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PROTOCOLS/METHODS

In vitro flowering of *indica* rice (*Oryza sativa* L. spp. *indica*)

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Abstract Nodal explants of rice cultivar Pathumthani 1 (PT1; short-day photoperiod insensitive) were collected, surface-disinfected, and cultured on modified MS medium under *in vitro* conditions for 90 d. A total of 60% nodal explants generated flowering plantlets (with one inflorescence per cluster). The net photosynthetic rate was greater, and soluble sugars (including glucose, fructose, and sucrose) accumulated to higher levels in the leaves of flowering as compared to non-flowering plants. In contrast, chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoid content were enriched to a greater degree in the leaves of non-flowering as compared to flowering plants. Also, growth performance parameters, including plant height, number of leaves per plant, leaf area, fresh weight, and dry weight of plantlets derived from seedlings were superior to those of plantlets derived from nodal explants. In addition, the protocol proved to successfully induce flowering in KDML 105, a short-day photoperiod-sensitive rice cultivar.

Keywords Booting stage · Net photosynthetic rate · Nodal explants · Pigments · Soluble sugar

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Introduction

Rice (*Oryza sativa* L.) is one of the major carbohydrate resources in many countries of the world, especially Asian countries, feeding more than 3 billion people and providing 50–80% of daily calorie intake (Khush 2005). Flowering, or heading date, of rice in paddy fields is well established, not only in terms of molecular biology (Hirose *et al*. 2006; Ishimaru *et al*. 2007; Chen and Wang 2008; Tsuji *et al*. 2008, 2011), but also as a phenotypic phenomenon (Tang *et al*. 2009). Rice cultivars have been classified into groups, including short-day photoperiod sensitive (SD) and short-day photoperiod insensitive. In SD rice, heading date 3a (Hd3a) protein is expressed during regulation of the SD-photoperiod-dependent flowering pathway, basically determined by expression of two essential flowering promotion genes, *Hd3a* and *RICE FLOWERING LOCUS T 1* (*RFT1*), in the leaf blades and the shoot apical meristem (Abe *et al*. 2005; Tsuji *et al*. 2008). Also, a rice florigen or flowering signal has been identified, and includes the Hd3a protein, which is orthologous with the *FLOWERING TIME* (*FT*) gene product in *Arabidopsis* (Kojima *et al*. 2002). The product of the *RFT1* gene, located on chromosome 6 (Izawa *et al.* 2003), and Hd3/FT protein signaling acts in the shoot apical meristem (Izawa *et al*. 2002). In the paddy field, developmental stages of rice cultivars have been well established, including seedling, vegetative, and reproductive development prior to grain harvesting (Counce *et al*. 2000).

In vitro flowering is a topic of interest that has already been investigated in monocotyledonous plants such as orchids (Hee *et al*. 2007; Sim *et al*. 2007; Tee *et al*. 2008), calla lily (Naor *et al*. 2004), date palm (Masmoudi-Allouche *et al*. 2010), bamboo and ginseng (Lin *et al*. 2003, 2005), *Kniphofia leucocephala* (Taylor *et al*. 2007), and *Spathiphyllum* (Dewir *et al*. 2007). From previous publications, many internal and

external factors which control flowering *in vitro* are evident, for example, the origin of explant material, plant growth regulators (auxins, cytokinins, gibberellins, and plant growth retardants), carbohydrate to nitrogen ratio (C/N ratio), and the *in vitro* environment (Ziv and Naor 2006). *In vitro* flowering is a powerful tool used in rice breeding programs, along with *in vitro* fertilization and *in vitro* breeding (Uchiumi *et al*. 2007; Jung and Müller 2009). In the case of rice, there have to date been no reports on *in vitro* flowering. In Thailand, Pathumthani 1 (PT1: SD photoperiod insensitive) is the most popular rice cultivar grown in the irrigated zone, whereas jasmine rice, or KDML 105, is a SD-photoperiod-sensitive (providing one cultivation per annual cycle) premium quality grain, which is well-known worldwide for cooking quality, long grain morphology, aroma, flavor, and soft texture (Ariyaphanphitak *et al*. 2005; Laohakunjit and Kerdchoechuen 2007). The aim of this study was to develop an efficient protocol for induction of *in vitro* flowering in the rice crop.

Materials and Methods

Plant materials and treatments. Seeds of Pathumthani 1 rice (*O. sativa* L. spp. *indica* cv. PT1) and KDML105 (*O. sativa* L. spp. *indica* cv. KDML105) were provided by the Pathumthani Rice Research Center (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative, Pathumthani, Thailand) and were manually de-husked, surface-disinfected once in 5% (*v/v*) Clorox® for 60 min, once in 30% Clorox® for 30 min, and then rinsed three times with sterile distilled water. Surface-disinfected seeds were germinated on 0.25% Phytagel®-solidified MS medium (Murashige and Skoog 1962) in 250-mL glass vessels, and then sub-cultured at monthly intervals. Alternatively, PT1 seeds were germinated (7–9 d after sowing) and then transferred to pots (15 cm diameter × 30 cm height) containing clay soil (EC=2.687 dS m^{-1}; pH=5.5; organic matter=10.4%; total nitrogen=0.17%; total phosphorus=0.07%; total potassium=1.19%) for 85 d (Fig. 1*A*). Experiments were conducted at the Thailand Science Park, Pathumthani, Thailand (latitude 14°01′12″ N; longitude 100°31′12″ E) between August and October 2009. In the booting stage [85 d after sowing], nodal explants (2±0.2 cm lengths) were collected, surface-disinfected once in 70% (*v/v*) ethanol for 2 min, once in 10% Clorox® for 10 min, and then rinsed three times with sterile distilled water. Surface-disinfected nodal segments were placed on MS medium (Fig. 1*B*). Two explant sources,

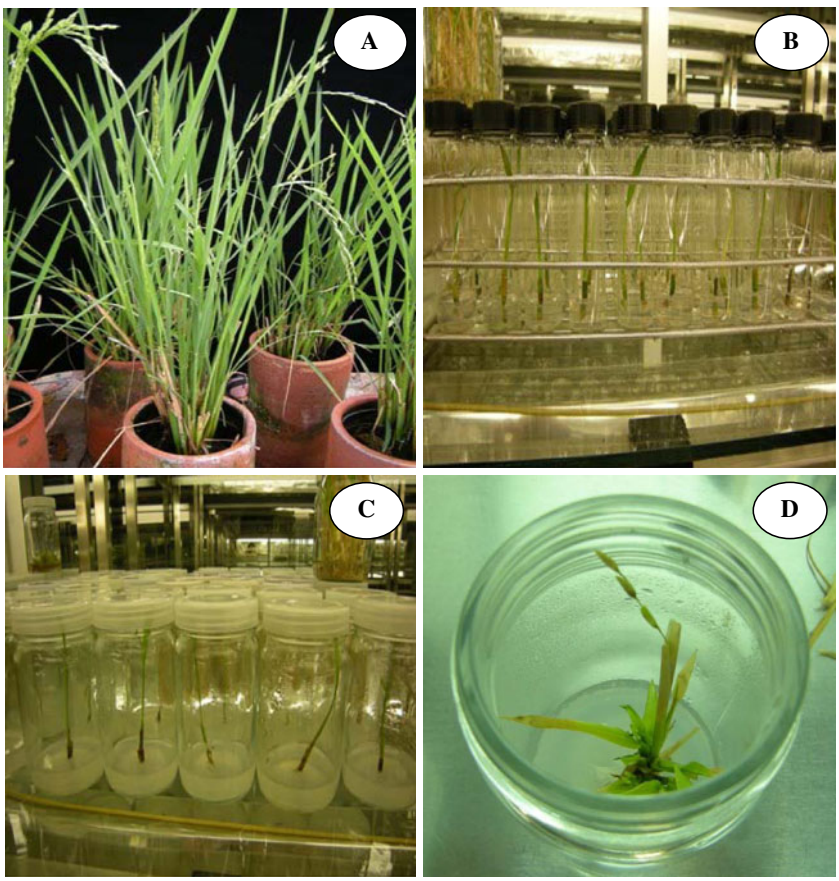

Figure 1. Protocol of *in vitro* flower initiation in PT1 rice (*O. sativa* L. spp. *indica* cv. Pathumthani 1); primary nodal explants (*A*), contamination checking (*B*), plantlet culture system (*C*), and flower developmental stage (*D*) after culture in modified MS medium for 90 d.

Table 1. Flowering percentage (FP), number of flowers per cluster (FN), plant height (PH), number of leaves per plant (LN), leaf area (LA), fresh weight (FW), and dry weight (DW) of plantlets derived from seedlings (without flowering), or nodal explants (with flowering) cultured in modified MS medium for 90 d

Explant	FP (%)	FN	PH (cm)	LN	LA (mm^2 $plant^{-1}$)	FW (mg)	DW (mg)
Seedling	0b	0b	9.57±0.60a	3.3±0.6a	411.6±9.4a	196.4±5.9a	41.1±3.2a
Nodal cutting	60±2a	1±0.2a	8.75±0.38b	2.5±0.5b	316.7±10.5b	175.7±4.3b	35.8±2.3b
ANOVA	–**	–*	–*	–*	–*	–*	–*

Values±SE with different *letters* in each *column* are significantly different at $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

seedling and node cuttings, were used as initial material for this experiment (Fig. 1*C*). The seedling and node cutting explants were cultured on modified MS medium containing 6.81 μM paclobutrazol, 4% (*w/v*) sucrose, and 1.5% (*w/v*) agar. The culture medium was adjusted to pH 5.7 before autoclaving. The plantlets were cultured *in vitro* under 25±2°C ambient temperature, 60±5% relative humidity and 60±5 μmol m^{-2} s^{-1} photosynthetic photon flux density, provided by fluorescent lamps with a 16 hd^{-1} photoperiod for 90 d (Fig. 1*D*). Data on photosynthetic pigments, net photosynthetic rate, sugar content, *in vitro* flowering, and growth characters were collected. The experiment was arranged as a completely randomized design with 20 replicates (n=20). Analysis of variance (ANOVA) was analyzed by SPSS software (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA).

Data collection. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (TC) were analyzed following the methods of Shabala *et al*. (1998), and total carotenoids (C_{x+c}) concentration was assayed according to Lichtenthaler (1987). One hundred milligrams of leaf material was collected and placed in 25-ml glass vials, along with 10 ml 95.5% acetone, and blended using a homogenizer. The glass vials were sealed with parafilm to prevent evaporation, and stored at 4°C for 48 h. Chl_a and Chl_b concentrations were measured using a UV–visible spectrophotometer at 662 and 644 nm wavelengths. C_{x+c} concentration was also measured by spectrophotometer at 470 nm. A solution of 95.5% (*v/v*) acetone was used as a blank comparator.

Net photosynthetic rate (P_n) was calculated by comparing the different concentrations of CO_2 inside (C_{in}) and outside (C_{out}) the glass vessel containing the rice plantlets. The CO_2 concentrations at steady state were measured by gas chromatography (Model GC-17A, Shimadzu Co. Ltd., Kyoto, Japan). The P_n of *in vitro* cultivated seedlings was calculated according to the method of Fujiwara *et al*. (1987).

Soluble sugars (sucrose, glucose, and fructose) in the leaf tissues were analyzed according to a modified Karkacier method (Karkacier *et al*. 2003). One hundred milligrams fresh weight tissue was ground with liquid nitrogen in a pre-cooled mortar, extracted with 1 ml nanopure water, vigorously shaken for 15 s, sonicated for 15 min, and then centrifuged at 12,000 rpm for 15 min. The supernatant was filtered through a 0.45-μm membrane and stored at −20°C prior to the measurement of the sugar content (sucrose, glucose, and fructose) using high-performance liquid chromatography (HPLC). A total of 50 μl of crude extracts were automatically injected into the HPLC system with a Waters 600 pump. On-line detection was performed using a Waters 410 differential refractometer detector, and data was analyzed with Empower software. The analytical column was a MetaCarb 87C equipped with a guard column. De-ionized water was used as the mobile phase with a 0.8-ml min^{-1} flow rate. Purified glucose, fructose, and sucrose were used as standards.

Flowering percentage, number of flowers per cluster, plant height (PH), number of leaves per plant (LN), leaf area (LA), fresh weight (FW), and dry weight (DW) of

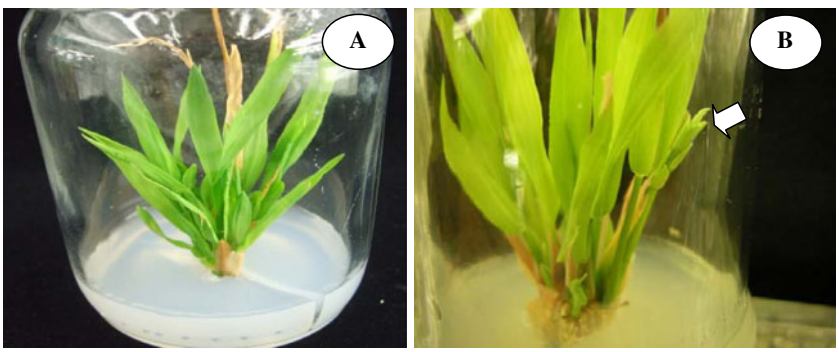

Figure 2. Morphological characteristics of PT1 plantlets derived from seedlings without flowering (*A*), or nodal explants with flowering (*B*; *arrow*) after culture in modified MS medium for 90 d.

Table 2. Chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll (TC), total carotenoid (C_{x+c}) content, and net photosynthetic rate (P_n) of plantlets derived from seedlings (without flowering) or nodal explants (with flowering) cultured in modified MS medium for 90 d

Explant	Chl_a (μg g^{-1} FW)	Chl_b (μg g^{-1} FW)	TC (μg g^{-1} FW)	C_{x+c} (μg g^{-1} FW)	P_n (μmol $CO_2 m^{-2}$ s^{-1})
Seedling	111.2±2.1a	99.7±4.3a	210.9±4.8a	30.2±1.2a	3.2±0.1b
Nodal cutting	92.1±3.7b	52.2±3.7b	144.3±5.2b	24.2±1.9b	4.5±0.2a
ANOVA	–**	–**	–**	–**	–**

Values±SE with different letters in each column are significantly different at $p \leq 0.01$

plantlets were recorded. Rice plantlets were dried at 80°C in a hot-air oven for 2 d, and then incubated in desiccators before the measurement of DW. LA was measured using a leaf area meter DT-scan.

Results

In this investigation, rice inflorescences successfully emerged on modified MS medium containing 6.81 μM paclobutrazol, 4% (*w/v*) sucrose, and 1.5% (*w/v*) agar, using nodal explants in the booting stage. A 60% level of *in vitro* flowering (inflorescence emergence) was observed for plantlets derived from nodal explants of rice (Table 1), and one florescence per cluster was demonstrated (Figs. 1*D* and 2*B*), while flowering was absent from plantlets derived from seedling explants (Fig. 2*A*, Table 1). Plant height, number of leaves per plant, leaf area, fresh weight, and dry weight of *in vitro* plantlets derived from nodal explants decreased significantly ($p \leq 0.05$), and were lower than in plantlets derived from seedlings by 8.57%, 24.24%, 23.06%, 10.54%, and 12.90%, respectively (Table 1). The growth performance of plantlets derived from seedlings was superior to that of plantlets derived from nodal explants. Moreover, the leaf color of the plantlets derived from seedling explants was a darker green (Fig. 2*A*) than that of the plantlets derived from nodal explants (Fig. 2*B*). Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll, and total carotenoids (C_{x+c}) content in the leaf tissues of plantlets derived from nodal explants were significantly reduced, by 17.18%, 47.64%, 31.58%, and 19.87%, respectively, when compared to plantlets derived from seedlings (Table 2). The photosynthetic pigments in the vegetative stage of *in vitro* plantlets were enriched to a greater degree than in the reproductive stage. In contrast, the net photosynthetic rate (P_n) of the plantlets derived from nodal explants was greater than that of plantlets derived from seedlings by a factor of 1.4 (Table 2), relative to sugar accumulation in the leaf tissues. Soluble sugars (including glucose, fructose, and sucrose) in the plantlets derived from nodal explants were manifestly enriched and at higher levels than in plantlets derived from seedlings (Fig. 3). Accumulated soluble sugars in the leaf tissues, major carbohydrate resource obtained from elevated photosynthetic rate and sucrose-applied medium, may play a key role in carbohydrate signaling for flowering initiation in rice plants. Also, P_n in the flowering stage was elevated when compared to the vegetative stage. *In vitro* inflorescence production was not only successfully induced in the PT1 rice cultivar (SD photoperiod insensitive), but also in KDML105 (SD photoperiod sensitive), as demonstrated in Fig. 4. In the present study, rice inflorescences were successfully elicited, although *in vitro* conditions promoting floral blooming, fertilization, and seed set in plant tissue culture are still to be determined. The environmental conditions of *in vitro* culture are quite different from those of a natural paddy field. As a consequence, control of plant tissue culture environment will be further undertaken to study floral emergence, pollen viability, *in vitro* fertilization, and seed development.

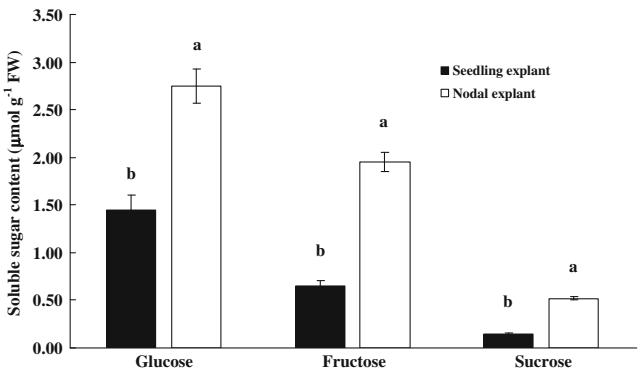

Figure 3. Glucose, fructose, and sucrose contents in the leaf tissues of PT1 plantlets derived from seedlings or nodal explants after culture in modified MS medium for 90 d. Different *letters* in each *bar* show a significant difference at $p \leq 0.01$.

Discussion

A key factor for *in vitro* flowering in rice is the use of nodal explants from the booting stage in natural conditions as the

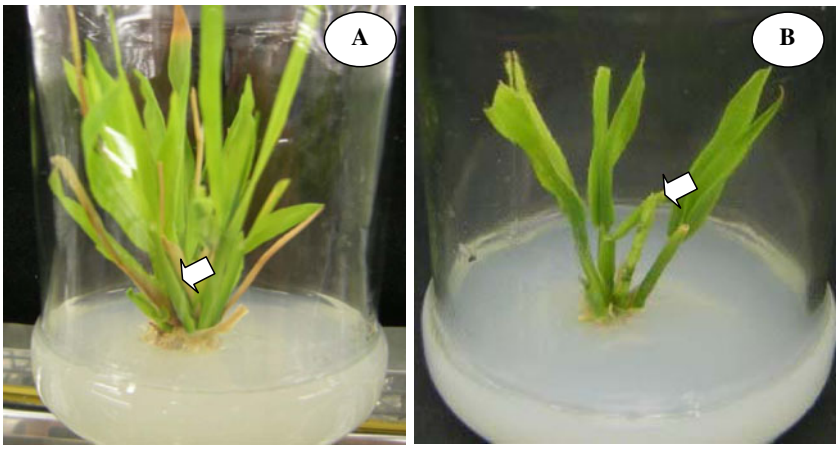

Figure 4. *In vitro* flowering of KDML105 (*O. sativa* L. spp. *indica* cv. KDML105); *arrows* indicate the booting (*A*) and flowering stages (*B*) after culture in modified MS medium for 90 d.

initial plant material. In the case of the explant material, nodal segments from plant species have been used effectively for inflorescence development in the *in vitro* culture system. In addition, plant height and leaf size of *in vitro* plantlets were controlled using 6.81 μM paclobutrazol or anti-gibberellic acid in the culture medium. In previous reports, the function of paclobutrazol as a growth retardant to produce a compact or dwarf form (Thompson *et al*. 2005; Ghosh *et al*. 2010), and alternatively to regulate the floral organs of plants (Qiao *et al*. 2009; Wang *et al*. 2009) has been demonstrated. For example, the proportion of *in vitro* flowering of *Saposhnikovia divaricata* was 26% in a culture medium supplemented with 1.02 μM paclobutrazol, while plant height was retarded to 50% of the control (Qiao *et al*. 2009). Also, the proportion of *in vitro* flowering of the orchid species *Dendrobium nobile* was 33.3% in the culture medium containing 1.72 μM paclobutrazol (Wang *et al*. 2009). In the present study, plant height of *in vitro* plantlets was consequently controlled by adding paclobutrazol to the medium.

In the present study, the levels of photosynthetic pigments (including Chl_a, Chl_b, TC, and C_{x+c}) in plantlets with no inflorescences (seedlings as initial explants) were higher than for flowering plantlets (booting nodal explants). In the reproductive stage of rice, photosynthetic pigments in the leaf tissues were generally reduced, depending on the number of days after flowering, leading to low P_n (Murchie *et al*. 2002; Jiao *et al*. 2003; Zhang *et al*. 2007; Kumagai *et al*. 2009). In contrast, P_n in this study was higher in the flowering plantlets in comparison to those with an absence of flowering. During *in vitro* culture, the major source of carbon is sugar in the culture medium (4% *w/v* sucrose), which is uptaken, translocated, and accumulated in the sink organs. So, the low level of P_n in vegetative plantlets may derived from sugar-feedback inhibition. Floral development in rice relies on direct access to the carbon sources from the leaf factory tissues, which are enriched with soluble sugars. In floral initiation, sugar may play a key role in transition signaling, and was hence applied exogenously in *Perilla frutescens* (Zhang 2007), *Spathiphyllum* (Dewir *et al*. 2007), and *K. leucocephala* (Taylor *et al*. 2007).

In conclusion, inflorescences of *in vitro* cultured rice were successfully initiated using nodal explants derived from the booting stage of plants in pot culture. A dwarf plantlet was obtained *in vitro* by control treatment with paclobutrazol. Glucose was enriched in the leaf tissues of flowering plantlets as a dominant soluble sugar, which was demonstrated as a key factor for flower initiation and development in the rice crop. This innovative protocol was effectively demonstrated for KDML 105, a SD photoperiod-sensitive rice cultivar, which naturally differs from the SD photoperiod-insensitive variety PT1.

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References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309:1052–1056

Ariyaphanphitak W, Chidthaisong A, Sarobol E, Bashkin VN, Towprayoon S (2005) Effects of elevated ozone concentrations on Thai jasmine rice cultivars (*Oryza sativa* L.). Water Air Soil Poll 167:179–200

Chen HJ, Wang SJ (2008) Molecular regulation of sink-source transition in rice leaf sheaths during the heading period. Acta Physiol Plant 30:639–649

Counce PA, Keisling TC, Mitchell AJ (2000) A uniform, objective, and adaptive system of expressing rice development. Crop Sci 40:436–443

Dewir YH, Chakrabarty D, Ali MB, Singh N, Hahn E-J, Paek K-Y (2007) Influence of GA_3, sucrose and solid medium/bioreactor culture on *in vitro* flowering of *Spathiphyllum* and association of glutathione metabolism. Plant Cell Tissue Organ Cult 90:225–235

Fujiwara K, Kozai T, Watanabe I (1987) Fundamental studies on environments in plant tissue culture vessels. (3) Measurements of carbon dioxide gas concentration in closed vessels containing tissue cultured plantlets and estimates of net photosynthetic rates of the plantlets. J Agr Met 43:21–30

Ghosh A, Chikara J, Chaudhary DR, Prakash AR, Boricha G, Zala A (2010) Paclobutrazol arrests vegetative growth and unveils unexpressed yield potential of *Jatropha curcas*. J Plant Growth Regul 29:307–315

Hee KH, Loh CS, Yeoh HH (2007) Early *in vitro* flowering and seed production in culture in *Dendrobium* Chao Praya Smile (Orchidaceae). Plant Cell Rep 26:2055–2062

Hirose T, Ohdan T, Nakamura Y, Terao T (2006) Expression profiling of genes related to starch synthesis in rice leaf sheaths during the heading period. Physiol Plant 128:425–435

Ishimaru K, Hirotsu N, Madoka Y, Kashiwagi T (2007) Quantitative trait loci for sucrose, starch, and hexose accumulation before heading in rice. Plant Physiol Biochem 45:799–804

Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K (2002) Phytochrome mediates the external light signal to repress FT orthologs in photoperiodic flowering of rice. Genes Dev 16:2006–2020

Izawa T, Takahashi Y, Yano M (2003) Comparative biology comes into bloom: genomic and genetic comparison of flowering pathways in rice and *Arabidopsis*. Curr Opin Plant Biol 6:113–120

Jiao D, Ji B, Li X (2003) Characteristics of chlorophyll fluorescence and membrane-lipid peroxidation during senescence of flag leaf in different cultivars of rice. Photosynthetica 41:33–41

Jung C, Müller AE (2009) Flowering time control and applications in plant breeding. Trends Plant Sci 14:563–573

Karkacier M, Erbas M, Uslu MK, Aksu M (2003) Comparison of different extraction and detection methods for sugars using amino-bonded phase HPLC. J Chromatog Sci 41:331–333

Khush GS (2005) What it will take to feed 5.0 billion rice consumers in 2030? Plant Mol Biol 59:1–6

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yono M (2002) Hd3a, a rice ortholog of the *Arabidopsis FT* gene, promotes transition to flowering downstream of Hd1 under short-day conditions. Plant Cell Physiol 43:1096–1105

Kumagai E, Araki T, Kubota F (2009) Characteristics of gas exchange and chlorophyll fluorescence during senescence of flag leaf in different rice (*Oryza sativa* L.) cultivars grown under nitrogen-deficient condition. Plant Prod Sci 12:285–292

Laohakunjit N, Kerdchoechuen O (2007) Aroma enrichment and the change during storage of non-aromatic milled rice coated with extracted natural flavor. Food Chem 101:339–344

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

Lin CS, Chen CT, Hisao HW, Chang WC (2005) Effects of growth regulators on direct flowering of isolated ginseng buds *in vitro*. Plant Cell Tissue Organ Cult 83:241–244

Lin CS, Chen CT, Lin CC, Chang WC (2003) A method for inflorescence proliferation. Plant Cell Rep 21:838–843

Masmoudi-Allouche F, Meziou B, Kriaâ W, Gargouri-Bouzid R, Drira N (2010) *In vitro* flowering induction in date palm (*Phoenix dactylifera* L.). J Plant Growth Regul 29:35–43

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

Murchie EH, Yang J, Hubbart S, Horton P, Peng S (2002) Are there associations between grain-filling rate and photosynthesis in the flag leaves of field-grown rice? J Exp Bot 53:2217–2224

Naor V, Kigel J, Ziv M (2004) Hormonal control of inflorescence development in plantlets of calla lily (*Zantedeschia* spp.) grown *in vitro*. Plant Growth Regul 42:7–14

Qiao Q, Xing F-W, Xiao Y-P, Chen H-F (2009) Somatic embryogenesis and *in vitro* flowering in *Saposhnikovia divaricata*. J Plant Growth Regul 28:81–86

Shabala SN, Shabala SI, Martynenko AI, Babourina O, Newman IA (1998) Salinity effect on bioelectric activity, growth, Na^+ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. Aust J Plant Physiol 25:609–616

Sim GE, Loh CS, Goh CJ (2007) High frequency early *in vitro* flowering of *Dendrobium* Madam Thong-In (Orchidaceae). Plant Cell Rep 26:383–393

Tang T, Xie H, Wang Y, Lü B, Liang J (2009) The effect of sucrose and abscisic acid interaction on sucrose synthase and its relationship to grain filling of rice (*Oryza sativa* L.). J Exp Bot 60:2641–2652

Taylor NJ, Light ME, van Staden J (2007) Monosaccharides promote flowering in *Kniphofia leucocephala in vitro*. Plant Growth Regul 52:73–79

Tee CS, Maziah M, Tan CS (2008) Induction of *in vitro* flowering in the orchid *Dendrobium* Sonia 17. Biol Plant 52:723–726

Thompson DI, Anderson NO, van Staden J (2005) Watsonias as container plants: using paclobutrazol for flowering and height control. S African J Bot 71:426–431

Tsuji H, Tamaki S, Komiya R, Shimamoto K (2008) Florigen and the photoperiodic control of flowering in rice. Rice 1:25–35

Tsuji H, Taoka K, Shimamoto K (2011) Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation. Curr Opin Plant Biol 14:45–52

Uchiumi T, Uemura I, Okamoto T (2007) Establishment of an *in vitro* fertilization system in rice (*Oryza sativa* L.). Planta 226:581–589

Wang ZH, Wang L, Ye QS (2009) High frequency early flowering from *in vitro* seedlings of *Dendrobium nobile*. Sci Hort 122:328–331

Zhang CJ, Chu HJ, Chen GX, Shi DW, Zuo M, Wang J, Lu CG, Wang P, Chen L (2007) Photosynthetic and biochemical activities in flag leaves of a newly developed superhigh-yield hybrid rice (*Oryza sativa*) and its parents during the reproductive stage. J Plant Res 120:209–217

Zhang T (2007) *In vitro* flowering of *Perilla frutescens*. In Vitro Cell Dev Biol 43:91–94

Ziv M, Naor V (2006) Flowering of geophytes *in vitro*. Prop Ornam Plant 6:3–16