Physiological and Molecular Traits Associated with Grain Weight Potential in Wheat

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Abstract

Grain weight is an important component of grain yield and quality in wheat. Despite many years of research into the determination of grain weight our understanding of this subject remains incomplete. Most of the research aimed at studying grain weigh was focused on the grain filling period; however, the importance of the phase immediately previous to anthesis, in addition to the post-anthesis period, has become apparent. The objective of the present study was to evaluate physiological and molecular variables at pre- and post-anthesis in order to better understand grain weight determination in wheat. In order to achieve this, two experiments were carried out under field conditions. In both experiments the effect of growing season, cultivars, and grain position on grain weight and associated traits were assessed. Final grain weight, grain volume, grain dimensions, carpel weight at anthesis, water and dry matter dynamics, number of endosperm and pericarp cells, and expression of expansin genes were measured. Final grain weight was associated with grain volume ($r= 0.93$; $p<0.01$). Both, grain weight and volume were linearly related with the length of grains ($r= 0.95$; $p<0.01$ and $r= 0.90$; $p<0.01$, respectively). Grain weight was also associated with other post-anthesis variables including endosperm and pericarp cell number ($r= 0.74$, $p<0.05$ and $r= 0.90$, $p<0.01$, respectively). Grain weight showed a close association with carpel weight at anthesis ($r= 0.89$; $p<0.01$). In addition to these traits, the expression of expansin A6 mimic the enlargement of grains which occurs early in the grain filling period, suggesting an associative role.

Media summary

Grain weight potential is associated with traits developed before and early after anthesis, which modulate the pericarp tissues of grains.

Keywords
Grain weight, carpels, expansins, pericarp, endosperm.

Introduction

Grain weight is an important component of grain yield and quality of wheat. Although a great effort has been dedicated to study how grain weight is determined the knowledge of genotype and environmental factors regulating grain weight is not completely understood. In wheat, it is generally accepter that anthesis is the starting point of grain weight determination. Therefore, most of the research aimed at studying this trait has been focused on the grain filling period. Among others, grain filling rate and duration, and the number of endosperm cells have been seeing as the most important traits associated with final grain weight in wheat (Brocklehurst, 1977; Sofield et al., 1977). However, the assumption that grain weight is exclusively determined from anthesis onwards has been challenged by reports published over the last years where manipulations of environmental conditions and source-sink ratio previous to anthesis significantly affected final grain weight (e.g., Calderini et al., 1999; Calderini and Reynolds, 2000). These findings were recently confirmed for wheat (Duggan and Fowler, 2006; Ugarte et al., 2007) and extended to other temperate cereals as barley and triticale (Ugarte et al., 2007). In addition, Ugarte et al. (2007) found that the booting-anthesis period was the most affecting pre-anthesis phase for grain weight determination. Regulation of grain weight by the pre-anthesis period could be ascribed to the carpels of florets which are growing fast between booting and anthesis. Associations between final grain weight and carpel weight at anthesis were found in barley (Scott et al., 1983) and wheat (Calderini and Reynolds, 2000).

Therefore, pericarp cells (developed when the carpels are growing) could be involved in grain weight determination. Pericarp grow is the result of the number of pericarp cells and the enlargement of these cells. It has been showed that plant cell enlargement is controlled by a family of proteins called expansins, which are involved in cell wall loosening (McQueen-Mason et al., 1992). Although little is known about expression of expansins in growing grains recent experiments found them in wheat grains (Calderini et al., 2006; Liu et al, 2007). In the present study, two experiments were carried out at field conditions aimed at identifying physiological and molecular traits associated with grain weight potential determination in wheat. The ultimate objective of this study is to improve a previously proposed model of grain weight determination in wheat (Calderini et al., 2001).

Materials and Methods

Two experiments were carried out under field conditions at the experimental field of Universidad Austral de Chile in Valdivia (39° 62' S, 73° 08' W). Experiment 1 consisted of 2 wheat cultivars (Bacanora and Kambara), contrasting in average grain weight, evaluated during 3 growing seasons. In experiment 2, 3 wheat cultivars were evaluated: Pandora and Huanil in the first season and Pandora and Huayun during the second season. Both experiments were arranged as a completely randomized design with 3 replicates. Plots were managed to avoid biotic and abiotic stresses. Traits associated with grain weight were measured both at pre- and post-anthesis. Grain weight was measures in the 4 grain positions of central spikelets (G1, G2, G3 and G4; where G1 is the grain position closest to the rachis) of spikes in both experiments. Different grain traits were measured depending on the experiment and the growing season. Briefly, grain volume was evaluated in all grain positions in experiment 1 and in G2 and G4 in experiment 2. Between booting and anthesis, flowers of 4 main grain positions of central spikelets of spikes were sampled to measure carpel weight and development in the 2nd. and 3rd. season of experiment 1. Water and dry matter dynamics of grains as well as grain dimension (length, width and height), were measure between anthesis and maturity in both experiments. In addition, endosperm and pericarp cell number were measured in experiment 2, while expansin expression was evaluated in experiment 1. The endosperm cells number was determined following Gleadow et al. (1982). To account pericarp cells, dorsal sections of grains were dissected and pericarp tissue extracted and dyed with *green methyl.* The histological preparations were measured by optical microscopy with digital camera incorporated. Each picture was analyzed using ImageJ Computational Software http://rsb.infonih.gov/ij/). To measure expansin expression, grains of the 2 position from spikelets of the middle of each spike were harvested. For semi-quantitative RT-PCR experiments grains were collected at 2, 5, 9, 12, 17, 20 DAA and immediately frozen with liquid nitrogen. RNA was isolated using TRIZOL and treated wit DNAse. For unique expansin sequences obtained in works before, specific PCR primer pairs were designed in order to discriminate different expansin transcripts. A fragment of the constitutively expressed 18S ribosomal RNA gene was used as a control. After the standardization of PCR conditions, RT-PCR products was evaluated semicuantitative by electrophoresis in 2% agarose gels with ethidium bromide staining.

Results

In both experiments a wide range of final grain weight was found, i.e. 31.6-72.1 and 36.3-58.4 mg in experiment 1 and 2, respectively. This trait was mainly affected by the genotype ($p<0.01$) and grain position ($p<0.01$). The highest difference of grain weight was due to grain position (63 and 38% between G2 and G4 in experiment 1 and 2, respectively) while cultivars showed lower, but still important, differences (averaged across grain positions, 24 and 11% in experiment 1 and 2, respectively). In agreement with previous results, a close association was found between grain weight and grain volume at harvest in experiment 1 (Fig. 1), and in evaluated grain positions (G2 and G4) of experiment 2 ($y= 1.20x + 2.79$. $R^2= 0.90$). The length of grains explained most of final volume of grains in experiment 1 (Fig. 1) and 2 ($y= 15.5x - 64.8$. $R^2= 0.65$). In addition, maximum water content was also linearly associated with final grain weight ($r= 0.97$; $p<0.01$ and $r= 0.96$; $p<0.01$, in experiment 1 and 2, respectively). Taking into account these results, and considering that the length of grains is set early in the grain filling period (by 30-35% of final grain weight), the association between grain weight, maximum water content and the length of grains was assessed at contrasting grain positions (G2 and G4) of experiment 1. Both, grain weight and maximum water content were linearly associated with grain length (Fig. 2). In addition, cellular traits of grains were evaluated in G2 and G4 grain positions of experiment 2.

Consistently, grain weight and grain volume was associated with both endosperm cell number (r= 0.76, p<0.05 and r= 0.81, p<0.05, respectively) and pericarp cell number (r= 0.74, p<0.05 and r= 0.90, p<0.01).

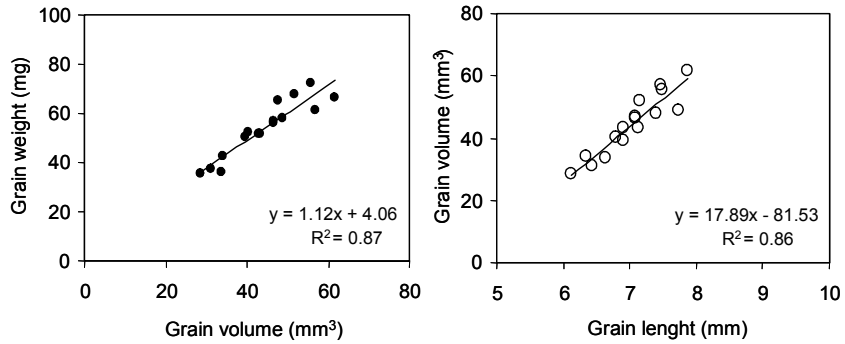

Figure 1. Relationship between grain weight and grain volume and grain volume and grain length in experiment 1.

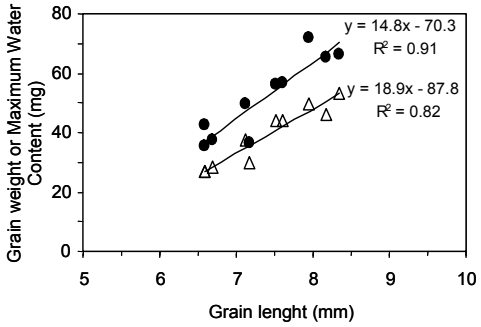

Figure 2. Relationship between grain weight (closed circles) or maximum water content (open triangles) and grain length in grain positions G2 and G4 of experiment 1.

More interestingly, final grain weight was associated with carpel weight of flowers at the timing of floret fertilization in experiment 1 (Fig. 3). These results show that differences in grain weight due to cultivar and/or grain position within the spikelet were already set at anthesis. In addition, expansin expression was identified in pericarp cells of growing grains of experiment 1. Among expansins, ExpA 6c showed similar pattern than the enlargement of grains (Fig. 4).

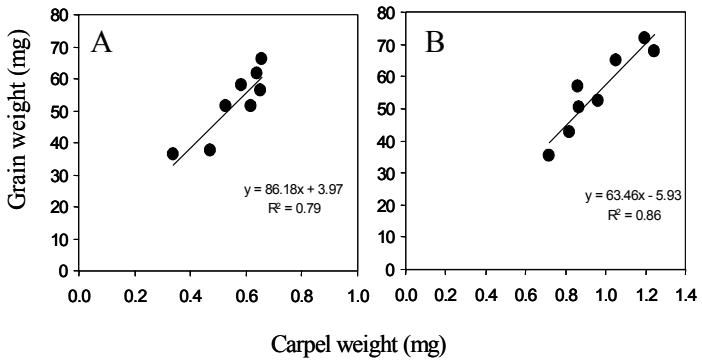

Figure 3. Relationship between final grain weight and carpel weight at anthesis in season 2 (A) and 3 (B) of experiment 1.

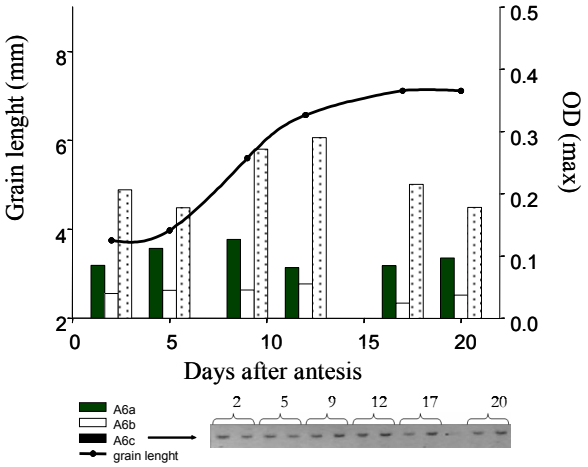

Figure 4. Grain length and expansin expression (OD) after anthesis. Three expansins are shown: ExpA 6a (closed bars), ExpA 6b (open bars) and ExpA 6c (dotted bars).

Conclusions

Final grain weight and its volume are closely associated with traits set immediately before (carpel weight) and early after (e.g., grain length, maximum water content). Most of them, related with the pericarp of grains. This component of grains and its growth seems to be associated with grain weight potential.

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