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Molina-Cano J.L. (ed.), Christou P. (ed.), Graner A. (ed.), Hammer K. (ed.), Jouve N. (ed.), Keller B. (ed.), Lasa J.M. (ed.), Powell W. (ed.), Royo C. (ed.), Shewry P. (ed.), Stanca A.M. (ed.).
Cereal science and technology for feeding ten billion people: genomics era and beyond

Zaragoza : CIHEAM / IRTA
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 81

2008
pages 453-455

Using cell-lineage tagging to decipher early differentiation and development in wheat endosperm

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SUMMARY – Wheat grain shape and size affects processing quality and is determined by the timing, location, number and orientation of cell divisions within the grain. We are using a transgenic approach to study cell lineage in wheat endosperm development by inducing expression of nuclear-targeted GFP in young endosperm cells using an Ac/Ds transposon-based system. The system will allow us to mark clonal sectors and follow early endosperm lineage events. The transgenic lines generated will provide a valuable resource for studying development in wheat tissues, including the endosperm, and the knowledge gained will assist in the design of strategies for improving cereal grain yield/quality. Here we report progress in the generation and testing of transgenic wheat lines.

Introduction

Following triple fertilization and free nuclear division cellularization of cereal endosperm begins with the formation of tube-like structures called alveoli. Nuclei within the alveoli divide in the periclinal plane and cell walls form between the daughter nuclei resulting in a peripheral layer of cells and a new layer of alveoli. This process continues until files of cells completely invade the central cell vacuole.

Cereal endosperms consist of 4 cell types: the embryo surrounding region, transfer cells, aleurone cells and starchy endosperm cells. Peripheral cells resulting from the first periclinal nuclear division in alveoli subsequently divide mainly in the anticlinal plane and become aleurone cells, whereas, after cellularization is complete, the orientation of cell division in those cells destined to become starchy endosperm cells is random. The paired appearance of aleurone cells indicating periclinal cell division, with the inner daughter cells dedifferentiating and becoming starchy endosperm cells, has been reported and observed in our laboratory (Morrison *et al*., 1975; Olsen, 2001).

In order to study cell division and differentiation events in early wheat endosperm development expression of nuclear-targeted GFP will be induced in cells of young endosperms using an Ac/Ds transposon-based system (Kurup *et al*., 2005). The system consists of an Ac transposase gene under the control of a heat-shock promoter (HSP; Raho *et al*., 1995) and nuclear-targeted GFP as the reporter gene downstream of a ubiquitous promoter but interrupted by a Ds element. Transgenic wheat containing both constructs will be heat-shocked to induce expression of the transposase which in turn will excise the Ds element causing expression of the reporter gene in a few cells in early wheat endosperms. All cells that derive from these cells will also express GFP allowing us to mark clonal sectors and follow early wheat endosperm cell lineages.

Materials and methods

All constructs were generated using standard cloning procedures. Transient expression experiments used immature (approximately 14 DAP) wheat embryos and endosperm (cv. 'Cadenza') and particle bombardment using a PDS 1000/He particle gun (Bio-Rad). Wheat plant cultivation, explant isolation, preculture and bombardments were according to Sparks and Jones (2004), except that embryo axes were not removed. Plates requiring heat shock were removed from the culture room 24 h after bombardment and incubated at 40℃ for 60 min. All plates were then cultured for a further 24 h at 22℃. GUS activity was visualized by hist ochemical staining at 37℃ for approximately 16 h before counting blue foci. GFP was visualized using a Leica MZFL111 epifluorescence stereo-microscope.

Results and discussion

Transgenic wheat with the *uidA* (GUS) reporter gene controlled by the heat-shock promoter (pHSPdGUS) is being used to optimize heat-shock conditions. The heat-shock promoter drives inducible expression of the *uidA* gene in heat-shocked immature wheat embryos/endosperm in a transient system and in transgenic seed (Fig. 1).

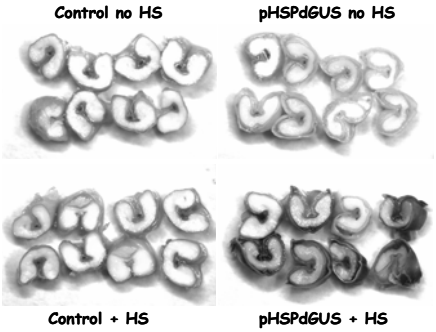

Fig. 1. Heat-shock induction of GUS expression in transgenic seed transformed with pHSPdGUS. Transgenic T1 seeds and control seeds were halved and one half subjected to heat-shock for 60 min at 40℃ before GUS assay.

We have demonstrated nuclear targeting of GFP to wheat scutella and endosperm nuclei using an Actin::H2B-GFP construct. The location of the Ds-element appears to be critical for interruption of gene expression (McElroy, *et al*., 1997), we therefore tested Ds-mediated interruption of nuclear-targeted GFP expression (driven by the rice actin promoter). When the promoter included the untranslated exon and first intron (pAct1-Ds-H2B-GFP) we observed no interruption of GFP expression, but GFP expression was interrupted after deletion of the exon and intron from the promoter (pActN-Ds-H2B-GFP) (Table 1). In these experiments we were unable to induce Ds transposition and GFP expression in the presence of pHSPdAc and after heat-shock (Table 1).

Table 1. Number of embryos with GFP fluorescent nuclei after bombardment with Ac/Ds constructs

Construct	Heat shock	Embryos with GFP foci†			
		0	1-10	11-50	>50
pAct1::Ds::H2B-GFP	-	33.8% +/- 5.97	23.6% +/- 3.30	23.7% +/- 2.44	19.0% +/- 6.01
pAct1::Ds::H2B-GFP + pHSPdAc	-	§19.6% +/- 2.10	§33.8% +/- 3.75	§28.4% +/- 8.35	§18.3% +/- 6.70
pAct1::Ds::H2B-GFP + pHSPdAc	+	§35.8% +/- 7.50	§20.8% +/- 5.80	§23.3% +/- 5.00	§20.5% +/- 2.80
pActN::Ds::H2B-GFP	-	99.4% +/- 0.60	0.6% +/- 0.60	0%	0%
pActN::Ds::H2B-GFP + pHSPdAc	-	99.6% +/- 0.40	0.4% +/- 0.40	0%	0%
pActN::Ds::H2B-GFP + pHSPdAc	+	99.4% +/- 0.40	0.6% +/- 0.40	0%	0%

†Numbers are the percentage of embryos in each category and are the mean of 5 experiments except § which are the mean of 2 experiments.

However, Ds excision is known to be associated with DNA replication in maize (Chen *et al*., 1992; Wirtz *et al*., 1997) and our constructs lack a eukaryotic origin of replication which has been shown to be required for transient testing of Ds constructs but results in only very low levels of Ds excision (McElroy *et al*., 1997; Takumi *et al*., 1999). We are currently generating stable transgenic lines with pActN-Ds-H2B-GFP and pHSPdAc separately, for crossing, and as double transformants by co-bombardment. These plants will provide a valuable resource for studying development in wheat tissues, including the endosperm, and the knowledge gained will assist in the design of strategies for improving cereal grain yield/quality.

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