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Expression of High Molecular Weight Glutenin Subunit Genes Correlated with Gluten Content in Common Wheat Varieties from the Highland of Ecuador

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In this study we analyzed the performance of three wheat varieties in relation to gluten content under high-altitude growing conditions in the Andes of Ecuador. A field experiment was conducted at 3058 meters above sea level during 2009 using adapted wheat cultivar Cojitambo, cv. Carnavalero, and cv. Sibambe. Transcript accumulations of High Molecular Weight Glutenin Subunits (HMW-GS) genes were also evaluated during grain development using qRT-PCR. We recorded the expression profile of HMW-GS genes during 41 days and showed a coordinated pattern of induction with significant higher levels at 82–86 days. Transcript accumulation of *1Dx5, 1Dy10, 1Bx7, 1Ax1,* and *1By9* genes were analyzed in more details during this period. The assay highlighted the specific contribution of *1Bx7, 1Dy10,* and *1Dx5* during gluten formation in Ecuadorian wheat varieties. Under Andean highlands conditions, cv. Carnavalero showed the higher values of total agglomerated protein upon hydration and higher levels of expression of particular HMW-GS genes. The data suggest a correlation between wet gluten content and HMW-GS genes expression. Our study contributes to understand gluten formation in wheat endosperm under high-altitudes conditions in the Andes.

Keywords: wheat, glutenin, HMW-GS genes, highlands of Ecuador

Introduction

Wheat (*Triticum aestivum* L.) is the most important crop in the world in terms of consumption and cultivation area (Tosi et al. 2009). It is widely used as feed for livestock and in food chains such as pasta or breadmaking. Viscoelastic properties of bread wheat flour reside primarily in the types and quantities of gluten proteins, being gliadins and glutenins the most abundant (Peña et al. 2005; Anjum et al. 2007). While gliadins are insoluble proteins that contribute to viscous flow and extensibility, glutenins are the ones that confer

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elasticity to wheat flour dough. In this context, the glutenin fraction of the complex is perhaps a major determinant of breadmaking performance, and responsible for variations in dough strength among wheat varieties (Shewry et al. 2003).

Molecular mass classification of glutenins polymers further distinguishes Low Molecular Weight Glutenin subunits (LMW-GS) and High Molecular Weight Glutenin subunits (HMW-GS). In bread wheat, genes encoding HMW-GS proteins resides at the orthologous loci *Glu-A1, Glu-B1* and *Glu-D1* (Shewry et al. 1995). Each loci contain at least two allelic variants encoding x- and y-type subunits (Shewry et al. 1995). Genetics, breeding, and transgenic studies suggest that presence/absence of specific HMW-GS alleles or allele ratios might impact on dough properties (Payne et al. 1981; Payne 1987; Barro et al. 1997; Wieser and Zimmermann 2000; Peña et al. 2005), making HMW-GS perfect candidates to study gluten performance in wheat varieties.

In terms of gene expression, HMW-GS are tightly regulated by a common signal during grain development (Altenbach et al. 2002; Greene 1983). Early after flowering, wheat cells start to accumulate HMW-GS transcripts (Altenbach et al. 2002). This event predates deposition of starch and gluten proteins in wheat endosperm. Even though grain filling is expected to occur after fertilization on wheat varieties that grow on temperate long-day climates, temporal regulation of HMW-GS-inducing signal appears to changes with environmental conditions (Altenbach et al. 2002) or wheat genetic background (Labuschagne et al. 2009).

In the Andean highlands of Ecuador, wheat varieties are adapted to high-altitude growing conditions, and typically planted between 2.500 and 3.200 meters above sea level. Under these conditions, gluten content has never been accessed in terms of breadmaking quality and variation in gene expression. In this report, we examine transcript accumulation from HMW-GS genes during grain development in relation to gluten content in three common wheat varieties from the highlands of Ecuador.

Materials and Methods

To determine whether local wheat varieties have differences in gluten content, we conducted a field experiment to estimated flour quality of three wheat cultivars by measuring wet gluten in wheat endosperm. The experiment was carried out in the highlands of Ecuador at the Instituto Nacional de Investigaciones Agropecuarias (INIAP) during January–July 2009. We planted 3.6 m^2-plots of three local wheat varieties, cv. Cojitambo, cv. Carnavalero, and cv. Sibambe. The experimental farm is located at 3058 meters above sea level with average annual temperature range from 11 to 15°C. After harvesting, a 250g of grain sample was collected from each wheat cultivar and wet gluten determined using the referential INEN 529 method (Jaya 2010).

In order to identify the temporal expression profile of HMW-GS genes under high-altitude conditions, we analyzed transcript accumulation of *1Dx5, 1Dy10, 1Bx7, 1Ax1,* and *1By9* genes using quantitative reverse transcription polymerase chain reaction (qRT-PCR) in the wheat cv. Cojitambo during a 41-day period. One hundred Cojitambo plants were growth in the greenhouse at 2510 meter above sea level at temperature be-

tween 13 and 16°C. Wheat spikes of three phenologically similar plants were harvested during 8 time points between day 56 and 97 after sowing. Samples were frozen in liquid nitrogen and kept at –80°C until use. Total RNA was extracted using the TRIzol reagent (Invitrogen) and DNA-purified with DNAase I (Invitrogen) according to manufacturer's instructions. RNA concentration and integrity was checked by spectrophotometry or assessed by electrophoresis on agarose gels. First-strand cDNA was synthesized from (2,5 μg/uL) of total RNA by the SuperScript III Reverse Transcriptase (Invitrogen). Primers for HMW-GS subunits and normalizing housekeeping genes were designed based on GenBank accession numbers: X12928 *(1Dx5),* X12929 *(1Dy10),* X13927 *(1Bx7),* X61009 *(1Ax1),* and X61026 *(1By9)*. We tested three genes for normalization in wheat: GAPDH (AY290728), Puroindoline (X69912), and Rubisco (M37477) (Broglie et al. 1983; Li et al. 2004; Paolacci et al. 2009).Transcript accumulation was performed using an AB 7300 Real-Time PCR System (Applied Biosystems) with brilliant SYBR GreenER qPCR SuperMix (Invitrogen). Gene expression levels were recorded as Ct values (Czechowski et al. 2004) and relative expression (RE) calculated using the formula: $RE = 2^{-(Ct\ GEN - Ct\ NOR)}$. To determine which genes contribute to gluten formation in local wheat varieties, we analyze transcripts accumulation of HMW-GS in cv. Cojitambo, cv. Carnavalero, and cv. Sibambe under same greenhouse conditions. This experiment was performed as described above with few modifications. We planted five plants per pot and 90 plants per variety. We collected three phenologically similar plants at six time points between day 74 and 86. Samples preparation, RNA extraction, cDNA synthesis and normalization were performed as described above.

Results

In this study, we found significant variation in wet gluten composition among wheat varieties adapted to high altitudes. Under field conditions, cv. Carnavalero showed the higher values with 21.5% of total protein that agglomerates upon hydration. Wheat cv. Cojitambo and cv. Sibambe showed smaller values with 19.9% and 18.2%, respectively (Fig. 1). We were also able to track transcript accumulation of HMW-GS genes in wheat endosperm during grain development across 41 days (day 56 to 97). Expression profile in cv. Cojitambo showed a coordinated pattern of induction of glutenin genes with significant higher levels at a period between 82 and 86 days (data not shown). Up-regulation of most HMW-GS genes began at day 77 and was undetectable at day 97. Therefore, we tested the specific contribution of each HMW-GS gene among three wheat cultivars during 18 days (Figs 2 and 3). We observed that genes *1Bx7*, *1Dy10*, and *1Dx5* were significantly induced during day 82 and 84 in all wheat cultivars (Fig. 2). Meanwhile Ct values for gene *1Ax1* and *1By9* could not be distinguished from the control gene at any time point during the experiment (not shown). Under greenhouse condition, transcript of *1Bx7, 1Dy10*, and *1Dx5* in wheat cv. Carnavalero and cv. Cojitambo showed significantly more accumulation that wheat cv. Sibambe (Fig. 3). Transcripts *1Bx7* and *1Dx5* showed more expression during day 84 in both cv. Carnavalero and cv. Cojitambo while transcript *1Dy10* was more abundant in the same cultivars at day 82 (Fig. 3).

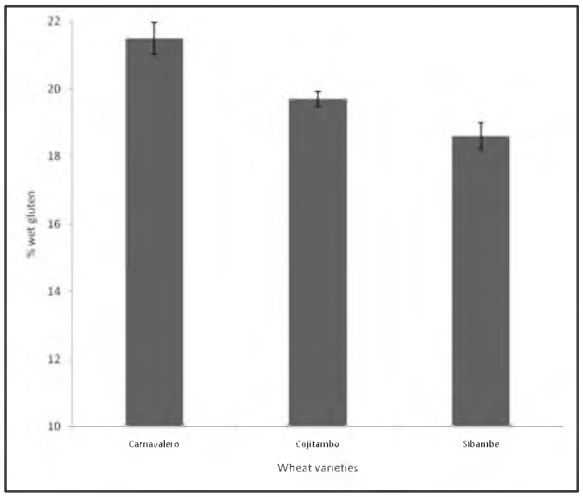

Figure 1. Wet gluten content of three local wheat varieties cultivated under short-day conditions in the highlands of Ecuador

Discussion

Our results suggest a correlation between wet gluten content among three wheat varieties and the transcripts level of HMW-GS genes under high-altitude growing conditions. In terms of grain yield, cv Cojitambo, cv. Carnavalero and cv. Sibambe have same average performance in the Andean highlands (Teran 2010). However, difference in gluten content among these varieties confirms the prevalence of cv. Carnavalero or cv Cojitambo as a choice for bread industry.

Temporal expression of most glutenin genes appears to be affected by environmental conditions (Altenbach et al. 2002). For that reason it was important to establish the expression profile of HMW-GS genes of wheat cultivars adapted to higher elevations. Our results are consistent with Altenbach et al. (2002) which report that transcript accumulation of HMW-GS occurred between 8 and 26 days post anthesis (DPA). Even though the expression pattern of our three cultivars are slightly different from temperate varieties, induction of genes occurred soon after flowering and was down regulated during grain filling.

Even though Johansson et al. (1993) showed that all HMW-GS can be expressed in a single wheat variety, not all might be critical for gluten formation since hexaploid wheat usually have 3 to 5 detectable glutenin subunits (Rooke et al. 1999; Radavanovic et al. 2002). Our data highlight the specific contribution of *1Bx7, 1Dy10,* and *1Dx5* during gluten formation of Ecuadorian wheat varieties. In concordance with our results, Yue et al. (2008) showed that RNAi-based silencing of *1Dx5* negatively affects expression of *1Bx7* and can cause substantial decrease in flour quality. If all HMW-GS genes appear to be regulated by a common signal (Altenbach et al. 2002; Grinwade et al. 1996), the reason why some genes remain silent might be explained by redundancy or genotype-specific factors.

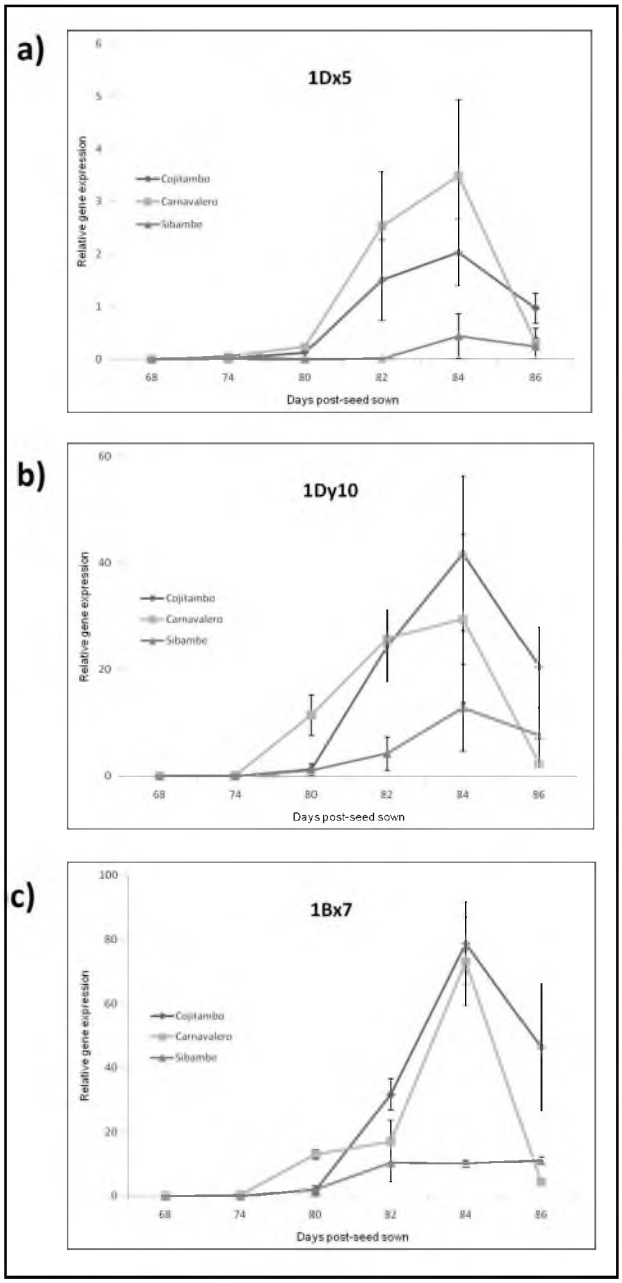

Figure 2. Relative expression of each HMW-GS gene a) *1Dx5*, b) *1Dy10*, and c) *1Bx7* calculated for the three wheat varieties cv. Cojitambo (rhombus), cv. Carnavalero (squares) and cv. Sibambe (triangles) between 68 and 86 days after sowing

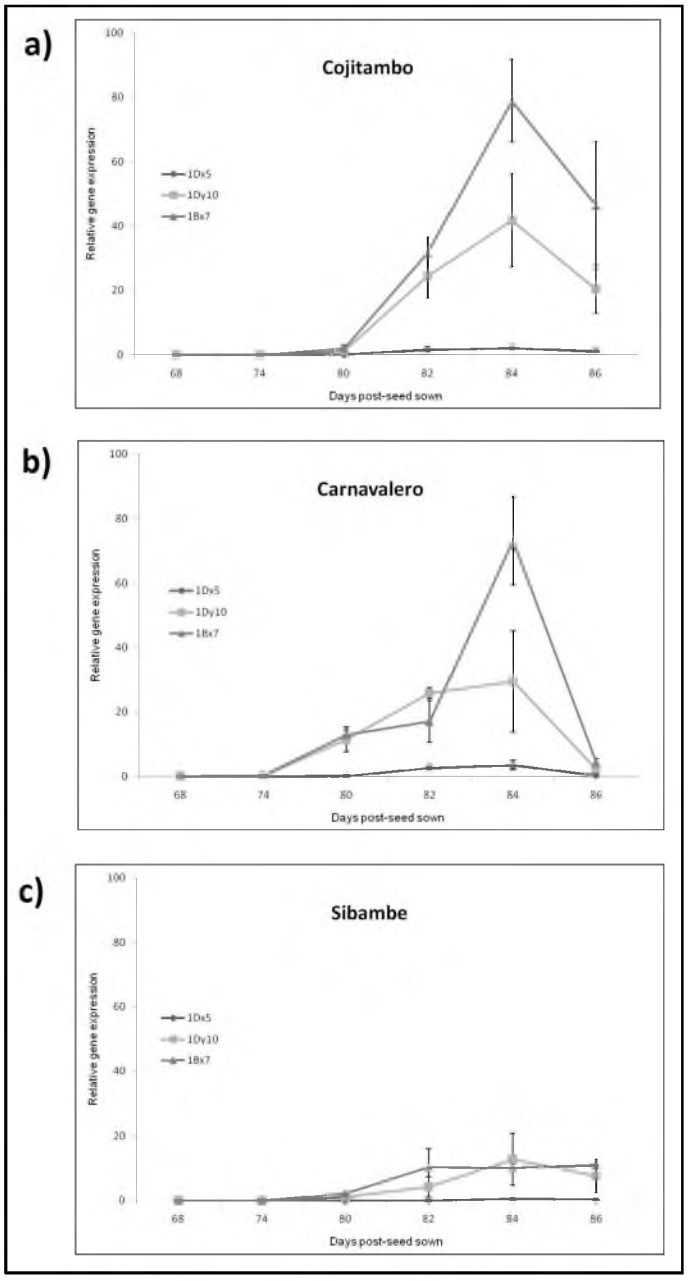

Figure 3. Relative expression of three HMW-GS genes in each of the local wheat varieties a) cv. Cojitambo, b) cv. Carnavalero, and c) cv. Sibambe under greenhouse conditions. Expression of genes *1Dx5* (rhombus), *1Dy10* (squares) and *1Bx7* (triangles) were recorded between 68 and 86 days after sowing

Our finding does not exclude that other factors may be influencing gluten content since allelic variation of specific HMW-GS or the amount and composition of LMG-GS were not addressed in this study. However, this is the first time that local wheat varieties are been assess for gluten content. Our study contributes to understand the expression pattern of wheat HMW-GS genes under high-altitude condition in the Andes.

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