Rapid PCD Process Promotes Early Maturity in Weedy Rice (*Oryza sativa* L. f. *spontanea*)

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Abstract

Background: Shorter grain-filling period and rapid endosperm development contributes to early maturity in weedy rice (*Oryza sativa* L. f. *spontanea*). However, the differences in programmed cell death (PCD) process and anti-oxidative enzymes system in the caryopsis between weedy and cultivated rice are largely unexplored.

Main Text: we selected four biotypes of weedy rice and associated cultivated rice (ACR, *Oryza sativa*) from different latitudes to conduct a common garden experiment. The difference of PCD process between weedy rice and ACR was compared by chemical staining, and the cell viability and nuclear morphometry of endosperm cells were observed by optical microscopy, and anti-oxidative enzymes activity were also measured during grain filling. We found that the PCD progress in weedy rice was more rapid and earlier than that in ACR. The percentage of degraded nuclei of weedy rice were 10%-83% higher than that of ACR. Endosperm cells in weedy rice lost cell viability 2-8 days earlier than that in ACR. The anti-oxidant enzymes activity of weedy rice were lower than that of ACR during grain filling. The ability of weedy rice to scavenge reactive oxygen species is weaker than that of ACR, which may contribute to the rapid PCD process in the endosperm cells of weedy rice.

Conclusion: The rapid PCD process and weaker ability to scavenge reactive oxygen species in endosperm cells lead to the shorter grain-filling period of weedy rice.

Introduction

Weedy rice (*Oryza sativa* L. f. *spontanea*) has become one of the most harmful weeds in paddy fields in the world. It is a plant of the genus *Oryza* that infests and competes with cultivated rice (*Oryza sativa*) in the world rice production area (Delouche et al. 2007; Azmi and Karim 2008). Weedy rice has many morphological and physiological characteristics related to weediness, such as rapid growth rate, high phenotypic plasticity, awnedness, early maturity, seed shattering, long seed dormancy and seed longevity, which facilitate seed dispersal and persistence in the paddy field, and weedy rice has been considered one of the three worst weeds in paddy fields worldwide (Azmi and Karim 2008; Dai et al. 2014, 2017; Burgos et al. 2014; Zhao et al. 2018, 2020). As cultivated and weedy rice share similar morphological and physiological traits, there is no selective chemical available to control weedy rice (Chauhan 2013). Our previous study found that the shorter grain-filling period promote early maturity in weedy rice compared with the associated cultivated rice. In addition, weedy rice has heavy shattering, which contribute to weedy rice escape from harvesting (Zhao et al. 2018). Furthermore, the rapid development of endosperm cells and starch grains leads to the shorter grain-filling period of weedy rice (Zhao et al. 2020). However, the relationship between the process of programmed cell death (PCD) in endosperm cells and the early maturity of weedy rice was unclear.

Programmed cell death (PCD) is a genetically determined physiological process which plays an important role in the development of tracheary element cells, aleurone layer cells and root cap cells (van Doorn et al. 2011; Pennell and Lamb 1997; Fukuda 2000; Xie et al. 2014; Fan et al. 2013). In the process of PCD of rice endosperm, the nucleus is the first to die out, and then the cells still maintain high physiological activity (Young and Gallie 1997, 1999). In wheat endosperm, PCD occurred randomly, while in rice, PCD first occurred in the middle of grain, and then gradually developed to the edge (Young et al. 1997; Lan et al. 2004; Li et al. 2018). Endosperm is the main material of rice seeds, accounting for 91% – 92% of the total weight of rice seeds. It stores a large amount of starch and a small amount of protein, which serves as the primary carbohydrate component in the diets of humans and livestock (Sabelli and Larkins 2009; Li et al. 2014). The endosperm cell development of rice is divided into four stages: coenocyte stage, cellularization stage, differentiation stage, and maturation stage during grain filling (Olsen 2004; Li et al. 2014), and there was programmed cell death during endosperm development (Olsen et al. 1995; Domínguez and Cejudo 2014). The development process of endosperm cells in rice was observed by electron microscope. The phenomena of nuclear deformation, nuclear membrane rupture, chromatin condensation and nucleocytoplasmic leakage were observed in endosperm cells, which indicated that PCD occurred during the development of endosperm (Wei et al. 2002; Li et al. 2004).

Evans blue is a macromolecular dye, which can dye the dead cells with membrane permeability loss into blue. TTC (2, 3, 5-triphenyl tetrazolium chloride) is a lipid soluble light sensitive complex, which can react with dehydrogenase in normal cells and turn red. The two staining methods were used to observe the process and pattern of PCD in endosperm cells (Young and Gallie 1999; Wang et al. 2004; Li et al. 2004; Yu et al. 2014; Wu et al. 2016a). Evans blue staining shows that PCD of endosperm cells

can occur shortly after anthesis until seed maturity (Young et al. 1997, Young and Gallie 1999). DAPI (4', 6-diamidino-2-phenylindole) is a kind of DNA fluorescent dye with high sensitivity and specificity, which has excellent fluorescence staining effect on nucleus and chromosome (Locato and De Gara 2018). Steedman's wax is a kind of low temperature wax, which can be miscible with ethanol, and can be used as paraffin section for serial section. Chen et al. (2012) and Wei et al. (2009) successfully used this method to observe the morphological changes of endosperm nucleus in wheat and barley during the process of PCD. Reactive oxygen species (ROS) could destroy the normal cellular metabolism through the oxidative damage to lipids, proteins, and nucleic acids, and cause growth impairment in plants. PCD in plant cells is mainly caused by the accumulation of reactive oxygen species (Breusegem and Dat 2006). To eliminate these ROS, plants have developed a complex anti-oxidative enzymes system (AES), including catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Nunez et al. 2003; Corpas et al. 2006; Zhang et al. 2015).

The PCD process of endosperm cells determines the length of cell cycle, and the PCD process in rice determines the length of grain filling period, and then determines the growth period of rice. There are many reports about the PCD process of cultivated rice, but the research of PCD process contribute to early maturity in weedy rice has not been reported. In previous research, we found that shorter grain-filling stages contribute to the early maturity of weedy rice, and the rapid development of endosperm cells and starch grains leads to the shorter grain-filling period of weedy rice (Zhao et al. 2018, 2020). In the present study, we used Steedman's wax embedded sections, DAPI staining, Evans blue staining and TTC staining to compare and analyze the difference of PCD process in endosperm cells between weedy rice and cultivated rice, and compare the difference of anti-oxidative enzymes system enzyme activity between weedy rice and cultivated rice during grain filling. Our objectives were to describe the rapid PCD process leading to a shorter grain-filling period in weedy rice. Our results on the differences in PCD process of endosperm cell between weedy rice and ACR may provide a new perspective for the control of weedy rice.

Materials And Methods

2.1 Experiment location and cultivation methods

Field trials were established at Jiangpu Experimental Farm (118°37'E, 32°02'N), Nanjing Agricultural University, China, in the summer cropping seasons (between May and November) of 2015. The field sites have clay-loam soil with medium fertility (organic matter 2.8%, N 97 mg kg⁻¹, available P 52 mg kg⁻¹, available K 161 mg kg⁻¹) and pH 7.1. *Echinochloa crus-galli*, *Leptochloa chinensis*, *Monochoria vaginalis* and *Cyperus difformis* were the dominant weeds in the field. The field that was left fallow before the summer season was subjected to rotary tilling. According to our previous studies on the genetic diversity and morphological characteristics of various weedy rice accessions (Dai et al. 2014; Zhao et al. 2018, 2020), we selected four weedy rice accessions of different geographic origins along with their associated cultivars at the collection site. The characteristics of the four weedy rice and their associated cultivars biotypes are listed in Table 1. The materials were previously described by Zhao et al. (2018). The local cultivars included Nangeng-5055 (TZCR) and Zhong Lian Hui-950 (YZCR), which are both widely cultivated in Jiangsu Province; Dangeng-17 (DDCR), which is widely planted in the Dandong City of Liaoning Province; and Yue Xin Zhan-2 (MMCR), which is from Maoming City of Guangdong Province. The experimental plots consisted of a 20 m² plot for each of the *Oryza* accessions, 50 cm spaces between the plots, sowing distances of 30 cm × 15 cm and a design of 20 rows × 20 columns. The individuals of each accession were planted in separate plots with three replications.

Table 1
Characteristics of the representative weedy rice (*Oryza sativa* L.) and cultivated rice (*Oryza sativa*) accessions used in the experiments

Types	District	Population number of rice	Cultivar or Accession	Origin (city, province)	Pericarp colour	Subspecies	Longitude	Latitude
Cultivated rice	Northeast China	WRLN004R1	Dangeng-17 (DDCR)	Dandong, Liaoning	White	<i>Typical-japonica</i>	124°17'E	39°58'N
	Estern China	WRJS023R1	Zhong Lian Hui-950 (YZCR)	Yangzhou, Jiangsu	White	<i>Typical-indica</i>	119°20'E	32°20'N
	Estern China	WRJS013R1	Nangeng-5055 (TZCR)	Taizhou, Jiangsu	White	<i>Typical-japonica</i>	119°57'E	32°26'N
	Southern China	WRGD008R1	YueXinzhan-2 (MMCR)	Maoming, Guangdong	White	<i>Typical-indica</i>	110°50'E	21°40'N
Weedy rice	Northeast China	WRLN004	DDWR	Dandong, Liaoning	Red	<i>Japonica</i>	124°17'E	39°58'N
	Estern China	WRJS023	YZWR	Yangzhou, Jiangsu	Red	<i>Indica</i>	119°20'E	32°20'N
	Estern China	WRJS013	TZWR	Taizhou, Jiangsu	Red	<i>Indica-clinous</i>	119°57'E	32°26'N
	Southern China	WRGD008	MMWR	Maoming, Guangdong	Red	<i>Indica</i>	110°50'E	21°40'N
TZWR: weedy rice from Taizhou; TZCR: cultivated rice from Taizhou; YZWR: weedy rice from Yangzhou; YZCR: cultivated rice from Yangzhou; MMWR: weedy rice from Maoming; MMCR: cultivated rice from Maoming; DDWR: weedy rice from Dandong; DDCR: cultivated rice from Dandong.								

2.2 Sample and data collection

2.2.1 Sampling and endosperm cell staining

A total of 320–340 panicles that headed on the same day were chosen and tagged for each plot. The flowering date of each upper spikelets on the tagged panicles was recorded. The tagged spikelets were sampled at 3, 5, 7, 9, 10, 11, 13, 15, 18, 20, 21, 25, 30, 35 days post anthesis (DPA). Kernels were collected from the upper region of each spikelet. Then grain samples from each replication were combined to form one sample per treatment. Approximately 200 sampled grains of weedy or cultivated rice were frozen in liquid nitrogen for 2 min before storing at -80 °C for measuring the activity of anti-oxidative enzymes.

TTC (2, 3, 5-tryphenyl tetrazolium chloride) stains viable cells or tissues, but not death cells (Lakon 1949). The TTC staining method was modified from Oberle and Watson (1953). Thin sections were made by hand of caryopses in different DPA (3, 5, 7, 9, 11, 13, 15, 18, 21 days post anthesis) and stained in 0.5% (w/v) 0.5% TTC (Aladdin, E104208-10 g, USA) for 30 minutes in 25°C. And photographed with a microscope (SMZ800, Nikon). At least five seeds per sample were observed. The term 'dead cell' should only be used for cells that are indicated to be dead by specific stains used as a viability assay, such as fluorescein diacetate (FDA) or Evans blue (van Doorn et al. 2011). Evans blue dye stains the cytoplasm of nonviable, but not viable, cells. The staining method was modified from Young and Gallie (1999). The caryopsis of 3, 5, 7, 9, 11, 13, 15, 18, 21 days post anthesis (DPA) were cut with sharp double-sided blade by hand after husking and stained in 0.1% (w/v) Evans blue (Aladdin, E104208-10 g, USA) for 2 min. Stained sections were washed with water for 1 hour and photographed with a stereo microscope (Zeiss Discovery V20, Germany). At least five seeds per sample were observed.

2.2.2 Steedman's wax embedding, DAPI staining and fluorescent observation

Taking 10 caryopsis of weedy rice and cultivated rice at 3, 5, 7 and 9 DPA, and removing both ends of caryopsis and leave 2–3 mm in the middle. Kernels were fixed in 2.0% glutaraldehyde (Sigma Chemical Company, St Louis, MO, USA) in 100 mM sodium phosphate buffer (pH 7.2) for 24 h at room temperature and then overnight at 4 °C. Samples were rinsed with the fixative solution again and dehydrated in a concentration series of ethanol solution. Tissues were embedded at 37 °C in Steedman's wax which was prepared from PEG 400 distearate (Sigma Chemical Company, St Louis, MO, USA) and 1-hexadecanol (Sigma Chemical Company, St Louis, MO, USA) (9:1) as described by Wei et al. (2009). The samples were left to polymerize at room temperature. Waxed kernel tissues were cut into approximately 8- μ m-thick sections on a rotary microtome (Leica RM2235, Germany), mounted on slides coated with glycerol albumin, and then dewaxed in absolute ethanol. Drop a drop of distilled water on the slide, float the slices on the distilled water for expansion, and bake the slices overnight at 30°C. After the slices were dewaxed overnight with 100% absolute ethanol, the next day they were dewaxed with fresh anhydrous ethanol for 1–2 times, 2–3 h each time, and then dried naturally for standby (He et al. 2002). Dewaxed glass slides containing kernel tissues were stained with DAPI (4', 6-diamidino-2-phenylindole, 1 μ g/mL) (Sigma Chemical Company, St Louis, MO, USA) and examined with a fluorescent microscope (Zeiss Discovery V20, Germany). Stained nuclei showed blue fluorescence with UV excitation.

2.2.3 Measurements of CAT, POD, and SOD activities

CAT activity was determined by following the consumption of H₂O₂ (extinction coefficient 39.4 mM⁻¹ cm⁻¹) at 240 nm for 3 min (Aebi 1984). POD activity was assayed by the method described by Cakmak and Marschner (1992). SOD activity was determined through measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), according to the method of Giannopolitis and Ries (1977).

1.5 Data analysis

The statistical analyses consisted of ANOVAs. Means were compared by the least significant difference (LSD) test at the 0.05 probability level. All statistical analyses were conducted using the SPSS software package (18.0; SPSS Inc., Chicago, IL, USA), and graphs were generated using Origin 8.0 (OriginLab, Hampton, MA, USA).

Results

2.1 Comparison of nuclear morphological changes in endosperm between weedy rice and cultivated rice

The process of PCD is often accompanied by degeneration of cell nuclei. The DAPI is a highly sensitive and specific DNA fluorescent dye, which has excellent fluorescence staining effect on the cell nucleus and chromosome. The endosperm cell nuclei of weedy rice and cultivated rice were in the coenocyte stage or cellularization stage, and the endosperm nucleus was small and regular spherical at 3 days post anthesis (DPA). Starch accumulated continuously in endosperm cells, and the nucleus of starch endosperm was extruded, gradually deformed and disintegrated at 5 to 9 DPA (Fig. 1).

Morphological and statistical results of starch endosperm cell nuclei of weedy rice and cultivated rice (normal nuclei, deformed nuclei and degraded nuclei) at 3, 5, 7 and 9 DPA were shown in Fig. 1 and Fig. 2. After DAPI staining, the nuclei of endosperm of weedy rice and cultivated rice in Taizhou were 100% normal nuclei at 3 DPA. The percentage of normal nuclei of TZWR was 3% lower than that of TZCR, and the percentage of deformed nuclei and degraded nuclei of TZWR were 2% and 1% higher than that of TZCR at 5 DPA, respectively. The percentage of normal nuclei and deformed nuclei of TZWR was 25%–48% lower than that of TZCR, and the percentage of degraded nuclei of TZWR were 74% and 63% higher than that of TZCR at 7 and 9 DPA, respectively (Figs. 1A1–A4, Figs. 1B1–B4; Fig. 2A).

After DAPI staining, the percentage of normal nuclei of YZWR was 44% and 15% lower than that of YZCR at 3 and 5 DPA, respectively, and the percentage of deformed nuclei and degraded nuclei of YZWR were 2%–34% higher than that of YZCR at 3 and 5 DPA. There were no normal nuclei in the endosperm cells of YZWR and YZCR, and the percentage of deformed nuclei of

YZWR was 83% and 3% lower than that of YZCR at 7 and 9 DPA, respectively. The percentage of degraded nuclei of YZWR were 83% and 3% higher than that of YZCR at 7 and 9 DPA, respectively (Figs. 1C1-C4, Figs. 1D1-D4; Fig. 2B).

The endosperm cells of MMWR and MMCR were normal nuclei at 3 DPA. From 5 DPA to 9 DPA, the percentage of normal nuclei and deformed nuclei of MMWR were 5%-49% lower than that of MMCR, and the percentage of degraded nuclei of MMWR was 14%-59% higher than that of MMCR (Figs. 1E1-E4, Figs. 1F1-F4; Fig. 2C). The percentage of deformed nuclei and degraded nuclei were 18% and 2% at 3 DPA in DDWR, respectively. However, the percentage of normal nuclei was 100% in DDCR at 3 DPA. From 5 DPA to 9 DPA, the percentage of normal nuclei and deformed nuclei of DDWR were 1%-47% lower than that of DDCR, and the percentage of degraded nuclei of DDWR were 10-70% higher than that of DDCR (Figs. 1G1-G4, Figs. 1H1-H4; Fig. 2D). Generally speaking, the PCD process of endosperm cell nuclei of weedy rice was faster than that of associated cultivated rice (Figs. 1, 2).

2.2 Comparison of endosperm cell viability between weedy rice and cultivated rice

Viability staining provides a means to follow the pattern and progression of cell death during endosperm development. Evans blue dye only stains cells which are no longer capable of excluding the dye, indicating a loss of membrane integrity and consequently viability. The embryos of weedy rice and cultivated rice could not be dyed blue by Evans blue, which means that the embryos were always active during endosperm development. The endosperm of weedy rice and cultivated rice was gradually dyed blue by Evans blue with the development of endosperm, that is, endosperm cells gradually lost membrane permeability and became dead cells (Fig. 3). All starch endosperm cells of TZWR were completely stained dark blue by Evans blue at 13 DPA, while those of TZCR were at 21 DPA. The starch endosperm cells of YZWR and MMWR were completely stained dark blue by Evans blue at 13 DPA, and the starch endosperm cells of YZCR and MMCR were completely stained dark blue by Evans blue at 15 DPA. Endosperm cells of DDWR were completely stained dark blue by Evans blue 4 days earlier than that of DDCR (Fig. 3). In all, the whole starch endosperm of weedy rice was completely dyed dark blue by Evans blue 2–8 days earlier than that of associated cultivated rice, that is, endosperm cells of weedy rice lost membrane permeability and became dead cells 2–8 days earlier than associated cultivated rice (Fig. 3).

The embryo of weedy rice and cultivated rice can be dyed red by TTC, which means that the embryo has strong viability during endosperm development. The endosperm of weedy rice and cultivated rice could not be dyed red by TTC with the development of endosperm, which indicated that endosperm cells gradually lost viability (Fig. 4). The endosperm cells of DDWR could not be dyed red at 9 DPA by TTC, while the endosperm cells of weedy rice in other three places could not be dyed red by TTC at 15 DPA. However, the endosperm cells of four cultivated rice varieties could not be dyed red by TTC at 18 DPA (Fig. 4).

2.4 Comparison of anti-oxidative enzymes system between weedy rice and cultivated rice

The anti-oxidative enzymes activity decreased gradually both in weedy rice and cultivated rice, and the CAT activity of weedy rice was significantly lower than that of associated cultivated rice (Fig. 5). The CAT activity levels of TZWR was 10.39–82.95 U/g lower than that of TZCR at 3–25 DPA, while similar at 30 DPA (Fig. 5A). The CAT activity of YZWR was 37.72–53.81 U/g lower than that of YZCR at 3–15 DPA, but there was no significant difference between YZWR and YZCR at 20–30 DAP (Fig. 5B). The CAT activity of MMWR was significantly lower than that of MMCR at 3–15 DPA, but there was no significant difference between MMWR and MMCR at 20–30 DPA (Fig. 5C). At 3 and 5 DPA, there was no significant difference between the CAT activity of DDWR and DDCR, and the CAT activity of DDWR was 23.62–42.20 U/g lower than that of DDCR at 10–30 DPA.

The change trend of SOD activity of weedy rice was similar to that of associated cultivated rice, and there was no significant difference between MMWR and MMCR. The decline rate of SOD activity of weedy rice in the other three areas was faster than that of associated cultivated rice (Fig. 6). The SOD activity of TZWR was the highest at 3 DPA, which was 3.45 U/mg higher than that of TZCR. The SOD activity of TZCR was the highest at 5 DPA, which were increased continuously after the anthesis, reached a maximum, and declined thereafter. The SOD activity of TZCR was 0.73–1.52 U/mg higher than that of TZWR at 10–20 DPA (Fig. 6A). The SOD activity of weedy rice and cultivated rice in Yangzhou showed a downward trend, but the SOD activity of YZWR was 0.99–1.96 U/mg significantly lower than that of YZCR at 3, 25 and 30 DPA (Fig. 6C). SOD activity of weedy rice and cultivated rice was higher from Dandong at 3 to 10 DPA, and there was no significant difference between them. The SOD activity

decreased at 15 to 30 DPA, but the decline rate of weedy rice was faster, which was significantly lower than that of cultivated rice by 1.25–2.50 U/mg (Fig. 6D).

The POD activity of weedy rice in Taizhou showed a downward trend, the highest at 3 DPA, and was 184.53 U/g higher than that of TZCR, the POD activity of TZCR reached the maximum at 15 DPA, and declined thereafter. The POD activity of TZCR was 357.52–559.19 U/g higher than that of TZWR at 15–30 DPA (Fig. 7A). The POD activity of YZWR and YZCR showed a downward trend, but the decline rate of YZWR was slower. The POD activity of YZWR was 103.25 U/g lower than that of YZCR at 5 DPA, and was significantly higher than that of cultivated rice by 218.56 U/g at 20 DPA (Fig. 7B). The POD activity of MMWR reached the highest at 5 DPA, which was 219.77 U/g higher than that of MMCR, while the POD activity of MMCR reached the highest at 3 DPA, the POD activity of MMCR was 154.35 U/g higher than that of MMWR at 20 DPA (Fig. 7C). There was no significant difference in POD activity between weedy rice and cultivated rice in Dandong (Fig. 7D).

Discussion

Programmed cell death (PCD) refers to the process of physiological natural cell death initiated and regulated by its own internal mechanism during the development of plants. PCD plays an essential role in plant development and responses to abiotic and biotic insults, just as it does in many other eukaryotic organisms (Kabbage et al. 2017). Generally speaking, the nucleus is the last organelle that disintegrates in the process of plant PCD, and then the cells lose their physiological activity (Pennell and Lamb 1997). The rice endosperm is a special PCD process, in which the nucleus disintegrates first, and the cells still maintain high physiological activity after nuclear disintegration, and the grain weight continues to increase (Wu et al. 2016b). The mature endosperm is composed of aleurone layer and starch endosperm. Aleurone layer is an active tissue that stores protein and lipid, while starch endosperm is an inactive tissue storing starch and protein (Zheng et al. 2017). During the development of endosperm cells, the accumulation of storage compounds is accompanied by endosperm PCD. All cells in the starchy endosperm are dead in mature seeds owing to programmed cell death (PCD) (Wang et al. 2012; Sabelli and Larkins 2009; Kobayashi et al. 2013). Li et al. (2018) found that cell viability of endosperm directly related to PCD, endosperm cells exhibited deformed nuclei and a loss of membrane integrity during early wheat grain filling. In current research, it was found that PCD occurred in endosperm cells of weedy rice and cultivated rice at the early stage of grain filling, and the process of PCD was basically completed in endosperm tissues at the late filling stage (Figs. 1–4). The endosperm of weedy rice and cultivated rice still maintained dehydrogenase activity and cell activity after nuclear disintegration (Figs. 1–4), which was consistent with previous studies. However, compared with cultivated rice, the nucleus of endosperm cells in weedy rice was deformed and disintegrated earlier than that in cultivated rice, and endosperm cells of weedy rice lost activity earlier than cultivated rice. This implied that the process of PCD in endosperm cells of weedy rice was faster than that of cultivated rice, and this may be one of the important physiological mechanisms of early maturity in weedy rice. Plant endogenous hormones are closely related to the process of PCD, altering endogenous hormone concentrations during grain filling could delay endosperm PCD, increasing grain filling time, such as abscisic acid (ABA), ethylene, and gibberellic acid (GA) can regulate PCD in the developing endosperm (Young and Gallie 1999, 2000; Li et al. 2018). However, the difference of hormone content between weedy rice and cultivated rice and its relationship with PCD during grain filling need to be further studied.

The occurrence of PCD in plant cells is mainly caused by the accumulation of reactive oxygen species (Breusegem and Dat 2006), anti-oxidative enzymes system such as SOD, POD and CAT can protect cells by scavenging reactive oxygen species, and their activities are closely related to plant anti-aging (Corpas et al. 2006). SOD as the first enzyme involved in the scavenging reaction of reactive oxygen species, can catalyze the disproportionation of superoxide to produce H_2O_2 , while CAT and POD can transform H_2O_2 into water and oxygen (Corpas et al. 2006). The activities of SOD and CAT were higher in the grain during rice endosperm development (Lan et al. 2004). We found that at least one antioxidant enzyme activity of weedy rice was lower than that of associated cultivated rice. Anti-oxidative enzymes activity is closely related to rice nature senescence and maturity. It has been reported that compared with rice varieties with a longer growth period, the activities of CAT and POD in leaves of rice varieties with a shorter growth period were lower, and senescence earlier (Wang et al. 2010). Therefore, we speculate that the rapid PCD process in the endosperm of weedy rice may be closely related to the activity of antioxidant enzymes. Under low antioxidant enzyme activity, cell can't effectively scavenge oxygen free radicals, cell macromolecules were poisoned, which accelerated the process of PCD in the endosperm of weedy rice. Compared with SOD and POD, CAT may play a more important

role in scavenging reactive oxygen species (Zhang et al. 2015). However, as the contents of reactive oxygen species and malondialdehyde (MDA) in endosperm of weedy rice and cultivated rice are not determined, the relationship between PCD process and ROS scavenging capacity of weedy rice and cultivated rice needs to be further verified. It has been reported that ascorbate peroxidase (APX), dehydroascorbic reductase (DHAR), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione (GSH), ascorbic acid (ASA) and other nonenzymatic substances can remove reactive oxygen species, which may play an important role in regulating grain filling and PCD process of endosperm cells (Yamauchi et al. 2001). The difference of these enzymes activity during endosperm development between weedy rice and cultivated rice will be the focus of the next study.

The PCD process is closely related to grain filling, and the factors affecting the grain filling can also affect the process of PCD. To date, research on PCD process and grain filling differences in rice has mostly been based on enzyme activity, hormone balance, and PCD-related genes expression (Yang et al. 2003; Yin et al. 2012). Many studies have shown that starch synthase activity and hormone level are the main causes of differences in grain filling (Zhang et al. 2015). Thirty-three major enzymes are reported to be involved in sucrose-to-starch conversion (SSC) during endosperm development in rice (Nakamura et al. 1989). Among these enzymes, sucrose synthase (SuSase, EC 2.4.1.13), acid invertase (AI, EC 3.2.1.26), ADP-glucose pyrophosphorylase (AGPase, EC 2.7.7.27), starch synthase (StSase, EC 2.4.1.21) and starch branching enzyme (SBE, EC 2.4.1.18) are considered to play key roles in this process (Nakamura et al. 1989; Wang et al. 2015). Reports have shown that many genes are involved in controlling the process of SSC, including *SuS2*, *SuS4*, *OsCIN2*, *OsINV2*, *AGPS1*, *AGPS2b*, *AGPL2*, *SSSIIa*, *SSSIIc*, *GBSSI*, and *SBEI* (Wang et al. 2015). The relationship between activities of key enzymes and expressions of genes involved in sucrose-to-starch conversion and PCD need to be further studied. Plant hormones, such as ABA and ethylene, play vital roles in regulating grain filling. An appropriate level of ABA can enhance the key enzyme activity and gene expression related to starch metabolism and improve grain-filling rate (Wang et al. 2015). Ethylene can enhance the active oxygen system and stimulate free radical production in grains, and ethylene-induced H₂O₂ can reduce grain weight and grain-filling rate (Zhang et al. 2015; Chen et al. 2013). Combined with this study, we can prolong the duration of PCD process and enhance the enzyme activities of antioxidant system by spraying some kind of chemical regulators, so that weedy rice has a longer filling period, which is later than cultivated rice. When the cultivated rice is mature and harvested, weedy rice is not mature, which eventually makes weedy rice difficult to disperse and spread, and finally control weedy rice. PCD is a physiological process determined by PCD related gene and plays an indispensable role in plant development (Schmid et al. 1999). In addition, the differences of PCD related genes (*Os02g48450*, *Os04g02120*, *Os04g08390*, *Os05g31570*, *Os06g17970*, *Os08g30634*, *Os09g14410*, *Os09g30220*, *Os11g13940*, *Os11g38440*, *Os11g38580*, and *Os12g14330*) (Yin et al. 2012), degradation of nuclear DNA and other indicators related to PCD process in endosperm cells between weedy rice and cultivated rice need to be further studied.

Conclusion

The endosperm cells of weedy rice degraded and lost viability earlier and more rapidly than those of ACR. The ability of scavenging reactive oxygen species by endosperm cells of weedy rice was weaker than that of ACR. The PCD process of endosperm cells in weedy rice was faster than that in cultivated rice. The rapid PCD process shortened the grain filling period of weedy rice, and eventually led to the early maturity of weedy rice. A better understanding of the mechanisms involved in PCD process of endosperm cells will improve the design of management strategies for weedy rice.

Abbreviations

ACR: associated cultivated rice; PCD: programmed cell death; TTC: 2, 3, 5-tryphenyl tetrazolium chloride; DAPI: 4', 6-diamidino-2-phenylindole; ROS: reactive oxygen species; CAT: catalase; POD: peroxidase; SOD: superoxide dismutase; DPA: days post anthesis.

Declarations

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Authors' Contributions

SLS and SQ conceived and designed the research. CZ and conducted the experiments and collected the data, CZ, WRX, LCM, WMD and ZZ conducted field trials. CZ, SQ and XLS analyzed the data, CZ and XLS wrote the manuscript.

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Availability of Data and Materials

All data generated or analyzed in this study are included in this article.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

All authors agree to the contents of manuscript.

Competing Interests

The authors declare that they have no conflict of interest. Reference

References

1. Aebi H. (1984) Catalase in vitro. *Methods in enzymology*. Academic Press 105: 121-126. doi:10.1016/S0076-6879(84)05016-3.
2. Azmi M, Karim SMR (2008) Weedy rice-biology, ecology and management. Kuala Lumpur, Malaysia: Malaysian Agricultural Research and Development Institute (MARDI), Kuala Lumpur, Malaysia, MARDI Publication, pp: 56-68.
3. Breusegem FV and Dat JF (2006) Reactive oxygen species in plant cell death. *Plant Physiol* 141:384-390. doi: 10.1104/pp.106.078295.
4. Burgos NR, Singh V, Tseng TM, Black H, Young ND, Huang Z et al (2014) The impact of herbicide-resistant rice technology on phenotypic diversity and population structure of United States weedy rice. *Plant Physiol* 166:1208-1220. doi: 10.1104/pp.114.242719.
5. Cakmak I, Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase ascorbate peroxidase and glutathione reductase in bean leaves. *Plant Physiol* 98:1222-1227. doi: 10.1104/pp.98.4.1222.
6. Chauhan BS (2013) Strategies to manage weedy rice in Asia. *Crop Prot* 48:51-56. doi:10.1016/j.cropro.2013.02.015
7. Chen L, Jin M, Zhang WL, Wang CX, Wu YB, Wang ZZ, Tang XY (2020) Research advances on characteristics damage and control measures of weedy rice. *Acta Agron Sin* 46:969-977. doi: 10.3724/SP.J.1006.2020.92064.
8. Chen TT, Xu YJ, Wang JC, Wang ZQ, Yang JC, Zhang JH (2013) Polyamines and ethylene interact in rice grains in response to soil drying during grain filling. *J Exp Bot* 64:2523–2538. doi: 10.1093/jxb/ert115.
9. Chen YF, Zhang J, Xie PS, Zhou WD, Chen JM, Wei CX (2012) Programmed cell death in wheat starchy endosperm during kernel development. *Afr J Agr Res* 7:6533-6540. doi: 10.5897/AJAR11.257.
10. Corpas FJ, Fernández-Ocaña A, Carreras A, Valderrama R, Luque F, Esteban FJ et al (2006) The expression of different superoxide dismutase forms is cell-type dependent in olive (*Olea europaea* L) leaves. *Plant Cell Physiol* 47:984-994. doi:10.1093/pcp/pcj071.
11. Dai L, Dai WM, Song XL, Lu BR, Qiang S (2014) A comparative study of competitiveness between different genotypes of weedy rice (*Oryza sativa* L) and cultivated rice. *Pest Manag Sci* 70:113–122. doi:10.1002/ps.3534.

12. Dai L, Song XL, He BY, Valverde BE, Qiang S (2017) Enhanced photosynthesis endows seedling growth vigour contributing to the competitive dominance of weedy rice over cultivated rice. *Pest Manag Sci* 73:1410-1420. doi:10.1002/ps.4471.
13. Delouche JC, Burgos NR, Gealy DR, de San Martin GZ, Labrada R, Larinde M, Rosell C (2007) Weedy rices: origin, biology, ecology and control. *FAO Plant Production and Protection Paper* 188. FAO, Rome, pp:3–15.
14. Domínguez F, Cejudo FJ (2014) Programmed cell death (PCD): an essential process of cereal seed development and germination. *Front Plant Sci.* 5:366. doi:10.3389/fpls.2014.00366.
15. Fan HY, Zhou ZQ, Yang CN, Jiang Z, Li JT, Cheng XX, Guo YJ (2013) Effects of waterlogging on amyloplasts and programmed cell death in endosperm cells of *Triticum aestivum* L. *Protoplasma* 250:1091-1103. doi:10.1007/s00709-013-0485-z.
16. Fukuda H (2000) Programmed cell death of tracheary elements as a paradigm in plants. *Plant Mol Biol* 44: 245–253. doi: 10.1023/A: 1026532223173.
17. Giannopolitis GN, Ries SK (1977) Superoxide dismutase I Occurrence in higher plants *Plant Physiol* 59:309–314. doi: 10.1104/pp.59.2.309.
18. He Q, You RL, Bao WM (2002) Preprophase band loses its function as a cytokinetic apparatus in mitosis of neck canal mother cell. *Protoplasma* 220:105–109. doi:10.1007/s00709-002-0029-4.
19. Kabbage M, Kessens R, Bartholomay LC, Williams B (2017) The life and death of a plant cell. *Annu Rev Plant Biol* 68:375-404. doi:10.1146/annurev-arplant-043015-111655.
20. Kobayashi H, Ikeda TM, Nagata K (2013) Spatial and temporal progress of programmed cell death in the developing starchy endosperm of rice. *Planta* 237:1393–1400. doi: 10.1007/s00425-013-1854-8.
21. Lakon G (1949) The topographical tetrazolium method for determining the germination capacity of seeds. *Plant Physiol* 24:389–394. doi: 10.1104/pp.24.3.389.
22. Lan SY, Zhong FX, Yang ZM, Jin DM, Xu ZX (2004) The starchy endosperm denucleation by a process of programmed cell death during rice grain development. *Shi Yan Sheng Wu Xue Bao* 37:34-44. doi:10.3321/j.issn:1673-520X.2004.01.006.
23. Li C, Li C, Wang BB, Zhang RQ, Fu KY, Gale WJ et al (2018) Programmed cell death in wheat (*Triticum aestivum* L) endosperm cells is affected by drought stress *Protoplasma*. 255, 1039-1052. doi:10.1007/s00709-018-1203-7.
24. Li DL, Li XG, Gu YJ, Wang Z (2014) Development of different rice varieties. *Sci Agric Sin* 47:3757-3768. doi: 10.3864/j.issn.0578-1752.2014.19.004.
25. Locato V, De Gara L (2018) Programmed cell death in plants: An Overview. In: De Gara L, Locato V (eds) *Plant Programmed Cell Death. Methods in Molecular Biology*, vol 1743. Humana Press, New York, NY. doi:10.1007/978-1-4939-7668-3_1.
26. Nakamura Y, Yuki K, Park SY (1989) Carbohydrate metabolism in the developing endosperm of rice grains. *Plant Cell Physiol.* 30:833–839. doi:10.1093/oxfordjournals.pcp.a077813
27. Nunez M, Mazzafera P, Mazorra LM, Siqueira WJ, Zullo MAT (2003) Influence of a brassinosteroid analogue on antioxidant enzymes in rice grown in culture medium with NaCl. *Biol Plant* 47: 67–70. doi: 10.1023/A:1027380831429.
28. Oberle GD, Watson R (1953) The use of 235-triphenyl tetrazolium chloride in viability test of fruit pollen. *Amer Soc Hort Sci Proc* 61:299-303.
29. Olsen OA (2004) Nuclear endosperm development in cereals and *Arabidopsis thaliana*. *Plant Cell* 16 (suppl 1): S214-S227. doi: 10.1105/tpc.017111
30. Olsen OA, Brow RC, Lemmon BE (1995) Pattern and process of wall formation in developing endosperm. *BioEssays* 17:803-812. doi: 10.1002/bies.950170910
31. Pennell RI, Lamb C (1997) Programmed cell death in plants. *Plant Cell* 9:1157-1168. doi: 10.1105/tpc.9.7.1157.
32. Sabelli PA, Larkins BA (2009) The development of endosperm in grasses. *Plant Physiol* 149:14–26. doi: 10.1104/pp.108.129437.
33. Schmid M, Simpson D, Gietl C (1999) Programmed cell death in castor bean endosperm is associated with the accumulation and release of a cysteine endopeptidase from ricinosomes. *Proc Nat Acad Sci USA* 96: 14159–14164. doi:10.1073/pnas.96.24.14159

34. van Doorn WG, Beers EP, Dangl JL, Franklin-Tong VE, Gallois P, Hara-Nishimura I et al (2011) Morphological classification of plant cell deaths, *Cell Death Differ* 18:1241–1246. doi:10.1038/cdd.2011.36.
35. Wang BQ, Fan XR, Xu GH, Shen QR (2010) Characteristics of flag leaf senescence among three rice cultivars with different nitrogen use efficiency. *J Nanjing Agric Univ* 33: 8-12. doi:10.7685/j.issn.1000-2030.2010.02.002.
36. Wang Z, Gu YJ, Hirasawa T, Ookawa T, Yanahara S (2004) Comparison of caryopsis development between two rice varieties with remarkable difference in grain weights. *Acta Bot Sin* 46:698-710. doi: 10.3321/j.issn:1672-9072.2004.06.008
37. Wang Z, Gu YJ, Zheng YK, Wang HH (2012) Ultrastructure observation of rice endosperm cell development and its mineral element analysis. *Chin J Rice Sci* 26:693–705. doi: 10.3969/j.issn.1001-7216.2012.06.009.
38. Wang ZQ, Xu YJ, Chen TT, Zhang H, Yang JC, Zhang JH (2015) Absciscic acid and the key enzymes and genes in sucrose-to-starch conversion in rice spikelets in response to soil drying during grain filling. *Planta* 241:1091–1107. doi: 10.1007/s00425-015-2245-0.
39. Wei CX, Lan SY, Xu ZX (2002) Ultrastructural features of nucleus degradation during programmed cell death of starchy endosperm cells in rice. *Acta Bot Sin* 44:1396-1402. doi:10.3321/j.issn:1672-9072.2002.12.002.
40. Wei CX, Zhang J, Xie PS, Zhou WD, Chen YF, Xu RG (2009) Studies on the programmed cell death in barley during starchy endosperm development. *Sci Agric Sin* 42:824–832. doi:10.3864/j.issn.0578-1752.2009.03.009
41. Wu X, Liu J, Li D, Liu CM (2016a) Rice caryopsis development II: dynamic changes in the endosperm. *J Integr Plant Biol* 58:786-798. doi:10.1111/jipb.12488.
42. Wu X, Liu J, Li D, Liu CM (2016b) Rice caryopsis development I: Dynamic changes in different cell layers. *J Integr Plant Biol* 58:772-785. doi:10.1111/jipb.12440.
43. Xie Y, Zhang C, Lai D, Sun Y, Samma MK, Zhang J, Shen W (2014) Hydrogen sulfide delays GA-triggered programmed cell death in wheat aleurone layers by the modulation of glutathione homeostasis and heme oxygenase-1 expression. *J Plant Physiol* 171:53–62. doi:10.1016/j.jplph.2013.09.018.
44. Yamauchi N, Kusabe A (2001) Involvement of Ascorbate-Glutathione Cycle in Senescence of Stored Broccoli (*Brassica oleracea* L.). *J Jpn Soc Hortic Sci* 70:704-708. doi:10.2503/jjshs.70.704.
45. Yang JC, Zhang JH, Wang ZQ, Zhu QS (2003) Hormones in the grains in relation to sink strength and postanthesis development of spikelets in rice. *Plant Growth Regul* 41:185–195. doi:10.1023/B:GROW.0000007503.95391.38.
46. Yin L, Xue H (2012) The MADS29 transcription factor regulates the degradation of the nucellus and the nucellar projection during rice seed development. *The Plant Cell* 24:1049-1065. doi: 10.1105/tpc.111.094854.
47. Young TE, Gallie DR (1999) Analysis of programmed cell death in wheat endosperm reveals differences in endosperm development between cereals. *Plant Mol Biol* 39: 915–924. doi: 10.1023/A:1006134027834.
48. Young TE, Gallie DR (2000) Programmed cell death during endosperm development *Plant Mol Biol* 44 283–301. doi: 10.1007/978-94-010-0934-8_4.
49. Young TE, Gallie DR, Demason DA (1997) Ethylene-mediated programmed cell death during maize endosperm development of wild-type and shrunken2 genotypes. *Plant Physiol* 115:737–751. doi: 10.1104/pp.115.2.737.
50. Young TE, Gallie DR, Demason DA (1997) Ethylene-mediated programmed cell death during maize endosperm development of wild-type and shrunken 2 genotypes. *Plant Physiol* 115:737-751. doi: 10.1104/pp.115.2.737.
51. Yu XR, Liang Z, Xiong F, Wang Z (2014) Structural and histochemical characterization of developing rice caryopsis. *Rice Sci* 21:142-149. doi:10.1016/S1672-6308(13)60176-6.
52. Zhang H, Liu K, Wang Z, Liu L, Yang JC (2015) Absciscic acid ethylene and antioxidative systems in rice grains in relation with grain filling subjected to postanthesis soil-drying. *Plant Growth Regul* 76:135-146. doi:10.1007/s10725-014-9983-z.
53. Zhao C, Xu W, Meng L, Qiang S, Dai W, Zhang Z et al (2020) Rapid endosperm development promotes early maturity in weedy rice (*Oryza sativa* f. *spontanea*) *Weed Sci* 68:168-178. doi: 10.1017/wsc.2020.5.
54. Zhao C, Xu W, Song X, Dai W, Dai L, Zhang Z et al (2018) Early flowering and rapid grain filling determine early maturity and escape from harvesting in weedy rice. *Pest Manag Sci* 74:465-476. doi: 10.1002/ps.4730.

Figures

Figure 1

DAPI staining of endosperm nucleus in weedy and cultivated rice. A1-A4: weedy rice from Taizhou, TZWR; B1-B4: cultivated rice from Taizhou, TZCR; C1-C4: weedy rice from Yangzhou, WZWR; D1-D4: cultivated rice from Yangzhou, YZCR; E1-E4: weedy rice from Maoming, MMWR; F1-F4: cultivated rice from Maoming, MMCR; G1-G4: weedy rice from Dandong, DDWR; H1-H4: cultivated rice from Dandong, DDCR.

Figure 2

Proportion of normal, deformed and degraded nuclei in endosperm cells of weedy and cultivated rice. A, weedy and cultivated rice from Taizhou; B, weedy and cultivated rice from Yangzhou; C, weedy and cultivated rice from Maoming; D, weedy and cultivated rice from Dandong. TW: weedy rice from Taizhou; TC: cultivated rice from Taizhou; YW: weedy rice from Yangzhou; YC: cultivated rice from Yangzhou; MW: weedy rice from Maoming; MC: cultivated rice from Maoming; DW: weedy rice from Dandong; DC: cultivated rice from Dandong. DPA, days post-anthesis. Different lowercase letters indicate statistical significance for the comparison between weedy rice and its associated cultivated rice (independent-sample t-test, $P < 0.05$).

Figure 3

Evans blue staining in developing grains of weedy and cultivated rice. TZWR: weedy rice from Taizhou; TZCR: cultivated rice from Taizhou; YZWR: weedy rice from Yangzhou; YZCR: cultivated rice from Yangzhou; MMWR: weedy rice from Maoming; MMCR: cultivated rice from Maoming; DDWR: weedy rice from Dandong; DDCR: cultivated rice from Dandong. The Roman numerals at the bottom of the picture represent the days post anthesis.

Figure 4

TTC staining in developing grains of weedy and cultivated rice TZWR: weedy rice from Taizhou; TZCR: cultivated rice from Taizhou; YZWR: weedy rice from Yangzhou; YZCR: cultivated rice from Yangzhou; MMWR: weedy rice from Maoming; MMCR: cultivated rice from Maoming; DDWR: weedy rice from Dandong; DDCR: cultivated rice from Dandong. The Roman numerals at the bottom of the picture represent the days post anthesis.

Figure 5

Changes in activities of CAT in weedy rice and cultivated rice. A, weedy and cultivated rice from Taizhou; B, weedy and cultivated rice from Yangzhou; C, weedy and cultivated rice from Maoming; D, weedy and cultivated rice from Dandong. TZWR: weedy rice from Taizhou; TZCR: cultivated rice from Taizhou; YZWR: weedy rice from Yangzhou; YZCR: cultivated rice from Yangzhou; MMWR: weedy rice from Maoming; MMCR: cultivated rice from Maoming; DDWR: weedy rice from Dandong; DDCR: cultivated rice from Dandong.

Figure 6

Changes in activities of SOD in weedy rice and cultivated rice. A, weedy and cultivated rice from Taizhou; B, weedy and cultivated rice from Yangzhou; C, weedy and cultivated rice from Maoming; D, weedy and cultivated rice from Dandong. TZWR: weedy rice from Taizhou; TZCR: cultivated rice from Taizhou; YZWR: weedy rice from Yangzhou; YZCR: cultivated rice from Yangzhou; MMWR: weedy rice from Maoming; MMCR: cultivated rice from Maoming; DDWR: weedy rice from Dandong; DDCR: cultivated rice from Dandong.

Figure 7

Changes in activities of POD in weedy rice and cultivated rice. A, weedy and cultivated rice from Taizhou; B, weedy and cultivated rice from Yangzhou; C, weedy and cultivated rice from Maoming; D, weedy and cultivated rice from Dandong. TZWR: weedy rice from Taizhou; TZCR: cultivated rice from Taizhou; YZWR: weedy rice from Yangzhou; YZCR: cultivated rice from Yangzhou; MMWR: weedy rice from Maoming; MMCR: cultivated rice from Maoming; DDWR: weedy rice from Dandong; DDCR: cultivated rice from Dandong.