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Down-regulation of the *OsPDCD5* gene induced photoperiod-sensitive male sterility in rice

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ABSTRACT

Programmed cell death (PCD) is a crucial process for plants during development and environmental stress. *OsPDCD5*, is an ortholog to mammalian programmed cell death 5 gene. Here we report that decreased expression of *OsPDCD5* caused by antisense technology could induce pollen sterility in photoperiod-sensitive rice. The male sterility of transgenic plants is reversible, determined mainly by photoperiod. Transgenic plants are sterile under long-day photoperiod (≥13.5 h sunlight) with postponed heading time, while they can restore fertility under short-day photoperiod (below 13 h sunlight). Analysis of tissue sections of anthers displays the tapetum cells of the anther wall retard PCD process in transgenic plants under long-day photoperiod. The retarded PCD was also confirmed by DNA fragmentation. In F_1 hybrids made from transgenic plants with antisense-*OsPDCD5* and japonica rice varieties, lower transcript inhibition could restore fertility, under certain photoperiod and temperatures. The surprising discovery of the photosensitive male fertility suggested that by using specific photoperiods, the male sterility (PGMS) could be used for commercial production of hybrid rice using a two-line breeding system. The transgenic hybrid strategy was easy to apply, reduced costs and shortened the breeding period. It provides great advantages for commercial applications in rice and other crops.

1. Introduction

Hybrid vigor or heterosis has been widely applied in the commercial production of many crops. The production of hybrid seeds is dependent on an effective pollination control system that will prevent unwanted self-pollination. Recently genetic engineering tools have been developed to make hybrid seed production easier, mainly through the generation of nuclear-encoded male sterility and its restoration. These strategies included the destruction of the tapetum or other anther-specific tissues during pollen development by expressing cytotoxic products [1–3], suppressing the pollen development pathway [4] and altering the levels of essential metabolites in pollen [5,6]. Because genetically engineered nuclear male sterility is dominant, development of genetically modified lines to restore hybrid fertility and maintain sterile plants is necessary. The process of propagating the male sterile lines and hybrids is labor intensive and becomes complicated. Some genetically engineered male sterility lines can propagate by crossing with normal rice, but selecting sterile lines from the progeny can be problematic [7]. An effective means of developing conditional, reversible male sterility would be preferable and practical.

There are several approaches for securing reversible male sterility, including chemical induction [8,9] and environmental modulation [10] of fertility. These strategies often produce extra protein which poses a potential contamination problem for both the plant and the environment in general. In addition, the efficiency of this system is relatively low and the cost of chemical induction can be high.

Here a new system for creation of inducible male sterile rice based on down-regulation of the *OsPDCD5* gene was demonstrated. Surprisingly the transgenic male sterile rice displayed PGMS (photoperiod-sensitive genetic male sterility [11]), which was influenced by the light/dark-hour ratio in the environment. Further the F_1 hybrids from transgenic rice and rice varieties could also restore fertility under long-day conditions.

2. Materials and methods

2.1. Construction of transgenic expression vectors

Fragment *OsPDCD5*, which was sub-cloned with the *Bst*EII and *Hind*III restriction sites at 5′ and 3′ ends, and the binary

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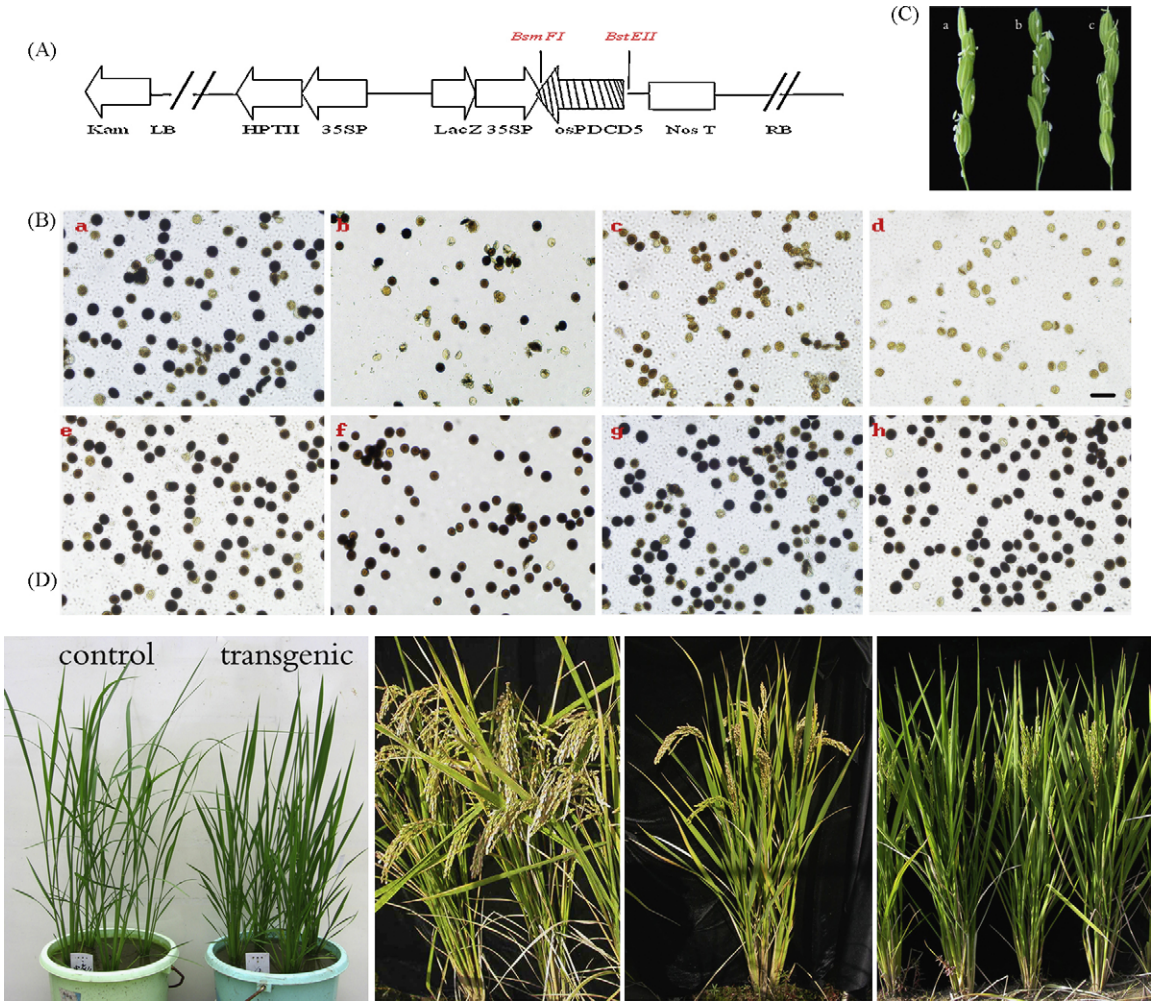

Fig. 1. Phenotype of 35s: antisense-*OsPDCD5* transgenic rice and the control Zhonghua 11 grown under natural long-day conditions. (A) Construction of a chimeric gene for the expression of antisense RNA. 35SP: CaMV 35S promoter; Nos T: nos terminator; HPTII: hygromycin; kam: kanamycin. (B) The fertility of pollen from plants grown under artificial conditions with different photoperiods. bars = 50 μm. a–d from transgenic rice grown under 11.5, 12.5, 13.5 and 14.5 h of light, respectively. h is the control. e–g are F1 generation from *antisense-OsPDCD5* transgenic rice crossed with Zhonghua 11, Wuxiangjing no. 14 and Zhendao 88, respectively, under natural long-day conditions in Shanghai (≥13.5 h sunlight). (C) Pollen grains of control (left), F1 (middle) and transgenic (right) rice. (D) Control (Zhonghua 11) and transgenic rice at tillering stage (left1). Photos of control Zhonghua 11 (left 2), transgenic rice (left 4) and their F1 (left 3) were taken at maturity in the field of Shanghai.

plasmid vector pCAMBIA1304 (donated by Dr. Jefferson RA) were both digested with *BsmF*I and *BstE*II. The inverted fragment *OsPDCD5* was sub-cloned into pCAMBIA1304 downstream of the CaMV 35S promoter, resulting in the recombinant plasmid pCAM-anti-*OsPDCD5* (Fig. 1A).

2.2. Plant material and transformation

Embryogenic calli from a weak photoperiod-sensitive *Japonica* rice cultivar 'Zhonghua 11' was used for transformation experiment. The calli were bombarded with tungsten particles coated with the pCAM-anti-*OsPDCD5* plasmid (gene gun transduction). Resistant calli were obtained from a selection medium, consisting of 30 mg/l hygromycin, where they were cultured for 2–3 weeks. The selected calli were transferred into regeneration medium and allowed to develop into plants before being moved into a greenhouse. Test chambers were from China National Rice Research Institute, automatically maintained at certain environmental conditions. The male parents of the hybrid F1 were 'Wuxiangjing no. 14', 'Zhendao 88' and 'C 418' varieties, which were donated the Shandong Academy of Agricultural Sciences.

2.3. PCR analysis to identify the antisense-OsPDCD5

Genomic DNA, extracted from leaf tissue, was used as a template. *Antisense-OsPDCD5* was checked by primers 35arp-4. (5′ CACTGACGTAAGGGATGACGCACAA 3′; 5′ AGATGGCTGACCCAGAGTTGGAAG 3′).

2.4. Southern blot analysis and real-time PCR analysis

Southern hybridization was manifested according to the manufacturer's standard protocol (Alkphos Direct labeling reagent, Amersham Biosciences). The probe for Southern hybridization was *OsPDCD5* fragment. Flag leaves of rice at the booting stage were collected and immediately frozen in liquid nitrogen. Total RNA was extracted using the RNAprep Plant Kit (Tiangen, China). cDNA was synthesized according to manufacturer's instructions (perfect real-time kit, TaKaRa, Japan). Samples were subjected to Real-Time PCR analysis using the Bio-Rad iCycler IQ Real-Time PCR Detection System (Bio-Rad, USA). The primers used for PCR amplification were Jpcd-1(5′ CTCACTTCTGGAGCAAATCAATAC3′; 5′ TGGAACAAGCCAAACCAAACT 3′), Actin-2 (5′ CACGCCATTCGCCTCCTTC3′; 5′ TTTGTCCCATCCCAACCATAA 3′). Relative mRNA quantification was performed by normalization to the *Actin* gene.