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ORIGINAL ARTICLE

Programmed cell death in wheat (*Triticum aestivum* L.) endosperm cells is affected by drought stress

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Abstract

Drought frequently occurs during wheat (*Triticum aestivum* L.) grain filling. The objectives of this study were (i) to investigate the effect of post-anthesis drought on programmed cell death (PCD) in wheat endosperm cells and (ii) to examine the role of ethylene (ETH) receptors and abscisic acid (ABA) in regulating wheat endosperm PCD. Two winter wheat cultivars ('Xindong 18' and 'Xindong 22') were used in this study. Grain samples were collected from normal and drought stressed plants at 5-day intervals between 5 and 35 days post-anthesis. The samples were then compared with respect to cell viability, nuclear morphometry, cell ultrastructure, DNA integrity, nucleic acid content, and nuclease activity. Analysis was also conducted about gene transcripts related to PCD, ETH receptors, and ABA biosynthesis and degradation. Drought stress reduced cell viability, accelerated nuclear deformation, and increased mitochondrial dissolution. The activity of nucleic acid hydrolase was greater, and the nucleic acid concentrations were less in the drought treatments than in the control. As a result, the peak in DNA fragmentation occurred earlier in the drought treatment. Drought stress significantly increased the expression of four genes related to ABA (*nced1*, *nced2*, *ao1*, *ao2*). In contrast, drought significantly reduced the expression of four genes related to ETH receptors (*ers1*, *ers2 etr1*, *etr2*) and one gene related to PCD (*dad1*). In summary, the results indicated that drought stress caused PCD to occur earlier in the endosperm of winter wheat.

Keywords Drought · Endosperm cell · Ethylene receptor · Programmed cell death · *Triticum aestivum L*

Abbreviations

ABA	Abscisic acid
DAPI	4′,6-Diamidino-2-phenylindole
DPA	Days post-anthesis
DT	Drought treatment
ETH	Ethylene
PCD	Programmed cell death

Introduction

As a staple for nearly one fifth of the global population, wheat (*Triticum aestivum* L.) is one of the world's most important crops (Farooq et al. 2014). Drought stress is the principal abiotic factor affecting wheat yield in arid and semiarid areas (Zadražnik et al. 2013). More than 50% of the world's wheat-growing area is affected by periodic drought (Rajaram 2001). Although drought may affect wheat growth during all growth stages, the reproductive and grain-filling phases are the most sensitive stages (Pradhan et al. 2012). Post-anthesis drought stress usually shortens the grain-filling period and reduces the grain-filling rate (Kobata et al. 1992; Yang et al. 2006). Farooq et al. (2014) reported that mild post-anthesis drought reduced wheat yields by 1–30%. More information is needed about the mechanism by which drought stress affects wheat grain filling.

Programmed cell death (PCD) is a genetically determined physiological process which plays an integral role in the development of multicellular organisms (Schmid et al. 1999). In plants, PCD is essential in the development of tracheary element cells (Fukuda 2000), aleurone layer cells (Xie et al. 2014), and root cap cells (Mao et al. 2001). In wheat, starchy endosperm accounts for 80–85% of grain weight (Li et al. 2015). Therefore, endosperm development (i.e., cell division,

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differentiation, and filling) has great influence on grain weight and quality (Muñoz and Calderini 2015). The primary function of starch endosperm cells is to synthesize starch and storage proteins. As they synthesize storage metabolites, starchy endosperm cells also initiate a programmed cell death that eventually affects all but the outermost cells of the endosperm. The outermost cells differentiate into the aleurone layer and remain living in the mature seed (Olsen and Becraft 1998; Wang et al. 1998; Young and Gallie 2000b).

Evans blue staining shows that PCD of starchy endosperm cells in wheat and maize (*Zea mays* L.) can occur from shortly after anthesis until seed maturity (Young et al. 1997, Young and Gallie 1999). There seems to be no pattern to the occurrence of PCD in starchy endosperm cells of wheat (Young and Gallie 1999). In contrast, PCD begins in the center or upper center of rice and maize endosperm and then spreads to the periphery (Young et al. 1997; Lan et al. 2004). The PCD process results in nucleus deformation, chromatin condensation, and nuclear membrane rupture (Wei et al. 2002). Li et al. (2004) observed that the endoplasmic reticulum, mitochondria, and amyloplast function normally as the nucleus disintegrates, so that starch and storage proteins continue to be synthesized and accumulate.

Young (1999) observed that nuclease activity increased during PCD, causing extensive degradation of nuclear DNA. The resulting DNA fragments were 180–200 bp long. The appearance of DNA laddering (visualized by gel electrophoresis) is an important indicator of PCD in wheat and maize but not in rice, which only has large DNA fragments (Wei et al. 2002; Li et al. 2004; Kobayashi et al. 2013). The nucleus normally disappears at the end of PCD; however, barley (*Hordeum vulgare* L.) endosperm was still alive after the nucleolus broke apart (Wei et al. 2009). Generally, necrosis is a typically acute cell death response that develops rapidly. However, PCD in starchy endosperm cells lasts for a long time (Fan et al. 2013). These observations indicate that PCD in starchy endosperm cells is unique and should be distinguished from common plant cell death.

Endogenous hormones, especially ethylene (ETH) and abscisic acid (ABA), have been shown to significantly affect endosperm PCD and the grain filling process (Young et al. 1997; Yang et al. 2006; Liu et al. 2008). Young et al. (1997) detected two peaks in ETH release during maize endosperm development. The first peak was associated with the initiation of PCD in the endosperm. The second peak was accompanied by increased nuclease activity and internucleosomal fragmentation of nuclear DNA. Ethylene perception requires membrane-localized receptors which are encoded by the genes *ers1*, *ers2*, *etr1*, *etr2*, and *ein4* in Arabidopsis (Chang et al. 1993; Hua et al. 1995, 1998a; Sakai et al. 1998). Ethylene receptors function as negative regulators of cell death and are inactivated following ethylene binding (Hua and Meyerowitz 1998b). In addition to ETH, ABA levels are also elevated during PCD. The ABA influences events during late kernel development, such as acquisition of desiccation tolerance and induction of embryo dormancy. Although ABA is not directly involved in the onset of PCD, alterations in the ABA biosynthesis pathway and in ABA perception affect the onset of PCD by causing changes in ethylene biosynthesis (Nguyen et al. 2007). Two viviparous (vp) mutants have implicated ABA as a negative regulator of ETH biosynthesis and action during maize endosperm development (Young and Gallie 2000a). Abscisic acid has also been shown to prevent the programmed death of aleurone cells in germinating barley seed (Wang et al. 1996; Bethke et al. 1999). The onset of cell death in endosperm appears to be achieved through a balance between ETH and ABA. A decrease in the ratio of ETH to ABA increases the grain filling rate (Yang et al. 2006). There is clear evidence of a close relationship between ETH and ABA in the regulation of endosperm PCD.

Many studies have been done about PCD in the endosperm of cereal grain. Some studies indicate that stresses such as water-logging accelerated wheat endosperm PCD, possibly due to increases in superoxide dismutase and catalase (Fan et al. 2013; Cheng et al. 2016). Few studies, however, have examined the effects of drought stress on PCD in wheat endosperm. This study compared PCD in developing wheat endosperm under either normal conditions or post-anthesis drought stress. The objectives were (i) to correlate changes in cell viability and organelle structure with the progression of DNA fragmentation, the induction of nuclease activity, and changes in DNA content, and (ii) to investigate the role of ETH receptors and ABA in regulating wheat endosperm PCD.

Materials and methods

Experimental site and cultivation methods

The experiment was performed at the Shihezi University Experimental Farm, Shihezi, China (44° 17′ N, 86° 03′ E) from October 2015 to June 2016. The soil at the site is classified by Chinese scientists as gray desert (Calcaric Fluvisol). Some characteristics of the 0–20-cm soil depth were as follows: available N, 63 mg/kg; available P, 15 mg/kg; and available K, 208 mg/kg. The plots had previously been cropped to sunflower (*Helianthus annuus*). Two winter wheat cultivars were used in this experiment: ‘Xindong 18’ and ‘Xindong 22’. Xindong 18 is an intermediate maturing cultivar, requiring about 273 days to reach maturity. Xindong 22 is an early maturing cultivar, requiring about 267 days to reach maturity.

The experiment consisted of a randomized complete block design with two drip irrigation treatments: normal irrigation