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Research Paper

A practical protocol to accelerate the breeding process of rice in semitropical and tropical regions

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Breeding of excellent rice varieties is essential for modern rice production. Typical breeding procedures to introduce and maintain valuable agricultural traits require at least 8 generations from crossing to stabilization, always taking more than 4–5 years of work. This long and tedious process is the rate-limiting step in the development of new varieties, and therefore fast culturing methods are in urgent need. Taking advantage of early flowering characteristics of light-sensitive rice under short-day conditions, we have developed a practical protocol to accelerate the breeding cycle of rice, which we have termed the “1 + 2”, “2 + 2”, “1 + 3”, and “0 + 5” methods according to the different rice varieties and different breeding purposes. We have also incorporated several techniques, including glume cutting, seed desiccation at 50°C in a drier seed dormancy breakage with low concentration of HNO_3, and direct seeding. Using the above strategy, we have shortened the life cycle of light-sensitive rice varieties to about 70 days, making it possible for several rice cultivars to proliferate 4–5 generations in a single calendar year. This protocol greatly accelerates the process of new variety breeding, and can be used in rice research for shortening the process of genetic analysis and the construction of mapping populations.

Key Words: *Oryza sativa*, variety breeding, life cycle, short-day, fast proliferation.

Introduction

Rice (*Oryza sativa*) is one of the most important crops in the world. In China, its culture area accounts for about 25% of the whole cereal culture area, and accounted for about 50% of total food supply in 2010. Excellent rice cultivars are essential to increase field yields and improve rice taste and cooking quality. The application of high quality seeds contributes to about 70% efficiency of total improvement methods (Cheng *et al.* 2007, Maninder *et al.* 2015). To produce excellent rice cultivars with high yield, high resistance to disease and insects, outstanding quality, and adaptation to a wide range of ecological niches are the goals of rice breeders (Khush 2001, Xing and Zhang 2010).

Traditional rice breeding procedures include several generations of crossing and backcrossing between two parental cultivars to combine and stabilize desired agricultural traits (Giri and Vijaya Laxmi 2000). Seven to eight generations are required from the start of crossing to final authorization, and more generations are necessary for distant hybridization. Medium-late *Japonica* rice, which covers the most culture area in China, can only be cultured one generation in its local area because of its high-sensitive to light, two generations could be accomplished in a year with one more generation in the tropical area. With normal breeding procedures and natural conditions in China, it always takes researchers five or more years to complete these tedious processes of crossing and selection. This long breeding period greatly decreases the efficiency of research and development of new varieties. Therefore, methods for fast rice culturing are in urgent need. Rice is an annual, warm, short-day crop, growing in environments with high temperature and high humidity (Sweeney and McCouch 2007). Rice is divided into two major subspecies, *Indica* rice and *Japonica* rice. *Indica* rice is tolerant to high temperature and strong light; in contrast, *Japonica* rice is tolerant to low temperature and weak light strength (Kovach *et al.* 2007, Sang and Ge 2007). Based on differences in response to light period, rice can be classified into early, medium, and late types (Wei *et*

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al. 2009). The late types are light-sensitive and flower only in short-day conditions, whereas the early and medium types are less sensitive to light and show no significant response to light-period changes. The growth period of rice is restricted by both light and temperature: it grows and develops faster under high temperature, while heading earlier under short-day conditions with enough vegetation growth. Therefore, an accelerated rice culturing method can be developed according to growth habits in terms of light and temperature.

Hainan Province (the southernmost province of China) is located between 18 and 20 degrees north latitude and has sufficient light and temperature sources for rice growth throughout the year (Gao *et al.* 2014). From September to the following May (the autumn, winter and spring seasons of the Northern Hemisphere), the light condition at Hainan Province is in short-day period, which induces rice to flower much earlier than that of normal growing time (from April to October) in Zhejiang Province (located in the semitropical region of China) (http://www.weather.com.cn/html/weather/101210301.shtml). Taking advantage of the short days and high temperature climate in Hainan Province, we performed some simple manipulations, which we have termed the "1 + 2", "2 + 2", "1 + 3", and "0 + 5" methods, to shorten rice life cycle to about 70–80 days. For the "1 + 2" and "2 + 2" methods, 2 generations were completed in Hainan Province and 1 or 2 generations in Zhejiang Province. For the "1 + 3" method, we cultured rice for 1 generation in Zhejiang Province and 3 generations in Hainan Province. For the "0 + 5" method, we cultured rice for 5 generations in Hainan Province with no breeding in Zhejiang Province. With these breeding methods, we cultured rice for four to five generations in one year, which observably promoted the breeding process. This practical proposal for shorting the rice breeding process could be used for reference in other areas located in temperate zones and semitropical areas, to increase generations of rice breeding.

Materials and Methods

Materials and growth conditions

To obtain general data of rice life cycle, twenty widely applied cultivars were chosen to represent four classificatory types, i.e., early *Indica*, medium *Indica*, medium *Japonica*, and late *Japonica*. Some of them are traditional cultivars and some are hybrid rice varieties or restored lines from different culturing regions of China or other countries (**Supplemental Table 1**). All varieties were cultured both in Jiaxing and Lingshui. Jiaxing, located in southeast China, has a typical semitropical climate, while Lingshui (in Hainan Province) stands in the southernmost area of China with a typical tropical climate (**Supplemental Fig. 1**, http://ditu.google.cn/maps).

Culture arrangement for different purposes

For cultivars whose yields and growth characteristics in Jiaxing needed to be determined, we planted them in Jiaxing to complete one generation from late May to late October. We then cultured them in Lingshui from October to the following May to complete another three generations. For plants used for genetic analysis, construction of a mapping population, making a recombinant inbred line (RIL), or continuous backcrossing, we planted them only in Lingshui to complete five generations in one year. Detailed manipulations are described in the following paragraphs.

Technique I: direct planting and subsequent culturing

To reduce wounding of seedlings during transplanting, all seedlings were directly planted in the field, without additional processes of culturing seedlings in a nursery and further transferring them to another place (**Fig. 1**D, **1**E, **1**F). To culture small samples, we planted germinated seeds in an 8 cm × 8 cm line-to-row ratio (36 seedlings) in a 50 cm × 50 cm square. For larger samples, we expanded the field to 90 cm × 90 cm to plant about 150 seedlings. A wood board was used to cover seedlings with mud. Irrigation and fertilization of seedlings were performed after the 3rd leaf appeared. Other field management was conducted in a normal manner.

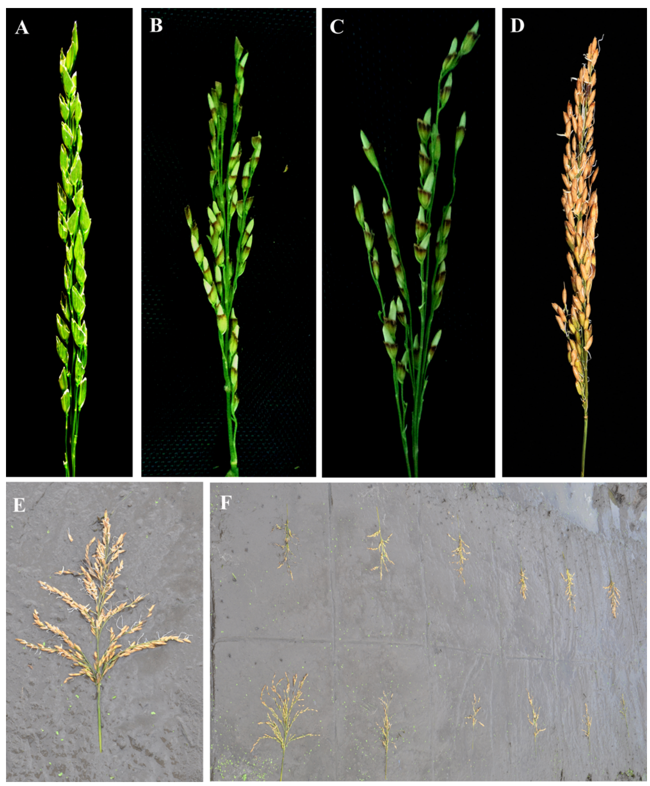

Fig. 1. Manipulations to shorten rice life cycle in Lingshui. A. Cutting half of the glumes of impregnated flowers. B. Unpolished seeds resulting from crossing. C. Unpolished seeds resulting from cutting the top 1/3 of glumes of male parent. D. Whole spikes with germinating seeds. E. Direct planting of whole spikes with germinating seeds. F. Direct planting of individual samples.

Technique II: crossing method

When rice opens flowers, choose inflorescences with some opened flowers from strong tillers to cross or back-cross. Remove the opened flowers and left about 25 impregnate flowers for each tiller in the afternoon, when the opened flowers close its glume two hours later. Then cut the top half of glumes to remove stamens with covering by envelopes (**Fig. 1**A). On the next day, pollination was performed with pollen from a different background. The crossing seeds were harvested (**Fig. 1**B) 15–18 days after pollination. For male-sterile lines which do not require artificial emasculation, the manipulations are the same as traditional rice crossing without removing stamens.

Technique III: treatment for promoting maturation

Firstly, rice was planted at a proper high density (90–100 plants/m^2). Seeding density is an important factor for rice yield (Lin *et al.* 2011, Wang *et al.* 2014). In our experience, high density seedlings can also promote rice maturation: growing rice seedlings at a proper high density always leads to the mimicking of a short-day effect, and direct planting associated with rational close planting can promote rice to flower earlier by 10–15 days than transplanting rice.

Secondly, the glumes were cut at rice florescence. According to our experience, hybrid seeds have germination ability 15 days after pollination. To make the maturation stage of parental plants consistent with hybrid rice, flowers at the lower part of the spike were removed, leaving several strong spikelets, and the top 1/3 of glumes were cut to promote maturing of male parent, which provides pollen in the next generation (**Fig. 1**C).

Thirdly, artificial short-day conditions were made to accelerate maturation. Day length plays an important role for late *Japonica* rice florescence (Ghose and Shastry 1954, Song and Luan 2012). There are long-day light conditions in Lingshui (from late April to late July) and Jiaxing (from March to early August). Light-sensitive cultivars will stay in the vegetative stage without growth-phase transition. To accelerate the early maturation of these cultivars, we placed artificial shades on rice seedlings at a growth stage of more than five leaves old. Using black plastic woven mesh to cover them at 5 p.m. and uncovering the mesh at 7 a.m. on the next morning resulted in 11 : 13 h of light: dark photoperiod. This treatment was performed continuously for about 14 days.

Technique IV: dormancy breaking and germination treatment

The freshly harvested seeds were treated with desiccation-HNO_3 treatment-germination acceleration to break seed dormancy and germination (Bewley 2013, Bradbeer 1988, Zhang *et al.* 2009). The unpolished seeds or the whole spikes were desiccated at 50°C for 24–30 hours in a drier. They were then subjected to HNO_3 solution treatment for 58–60 hours to thoroughly break dormancy. These seeds were washed with clear water and put in an incubator for germination for 40–50 hours. When 70% of the seeds were germinated, seedlings were properly transferred to the field to prevent their roots from growing too long to separate from each other (**Fig. 1**C). To make the HNO_3 solution, we added 3.5 ml 36% HNO_3 (Sinapharm, China) to 1 L water for grains while adding 2.5 ml 36% HNO_3 to 1 L water for unpolished seeds. The HNO_3 solution should be newly made to prevent HNO_3 volatilization.

Results

Determination of rice life cycles

From 2001 to 2011, we observed and determined the growth and development characteristics of more than 20 *Indica* and *Japonica* varieties in Jiaxing and Lingshui. Normally, rice can grow two generations from mid-April to late October in Jiaxing, with a lifespan of about 95 (early *Indica* rice) and 155 (late *Japonica* rice) days for each generation. As shown in **Table 1**, the life cycle of early *Indica* and late *Japonica* is about 70 days in the autumn and spring seasons in Lingshui, which is remarkably shorter than that in Jiaxing in the summer season. Interestingly, the life cycle of these cultivars in the autumn and spring seasons is much shorter than that in the winter season (about 100 days), corresponding to low-temperature conditions of Lingshui in the winter. On the other hand, some cultivars of medium *Indica* and *Japonica* (such as BG367-4, IR54, and Minghui63) have a long basic vegetative growth period (more than 115 days for each generation) in both places. Therefore, it is difficult to use this protocol to shorten their life cycle. To obtain repeatable and reliable results, we recorded related light and temperature parameters for every breeding generation (data not shown).

Effectiveness of the treatments for shortening rice life cycles

To evaluate the effectiveness of treatments for shortening rice life cycles, two late *Japonica* rice varieties, '*Jia58*' and '*Jia33*', were planted in Jiaxing and Hainan between 2012 and 2013.

The '*Jia33*' was planted in Hainan for two generations between 11/25/2012 and 05/18/2013; the high-density direct seeding method (90–100 plants/m^2) and normal seeding method (transplanting at a density of 30–37 plants/m^2) were used for comparison. The results indicated that 12 and 14 days of the rice life cycles were shortened with the high-density direct seeding method (**Table 4**). The '*Jia58*' and '*Jia33*' planted with the normal seeding method were used to compare the effectiveness of cutting glumes. '*Jia33* × *Jia58*' crossing was also carried out to compare the maturation process of hybridization seeds and normal seeds. The results showed that the growth period from flowering to maturation could be shortened to about 15 days, and shortened to 12 days using unpolished rice for germination (**Table 5**). The results also indicated that the growth period showed no significant difference between flowers with or

Table 1. Life cycle record of varieties in different seasons in Jiaxing and Lingshui. 2001–2011

#	Cultivar name	Summer season in Jiaxing (April to Oct.)*	Autumn season in Lingshui (Sep. to Dec.)	Winter season in Lingshui (Dec. to March)	Spring season in Lingshui (Feb. to May)	Early summer season in Lingshui (March to June)
1	*Jiaxing aromatic rice*	95 ± 5	80 ± 5	90 ± 5	75 ± 5	70 ± 5
2	*Jiaxing8*	90 ± 5	80 ± 5	90 ± 5	75 ± 5	69 ± 5
3	*Jiashao1*	90 ± 5	75 ± 5	85 ± 5	72 ± 5	68 ± 5
4	*Jiayu293*	93 ± 5	80 ± 5	90 ± 5	75 ± 5	70 ± 5
5	*Xieqingzao*	100 ± 5	85 ± 5	100 ± 5	80 ± 5	75 ± 5
6	*Minghui63*	125 ± 5	95 ± 5	110 ± 5	100 ± 5	90 ± 5
7	*Pei'ai64s*	115 ± 5	90 ± 5	100 ± 5	95 ± 5	85 ± 5
8	*Lemont*	112 ± 5	96 ± 5	105 ± 5	99 ± 5	86 ± 5
9	*Rico No 1*	110 ± 5	95 ± 5	105 ± 5	97 ± 5	88 ± 5
10	*Jiaxian hui82*	125 ± 5	98 ± 5	118 ± 5	100 ± 5	90 ± 5
11	*Zhendao88*	130 ± 5	81 ± 5	91 ± 5	72 ± 5	70 ± 5
12	*Jiahui47*	130 ± 5	80 ± 5	92 ± 5	70 ± 5	70 ± 5
13	*Jiahui67*	120 ± 5	85 ± 5	97 ± 5	70 ± 5	73 ± 5
14	*Liaojing9*	110 ± 5	70 ± 5	85 ± 5	70 ± 5	70 ± 5
15	*Koshihikari*	105 ± 5	70 ± 5	78 ± 5	70 ± 5	70 ± 5
16	*Wuyujing7*	140 ± 5	81 ± 5	95 ± 5	75 ± 5	76 ± 5
17	*Jia06-64*	155 ± 5	92 ± 5	98 ± 5	80 ± 5	76 ± 5
18	*Jia33*	155 ± 5	80 ± 5	95 ± 5	75 ± 5	76 ± 5
19	*Jiayou2*	150 ± 5	78 ± 5	92 ± 5	75 ± 5	76 ± 5
20	*5088s*	150 ± 5	80 ± 5	92 ± 5	75 ± 5	73 ± 5

1–10 were *Indica* cultivars, whose data were the average over four years from 1996 to 1999. 11–20 are *Japonica* cultivars, whose data were the average over four years from 2006 to 2009.
*: In Jiaxing, *Indica* cultivars were planted in the middle ten days of April while *Japonica* cultivars were planted in the last ten days of May.

without artificial pollination after cutting glumes.

The artificial short-day conditions could accelerate the early maturation of light-sensitive cultivars. We placed artificial shades on rice seedlings at a growth stage of more than five leaves old for about 14 days. With this treatment, the rice growth period was shortened to about 50 days, and life cycles could be completed in 100–110 days (**Table 6**). As freshly harvested rice seeds remain dormant, breaking dormancy treatment, such as H_2O_2, GA_3, KNO_3, or HNO_3, should be applied to accelerate rice breeding (Lei *et al.* 2004, Zhang *et al.* 2009). Low content of HNO_3 solution was used to break dormancy in this protocol. The germination percentage reached about 85% after 5 days of acceleration, while 15 days will be needed by the contrast (**Fig. 2**).

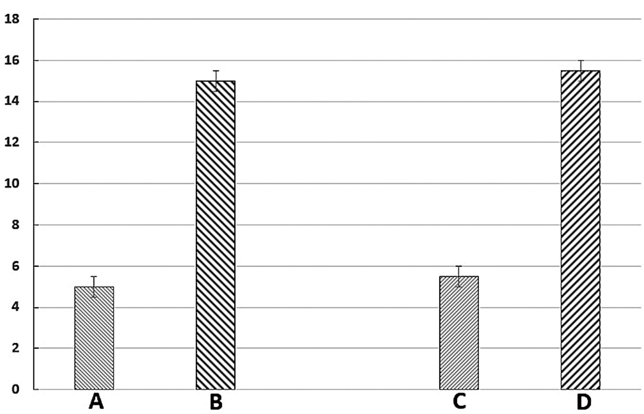

Fig. 2. Days needed for rice germination rate to reach 85% with dormancy breaking treatment and contrast. A, C: '*Jia58*' and '*Jia33*' seeds treated with desiccation-HNO_3 treatment-germination acceleration; B, D: '*Jia58*' and '*Jia33*' seeds treated with desiccation-germination acceleration.

Culture arrangement and application examples

Taking advantage of the treatments for shortening rice life cycles, while considering breeding purpose, we proposed four methods which we have termed "*1 + 2*", "*2 + 2*", "*1 + 3*", and "*0 + 5*". Detailed operation methods and application examples are as follows.

"1 + 2" and "2 + 2" methods for two generations in tropical regions

The "1 + 2" method is suitable for *Japonica* rice varieties from the Huang-huai-hai region and the northeast area of China with a life cycle of more than 150 days. The "2 + 2" protocol is suitable for double-cropping early *Indica* rice from the middle and lower regions of the Yangtze River. After complete production in its original region, two generations were cultured in a tropical region from early October to late March. For the first generation, seeding at early October and harvest at mid-December; From late December to late March for the second generation.

Take the breeding of "Shaojia1" which is an early *Indica* rice for example. The F_2 group named "199706" was planted by direct seeding in Lingshui on October 20, 1997; 113 spikes with desirable agronomic characteristics were selected on January 3, 1998. The 113 spikes, named "F_3-1–113", were divided into two groups, one for direct seeding on January 11, and the other for rice blast resistance detection. On April 7, 71 of 113 spikes with good agronomic characteristics and

Table 2. The construction process of "*Jiahui30*" *IPA1* near-isogenic line

Generation	Area	Seeding date (month/day/year)	Materials	Crossing date (month/day/year)	Manipulation
1	Lingshui	12/25/2010	e6 and JH30	2 /12/2011	e6 × JH30
2	Lingshui	3 / 6 /2011	F_1 and JH30	4 /26/2011	F1 × JH30
3*	Jiaxing	5 /25/2011	BC_1F_1 and JH30	8 / 1 /2011	BC_1F_1 × JH30
4	Lingshui	8 /30/2011	BC_2F_1 and JH30	10/14/2011	BC_2F_1 × JH30
5	Lingshui	11/ 8 /2011	BC_3F_1 and JH30	1 /23/2012	BC_3F_1 × JH30
6	Lingshui	2 /20/2012	BC_4F_1	–	BC_4F_1⊗
7	Jiaxing	5 /25/2012	BC_4F_2	–	BC_4F_2⊗
8	Lingshui	9 /30/2012	*J335A*, *J60A*, *J57A*and BC_4F_3	11/15/2012	*J335A*, *J60A*, *J57A* × BC_4F_3 separately
9	Lingshui	12/10/2012	3 hybrid combinations		Hybrid rice test and regional test

* The *IPA1* gene was tested in every generations from BC_1F_1 to "*Jiahui30*" NIL completed.

Table 3. The construction process of "*Jia33*" near-isogenic line

Generation (address)	Seeding date (month/day)	Materials	Heading date (month/day)	Manipulation	Harvest date (month/day)	Remarks	Growth period (days)
1 (Jiaxing)	5/25	P_1 (*Jia64*) and P_2 (*Jia33*)	8/25	$P_1 \times P_2$	9/15	Harvest F_1 seeds	114
2 (Hainan)	9/22	F_1	11/ 8	Select 250 spikelets, cutting top glume	11/24	Harvest 189 F_2 seeds	64
3* (Hainan)	11/28	F_2 and P_2	1/26	Glabrous Plant × P_2	2/15	Harvest B_1F_1 27 seeds	80
4 (Hainan)	2/20	B_1F_1	4/6	Select 300 spikelets of different plants, cutting top glume	4/25	Harvest B_1F_2 281seeds	65
5 (Hainan)	4/28	B_1F_2 and P_2	6/16	Short-day treatment for 12 days on 5/14, Select Glabrous Plant × P_2	7/5	Harvest B_2F_1 seeds	69
6 (Hainan)	7/9	B_2F_1	8/26	Select 300 spikelets of different plants, cutting top glume	9/13	Harvest B_2F_2 seeds	67

* The third generation in Hainan was planted in a plastic greenhouse to promote rice growth.

high rice blast resistance were selected. This series was then planted in Jiaxing where its original region on 20th April, and finally the "*Shaojia1*" variety was developed after F_6–F_7 generation breeding.

"1 + 3" method for three generations in a tropical region

This method carries one generation in the original region from late May to late September and three generations from early October to late May in Hainan Province. More specifically, seeding was performed in late May and harvesting of seeds with germination capacity in late September. Then take three generations from early October to early December, mid-December to early March, mid-March to late May, separately.

This method is a typical method for adding a generation of late *Japonica* rice. Take the breeding of "*Jia58*" for example. Three generations were completed from October 5, 2006 to May 20, 2007 in Hainan Province. For the first generation, the F_1 seeds of "Jia33 × Jia0664" were planted by the direct seeding method on October 5, 2006; the seeds of F_2 were harvested on December 6, 2006. For the second generation, the harvested seeds of the first generation were planted on December 15, and then the F_3 seeds were harvested on March 8, 2007. For the third generation, planting was on March 11 and the F_4 seeds were harvested on May 20, 2007. The late *Japonica* rice "*Jia58*" was developed after an additional generation in Jiaxing and two generations in Hainan Province from the years 2007 to 2008, and then it was authorized in Zhejiang Province in 2013 after regional tests in Hangzhou City and Zhejiang Province.

To produce rice varieties with ideal plant architecture, we carried out molecular marker-assisted selection breeding using the "*IPA1*"gene (Jiao *et al.* 2010). A near-isogenic line (NIL) of "*Jiahui30*" with the *IPA1* gene was developed in 22 months with this protocol (**Table 2**).

"0 + 5" method for five generations in a tropical region

For late *Japonica* from the middle and lower regions of the Yangtze River, and *Japonica* from the Yellow River and northeast China, the "0 + 5" protocol was used for genetic analysis, construction of near-isogenic lines (NIL), continuous backcrossing to generate male-sterile lines, and testing of compatibilities between different lines of hybrid rice. One generation was completed in about 70 days.

Five generations could be completed in one year for light-sensitive *Japonica* rice and early *Indica* rice in Hainan Province. Taking the construction of "*Jia33*" NIL as an example (**Table 3**), 5 generations were completed from September 22, 2009 to late July 2010.

Discussion

Based on the records of life cycle of different cultivars in Jiaxing and Lingshui from 1996 to 2014, we developed a fast rice culturing protocol for breeding. This protocol combines four major manipulations to shorten the rice life cycle

Table 4. Effectiveness of high-density direct seeding

Planting address	Material	Planting method	Seeding date (month/day/year)	Heading date (month/day/year)	Maturation date (month/day/year)	Growth period (days)
Hainan	*Jia33*	Normal seeding	11/25/2012	02/04/2013	03/01/2013	95
Hainan	*Jia33*	High-density direct seeding	11/25/2012	01/23/2013	02/16/2013	83
Hainan	*Jia33*	Normal seeding	03/01/2013	04/26/2013	05/18/2013	79
Hainan	*Jia33*	High-density direct seeding	03/01/2013	04/14/2013	05/04/2013	65

Table 5. Effectiveness of cutting glumes

Planting address	Material	Glume treatment	Crossing date (month/day/year)	Flowering date (month/day/year)	Maturation date (month/day/year)	Growth period from flowering to maturation (days)
Hainan	*Jia33*	Cutting glumes	–	04/30/2013	05/15/2013	15
Hainan	*Jia58*	Cutting glumes	–	04/30/2013	05/16/2013	16
Hainan	*Jia33*	Cutting glumes	*Jia33* × *Jia58*	04/30/2013	05/13/2013	13
Hainan	*Jia33*	Without treatment	–	04/30/2013	05/30/2013	30
Hainan	*Jia58*	Without treatment	–	04/30/2013	05/31/2013	31

Table 6. Effectiveness of artificial short-day conditions

Planting address	Material	Seeding date (month/day/year)	Short-day treatment starting date (month/day/year)	Short-day treatment ending date (month/day/year)	Maturation date (month/day/year)	Growth period (days)
Jiaxing	*Jia58*	5/25/2013	–	–	11/01/2013	160
Jiaxing	*Jia58*	5/25/2013	6/29/2013	7/13/2013	8/26/2013	93
Jiaxing	*Jia33*	5/25/2013	–	–	10/28/2013	156
Jiaxing	*Jia33*	5/25/2013	6/27/2013	7/11/2013	8/23/2013	90

to about 70 days. This fast-breeding protocol can be applied in genetic analysis, construction of mapping populations and near-isogenic lines (NIL), and continuous crossing and backcrossing in breeding procedures. With this protocol, we have generated six authorized cultivars in only three years, which is much faster than the average efficiency of new cultivar development.

The fast-breeding protocol was developed according to rice growth characteristics, mainly on the short-day feature. The rice flowering phase is controlled by a number of environmental factors, such as day length, temperature, and water supply. Day length plays an important role in flowering time for late *Japonica* rice of the strong light-sensitive type (Ghose and Shastry 1954, Song and Luan 2012). The artificial short-day conditions could be manipulated in two ways, one by shading with black plastic woven mesh at the five-leave-old growth stage, and the other by high-density direct seeding. We found different treating days should be applied to different growth stages, 14 days for five-leave-old seedlings, 12 days for seven-leave-old seedlings, and 10 days for nine-leave-old seedlings (Li *et al.* 2010, China Rice, 16: 45–46). Rational close seeding is one of the important techniques for superior quality and high yield of rice (Lin *et al.* 2011). It has been suggested that a seeding density of 25 cm × 17 cm is suitable for high-yielding hybrid rice (Wang *et al.* 2014). However, a higher density (90–100 plants/m^2) has been suggested in order to create short-day conditions and speed up the breeding process. Light-sensitive rice always shows 'over-optimum age' phenotype and heads earlier under short-day conditions. In this study, we were able to head rice 10–15 days earlier by high-density direct seeding.

In our experience, cutting glumes can promote maturation. Self-fertilization seeds can have germination ability at 15 days after cutting the top 1/3 of glumes, which seems like hybrid seed, because only strong spikelets were left and more nutrients could be used for seed development. It will take more than 30 days from flowering to maturation for inflorescence without treatment. Thus, a reduction of 10–15 days can be achieved using unpolished rice for germination. Freshly harvested rice seeds remain dormant, but it has been reported that many methods can be used to break seed dormancy, such as chilling, dry storage, light, and exposure to chemicals. (Bewley 2013, Bradbeer 1988). H_2O_2, GA_3, KNO_3, and HNO_3 have been reported to be effective chemicals in breaking rice dormancy (Lei *et al.* 2004, Zhang *et al.* 2009). Rice can reach an 88% seed germination rate after 24 h of soaking in 0.5% HNO_3 solution (Zhang *et al.* 2009). In this study, we proposed two concentrations of HNO_3 solution for grains and unpolished seeds. The seed germination rate could reach about 85% after 5 days of germination treatment. Therefore, HNO_3 solution treatment can be an effective method to break seed dormancy.

From 1993 to 2013, we successfully bred 18 new varieties and 4 male-sterile lines. Fifteen varieties were characterized in one province, and 3 varieties were authorized in more than two provinces or were authorized by state organizations (**Supplemental Table 2**). During our early stage (1993–2003), we only generated 4 traditional rice cultivars, without any hybrid rice or male-sterile line. The average time consumed for each cultivar was more than 5 years, and the total culturing area was no more than 100,000 hectares. However, after we gradually developed this method for multiple-generation breeding and adopted it from 2003, 14 cultivars were generated and characterized. More remarkably, we developed 8 new varieties within only four years from 2007 to 2010. Two of these outstanding varieties, "*Jia58*" (authorized in Zhejiang) and "*Jia33*" (authorized in Jiangsu, Zhejiang, and Shanghai) have been planted for more than 335,000 hectares. These new varieties not only display excellent quality, high yield, and high resistance to various kinds of pathogens, but also are suitable for planting under different climate conditions and environments in China. Recently, we adopted this protocol to introduce the *IPA1* gene to Jiaxing local varieties and successfully obtained two lines showing remarkable high field production within 18 months (unpublished data). Thus, this fast culturing protocol has shown great advantage in improving current breeding courses and procedures and has brought much benefit to modern agriculture production.

Arabidopsis is adopted as a model organism in a wide range of theory and application studies, owing to its small size, simple genome, multiple genetic resources, and especially, for its fast growth speed (Arabidopsis: a laboratory manual, 2002). In a typical greenhouse with long-day conditions, Arabidopsis completes its life cycle in about two months; thus, it can reproduce more than 6 generations in one year (Arabidopsis: a laboratory manual, 2002; Li *et al.*, unpublished data). As Arabidopsis grows fast, researchers can easily and quickly obtain genetic analysis data, construct mapping populations, gain homozygote transgenic plants, and make double or even higher multiple mutants. Rice has been adopted as a model organism for its absolute importance in the global food supply (Cheng *et al.* 2007). Originally, rice was a short-day organism with a long basic vegetative growth period; the life cycle of most rice varieties is more than 120 days, and even 160 days. The biggest obstacle in conducting genetic analysis or breeding studies on rice is its long period of growth and development. To accelerate the breeding process, breeding researchers have to culture rice one generation in a native region and one generation in winter in a tropical region (e.g., Hainan in the south of China). With this protocol, we have greatly shortened the rice life cycle to about 70 days, which is comparable to that of Arabidopsis. We can arrange rice experiments or breeding procedures that are the same as those for Arabidopsis, which remarkably increases our efficiency in development of new cultivars. This protocol is suitable for not only light-sensitive varieties or those having a short basic vegetative growth period from East Asia but also early *Indica* cultivars from South China. For varieties displaying a long basic vegetative growth period, such as late maturing medium *Japonica* and late maturing medium *Indica* from South China or Southeast Asia, this protocol cannot evidently promote their growth. In addition, this protocol will also provide some clues to the promotion of breeding processes of other light-sensitive organisms, such as maize and soybean.

Furthermore, cultivars developed with this protocol show enhanced light sensitivity, high-temperature tolerance, and a little earlier maturation than other varieties generated in native regions (Li *et al.*, unpublished data). These excellent characteristics provide two important advantages. First, those cultivars showing less light sensitivity can complete their life cycle before the cold wave comes and grow well in an even higher temperature climate, leading to stable high yields and wider applications from north China to south China. Second, the flowering date of these cultivars is steady and the flowering times of restored lines and male-sterile lines can coincide properly, which will greatly increase the production of hybrid seeds of rice.

In applying this protocol, we advise noting the following additional points. First, researchers should choose appropriate and fertile fields with good irrigation. Second, to prevent the interference of previous rice seedlings growing in the same field, other crops or vegetables should be cultured before planting rice. Third, as the roots of directly planted rice grow on the soil surface, irrigation and fertilization should be managed properly. Finally, high-density direct seeding should be of proper density (90–100 plants/m^2), or the plants will be too weak and easily falling by seeding too close.

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