ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Journal of Agronomy 6 (1): 68-74, 2007
ISSN 1812-5379

An Alternative Protocol for Flowering Induction of Thai indica Rice (*Oryza sativa* L. sp. *indica*) Using a Short-Day Photoperiod Treatment

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Abstract: The aim of this study is to develop a novel technique for reproductive induction of Thai indica rice grown under environmental control system. Flower time of aroma rice cultivars, jasmine (KDML 105) and Homjan (HJ) were directly induced by 8 h photoperiod flux in both a month exposure and long-term exposure as short-day photoperiod sensitive. The growth characteristics, including plant height, leaf length, leaf area and flag leaf area, were highly regulated by short-day photoperiod. As well as, the Short-Day (SD) photoperiod (8-10 h day^{-1}) was strongly enhanced on rice flowering over crop maturity and positively related to flag leaf area. On the other hand, the flower time of Pathumthani 1 (PT1) rice was initiated in all treatments as SD insensitive or day natural rice. In addition, the maturity or prolong the Day after Plantation (DAP) strongly stimulated on PT1 rice flowering over photoperiod treatments. The basic knowledge of this investigation was successfully applied to induce the flower in 7 lines of F_1 salt-tolerant rice population. It should be further used for rice pure-line production in a short period of breeding program.

Key words: Breeding program, environmental control system, F_1 population, photoperiod sensitive, maturity

INTRODUCTION

Rice is one of the most staple cereals, which is supply as carbohydrate sources over a half the world population (Cassman, 1999; Khush, 2005). Rice yield has been dramatically improved for global food production (Nguyen and Ferrero, 2006) because the world population is increase to 8 billion in 2025, especially in Asia (60% earths' people live). There are many research topics to increase on rice crop yield since Green Revolution, including hybrid rice, super hybrid rice and transgenic C_4 rice (Yuan, 1997; Yang *et al*., 2002; Miyao, 2003; Virmani and Kumar, 2004; Li-jiao *et al*., 2006; Suzuki *et al*., 2006; Takai *et al*., 2006). In conventional rice breeding, the reproductive or flowering is an important stage for rice crop improvement, which is an attractive topic. There are many factors to induce on rice flowering such as photoperiod, light quality, light intensity, plant hormones and low temperature (Summerfield *et al*., 1992; Laurie, 1997; Adams *et al*., 2001; Yano *et al*., 2001; Kojima *et al*., 2002; Craufurd *et al*., 2003; Fujino, 2003; Hayama *et al*., 2003; Izawa *et al*., 2003; Boss *et al*., 2004; Laurie *et al*., 2004; Baurle and Dean, 2006; Izawa *et al*., 2006). The photoperiodic control of rice flowering has been intensively studied in molecular level, including Quantitative Trait Loci (QTLs) or gene(s) mapping (Laurie, 1997; Yano *et al*., 2001; Nakagawa *et al*., 2005) and flowering time gene(s) control (Yano *et al*., 2001; Izawa *et al*., 2002; Kojima *et al*., 2002; Fujino, 2003; Hayama *et al*., 2003; Izawa *et al*., 2003; Cui *et al*., 2004; Doi *et al*., 2004; Ma *et al*., 2004; Laurie *et al*., 2004). In Thailand, aromatic rice cultivars (*Oryza sativa* L. sp. *indica*) have been popularly cultivated in several areas, especially in north and northeastern regions as a high cooking quality, long grain and jasmine flavor. These varieties have been classified into two groups, short-day photoperiod sensitive and day natural rice (insensitive day-length). Jasmine (KDML 105) and Homjan (HJ) rice cultivars are reported as a short-day plant (SDP), while Pathumthani 1 is a SD photoperiod insensitive (Ariyaphanphitak *et al*., 2005; Leohakunjit and Kerdchoechuen, 2007). In addition, HJ rice is identified as salt-tolerant variety (Cha-um *et al*., 2007), which is an effective parental line for salt-tolerant improvement. A short-cut conventional breeding initially depends on matching time of parent flowering stages. In present study, the investigation of flowering induction is to be controlled by short-day environmental factors and crop maturity, leading to produce a flower for reciprocal-cross breeding in KDML105×HJ and PT1×HJ.

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MATERIALS AND METHODS

Effect of photoperiod flux on rice flowering: Three varieties of aromatic rice, Thai jasmine (KDML105), Homjan (HJ) and Pathumthani 1 (PT1), were used as initial material. Seeds of rice varieties were germinated and then planted in pot containing clay soil and water. One-month-old rice plants were divided to three groups, including Day Natural condition (DN), short-exposure (SESD) in a month and Long-Exposure Shot-Day photoperiod (LESD) prior to flowering date. The rice plants were treated short-day photoperiod in Walk-in Incubator (NK System Model LP-3PH, Nipon Medical Chemical Instruments Co Ltd., Tokyo Japan) at 28±2°C air temperature, 70±5% relative humidity (RH), 120±10 μmol m^{-2} sec^{-1} photosynthetic proton flux (PPF) with 8 h photoperiod provided by fluorescence lamps. Plant height, leaf length, leaf area, flag leaf length and flag leaf area were measured as morphological characteristics in flowering stage. The leaf area was measured using a Leaf Area Meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., UK). The experiment was designed as Completely Randomized Design (CRD) with 6 replications and 10 plantlets per replication. The means of parameters in all treatments were compared by Duncan's New Multiple Ranged Test (DMRT) and analyzed using SPSS software.

Plant maturity and photoperiod flux on rice flowering: Seeds of KDML105, HJ and PT1 rice varieties were germinated and then planted in pot containing clay soil and water. Rice seedlings in different days after planting (DAP) at 28, 42 and 56 days were treated by 8, 10 and 14 h photoperiod in Walk-in Incubator at 28±2°C air temperature, 70±5% relative humidity (RH), 120±10 μmol m^{-2} sec^{-1} PPF provided by fluorescence lamps for a month and then transferred to natural day-length photoperiod. Plant height, leaf length, leaf area, flag leaf length and flag leaf area were measured as morphological characteristics in flowering stage. The experiment was designed as 3×3 factorial in CRD with 6 replications and 10 plantlets per replication. The means of parameters in all treatments were compared by DMRT and analyzed using SPSS software.

Photoperiod sensitive assay in F_1 population: F_1 population of reciprocal-cross breeding in KDML105× HJ (D001, D002 and D003) and PT1×HJ (D004, D005, D006 and D007) was treated by a short-day photoperiod in Walk-in Incubator as previous description. Plant height, leaf length, leaf area, flag leaf length and flag leaf area were measured as morphological characteristics in flowering stage. The experiment was designed as CRD with 6 replications and 10 plantlets per replication. The means of parameters in all treatments were compared by DMRT and analyzed using SPSS software.

RESULTS

Effect of photoperiod flux on rice flowering: In day natural condition (long-day photoperiod or >11 h day^{-1} photoperiod), Jasmine (KDML105) and Homjan (HJ) rice cultivars showed the vegetative or juvenile stage, while Pathumthani 1 (PT1) rice produced a flower after plantation for 110 days. Flower induction in all cultivars was significantly stimulated by a month exposure time (SESD) and a long-exposure time (LESD) of short-day photoperiod (Table 1). It should be confirmed that the flower induction of KDML105 and HJ was related to a short-day photoperiod. Plant height, leaf area and flag leaf area in flowering stage of KDML105 rice treated by LESD were higher than those grown under SESP for 1.2, 1.1 and 1.4 folds, respectively. In addition to, plant height, leaf length, leaf area, flag leaf length and flag leaf area of HJ rice were expressed in similar pattern to KDML 105 (Table 2). There was a positive relation between whole leaf area and flag leaf area in both KDML105 (r^2 = 0.60) and HJ (r^2 = 0.68) rice cultivars (Fig. 1A and B). On the other hand, PT1 rice was a SD photoperiod insensitive that significantly showed the highest leaf area and flag leaf area in LESD treatment at 111.49 and 32.35 cm^2, respectively (Table 2). It was non-correlation between leaf area and flag leaf area in PT1 (r^2 = 0.15) rice (Fig. 1C).

Plant maturity and photoperiod flux on rice flowering: KDML105 and HJ rice cultivars were significantly displayed as SD photoperiod sensitive in both 8 and 10 h treatments. In 14 h photoperiod, those cultivars were still expressed on vegetative growth and unpredicted the reproductive parameters, especially flag leaf length and flag leaf area. Plant height, leaf length and flag leaf length of KDML105 rice were significantly enhanced by long day after planting and 10 h photoperiod, whereas whole leaf area and flag leaf area does not effects by Day after Planting (DAP). Flag leaf length of 56 days after planting

Table 1: Flower induction of Thai jasmine, Homjan and Pathumthani 1 rice cultivars in day-natural (control), short-exposure (SESD) and long-exposure shot-day photoperiod (LESD) treatments

Rice cultivars	Day-natural (DN)	SESD	LESD
Jasmine rice	-	+	+
Homjan	-	+	+
Pathumthani 1	+	+	+

-; Non flowering +; Flower induction

Table 2: Plant height, leaf length, leaf area, flag leaf length and flag leaf area of Thai jasmine, Homjan and Pathumthani 1 rice cultivars in day-natural (control), short-exposure (SESD) and long-exposure shot-photoperiod (LESD) treatments

Rice cultivars	Height (cm)	Leaf length (cm)	Leaf area (cm^2)	Flag leaf length (cm)	Flag leaf area (cm^2)
Jasmine rice					
Day-natural	0.00c	0.00b	0.00c	0.00b	0.00c
SESD	90.55b	46.63a	96.43b	57.38a	24.48b
LESD	105.47a	47.33a	105.41a	60.00a	34.06a
ANOVA	**	**	**	**	**
Homjan					
Day-natural	0.00c	0.00c	0.00c	0.00c	0.00c
SESD	105.80b	47.97b	58.14b	67.05b	40.33b
LESD	128.57a	56.53a	90.77a	81.20a	63.11a
ANOVA	**	**	**	**	**
Pathumthani 1					
Day-natural	104.82	41.15	99.28a	57.58	27.06b
SESD	96.33	44.63	72.15b	60.72	26.49b
LESD	98.47	45.13	111.49a	61.93	32.35a
ANOVA	NS	NS	**	NS	**

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's Multiple Range Test (DMRT). Non-significant and highly significant in statistics are represented by NS and **, respectively

Table 3: Plant height, leaf length, leaf area, flag leaf length and flag leaf area of Thai jasmine rice cultivars in different days after planting (DAP) at 28, 42 and 56 days and treated by 8, 10 and 14 h photoperiod in Walk-in Incubator

Treatments	Height (cm)	Leaf length (cm)	Leaf area (cm^2)	Flag leaf length (cm)	Flag leaf area (cm^2)
28 DAP					
8 h photoperiod	89.72ab	42.61b	113.51ab	44.79b	22.26ab
10 h photoperiod	86.61abc	42.85b	91.80b	44.80b	17.14b
14 h photoperiod	0.00c	0.00c	0.00c	0.00d	0.00c
42 DAP					
8 h photoperiod	86.71abc	45.89a	97.52ab	44.02b	19.50ab
10 h photoperiod	93.13a	47.57a	117.76a	53.36a	24.31a
14 h photoperiod	0.00d	0.00c	0.00c	0.00d	0.00c
56 DAP					
8 h photoperiod	83.00bc	43.14b	98.52ab	41.09c	20.97ab
10 h photoperiod	81.92c	47.41a	100.46ab	54.74a	20.06ab
14 h photoperiod	0.00d	0.00c	0.00 c	0.00d	0.00c
Significant level					
DAP	**	**	NS	**	NS
Photoperiod	**	**	**	**	**
DAP×Photoperiod	*	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's Multiple Range Test (DMRT). Non-significant, significant and highly significant in statistics are represented by NS, * and **, respectively

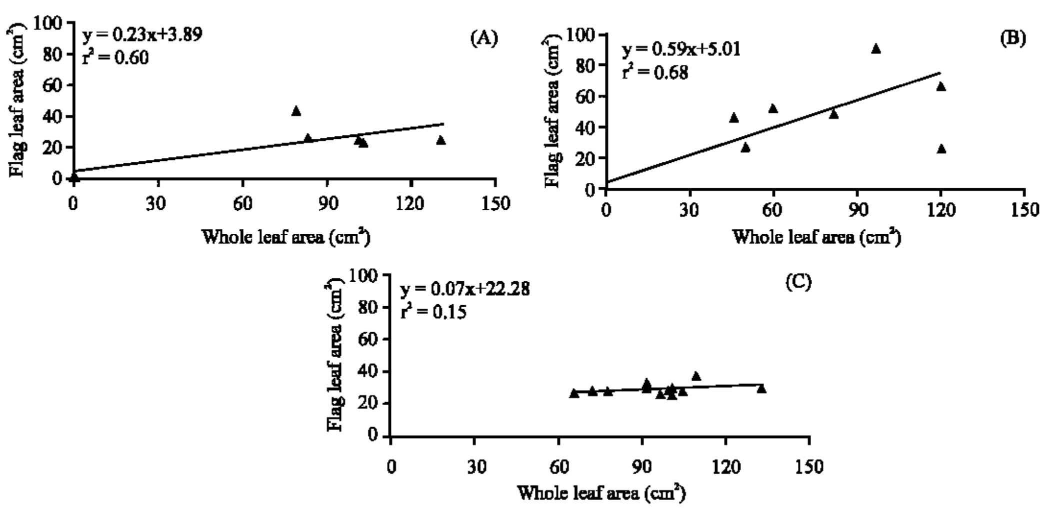

Fig. 1: Relationship between whole leaf area and flag leaf area in Thai jasmine (A), Homjan (B) and Pathumthani 1 (C) rice cultivars in day-natural (control), short-exposure (SESD) and Long-Exposure Shot-photoperiod (LESD) treatments

in KDML105 rice treated by 10 h photoperiod showed the highest at 54.74 cm (Table 3). Likewise, day after planting and photoperiod factors were strongly affected on all parameters of HJ rice (Table 4). It should be noted that the 8-10 h SD photoperiod at was directly stimulated on flowering induction of KDML105 and HJ rice cultivars. There were a positive correlation between photoperiod and flag leaf area (r^2 = 0.87 and r^2 = 0.78) (Fig. 2A and 2B). In addition to, the whole leaf area of SD photoperiod sensitive cultivars was positively related to flag leaf area (r^2 = 0.99 and r^2 = 0.96) (Fig. 3A and 3B). Alternatively, PT 1 rice was defined as photoperiod insensitive that the flowering time was depended on day after plantation or maturity. The photoperiod treatment in PT1 rice was unaffected on flowering time (Table 5 and Fig. 2C). As well as, it was un-correlation between whole leaf area and flag leaf area in PT1 rice (Fig. 3C).

Photoperiod sensitive assay in F_1 population: The basic knowledge of previous studies in SD photoperiod

Table 4: Plant height, leaf length, leaf area, flag leaf length and flag leaf area of Homjan rice cultivars in different days after planting (DAP) at 28, 42 and 56 days and treated by 8, 10 and 14 h photoperiod in Walk-in Incubator

Treatments	Height (cm)	Leaf length (cm)	Leaf area (cm^2)	Flag leaf length (cm)	Flag leaf area (cm^2)
28 DAP					
8 h photoperiod	98.95ab	44.82b	80.54c	51.16cd	16.39c
10 h photoperiod	90.70b	44.22b	89.41bc	48.35de	22.08abc
14 h photoperiod	0.00c	0.00c	0.00d	0.00f	0.00d
42 DAP					
8 h photoperiod	102.95a	46.21ab	95.10b	54.46bc	26.86a
10 h photoperiod	102.31a	49.39a	111.52a	59.97a	24.17ab
14 h photoperiod	0.00c	0.00c	0.00d	0.00f	0.00d
56 DAP					
8 h photoperiod	90.71b	43.47b	78.99c	45.56e	19.88bc
10 h photoperiod	103.85a	46.88ab	91.10bc	56.50ab	24.77ab
14 h photoperiod	0.00c	0.00c	0.00d	0.00f	0.00d
Significant level					
DAP	*	**	**	**	*
Photoperiod	**	**	**	**	**
DAP×Photoperiod	**	*	*	**	*

Different letter(s) in each column show significant difference at p≤0.01 by Duncan's Multiple Range Test (DMRT). Significant and highly significant in statistics are represented by * and **, respectively

Table 5: Plant height, leaf length, leaf area, flag leaf length and flag leaf area of Pathuthani 1 rice cultivars in different days after planting (DAP) at 28, 42 and 56 days and treated by 8, 10 and 14 h photoperiod in Walk-in Incubator

Treatments	Height (cm)	Leaf length (cm)	Leaf area (cm^2)	Flag leaf length (cm)	Flag leaf area (cm^2)
28 DAP					
8 h photoperiod	87.72a	40.92a	66.27bc	40.97ab	20.46ab
10 h photoperiod	85.52a	39.28ab	77.78abc	41.60ab	22.55a
14 h photoperiod	87.33a	37.15cd	79.10abc	40.47ab	16.43b
42 DAP					
8 h photoperiod	82.77a	38.90abc	74.84abc	42.07ab	19.41ab
10 h photoperiod	84.55a	40.49ab	81.88ab	44.20a	19.55ab
14 h photoperiod	84.51a	35.08e	90.94a	41.18ab	15.13b
56 DAP					
8 h photoperiod	87.36a	38.84bc	64.55bc	40.4ab	20.66ab
10 h photoperiod	74.92b	36.83de	58.78c	34.60c	17.05ab
14 h photoperiod	84.48a	36.29de	79.10abc	38.47bc	16.43b
Significant level					
DAP	**	**	**	**	**
Photoperiod	**	**	**	NS	NS
DAP×Photoperiod	**	**	*	**	NS

Different letter(s) in each column show significant difference at p≤0.01 by Duncan's Multiple Range Test (DMRT). Non-significant, significant and highly significant in statistics are represented by NS, * and **, respectively

Table 6: Plant height, leaf length, leaf area, flag leaf length and flag leaf area of F_1-population rice varieties treated by 8 h photoperiod in Walk-in Incubator

Rice population	Height (cm)	Leaf length (cm)	Leaf area (cm^2)	Flag leaf length (cm)	Flag leaf area (cm^2)
D001	72.63abc	37.54a	64.07b	37.81bc	17.66
D002	78.12a	34.35cd	65.79b	40.58a	17.50
D003	74.33ab	36.01b	84.62a	39.28ab	18.61
D004	72.09bc	35.47bc	64.78b	38.38abc	17.30
D005	74.42ab	34.46cd	69.51b	39.96ab	18.05
D006	68.33c	34.24cd	74.27ab	38.07abc	19.35
D007	69.16bc	33.51d	67.99b	36.17c	18.17
ANOVA	**	**	**	*	NS

Different letter(s) in each column show significant difference at p≤0.01 or p≤0.05 by Duncan's Multiple Range Test (DMRT). Non-significant, significant and highly significant in statistics are represented by NS, * and **, respectively

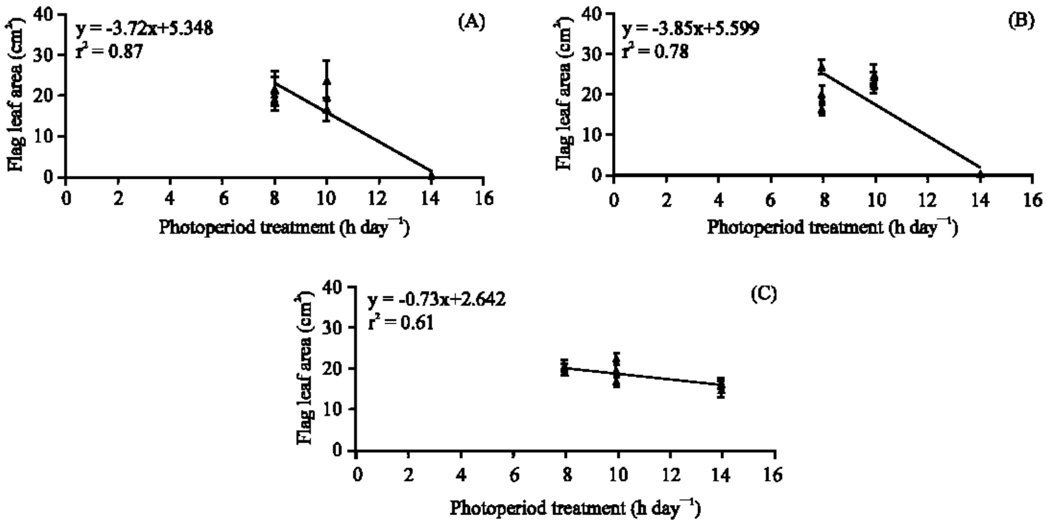

Fig. 2: Relationship between photoperiod and flag leaf area in Thai jasmine (A), Homjan (B) and Pathumthani 1 (C) rice cultivars in different kay After Planting (DAP) at 28, 42 and 56 days and treated by photoperiod fluxes for 8, 10 and 14 h d^{-1} in Walk-in Incubator

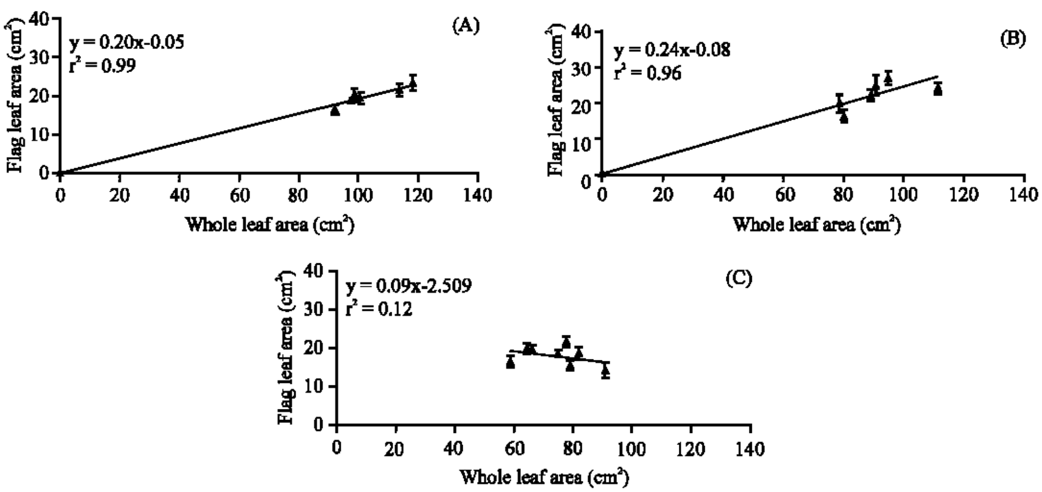

Fig. 3: Relationship between whole leaf area and flag leaf area in Thai jasmine (A), Homjan (B) and Pathumthani 1 (C) rice cultivars in different Day After Planting (DAP) at 28, 42 and 56 days and treated by photoperiod fluxes for 8, 10 and 14 h day^{-1} in Walk-in Incubator

sensitive should approve by reciprocal F_1 population of conventional breeding. The results showed that all lines of reciprocal F_1 population, D001-D007, were sensitive to SD photoperiod treatment (Table 6). It means that the photoperiod sensitive may be a dominant gene control that directly transferred from KDML105 or HJ to F_1 population. As well as, this investigation may be played as an alternative way to assay the photoperiod sensitive in mass population in either rice crop or other cereal species.

DISCUSSION

Short-Day (SD) photoperiod is one of the most environmental factors to stimulate on rice flowering. There are many reports to classify the cultivars and wild rice responses to SD in term of SD photoperiod sensitive and photoperiod insensitive (Ganashan and Whittington, 1976; Summerfield *et al*., 1992; Adams *et al*., 2001; Craufurd *et al*., 2003). KDML105 and HJ cultivars are represented as SD photoperiod sensitive, whereas PT1 rice is a SD photoperiod insensitive. The SD photoperiod treatment is not only early flowering time (Craufurd *et al*., 2003) but also plant height reduction (Ganashan and Whittington, 1976) in 6 rice varieties and their hybrid population. Moreover, low temperature or vernalization treatment is an alternative topic to induce rice flower together with SD photoperiod. The couple factors are positively related to early flowering induction in wild rice (*Oryza glaberrima*) and cultivated rice (*Oryza sativa* L. sp. *indica* and *japonica*) (Summerfield *et al*., 1992; Craufurd *et al*., 2003). Alternatively, the maturity is a strong factor for flowering initiation of SD insensitive variety as BR11 (Halder *et al*., 2004). It is similar pattern to PT1 rice in our experiment that defined as SD photoperiod insensitive variety. Flag leaf in reproductive stage of rice plays a key role on carbohydrate biosynthesis and sink-source transition, leading to grain yield efficacy (Nakano *et al*., 1995; Murchie *et al*., 2002; Ishimaru *et al*., 2004; Bing *et al*., 2006; Yang *et al*., 2007). The flag leaf is potentially index for reproductive stage development in rice crop to mention on present study. The SD photoperiod treatment in a month exposure time should be a novel tool to further apply for flowering induction in conventional rice breeding program.

CONCLUSIONS

Short-day photoperiod flux is a powerful factor to control the rice flowering time in sensitive cultivars, KDML105 and HJ, while insensitive cultivars (PT1) dose not influence. It was confirmed that the short-day photoperiod treatment was directly stimulated on KDML105 and HJ rice flowering. In addition, the maturity in PT1 rice would be played an important role in flowering initiation. This investigation was applied to assay the photoperiod sensitive in F_1 population of reciprocal conventional breeding as well as mass population of rice breeding program.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Sittiruk Roytrakul for critical reading manuscript, Dr. Teeraporn Boozaya-angoon for rice seed providing and conventional reciprocal-cross and National Center for Genetic Engineering and Biotechnology (BIOTEC Grant number BT-B-02-RG-BC-4702) for funding sources.

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