Journal of Cereal Science 58 (2013) 472–478

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

The role of sensitivity to abscisic acid and gibberellin in pre-maturity α-amylase formation in wheat grains

K.R. Kondhare [a], P.S. Kettlewell [a,*], A.D. Farrell [b], P. Hedden [c], J.M. Monaghan [a]

[a] Harper Adams University, Department of Crop and Environment Sciences, Newport TF10 8NB, UK
[b] The University of the West Indies, Department of Life Sciences, St. Augustine, Trinidad, West Indies
[c] Rothamsted Research, Department of Plant Biology and Crop Science, Harpenden, Hertfordshire AL5 2JQ, UK

ARTICLE INFO

Article history:
Received 3 July 2013
Received in revised form
20 September 2013
Accepted 23 September 2013

Keywords:
Wheat
α-Amylase
Rht
1BS/1RS

ABSTRACT

To study the role of abscisic acid (ABA) and gibberellin (GA) sensitivity in regulating pre-maturity α-amylase (PMA) in wheat grains, plants were grown in a glasshouse under cold-shock and ambient conditions. α-amylase activity in response to applied ABA and GA was measured in detached-grains with the embryo removed (*in vitro*) and in intact-grains attached to the plant (*in situ*). The *in vitro* experiment was conducted using Spark (low PMA-susceptible genotype) and Rialto (highly PMA-susceptible genotype), with the aim of defining the time point for GA-sensitivity. The results showed an increase in GA-sensitivity at about 640 degree days after anthesis (DAA) in Rialto. There was no evidence for a change in ABA-sensitivity in either variety. The *in situ* experiments were conducted using genotypes from a Spark × Rialto doubled haploid population segregating for the *Rht-D1a* (tall) or *Rht-D1b* allele and for the presence or absence of 1BS/1RS. For *Rht-D1a* (tall) or *Rht-D1b* genotypes with or without 1BS/1RS, the cold-shock significantly increased GA-sensitivity, whereas there was no significant change in ABA-sensitivity. These results show PMA is related to an increase in GA-sensitivity that occurs in the aleurone at around 640 degree DAA, and can be enhanced by environmental factors (e.g. cold-shock).

1. Introduction

1.1. Pre-maturity α-amylase (PMA) and hormones in wheat (Triticum aestivum, L) grains

In the United Kingdom (UK), PMA is the second major cause of elevated high isoelectric point (pI) α-amylase formation in mature wheat grains prior to germination (the first being pre-harvest sprouting) (Lunn et al., 2001). PMA is also referred to as late maturity α-amylase or late maturity endosperm α-amylase (Lunn et al., 2001). Susceptible genotypes show high levels of α-amylase in sound grains that result in a low Hagberg Falling Number (HFN) and render grain unsuitable for bread-making (Mares and Mrva, 2008). A cold-shock applied during the window of sensitivity, i.e. 26–30 days after anthesis (DAA), can induce PMA (Farrell and Kettlewell, 2008; Mrva and Mares, 2001). PMA is thought to be controlled by 1 or 2 recessive genes and a number of significant QTL have been identified (Barrero et al., 2013; Mares and Mrva, 2008).

The plant hormones abscisic acid (ABA) and gibberellins (GA) are important in the regulation of α-amylase during germination and pre-harvest sprouting (Bethke et al., 1997; Gerjets et al., 2010; Hader et al., 2003 and references therein). In germinating wheat grains, GA stimulates and ABA inhibits α-amylase formation (at least in dormant grain) (Hader et al., 2003). A similar role for ABA and GA may therefore be anticipated in developing grains during PMA-induction. The presence of GA-insensitive (GAi) dwarfing alleles, such as *Rht-B1b*, *Rht-D1b* and *Rht-B1c* (also known as *Rht1*, *Rht2* and *Rht3*, respectively), significantly reduces PMA in wheat, indicating the involvement of GA-sensitivity in PMA-induction (Gold and Duffus, 1993; Mrva and Mares, 1996; Mares and Mrva, 2008). The perception of GA by the GID1 receptor (GA-insensitive dwarf 1; nuclear based GA receptor; Ueguchi-Tanaka et al., 2005), which is mediated through DELLA (*Rht*, growth repressor) and the SCF complex, induces the expression of the GAMYB (promoter of α-amylase genes) and hence leads to α-amylase synthesis (Gilroy

Abbreviations: ABA, abscisic acid; CV, coefficient of variation; DAA, days after anthesis; d.f., degrees of freedom; GA, gibberellins; GAi, gibberellin-insensitive; GA_3, gibberellic acid (active GA produced by one branch of the biosynthetic pathway used for endogenous applications); PMA, pre-maturity α-amylase; QTL, quantitative trait locus; *Rht*, reduced height genes; SEM, standard error of mean; UK, United Kingdom; ZGS, Zadoks growth stage; 1BS/1RS or 1B/1R, wheat-rye chromosome translocation where the short arm of the 1B chromosome in wheat is replaced by the short arm of the 1R chromosome from rye.

* Corresponding author. Tel.: +44 (0)1952 815299; fax: +44 (0)1952 814783.
E-mail address: pskettlewell@harper-adams.ac.uk (P.S. Kettlewell).

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http://dx.doi.org/10.1016/j.jcs.2013.09.009

and Jones, 1994). Recently, Barrero et al. (2013) found that several GA (e.g. GA_8, GA_{19}, GA_{24}, GA_{29} and GA_{44}) were more abundant in PMA-susceptible genotypes than in PMA-resistant genotypes. *In vitro* studies by Bethke et al. (1997) with detached cereal grains investigated the response of the aleurone tissue to applied ABA. They showed that applied ABA inhibited high pI α-amylase formation. However, the role of ABA-sensitivity in developing grains has not been fully explored.

1.2. ABA- and GA-sensitivity of the aleurone using detached, developing grains (in vitro study)

Several factors are involved in regulating PMA formation in developing grains, including genotype, agronomy, and environmental conditions (Barrero et al., 2013; Farrell and Kettlewell, 2008, 2009; Mrva and Mares, 2006). In particular, a cold-shock applied during the window of sensitivity can induce PMA (Mrva and Mares, 2001; Farrell and Kettlewell, 2008). Our earlier work showed that a cold-shock applied during mid-grain development (i.e. from 520 to 680 degree DAA) increased GA-sensitivity in developing grains of a highly PMA-susceptible variety – Rialto, but not in a less PMA-susceptible variety – Spark (Kondhare et al., 2012). There was no evidence for a change in ABA-sensitivity during PMA-induction in either variety. However, from these results, it was unclear if this increase in GA-sensitivity in Rialto grains occurred during the cold-shock or following the cold-shock in the later stages of ripening. Hence, a major objective of the current study is to define more precisely the time point at which GA-sensitivity is increased in developing grains of induced Rialto plants.

1.3. Influence of reduced height (Rht) or dwarfing genes on the aleurone sensitivity of intact, developing grains

Dwarfing genes are used worldwide in agriculture not only for improving yield but also for increasing lodging resistance. Most modern bread-wheat cultivars carry either the *Rht-B1b* or the *Rht-D1b* semi-dwarfing allele (Hedden, 2010), which encode mutant forms of a DELLA protein (Peng et al., 1999). Wheat plants with the *Rht-B1a* or *Rht-D1a* allele are GA-sensitive and are mostly tall (Peng et al., 1999), with a few exceptions (Pearce et al., 2011). Genotypes that carry these tall alleles in combination with PMA QTL produce a high level of PMA (Barrero et al., 2013; Gold and Duffus, 1993; Mrva and Mares, 1996). Those plants with the *Rht-B1b* or *Rht-D1b* allele are GA-insensitive and semi-dwarf (Peng et al., 1999), if the PMA QTL are present, these genotypes may still produce PMA sporadically or when induced (Farrell et al., 2013; Gold and Duffus, 1993; Mrva and Mares, 1996, 2001). In the current study (*in situ* experiments), we seek to provide a better understanding of how *Rht-D1b* influences PMA by investigating its effect on PMA with respect to ABA- and GA-sensitivity.

1.4. The 1BS/1RS translocation and its effect on PMA

Despites producing several beneficial agronomic effects such as high yield, disease resistance, and good performance in adverse conditions, the 1BS/1RS wheat-rye chromosome translocation has been linked to inferior bread-making quality. The loss of quality seems to be due to genes present on the short arm of the 1R chromosome from rye, but the mechanism involved remains to be determined (Biliaderis et al., 1992; Dhaliwal et al., 1986). There is also evidence that the presence of the 1BS/1RS translocation increases the occurrence of PMA (Farrell et al., 2013; Mares and Mrva, 2008; Mrva et al., 2008). Farrell et al. (2013) found that 1BS/1RS had an extensive influence, increasing PMA occurrence in tall and semi-dwarf genotypes, under both inductive and non-inductive conditions.

Here we carry out an *in vitro* experiment investigating the timing of changes in ABA- and GA-sensitivity during PMA-induction in Spark and Rialto, and two *in situ* experiments investigating the extent of changes in ABA- and GA-sensitivity in selected Spark × Rialto genotypes. The findings should help explain the nature of PMA-induction and how it is regulated by the GA-insensitive *Rht-D1b* semi-dwarfing allele and the 1BS/1RS chromosome translocation.

2. Materials and methods

2.1. In vitro experiment

2.1.1. Plant materials and growing conditions

Seeds of Spark and Rialto were planted on 28 February 2010 in a glasshouse at Harper Adams University (Newport, Shropshire, UK). Plants were grown according to the PMA-induction protocol (as described in Farrell and Kettlewell, 2008; Kondhare et al., 2012). Plants were grown in a glasshouse bay and were grouped by variety. Plants were tagged at early anthesis (i.e. Zadoks Growth Stage 61 [ZGS 61]) (Zadoks et al., 1974) and subjected to two temperature conditions: cool-shock and ambient. The cold-shock induction was conducted during mid-grain development from 520 to 680 degree DAA (i.e. ZGS 75–77), which occurred in late July 2010.

2.1.2. Sampling methodology

Grains from plants under ambient and cold-shock conditions were harvested at three different time points: 580, 640 and 720 degree DAA (Fig. 1). More details about the degree DAA under non-induced and induced conditions for three time points with corresponding grain moisture content are given in Table 1. The main spike from 20 replicate plants were harvested at the time point: 580 degree DAA ($n = 20$), whereas spikes from 10 replicate plants were harvested at the two time points: 640 degree DAA ($n = 10$) and 720 degree DAA ($n = 10$). Higher replication was used for the first time point to ensure initial, smaller effects on GA-sensitivity would still be detectable. Samples (10 embryoless distal half-grains from the middle region of each replicate spike) were prepared and incubated *in vitro* in hormone solutions according to the protocol described in Kondhare et al. (2012), except that the ABA + GA_3 treatment was omitted. The concentrations of hormones used were also the same as used in Kondhare et al. (2012): solvent only control (10% ethanol), ABA (100 μM) and GA_3 (50 μM). Following incubation, the remaining incubation buffer was removed and samples were freeze-dried (Edwards Modulyo F101, UK) for 4 days. Subsequently, dried samples were ground with one distal half-grain per well in a 96-well block using a TissueLyser II (Qiagen, UK). α-amylase activity in flour was measured by the Megazyme assay (Megazyme International Ireland Ltd., Ireland) as follows.

The 96-well block with flour was incubated in a 60 °C water bath for 30 min. Buffer A (sodium maleate [100 mM, pH 6.0] plus calcium chloride [5 mM] and sodium azide [0.02%]) (pre-heated to 60 °C) was added to each well (250 μl) and the contents mixed by pipetting up and down five times. The extraction was continued for exactly 5 min at 60 °C. The amylazyme substrate solution (50 μl of one amylazyme tablet suspended in 1 ml of buffer A; Megazyme International Ireland Ltd., Ireland) was added to each well and the reaction continued for a further 5 min at 60 °C. Samples were removed from the water bath and immediately 300 μl of STOP solution (2% w/v TRISMA Base, pH 9.5) was added to terminate the reaction. The contents of the block were mixed by shaking the block several times by hand and were held at room temperature for