox8* and WT plants are shown as difference. Mean CO_2 uptake of wild-type plants was 2.147±0.067 μmol CO_2 s^{-1}/plant. **a** Schematic illustration of the continuous flow canopy chamber system PMK1 for measuring whole plant gas exchange. **b** Photosynthetic activity of whole plants was investigated at flowing stage and light intensity was 200 μmol quanta m^{-2} s^{-1}. Each *bar* is the mean ± SE of 25 independent measurements

chlorophylls content and enhanced the photosynthesis capacity phenotypes in *B. napus* L. In addition, the contents of GA_1 and GA_4 markedly were reduced, but the pathway of GA biosynthesis was not affected in transgenic plants. Importantly, those phenotypes could reverse to normal by treating with GA_3. It suggested that those phenotypes were due to the decreased levels of bioactive GA in transgenic plants.

The previous study indicated that the seed germination is subject to many factors, such as the growth habitat, the growth season, the environmental factors, the seed coating and the hormonal (Finch-Savage and Leubner-Metzger 2006). At present, the most important hormonal concerned seed germination, ABA and GA, are relatively well understood. ABA is major involved in establishing and maintaining seed dormancy, whereas GA is involved in breaking seed dormancy and promoting germination (Finch-Savage and Leubner-Metzger, 2006). Several lines of evidence demonstrated the importance of GA biosynthesis and signaling for stimulating seed germination in *Arabidopsis*. The role of GA in promoting various stages in *Arabidopsis* growth and development is demonstrated by the phenotypes of GA biosynthesis mutants like *ga1-3*, including severe dwarfism, male sterility, and strong coat-imposed seed dormancy (reviewed in Olszewski et al., 2002). GA application rescues all of these defects. Many GA biosynthetic genes are upregulated in seed tissues during seed germination (Yamaguchi et al., 2001). GA signaling is also required for efficient seed germination in *Arabidopsis.* Gibberellin 2-oxidases (GA2oxs) regulate plant growth by inactivating endogenous bioactive GAs. The past study indicated that the GA2oxs identified in various plant species can hydroxylate the C-2 of active C_{19}-GAs (GA_1 and GA_4) or C_{19}-GA precursors (GA_{20} and GA_9) to produce biologically inactive GAs (GA_8, GA_{34}, GA_{29}, and GA_{51}, respectively; Sakamoto et al. 2004). Sakamoto et al. (2001) created a dwarf mutant by expression *osGA2ox1* in rice. Schomburg et al. (2003) create a dwarf plant by overexpression of *AtGA2ox7* and *AtGA2ox8* in *Arabidopsis.* The experiment of Agharkar et al. (2007) indicated that overexpression of *AtGA2ox1* in low-input turfgrass (*Paspalum notatum* Flugge) resulted in semi-dwarf plants, reduced steminess, and increased number of tillers per area significantly, which enhanced the quality of the transgenic bahiagrass under weekly moving. Kloosterman et al. (2007) reported that *StGA2ox1* fulfills a central role in the transition from longitudinal stolon growth to tuber initiation by regulating GA levels in the subapical stolon region facilitating radial growth. Lee and Zeevaart (2005) cloned the GA2-oxidase3 of spinach, and its expression in *Nicotiana sylvestris* created a dwarf mutant. Lo et al. (2008) reported that the T-DNA mutant of *osGA2ox6* shown a dwarf phonotype. Huang et al. (2010) overexpression of *osGA2ox6* in rice created a dwarf plant. They all studied the dwarf plants caused by inactivating endogenous bioactive gibberellins using overexpression of GA2-oxidase. Similarly, our results here showed that overexpression of *AtGA2ox8* gene in *B. napus* L. not only regulated the plant height but also delayed seed germination.

An increased chlorophyll concentration, as found in the present study, is commonly associated with reduced GA content (Biemelt et al. 2004; Dijkstra et al. 2008). As shown above, the decreased GA content was produced by overexpression of a heterologous *AtGA2ox8* gene in *B. napus* L. in our study. Identically, the total chlorophyll increased 28.7–36.3%, and the photosynthesis capacity of a single leaf enhanced about 18.7% in transgenic plants compared with WT (Fig. 4c). Moreover, the photosynthesis capacity of the whole plants enhanced 35.7–48.6%, which mainly resulted from the increased leaves and branches. These results were similar to Huerta et al. (2008) reported to increase the photosynthetic capacity by expression of *CcGA20ox1* in citrus.

In conclusion, we created a dwarf plants, delayed the seed germination, and increased the photosynthesis capacity by heterologous expression of an *Arabidopsis* GA2-oxidase in *B. napus* L.

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References

Agharkar M, Lomba P, Altpeter F, Zhang HN, Kenworthy K, Lange T (2007) Stable expression of *AtGA2ox1* in a low-input turfgrass (*Paspalum notatum* Flugge) reduces bioactive gibberellin levels and improves turf quality under field conditions. Plant Biotechnol J 5:791–801

Ariizumi T, Steber CM (2007) Seed Germination of GA-Insensitive sleepy1 Mutants Does Not Require RGL2 Protein Disappearance in Arabidopsis. The Plant Cell 19:791–804

Biemelt S, Tschiersch H, Sonnewald U (2004) Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic tobacco plants. Plant Physiol 135:254–265

Dijkstra C, Adams E, Bhattacharya A, Page AF, Anthony P, Kourmpetli ÆS, Power JB, Lowe KC, Thomas SG, Hedden P, AL PÆ, Davey MR (2008) Over-expression of a gibberellin 2-oxidase gene from *Phaseolus coccineus* L. enhances gibberellin inactivation and induces dwarfism in *Solanum* species. Plant Cell Rep 27:463–470

Dunfield K, Srivastava S, Shah S, Kav NNV (2007) Constitutive expression of ABR17 cDNA enhances germination and promotes early flowering in *Brassica napus*. Plant Sci 173:521–532

Durley RC, Pharis RP (1973) Interconversion of gibberellin A_4 to gibberellins Al and AM by dwarf rice, cultivar Tanginbozu. Planta 109:357–361

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501–523

Huerta L, Forment J, Gadea J, Fagoaga C, Peña L, Pérez-amador M, García-martínez JL (2008) Gene expression analysis in Citrus reveals the role of gibberellins on photosynthesis and stress. Plant Cell Environ 31:1620–1633

Harberd NP, King KE, Carol P, Cowling RJ, Peng JR, Richards DE (1998) Gibberellin: inhibitor of an inhibitor of …? BioEssays 20 (12):1001–1008

Hedden P, Graebe J (1985) Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. J Plant Growth Regul 4:111–122

Hedden P, Proebsting WM (1999) Genetic analysis of gibberellin biosynthesis. Plant Physiol 119:365–370

Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci 5:523–530

Huang J, Tang D, Shen Y, Qin BX, Hong LL, You AQ, Li M, Wang X, Yu HX, Gu MB, Cheng ZK (2010) Activation of gibberellin 2-oxidase 6 decreases active gibberellin levels and creates a dominant semi-dwarf phenotype in rice (*Oryza sativa* L.). J Genet Genomics 37:23–36

Iglesias-Fernández R, Matilla AJ (2010) Genes involved in ethylene and gibberellins metabolism are required for endosperm-limited germination of *Sisymbrium oycinale* L. seeds germination in *Sisymbrium oycinale* L. seeds. Planta 231:653–664

Kende H, Zeevaart JAD (1997) The five "classical" plant hormones. Plant Cell 9(1):197–1210

Kloosterman B, Navarro C, Bijsterbosch G, Lange T, Prat S, Visser RGF, Bachem CWB (2007) StGA2ox1 is induced prior to stolon swelling and controls GA levels during potato tuber development. Plant J 52:362–373

Koshioka M, Douglas TJ, Ernst D, Huber J, Pharis RP (1983) Metabolism of [3H]gibberellin A_4 in somatic suspension cultures of anise. Phytochemistry 22:1577–1584

Kucera B, Cohn MA, Leubner-Metzger L (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15:281–307

Lee DJ, Zeevaart JAD (2005) Molecular cloning of GA 2-oxidase3 from spinach and its ectopic expression in *Nicotiana sylvestris*. Plant Physiol 138:243–254

Lo SF, Yang SY, Chen KT, Hsing YI, Zeevaart JAD, Chen LJ, Yu SM (2008) A novel class of gibberellin 2-oxidases control semidwarfism, tillering, and root development in rice. Plant Cell 20:2603–2618

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Meth Enzymol 148:350–382

Lin JZ, Zhou B, Yang YZ, Mei J, Zhao XY, Guo XH, Huang XQ, Tang DY, Liu XM (2009) Piercing and vacuum infiltration of the mature embryo: a simplified method for *Agrobacterium*-mediated transformation of indica rice. Plant Cell Rep 28:1065–1074

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays of tobacco tissue cultures. Physiol Plant 15:473–497

Nykiforuk CL, Johnson-Flanagan AM (1998) Low temperature emergence in crop plants: biochemical and molecular aspects of germination and early seedling growth. J Crop Prod 1:249–273

Norusis MJ (1993) Documentation SPSS for Windows. SPSS, Chicago

Oikawa T, Koshioka M, Kojima K, Yoshida H, Kawata M (2004) A role of OsGA20ox1, encoding an isoform of gibberellin 20-oxidase, for regulation of plant stature in rice. Plant Mol Biol 55:687–700

Olszewski N, Sun TP, Gubler F (2002) Gibberellin signaling: biosynthesis, catabolism, and response pathways. Plant Cell 14 (suppl):S61–S80

Rodríguez-Gacio MC, Matilla AJ (2009) Seed dormancy and ABA signalling: the breakthrough goes on. Plant Signal Behav 4:1035–1048

Rood S, Hedden P (1994) Convergent pathways of gibberellin Al biosynthesis in *Brassica*. Plant Growth Regul 15:241–246

Rood SB, Pharis RP, Koshioka M (1983) Reversible conjugation of gibberellins in situ in maize. Plant Physiol 73:340–346

Rood SB, Pearce D, Williams PH, Pharis RP (1989) A gibberellin-deficient *Brassica* mutant—rosette. Plant Physio 189:482–487

Schomburg FM, Bizzell CM, Lee DJ, Zeevaart JAD, Amasino RM (2003) Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. Plant Cell 15:151–163

Sakamoto T, Kobayashi M, Ihoh H, Tagiri A, Kayano T, Tanaka H, Iwahori S, Matsuoka M (2001) Expression of a gibberellin 2-oxidase gene around the shoot apex is related to phase transition in rice. Plant Physiol 125:1508–1516

Sakamoto T, Miura K, Itoh H, Tatsumi T, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Agrawal GK, Takeda S, Abe K, Miyao A, Hirochika H, Kitano H, Ashikari M, Matsuoka M (2004) An overview of gibberellin metabolism enzyme genes and their related mutants in rice. Plant Physiol 134:1642–1653

Sayed OH (2003) Chlorophyll fluorescence as a tool in cereal research. Photosynthetica 41:321–330

Sun TP, Gubler F (2004) Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Biol 55:197–223

Takahashi M, Kamiya Y, Takahashi N, Graebe JE (1986) Metabolism of gibberellins in a cell-free system from immature seeds of *Phaseolus vulgaris* L. Planta 168:190–199

Talon M, Zeevaart JA (1990) Gibberellins and stem growth as related to photoperiod in *Silene armeria* L. Plant Physiol 92:1094–1100

Thomas SG, Phillips AL, Hedden P (1999) Molecular cloning and functional expression of gibberellin 2-oxidases, multifunctional enzymes involved in gibberellin deactivation. Proc Natl Acad Sci USA 96:4698–4703

Wang H, Caruso LV, Downie AB, Perry SE (2004) The embryo MADS domain protein AGAMOUS-like 15 directly regulates expression of a gene encoding an enzyme involved in gibberellin metabolism. Plant Cell 16:1206–1219

Yamaguchi S, Kamiya Y, Sun T (2001) Distinct cell-specific expression patterns of early and late gibberellin biosynthetic genes during *Arabidopsis* seed germination. Plant J 28:443–453

Yamaguchi S, Kamiya Y, Nambara E (2007) Regulation of ABA and GA levels during seed development and germination in *Arabidopsis*. Blackwell, Oxford

Yamane H, Murofushi N, Osada H, Takahashi N (1977) Metabolism of gibberellins in early immature bean seeds. Phytochemistry 16:831–835

Yang J, Zhang J, Wang Z, Zhu Q, Wang W (2001) Hormonal changes in the grains of rice subjected to water stress during grain filling. Plant Physiol 127:315–323

Zeevaart JA, Gage DA, Talon M (1993) Gibberellin A1 is required for stem elongation in spinach. Proc Natl Acad Sci USA 90:7401–7405

Zhao XY, Yu XH, Foo E, Symons GM, Lopez J, Bendehakkalu KT, Xiang J, Weller JL, Liu XM, Reid JB, Lin CT (2007) A study of gibberellin homeostasis and cryptochrome-mediated blue light inhibition of hypocotyl elongation. Plant Physiol 145:106–111