Journal of Cereal Science 60 (2014) 210–216

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Use of the hormone-biosynthesis inhibitors fluridone and paclobutrazol to determine the effects of altered abscisic acid and gibberellin levels on pre-maturity α-amylase formation in wheat grains

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

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

ARTICLE INFO

Article history:
Received 21 December 2013
Received in revised form
26 February 2014
Accepted 7 March 2014
Available online 12 April 2014

Keywords:
α-Amylase
Abscisic acid
Fluridone
Gibberellins
Paclobutrazol
Wheat

ABSTRACT

During germination of cereal grain, α-amylase formation is known to be inhibited by abscisic acid (ABA) and stimulated by gibberellins (GA). The role of these hormones in pre-maturity α-amylase (PMA) formation in wheat grains is less well understood. Our previous work with ABA and GA exogenously applied to grains demonstrated a clear stimulatory effect of GA, with little effect of ABA. Here, in glasshouse experiments, fluridone (ABA biosynthesis inhibitor; FD [20 μM]) or paclobutrazol (GA biosynthesis inhibitor; PB [20 μM]) were applied to intact, developing grains of the PMA-susceptible variety Rialto at 480 days after anthesis (DAA) to assess if a reduction in endogenous ABA and/or GA alters PMA formation. The experiments were conducted under non-PMA-inducing (ambient) and PMA-inducing (cold-shock) conditions. In solvent-only treated grains, a cold-shock significantly reduced the ABA content but increased GA and α-amylase activity. FD increased GA levels and α-amylase activity under ambient conditions, but decreased GA levels and α-amylase activity under cold-shock conditions, with no effect on ABA levels under either condition. PB had no effect under ambient conditions, but reduced GA levels and α-amylase under cold-shock conditions. These results indicate an association between GA levels at mid-grain development and PMA formation in wheat.

1. Introduction

The occurrence of pre-maturity α-amylase (PMA) involves excessive synthesis of high isoelectric point (pI) α-amylase prior to germination, which is retained in mature wheat grains (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 and render grains unsuitable for bread-making (i.e. low Hagberg Falling Number [HFN]) (Mares and Mrva, 2008). In the United Kingdom (UK), PMA is the second major cause of a low HFN, the first being pre-harvest sprouting (PHS). PMA is thought to be controlled by 1 or 2 recessive genes and a number of significant quantitative trait loci (QTL) have been identified (Barrero et al., 2013; Mares and Mrva, 2008). Several factors are involved in regulating PMA formation in developing grains, including genotype, agronomy, and environmental conditions (Barrero et al., 2013; Farrell et al., 2013; Farrell and Kettlewell, 2008; Mrva and Mares, 2006). In particular, a cold-shock applied during 26–30 days after anthesis (DAA) can induce PMA in susceptible genotypes (Farrell and Kettlewell, 2008; Mrva and Mares, 2001).

Abscisic acid (ABA) and gibberellins (GA) are important in the regulation of α-amylase during germination and PHS (Bethke et al., 1997). When ABA and GA were applied to germinating grains, GA stimulated whereas ABA inhibited α-amylase formation (Bethke et al., 1997). Normally during wheat grain development and maturation, peak levels of GA occur around 15–20 DAA and that of ABA occurs later and across a wider range, i.e. 25–40 DAA (McWha,

Abbreviations: ABA, abscisic acid; CV, coefficient of variation; DAA, days after anthesis; d.f., degrees of freedom; FD, fluridone; GC–MS, gas chromatography-mass spectrometry; GA_3, gibberellic acid; GA, gibberellins; MC, Moisture content; PB, paclobutrazol; PHS, pre-harvest sprouting; PMA, pre-maturity α-amylase; QTL, quantitative trait locus; SEM, standard error of mean.

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

http://dx.doi.org/10.1016/j.jcs.2014.03.001
0733-5210/

1975). The occurrence of high ABA and low bioactive GA levels in the later stages of grain maturation should ensure that mature grains have low levels of α-amylase. However, in the case of PMA, a high level of α-amylase in mature grains is observed in susceptible genotypes.

Paclobutrazol (α-tert-butyl-β-(4-chlorobenzyl)-1H-1,2,4-triazole-1-ethanol), commonly abbreviated as PB, is a triazole type inhibitor of GA biosynthesis which decreases plant growth and development. PB is generally used in agronomic and horticulture crops to reduce undesirable longitudinal shoot growth without affecting plant productivity (Rademacher, 1995). PB action inhibits the activity of *ent*-kaurene oxidase, which is an enzyme in the GA biosynthesis pathway that catalyses the oxidation of *ent*-kaurene to *ent*-kaurenoic acid (Hedden and Graebe, 1985). Fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl) phenyl]-4-(IH)-pyridinone), commonly abbreviated as FD, is an ABA biosynthesis inhibitor that inhibits the activity of phytoene desaturase I, which catalyses the conversion of phytoene to phytofluene (Fong et al., 1983). Thus, it reduces the synthesis of carotenoids which are the precursors for ABA biosynthesis in plants. The sites of action of FD and PB in the ABA and GA biosynthesis pathways, respectively, are shown in Supplemental Fig. 1.

ABA and GA influence various aspects of grain development. Applied FD significantly reduces seed dormancy and initiates germination in wheat, supporting the association of ABA with seed dormancy (Kawakami et al., 1997). Yang et al. (2004) studied the role of ABA in wheat grain filling through the application of FD. When the spikes were sprayed with FD solution at 9 DAA, the activities of four enzymes of starch biosynthesis (i.e. sucrose synthase, ADP Glucose pyrophosphorylase, starch synthase and starch branching enzyme) in grains were significantly reduced and therefore, resulting in significantly lower grain filling rate in FD-treated plants compared to solvent-only treated plants (Yang et al., 2004), and confirming a role for ABA in grain filling. Pagano et al. (1997) showed that when sorghum panicles were sprayed with FD solution soon after anthesis, there was a significant increase in α-amylase activity in mature caryopses. In contrast, a significant decrease in α-amylase activity was observed when panicles were sprayed with PB solution (Pagano et al., 1997). Therefore, spraying intact wheat grains with PB solution just before mid-grain development is expected to inhibit the subsequent synthesis of α-amylase due to reduced endogenous GA. On the other hand, spraying intact grains with FD solution is expected to increase grain α-amylase by minimising the inhibitory effect of ABA towards α-amylase synthesis. However, the response to both hormone inhibitors would depend on how efficiently they are being taken up by developing grains.

Over the last two decades, manipulating hormone levels (using biosynthesis mutants or by applying chemical inhibitors) or hormone signalling (by altering the hormone sensitivity) has been a widely used approach to understand the hormonal mechanism of dormancy induction in developing seeds (Le Page-Degivry, 1997). Since developing cereal grains are a rich source of ABA and GA, hormone inhibitors such as FD and PB were used in the past to study the role of ABA and GA in various aspects of grain development, and provide a useful tool to study the role of ABA and GA on α-amylase synthesis in intact developing wheat grains.

Farrell and Kettlewell (2008) and Mares and Mrva (2008) suggested that a cold-shock applied during mid-grain development to induce PMA does so through altered ABA/GA sensitivity of the aleurone and/or ABA/GA levels in developing grains. Our earlier work used exogenous hormones to study the role of ABA- and GA-sensitivity of the aleurone of intact or detached developing wheat grains in PMA-induction by a cold-shock, and showed that PMA-induction is related to an increase in GA-sensitivity in susceptible genotypes whereas a change in ABA-sensitivity is less important (Kondhare et al., 2012, 2013). The present study tests the hypothesis that endogenous hormone levels are important for regulating PMA by reducing GA and ABA content in developing grains using fluridone (ABA biosynthesis inhibitor; FD) and paclobutrazol (GA biosynthesis inhibitor; PB). Three experiments were conducted in a glasshouse in which grains of the high PMA-susceptible genotype, Rialto, were treated with the inhibitors *in situ*. The first experiment studied the effects of applied FD and PB on α-amylase activity at the three time points (580, 720 and 1200 DAA) in grain development under ambient and cold-shock conditions. In the second experiment, the effects of applied FD and PB on α-amylase activity, ABA and GA levels at 580 DAA were determined under ambient and cold-shock conditions. In the third experiment, after FD and PB solutions were applied to grains to reduce endogenous ABA and GA levels, the sensitivity of the grains to subsequently applied hormones was studied under ambient and cold-shock conditions.

2. Materials and methods

2.1. Plant material and growing conditions

Rialto seeds were sown in trays filled with John Innes No. 2 Compost in a glasshouse at Harper Adams University (Newport, Shropshire, UK). Plants were grown according to the PMA induction protocol (as described previously by Farrell and Kettlewell, 2008; Kondhare et al., 2012). The main spike on each plant was tagged with coloured tape at early anthesis.

2.2. In situ application of inhibitors

One day before the inhibitor applications, the crease regions of developing grains within the main spike (per replicate plant) were made visible by opening the lemma with forceps. Grains were sprayed uniformly with FD (20 μM) or PB (20 μM) solutions using a hand-sprayer (0.5 L, Hozelock SprayMister, UK) at the rate of about 5 ml per spike (Supplemental Fig. 2a). In the current study, the inhibitor solutions were sprayed on three subsequent days starting at 480 DAA (Table 1). As a control, plants were sprayed with the same volume of 10% methanol. The whole spikes were sprayed with the inhibitor solutions or solvent between 5 and 7 pm as stomata on grains were expected to be open, which would facilitate the uptake of these inhibitors by the grains.

After application of the inhibitors, half of the plants remained in a glasshouse heated to 25/15 °C until maturity (ambient conditions), while the other half were transferred at 520 DAA to an air-conditioned glasshouse cooled to provide a constant temperature of 12 °C for 8 days (cold-shock conditions) (as described in Kondhare et al., 2012). Plants subjected to cold-shock conditions were returned to a warm glasshouse heated to 25/15 °C, where they remained until grain maturity.

2.3. Experiment 1: effects of inhibitors applied in situ on α-amylase activity at three time points

In this experiment, the effects of inhibitors (FD and PB) applied *in situ* to developing grains on α-amylase activities were assessed at three time points under ambient and cold-shock conditions. Entire spikes from plants were harvested in liquid nitrogen at three time points: 580, 720 and 1200 DAA, and stored in a −70 °C freezer. Samples were then freeze-dried over four days, before being analysed. More details about the degree DAA under cold-shock and ambient conditions for the three time points with corresponding grain moisture content are given in Table 1.

The two outermost grains from spikelets 7 and 9 (counting acropetally) of the main spike were selected and ground in a 96-