AN ABSTRACT OF THE THESIS OF

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Title: Influence of Heat Stress on Grain Yield, Grain Quality, and Protein Composition of Spring Wheat.

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C. James Peterson

Wheat (*Triticum aestivum* L.) plants exposed to higher than usual temperatures during ripening show altered agronomic and grain quality characteristics. Given that seasonal variation in quality creates difficulties in the marketing and processing of grain, improving the genetic adaptation of wheat cultivars to heat stress is an important objective in breeding programs. Some genotypes have been reported to have a thermo tolerant response and could be used as genetic sources for heat tolerance. Six spring wheat cultivars and four elite experimental lines were evaluated in Uruguay. Two field experiments were conducted in years 2001 and 2002 to determine response under natural heat stress conditions, and two greenhouse experiments were conducted to vary duration and timing of heat stress. Grain protein concentration increased with moderate (field conditions) and high heat stress (controlled environment). Heat stress imposed early in grain fill had the greater effect. In field conditions, moderate to high heat stress at mid-grain fill increased test weight and thousand kernel weight. Higher heat stress under controlled environment caused a decrease in thousand kernel weight, without any

difference in relation to duration or timing of stress. Rheological properties were affected by heat stress in field conditions. While moderate heat stress throughout grain fill caused stronger dough, moderate to high heat stress at mid-grain fill produced weaker dough. These results suggest a curvilinear response to increasing heat stress for both thousand kernel weight and rheological properties. Impact of heat stress under field conditions was inconsistent on protein molecular weight distribution. Moderate to high heat stress at mid-grain fill lowered level of monomeric proteins, and increased the ratio soluble polymeric proteins/monomeric proteins. Moderate heat stress throughout grain fill decreased percentage of soluble polymeric proteins, and increased percentage of low molecular weight albumins and globulins. No effect of heat stress was detected on protein molecular weight distribution in controlled environment. However, with longer duration of stress, significant genotype x treatment interaction was detected. Cultivars with relatively stable agronomic and quality characteristics were identified and could be used as genetic sources for improving resistance to heat stress.

Influence of Heat Stress on Grain Yield, Grain Quality, and Protein Composition of Spring Wheat.

by
Marina Castro Derényi

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Marina Castro Derényi, Author

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IN DEDICATION

To

my husband Carlos A.

our children

Manuel, Joaquín and Francisco

my mother Margarita

my grandmother Klára M.

and

to the memory of my father Oscar E.

INFLUENCE OF HEAT STRESS ON GRAIN YIELD, GRAIN QUALITY AND PROTEIN COMPOSITION OF SPRING WHEAT

GENERAL INTRODUCTION

Wheat (*Triticum aestivum* L.) is grown worldwide as a major contributor to human nutrition. Wheat grain production in Uruguay, South America, is 0.3 to 0.5 million tons per year. Approximately 0.4 million tons are used for local human consumption. More than 70% of this amount is used for French-type bread, 25% for pan bread, and 5% is used for other baking products (Anonymous 2001). Only sub-products from the milling industry, like bran, are used for animal feed. This agro industrial chain represents 100-million US dollars/year. In the last years, Uruguay has increased the import of wheat grain, flour and final products from the countries of the MERCOSUR (Southern Common Market) from 30 to 80 US million dollars (Arbeletche and Gutiérrez 2003), and has decreased the exports of same products from 75 to 10 US million dollars (Souto 2001, Arbeletche and Gutiérrez 2003). In this context, Uruguayan wheat needs to be competitive with those of high industrial quality from other countries, like Argentina, to be able to satisfy the demands of both processors and consumers for flour and bread products with superior and consistent quality. The National Institute for Agriculture Research (INIA) from Uruguay has been working on this problem for many years. Wheat germplasm with improved quality characteristics, together with the necessary adjustments in technological aspects of the industry, will allow farmers, millers and bakers to have a secure place for their production in the local market, but also have more opportunities to export grain.

Wheat is best adapted to cool growing conditions. Grain yields obtained in cooler temperate regions of Europe and South America, like Chile, are generally higher than in warmer regions like Australia and India, where temperatures of 35 to 40°C are likely to occur during the grain filling period. Low temperatures during kernel maturation contribute to high individual kernel weight, as well as a higher grain fertility, which are important components of yield. Chowdhury and Wardlaw (1978) reported that the optimal temperature regimen for maximizing kernel dry weight was between 15/10°C (day/night) and 18/13°C.

Wheat grain is composed mainly of starch and protein, and to a lesser extent, by other polysaccharides, minerals, lipids, and vitamins. An early classification made by Osborne in 1907 used sequential extraction with different solutions to separate protein fractions. The Osborne procedure, with minor modifications, is considered to be a useful but flawed basis for the separation of wheat proteins into their main groups. Four main groups of proteins have been defined according to their solubilization pattern: albumins (water-soluble), globulins (soluble in salt solution), gliadins (70% aqueous ethanol soluble), and glutenins (insoluble in either salt or 70% ethanol; soluble in alkaline solution). The last two types are gluten-forming proteins, which confer unique quality attributes to wheat flour. Albumins and globulins are not part of the gluten complex, and are solubilized and washed away into the water-saline soluble fraction upon gluten isolation. They consist primarily of numerous metabolic and hydrolytic enzymes synthesized during seed development (Wrigley and Bietz 1988). Solubility fractionation is not entirely satisfactory, however, because of the overlap between the different fractions (MacRitchie 1992).

Wheat is unique among cereal grains because of the viscoelastic properties of the gluten proteins in the endosperm which contribute to produce leavened bread and a diversity of other foods. The balance among gluten forming proteins is basic to determine wheat flour functional properties. Two types of proteins compose the gluten fraction: polymeric glutenins and monomeric gliadins. Glutenin proteins are divided into high molecular weight glutenin subunits (HMW-GS) or HMW prolamins, and low molecular weight glutenin subunits (LMW-GS), or sulfur-rich prolamins. HMW-GS account for 20% of total glutenins, while LMW-GS represent 80% of total glutenins (Bietz and Wall 1973, Peña 2002). These proteins are heterogeneous mixtures of polymers formed by disulfide-bonded linkages of polypeptides, and they can reach molecular weights of 5,000,000 Daltons (Da). According to their electrophoretic mobility in SDS-PAGE after reduction of the disulfide-bonds, the glutenin subunits can be classified in four groups: A-group (80,000 to 120,000 Da) corresponds to the HMW-GS; B-group (42,000 to 51,000 Da) and C-group (30,000 to 40,000 Da) are LMW-GS, distantly related to γ- and α-gliadins; and D-group, which is highly acidic and related to ω gliadins, also belongs to the LMW-GS (Gianibelli et al. 2001a).

Wheat is a hexaploid species containing three related genomes ($2n = 6x = 42$, AABBDD). Glutenin subunits are encoded by genes on the chromosomes from homeologous group 1 in hexaploid wheats. The loci where the HMW-GS genes are located are designed *Glu-A1*, *Glu-B1* and *Glu-D1*, and have been mapped to the long arms of chromosomes 1A, 1B, and 1D, respectively (MacRitchie 1992). Each locus includes two linked genes encoding two different types of HMW-GS: x-type (higher molecular weight) and y-type (lower molecular weight) subunits. The subunits notation is

done by identifying the genome from which the subunit is derived, the indication of whether it is x-type or y-type, and the assigned number. *Glu-1* loci present multiple alleles. Twenty four subunits have been described (Payne et al. 1981a, Ng and Bushuk 1989), including three encoded at *Glu-A1*, fourteen at *Glu-B1,* and seven at *Glu-D1* loci. Many more alleles have been described since then, and are reported in Graingenes (http://wheat.pw.usda.gov/GG2). In theory, each hexaploid wheat genotype could contain six different subunits, but due to gene silencing, only three, four or five subunits are present (Shewry et al. 1992).

HMW-GS are minor components in terms of quantity of the gluten complex, but they are key factors in the process of bread making. They determine gluten elasticity by promoting the formation of larger glutenin polymers. Certain allelic subunits impart different effects on gluten quality characteristics (Payne et al. 1981b). Examples of allelic variation at the *A1* locus are subunits 1 and 2* (contributing good quality) and the null allele (giving poorer quality). The same occurs at the *B1* locus, where pairs of subunits 17 + 18 are contrasted for higher resistance to extension with 13 + 16 or 7 + 8; and at *D1* locus, where pairs of subunits 5 + 10 stand for higher dough strength than do subunits 2 + 12 (MacRitchie 1992).

Genes on the short arms of chromosomes 1A, 1B, and 1D encode LMW-GS subunits. These loci are designed *Glu-A3*, *Glu-B3*, and *Glu-D3*. Recently, two new LMW-GS, with molecular weights of 30,000 to 31,000 Da (*Glu-D4* locus, located on chromosome 1D) and 32,000 Da (*Glu-D5* locus, located on chromosome 7D) were reported (Sreeramulu and Singh 1997). However, their exact locatization within the chromosome has not been established. Gupta and Shepherd (1990), studying 222

hexaploid wheats from 32 countries, have detected 20 different band patterns (LMW-GS blocks). Six correspond to the *Glu-A3* locus, nine to the *Glu-B3* locus, and five to the *Glu-D3* locus. A preliminary approach to rank LMW-GS alleles in order of quality has been reported by Gupta et al. (1991). Variation in gluten strength (Gianibelli et al. 2001a), and extensibility (Peña 2002) due to allelic polymorphism at LMW-GS loci has been reported.

Gliadins are heterogeneous mixtures of single-chained polypeptides. According to their mobility in acid-PAGE, they are divided into four groups: α-, β-, γ-, and ω-gliadins (from the fastest to the slowest mobility). However, genetic and chemical studies involving amino acid analysis and N-terminal sequences showed that gliadins can be arranged into three major groups of α/β-, γ-, and ω-gliadins. Their molecular weight range is 30,000 to 75,000 Da. It has been estimated that each wheat genotype can produce up to 50 different gliadins (Gianibelli et al. 2001b).

Genes coding for gliadin proteins are tightly linked and located at three homologous loci on the short arms of group 1 chromosomes (*Gli-A1*, *Gli-B1*, and *Gli-D1*) and group 6 chromosomes (*Gli-A2*, *Gli-B2*, and *Gli-D2*) (MacRitchie 1992). They also show multiple allelism (Wrigley and Shepherd 1973). *Gli-1* genes code for all the ω- and most of the γ-gliadins, and *Gli-2* genes code for all the α-, most of the β-, and some of the γ-gliadins. Metakovski (1991), based on the analysis of 360 wheat cultivars and 45 crosses, reported 111 gliadin alleles mapping to the 6 gliadin loci. Some reports show a relationship between certain gliadin alleles and gluten strength, but there could be a confounding effect due to linkages with the LMW-GS (Payne et al. 1984).

During grain filling, three growing phases can be defined: from anthesis to 15-16 days after anthesis (DAA) is the cell division phase; from 16 to 37 DAA, the cell enlargement and dry matter accumulation phase; and from 38 DAA to maturity, the grain dehydration or maturation phase (Wang and Gifford 1995). Timing and rate of deposition of gluten proteins in the wheat grain varies during the grain filling period. The gliadins start being synthesized earlier than the glutenins, while the highest synthesis rate for both protein classes takes place early in the grain filling period (Panozzo 1997). This researcher, working with Australian wheats, found that HMW-GS deposit before the low molecular weight glutenin subunits (LMW-GS) and that polymerization of glutenin aggregates occurs late in grain filling to form large macro-molecules. Carceller and Aussenac (2001), working with wheat cultivar Soissons, found that total protein accumulation occurred from anthesis to 38 DAA, then remained constant until maturity. Concentrations of monomeric proteins (gliadins, albumins and globulins) increased gradually from 10 DAA, reaching a maximum at 30 DAA. Polymeric proteins (glutenins) started to accumulate at 10 DAA and increased gradually until 37-39 DAA. The maximum accumulation rate for total proteins was reached at 20-21 DAA, and for monomeric proteins, at 22 DAA, both during cell enlargement phase. On the other hand, polymeric proteins showed the maximum accumulation rate about 35-37 DAA, toward the end of the cell enlargement phase. Within the polymeric proteins, the SDS-insoluble polymers (bigger than the soluble polymers) formation occurred during the late cell enlargement phase and was maximum when there was a rapid water loss of the grain. The polymer distribution is highly correlated with the percentage of the high molecular weight glutenins (HMW-GS) within these proteins (Carceller and Aussenac 1999).

Wheat bread quality is related to many quantitative gluten proteins-related parameters. They include: flour total protein content, glutenin solubility, glutenin content of the gluten, flour glutenin content, flour polymeric glutenin, polymer molecular weight distributions, glutenin/gliadin ratio, and insoluble glutenin/soluble glutenin ratio (Gupta et al. 1995, Dupuis et al. 1996, Weegels et al. 1996, Southan and MacRitchie 1999, Uthayakumaran et al. 1999, Lafiandra et al. 2000, Gianibelli et al. 2001a). To assess these parameters, high performance liquid chromatography, either by size exclusion (SE-HPLC) or reverse phase (RP-HPLC), and protein fractionation by solubility, have been used (Fu and Sapirstein 1996, Ciaffi et al. 1996b, Dupuis et al. 1996, Bangur et al. 1997, Sapirstein and Fu 1998, Morel et al. 2000).

There is no consensus among researchers about the relationship of gluten strength with different biochemical measures of protein properties. For example, Southan and MacRitchie (1999) suggested that the quantitative parameter that most influences gluten strength is the polymer molecular weight distribution. In this sense, Zhu and Khan (2001), working with six hard red spring wheat genotypes in North Dakota, found that SDS-insoluble glutenin polymers were a better determinant of dough mixing properties than SDS-soluble glutenin polymers. Weegels et al. (1996) suggested that glutenin/gliadin ratio is more related to gluten strength. Lafiandra et al. (2000) proposed to take into account several parameters at the same time: monomer/polymer ratio, polymer size, and high molecular weight glutenin subunits/low molecular weight glutenin subunits (HMW-GS/LMW-GS) ratio. Many of these differences among authors could result from use of different sample sets, wheat populations, or the methods used for assessing wheat quality parameters.

The increasing world population has lead to the necessity of expanding the agriculture frontiers to produce more food. The interest in wheat response to supra-optimal temperature has increased as wheat is being planted outside the optimal temperature (Shpiler and Blum 1986). There are 36 million ha (40% of temperate environments) of wheat around the world that suffer terminal heat stress in the growing season (Reynolds et al 2001). Also the importance of high temperature stress is likely to increase in future years if forecasts of general global warming continues (Adams et al. 1990). Uruguay is situated between 30° – 35° South latitude; thus the grain filling stage of wheat crops can be subjected to fairly high temperatures.

It is difficult to define heat stress in plants because response depends on thermal adaptation, duration of exposure, and stage of growth. Two major structures directly affected by high temperatures are proteins and membranes (Gusta and Chen 1987). Enzyme inactivation at high temperature is a major cause of growth reduction, while membrane disruption may alter water, ion and organic solute movement, photosynthesis, and respiration. Shpiler and Blum (1986) found that the duration of all developmental stages in wheat was reduced by high temperature, generally producing a decrease in yield. Tolerant varieties, those which maintain the highest yield in hot environments, maintain the longest duration of the growth stage between double ridge and anthesis, and the highest number of grain per spike. Several winter and spring wheat genotypes have shown a steady reduction in the grain filling period as temperature increased over the optimal (Wiegand and Cuellar 1981). Kernel dry weight is also affected by heat stress causing grain yield decrease. Higher temperatures than the optimal for grain growth produce a decline in kernel dry weight (Chowdhury and Wardlaw 1978, Wiegand and

Cuellar 1981, Wardlaw et al. 1989, Calderini et al 1999, Wardlaw et al. 2002). Research done with a heat sensitive wheat genotype (cv. Oxley), showed that a brief heat shock (40/16°C day/night) for five days starting 15 days after anthesis reduced mature individual kernel mass by 17% (Stone et al. 1995).

One strategy that could be used is simply to avoid heat stress. It would be desirable that genotypes grown in environments where heat stress often occurs show high grain filling rates with short to medium grain filling duration. Bruckner and Frohberg (1987), working with 20 spring wheat genotypes in the Northern Great Plains, US, found highly significant correlation coefficient (0.83, $P<0.01$) for grain filling rate and kernel weight. But, only one (ND584 sib) out of the 20 genotypes tested had both characteristics. This genotype could be exploited in breeding programs as source of stress tolerance resulting from high grain filling rate and short to medium grain filling duration.

Many factors can affect consistency of dough properties in a given wheat genotype. Heat stress, even for a few days during grain filling, has been found to be one of them. Protein quality, as measured by SDS sedimentation volumes and size-exclusion high-performance liquid chromatography, is highly influenced by the frequency of high temperatures during grain filling and by the relative humidity (Graybosch et al. 1995). Also, heat stress correlates negatively with loaf volume in many wheat cultivars (Finney and Fryer 1958, Blumenthal et al. 1991b). The timing and duration of heat stress during this developmental stage is an important source of variation in dough properties (Blumenthal et al. 1993; Stone and Nicolas 1995a, 1996, Corbellini et al 1997, Wardlaw et al. 2002). Experiments in controlled environments and in the field have shown that

dough strength and grain protein content of wheat increases with increasing temperatures up to about 30°C (Randall and Moss 1990, Stone et al. 1997, Corbellini et al. 1998).

Correll et al (1994), studying wheat data from 109 silos in South Australia from the years 1971-1991, found that the number of days in October above 30°C were positively correlated with grain-protein content. However, Bhullar and Jenner (1985), working with four wheat cultivars exposed to short episodes of heat stress, demonstrated that increase in percentage grain nitrogen was due to a reduction in starch content of the grain rather than to a change in the quantity of nitrogen. Lower activity of the enzyme soluble starch synthase, possibly due to thermal denaturation, may be responsible for the reduced starch deposition in the range of temperatures above 30°C (Jenner 1994).

Very high temperatures (e.g. short episodes of a few days at > 35°C) produces grain with weaker-than-expected dough properties (Randall and Moss 1990, Blumenthal et al. 1991a, 1993; Wrigley et al. 1994; Borghi et al 1995, Stone et al. 1997; Corbellini et al. 1998). Guedira et al. (2002) found this same effect even with temperatures of 30°C imposed to wheat cultivar Len from 10 DAA until ripening. There is evidence from field and greenhouse experiments that the resulting weaker dough could be due to a higher ratio gliadin:glutenin, with the hypothesis that gliadin synthesis continues at a greater rate than glutenin synthesis as part as the heat shock response (Blumenthal et al. 1991a, 1993). According to Blumenthal et al. (1990a, 1991a, 1993, 1994, 1995a), and Stone et al. (1997), there is an altered gliadin:glutenin ratio after heat stress, either because of a higher rate of monomeric protein synthesis, or by the reduction in the synthesis of glutenins. The effects of temperature on gliadin synthesis rate was found to be greater on the α-, β-gliadins than on the γ-, and ω-gliadins (Daniel and Triboï 2001). Stone et al.

(1997) reported that moderately high temperatures during grain filling of heat sensitive wheat cultivar Oxley decreased the ratio polymer:monomer due to a lesser accumulation of the polymer than that of the monomer. Variations in the assembly of protein polymers could be responsible for the reported poor dough properties after heat stress. These results agree with those found by Blumenthal et al. (1994), Corbellini et al. (1998) and Wardlaw et al. (2002). Reduction in dough strength and mixing tolerance after heat stress found by Guedira et al. (2002) was associated with changes in the unextractable polymeric protein. Heat stress during grain filling may affect the synthesis and accumulation of gluten proteins, and was found to be responsible for modifications in the polymeric fraction composition (soluble/insoluble polymers protein ratio) (Ciaffi et al 1996a).

Adaptation to high temperatures has been well characterized in wild plant species, but little information is available concerning the adaptation of crop plants. Certain crop plants can acquire heat tolerance by exposure to a gradual increase in temperature (Gusta and Chen 1987). Acquired thermo-tolerance may be defined as the ability of an organism to survive exposure to a normally lethal temperature, due to prior exposure to a sub-lethal temperature during which heat-shock-proteins (hsps) are induced. Blumenthal et al. (1990a) reported that the range of hsps of wheat is similar to those generally recognized: the low-molecular weight group (15-30 kDa) and those of high molecular weight (64-90 kDa). Their presence has been correlated with the acquisition of thermo-tolerance by the organism (Blumenthal et al. 1990b; Treglia et al. 1999). Blumenthal et al. (1998) found that the concentration of a specific hsps (HSP 70) remaining in mature grain increased as a result of a few days heat stress of wheat plants. However, the amount of HSP 70 in

mature grain samples from heat-stressed plants of 45 genotypes was not strongly correlated with loss of dough strength.

Given that seasonal variation in quality creates difficulties in the marketing and processing of grain (Peterson et al. 1992, 1998), stability of quality to heat stress is an important goal. There are reports (Blumenthal et al. 1994, 1995a; Wrigley et al. 1994; Stone and Nicolas 1995a, 1998a, 1998b) that indicate that there is some variation in wheat in the response to heat stress, with a few genotypes showing either a very small change or a decrease in the gliadin:glutenin ratio. Such lines indicate a likely source of parental lines for further examination as genetic sources for heat tolerance. Krishnan et al. (1989) reported that two-dimensional electrophoretic analysis revealed unique proteins (16, 17, and 26 kDa) in the thermo-tolerant wheat variety Mustang that were absent in the more thermal sensitive variety Sturdy. Vierling and Nguyen (1992), using diploid wheat genotypes, reported similar results. These studies provide a correlation between the synthesis of specific low molecular weight hsps and the degree of thermo-tolerance expressed following exposure to elevated temperatures. Also, they are a molecular basis for further genetic analysis of the role of hsps genes in thermal tolerance in wheat (Weng and Nguyen 1992).

CHAPTER 1

Influence of Heat Stress During Grain Fill on Agronomic, Grain Quality Characteristics and Protein Composition, in Controlled Environment

Introduction

Wheat (*Triticum aestivum* L.) is grown worldwide as a major source of protein and carbohydrates in the human diet. It is best adapted to cool growing conditions, obtaining higher grain yields in temperate regions of Europe and South America, as compared with regions such as Australia and India, where temperatures of 35 to 40°C are likely to occur during the grain filling period. Kernel characteristics and biochemical composition are affected by high growing temperatures.

Wheat is unique among cereal grains because of the viscoelastic properties of gluten proteins which are important for producing leavened bread and other foods. Gluten is comprised of two types of proteins: polymeric glutenins and monomeric gliadins. Albumin and globulin proteins are also present in the wheat grain, but are not considered as part of the gluten complex. These consist primarily of metabolic and hydrolytic enzymes synthesized during seed development and used as a source of nutrients for the future embryo growth (Wrigley and Bietz 1988). Glutenins, especially high molecular weight glutenins (HMW-GS), are key factors in the process of bread making. They determine gluten elasticity by forming large polymers (MacRitchie 1992). Gliadins contribute to dough extensibility and decrease dough mixing strength (Daniel and Triboi, 2001). The balance among gluten proteins is fundamental to determine wheat flour functional properties.

During grain filling, three growing phases can be defined: from anthesis to 15-16 days after anthesis (DAA) is the cell division phase; from 16 to 37 DAA, the cell enlargement and dry matter accumulation phase; and from 38 DAA to maturity, the grain dehydration or maturation phase (Wang and Gifford 1995). Timing and rate of deposition of gluten proteins in the wheat grain varies during the grain filling period. The gliadins start being synthesized earlier than the glutenins, while the highest synthesis rate for both protein classes takes place early in the grain filling period (Panozzo 1997). This researcher, working with Australian wheats, found that HMW-GS deposit before the low molecular weight glutenin subunits (LMW-GS), and that the polymerization of glutenin aggregates occurs late in grain filling, to form large macro-molecules. Carceller and Aussenac (2001), working with wheat cultivar Soissons, found that total protein accumulation occurred from anthesis to 38 DAA, then remained constant until maturity. Concentrations of monomeric proteins (gliadins, albumins and globulins) increased gradually from 10 DAA, reaching a maximum at 30 DAA. Polymeric proteins (glutenins) started to accumulate at 10 DAA and increased gradually until 37-39 DAA. The maximum accumulation rate for total proteins was reached at 20-21 DAA, and for monomeric proteins, at 22 DAA, both during cell enlargement phase. On the other hand, polymeric proteins showed the maximum accumulation rate about 35-37 DAA, toward the end of the cell enlargement phase.

Shpiler and Blum (1986) found that high temperature reduced the duration of all developmental stages in wheat, and generally resulted in decreased yield. There is a steady reduction in the grain filling period of winter and spring wheat genotypes as temperatures are increased over the optimal (Wiegand and Cuellar 1981). Kernel weight

also is affected by high temperatures, which further contributes to a decrease in grain yield (Chowdhury and Wardlaw 1978, Wiegand and Cuellar 1981, Wardlaw et al. 1989, Calderini et al 1999, Wardlaw et al. 2002).

Many factors can affect consistency of dough properties in a given wheat genotype. Heat stress, even for a few days during grain filling, can have a major impact on protein composition. Graybosch et al. (1995) showed that protein quality, as measured by SDS sedimentation volumes and size-exclusion high-performance liquid chromatography, is highly influenced by high temperatures and relative humidity during grain filling. Timing and duration of heat stress during grain filling are shown to be important sources of variation in dough properties (Blumenthal et al. 1993; Stone and Nicolas 1995a, 1996, Corbellini et al 1997, Wardlaw et al. 2002). Experiments in controlled environments and in the field have shown that dough strength and grain protein content of wheat increase as temperatures increase to about 30°C (Randall and Moss 1990, Stone et al. 1997, Corbellini et al. 1998). Very high temperature (e.g. short episodes of a few days at > 35°C) produces grain with weaker-than-expected dough properties (Randall and Moss 1990, Blumenthal et al. 1991, 1993; Wrigley et al. 1994; Borghi et al 1995, Stone et al. 1997; Corbellini et al. 1998). Guedira et al. (2002) found weaker-than-expected dough properties when temperatures of 30°C were imposed to wheat cultivar Len from 10 DAA until ripening.

Heat stress during grain filling may affect the synthesis, accumulation and/or assembly of gluten proteins. There is evidence from field and greenhouse experiments that the weaker dough could be due to a higher ratio of gliadin:glutenin. The hypothesis is that gliadin synthesis continues at a greater rate than glutenin synthesis at high

temperatures as part of the heat shock response (Blumenthal et al. 1991, 1993). According to Blumenthal et al. (1990, 1991, 1993, 1994, 1995a), and Stone et al. (1997), there is an altered gliadin:glutenin ratio after heat stress, either because of a higher rate of monomeric protein synthesis, or by the reduction in the synthesis of glutenins. Stone et al. (1997) reported that moderately high temperatures during grain filling decreased the ratio of polymer:monomer in the heat sensitive wheat cultivar Oxley. Heat stress was found to be responsible for modifications in the ratio of soluble/insoluble polymeric proteins, that contributes to dough properties (Ciaffi et al 1996).

Given that seasonal variation in quality creates difficulties in the marketing and processing of grain (Peterson et al. 1992, 1998), stability of quality is a very important goal. There are reports (Blumenthal et al. 1994, 1995a; Wrigley et al. 1994; Stone and Nicolas 1995a, 1998a, 1998b) that genetic variation in response to heat stress among varieties has been observed. A few genotypes show either a small change or a decrease in the gliadin:glutenin ratio. Such lines would be expected to have some consistent dough properties and may be a genetic source for further improving heat tolerance.

The purpose of this research was to assess changes in grain quality and gluten composition induced by timing and duration of heat stress during the grain filling period of selected wheat genotypes. The results will contribute to better understand the impact of stress on processing quality and lead to identification of heat tolerant wheat genotypes with more stable and consistent industrial quality.

Materials and Methods

Plant material

Six spring wheat (*Triticum aestivum* L.) cultivars and four elite experimental lines from National Institute for Agriculture Research (INIA) La Estanzuela Wheat Breeding Program, Uruguay, were selected to carry out this study. The genotypes represent a broad range of genetic diversity in grain yield and bread-making quality. Four cultivars from other programs were included (cultivars Trigo 3, Debeira, Ventnor and Pavón 76) based on their previous agronomic or quality characteristic performance under heat-stress conditions. Pedigree and high molecular weight glutenin subunits (HMW-GS) of the studied wheat genotypes are listed in Table 1.1.

Plant growing conditions

Three seeds of each wheat genotype were sown in 1.5 L opaque plastic containers, filled with a mixture of equal parts of soil, sand and substrate (Plantamax, Eucatex, Brazil), which contained expanded vermiculite and organic matter. Plants were well watered, and fertilized weekly with NPK (foliar fertilizer ISUSA NPK + micronutrients, 12-8-5) applied as soil drench. Plants were grown in a greenhouse, with a daytime maximum temperature of 24°C and a nighttime minimum temperature of 16°C. Natural light was supplemented for 6 to 8 hours daily (high pressure sodium SON lamps, 400 W, Philips, Belgium) during the fall and winter months to maintain 16-hr photoperiod.

Table 1.1. Wheat genotypes studied, their progenitors and HMW-GS.

Genotypes	Pedigree	HMW-GS		
		Glu-A1	*Glu-B1*	*Glu-D1*
Debeira	HD2160/5/TOBARI/CNO 67// BB/3/NAINARI 60 *2// TT/SONORA 64/4/HD1954	2*	7+8	2+12
Pavón 76	VCM//CNO/7C/3/KAL/BB	2*	17+18	5+10
Trigo 3	Unknown	2*	7+8	2+12
Ventnor	Unknown		7+8	5+10
Estanzuela Cardenal	KVZ/BUHO'S'//KAL/BB	1	7+9	5+10
Estanzuela Pelón 90	KVZ/TRM	1	13+16	5+10
INIA Mirlo	CAR853/COC//VEE5/3/URES	1	7+9	5+10
INIA Boyero	MN72131/BOBWHITE	2*	7+9	5+10
INIA Caburé	EFED//BUCK6/MR74507		7+8	5+10
INIA Churrinche	EFED/ECOL	2*	7+8	5+10
LE 2262	IA558/4/KAL/BB//CJ71/3/ALD/5/BOW	2*	17+18	5+10
LE 2265	PGO//CHEN/AE. SQUARROSA (224) /3/WEAVER	1	7+8	2+12
LE 2290	LE 2169/BUN'S'	1	7+8	2+12
LE 2294	IBOY//CLEO/INIA66	2*	7+9	5+10

The cultivar Ventnor required some vernalization. At mid-tillering, plants of this cultivar were placed in controlled environment chambers (model PGW36, Conviron, Winnipeg, Manitoba, Canada) for 20 days, at 8°C and 10-hr photoperiod. After this period, plants were returned to the greenhouse.

Heads were tagged to record the date of anthesis. At different days after anthesis (DAA), containers of each wheat genotype were assigned to high temperature treatments in controlled environment chambers. Other containers remained in the greenhouse in normal growing conditions as control treatments. Treatments are described in Table 2. Each treatment consisted of one container with three heads. Air temperature in the chambers and greenhouse was monitored continuously with a Minicube Hygrothermograph (model 08369-70, Oakton, Chicago, IL, USA).

Two replicated experiments were conducted (Table 1.2). In Experiment 1, planted in year 2001, containers of each genotype were exposed to high temperatures for either one or two weeks starting at 25 DAA. Its purpose was to examine impact of the duration of heat stress. In Experiment 2, planted in year 2002, plants were exposed to the heat treatment for one week, either at 15 DAA or 25 DAA. This was intended to examine the influence of heat stress at different stages of kernel development. After the exposure of plants to the high temperature regimen ended, they were placed again in the greenhouse with the control plants until maturity.

Table 1.2. Growth regimes for plants of different wheat genotypes during grain development.

Experiment	Treatment	Regimen	Temperature (°C)[a]
1	Control	Moderate daytime temperature	24/16
	25 DAA (1w)	One week high daytime temperature starting 25 DAA	35/25
	25 DAA (2w)	Two weeks high daytime temperature starting 25 DAA	35/25
2	Control	Moderate daytime temperature	24/16
	15 DAA (1w)	One week high daytime temperature starting 15 DAA	35/25
	25 DAA (1w)	One week high daytime temperature starting 25 DAA	35/25

[a] Day/night maximum; 24/16 regimen maintained for 6 hr (day) and 12 hr (night), separated by intermediate 3-hr periods of gradual increase or decrease of temperature in the range of 16 to 24°C. 35/25 regimen maintained for 5 (day) and 13 hr (night), separated by intermediate 3-hr periods of gradual increase or decrease of temperature (range of 25 to 35°C) and intensity of artificial light.

Kernel physical characteristics

Date of physiological maturity was recorded when the peduncle of the spike started to change color from green to light yellow. Grain filling period (GF) was calculated as number of days between anthesis and physiological maturity. Spikes were hand-harvested when the grain was ripe and threshed individually. Grain from spikes in each container that had a similar anthesis date was bulked, and grain protein concentration was measured on the resulting grain. Grain for each spike was counted and weighed to obtain thousand kernel weight (TKW). The results were expressed in grams.

Grain protein concentration

Grain protein concentration (GP) was measured in the bulked whole grain samples by near infrared spectrophotometer (NIRSystem 6500, Foss Inc., Silver Spring, MD, USA), which was previously calibrated by Kjeldahl (method AACC 46-11, with modifications). Grain protein concentration was reported on a 13.5 % moisture basis.

Ground whole wheat protein molecular weight distribution

A subset of six genotypes (Debeira, Estanzuela Pelón 90, INIA Churrinche, LE 2294, Pavón 76, and Trigo 3) were used to analyze ground whole wheat protein composition. They were chosen based on previous information regarding stability of their quality attributes over different growing environments. Bulked grain was milled into ground whole wheat using an UDY-Cyclone Sample Mill (model MS, UD Corporation,

Boulder, Colorado, USA) equipped with a 1 mm opening size screen. A 150 μm nitrex sieve was used with the ground samples to obtain uniform particle size.

Ground whole wheat protein molecular weight distribution (MWD) was measured by size-exclusion high-performance liquid chromatography (SE-HPLC) (Dalla Rizza et al. 2005). Protein extraction was carried out as described by Larroque et al. (2000), with modifications. Proteins from flour (100 mg) were extracted with 10 ml of 0.5% SDS-0.05*M* sodium phosphate buffer, pH 6.9, and sonicated at 20 W for 15 sec, using an Ohtake Works sonicator (Tokyo, Japan) with a 9-mm diameter probe. The supernatant obtained by centrifugation of the samples at 16,000 x g for 10 min was filtered through a 0,45 μm polyvinylidene diflouride filter membranes (Durapore, Millipore Corp., Ireland) and heated for 2 min at 80°C in a water bath.

The SE-HPLC was conducted using an HPLC Äkta Purifier System (Amersham Pharmacia Biotech, Uppsala, Sweden). A Superdex 200 HR 10/30 size exclusion analytical column (10 x 300 nm) that has dextran covalent bonding to highly cross-linked porous agarose beads was used. Protein extract (20 μL) was injected into the column. The same extraction buffer was used for sample elution, which was done at a flow rate of 0.5 mL/min at room temperature. Absorbance was measured at 214 nm. All HPLC measurements were run in duplicate and means values were used. The elution profiles were integrated by the HPLC software.

Protein fractions were calculated as described by MacRitchie and Gupta (1993). The percentages in protein of soluble polymeric protein (SPP) (mostly glutenin), monomeric protein (MP) (gliadin), and low molecular weight albumins and globulins (LMWAG) were determined from the total protein profile:

$$\% \text{ SPP} = \text{(area peak 1/total area profile)} \times 100 \quad (1)$$

$$\% \text{ MP} = \text{(area peak 2/total area profile)} \times 100 \quad (2)$$

$$\% \text{ LMWAG} = \text{(area peak 3/total area profile)} \times 100 \quad (3)$$

Experimental design and Statistical analysis

Treatments were arranged in Split-plot designs with three replications. Only two replications were used for ground whole wheat protein MWD (subset of six genotypes). The main plots were the temperature treatments, and the sub-plots, the wheat genotypes. Statistical analyses were performed using SAS computer software (SAS Institute, Cary, NC). Analysis of variance for all traits and orthogonal contrasts for main treatments were performed with the General Linear Model (GLM) procedure. The contrast comparisons were made to find out statistical differences between Control treatment versus the average of the high temperature treatments, and between the heat treatments themselves. Mixed Model procedure was used for calculating orthogonal contrasts for the interaction treatment x genotypes.

Results

Experiment 1: Duration of heat stress

Response to duration of heat stress

Heat stress treatments applied at a fixed grain growth stage [25DAA (1w), and 25 DAA (2w) (Table 1.2)] affected agronomic, grain quality, and grain protein composition (Table 1.3). Significant differences between the control and heat stress treatments were observed for grain filling period (GF), thousand kernel weight (TKW), and grain protein concentration (GP). The grain filling period was significantly reduced ($P<0.01$) by high temperatures regime at 25 DAA. On average, heat stress reduced the grain filling period by 9 days. There were significant differences related to duration of heat stress, with the exposure to two weeks of stress being more detrimental than one week for GF. Thousand kernel weight was reduced by 9.8 % ($P<0.05$) when exposed to heat stress, but no difference was found for duration of the stress. GP increased from 15.2 to 16.2 % ($P<0.05$) due to heat stress, but as occurred with TKW, duration of stress did not have an impact on grain protein.

There was no evidence of a change in protein MWD due to heat stress. However, in three (SSP, MP, and SPP/MP ratio) out of the four of these variables, there was a significant treatment x genotype interaction ($P<0.05$, $P<0.01$, and $P<0.05$, respectively) (Table 1.3).

Table 1.3. Experiment 1. Analysis of variance and observed least square means for grain filling period (GF), thousand kernel weight (TKW), grain protein concentration (GP), soluble polymeric protein (SPP), monomeric protein (MP), low molecular weight albumins and globulins (LMWAG), and ratio SPP/MP.

Source of variation	df	GF (days)	TKW (grs)	GP (%)	Source of variation	df	SPP (%)	MP (%)	LMWAG (%)	SPP/MP
		Mean squares					*Mean squares*			
Rep	2	84.1 †	45.6	0.5718	Rep	1	5.145	2.093	0.67	0.002008
Treatment (T)	2	1668.5 **	179.8 *	11.5902 †	Treatment (T)	2	0.662	0.311	1.26	0.000050
Control vs 25 DAA^{++}	1	2351.6 **	350.8 *	23.1533 *	Control vs 25 DAA^{++}	1	0.012	0.592	0.76	0.000013
25 DAA, 1w vs 2w	1	1055.6 **	5.6	0.0030	25 DAA, 1w vs 2w	1	1.311	0.030	1.77	0.000092
Error a	4	44.3	24.7	1.8737	Error a	2	11.121	9.761	0.37	0.007720
Genotype (G)	13	440.2 **	257.5 **	18.9080 **	Genotype (G)	5	69.242 **	74.096 **	9.35 **	0.051407 **
T x G	26	57.2 *	19.1	0.7208	T x G	10	9.139 *	10.967 **	0.82	0.007140 *
Error b	74	33.3	19.4	0.8988	Error b	15	2.859	2.379	0.73	0.0001955
Total	121				Total	35				
		Least square means					*Least square means*			
Treatment [A]					Treatment [A]					
Control		47	41	15.2	Control		32.3	57.9	9.8	0.564
25 DAA $^{++}$		38	37	16.2	25 DAA $^{++}$		32.4	58.2	9.5	0.563
25 DAA (1 w)		41	37	16.2	25 DAA (1 w)		32.6	58.2	9.2	0.565
25 DAA (2 w)		34	37	16.2	25 DAA (2 w)		32.1	58.1	9.8	0.561

25 DAA $^{++}$ = Average of heat treatments: 25 DAA (1w) and 25 DAA (2w)

[A] See Table 2

Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$.

Response of cultivars to duration of heat stress

Wheat cultivars used in this study were genetically diverse and variable for agronomic traits and end-use quality. The cultivars Trigo 3, Debeira, Ventnor and Pavón 76 were chosen based on previous information regarding tolerance of agronomic or quality characteristic performance to heat-stress conditions. Pedigree and high molecular weight glutenin subunits (HMW-GS) of the studied wheat genotypes are listed in Table 1.1.

Without heat stress, average grain filling period for all cultivars was 47 days, which resembles what may occur in the field in an early planting date in Uruguay. Debeira and LE 2290 had longer GF of 61 days, while INIA Caburé and LE 2265, had GF of only 34 days (Table 1.4a). Almost all the genotypes responded to heat stress with a decrease in GF. A significant treatment x genotype interaction for GF ($P<0.05$) was related to differences in the magnitude of this decrease (Table 1.4a). Three of the fourteen genotypes had a non-significant decrease in GF under stress conditions (INIA Caburé, LE 2265, and LE 2262). Not surprisingly, INIA Caburé and LE 2265 were the ones with the shorter GF under control conditions.

There were significant differences in GF among genotypes when examining duration of heat stress. Two weeks of stress resulted in shorter GF for Debeira, Estanzuela Cardenal, INIA Churrinche, LE 2290, Pavón 76, and Trigo 3.

The average TKW for normal growing conditions was 40 g. The genotype with the higher TKW was LE 2262 (54 g), while INIA Churrinche had the lowest (31 g) (Table 1.4a). The experimental line LE 2262 was selected from a cross called 'Tinamou',

Table 1.4a. Experiment 1. Least square means for grain filling period (GF) (days), thousand kernel weight (TKW) (grams), and grain protein concentration (GP) (%) for the response to the duration of heat stress (one or two weeks) starting 25 DAA, in selected wheat genotypes.

Genotypes	Treatment [A]	GF (days)	TKW (grs)	GP (%)
Debeira	**Control**	61	36	15.5
	25 DAA [++]	47 **	36	16.5
	25 DAA (1w)	55	34	16.5
	25 DAA (2w)	38 **	37	16.5
Estanzuela Cardenal	**Control**	35	46	15.0
	25 DAA [++]	38	40 †	16.7 *
	25 DAA (1w)	44	40	17.6
	25 DAA (2w)	31 **	39	15.7 *
Estanzuela Pelón 90	**Control**	50	47	14.5
	25 DAA [++]	33 **	46	15.6
	25 DAA (1w)	33	44	15.5
	25 DAA (2w)	32	47	15.6
INIA Boyero	**Control**	51	45	15.8
	25 DAA [++]	37 **	40 †	17.2 †
	25 DAA (1w)	40	38	17.1
	25 DAA (2w)	34	41	17.2
INIA Caburé	**Control**	34	34	14.3
	25 DAA [++]	30	35	14.8
	25 DAA (1w)	30	34	15.0
	25 DAA (2w)	29	35	14.5
INIA Churrinche	**Control**	45	31	16.8
	25 DAA [++]	42	27	17.8
	25 DAA (1w)	48	25	17.2
	25 DAA (2w)	35 **	29	18.4
INIA Mirlo	**Control**	44	41	16.5
	25 DAA [++]	34 *	36	17.0
	25 DAA (1w)	35	36	16.8
	25 DAA (2w)	33	36	17.1

[A] See Table 2

25 DAA [++] = Average of heat treatments: 25 DAA (1w) and 25 DAA (2w)

Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 25 DAA [++] ,and 25DAA (1w) vs 25DAA (2w).

Table 1.4a. (Continued)

Genotypes	Treatment [A]	GF (days)	TKW (grs)	GP (%)
LE 2262	**Control**	40	54	15.3
	25 DAA [++]	34	47 *	16.5 †
	25 DAA (1w)	36	44	16.2
	25 DAA (2w)	32	49	16.8
LE 2265	**Control**	34	43	12.2
	25 DAA [++]	31	41	14.0 *
	25 DAA (1w)	31	42	14.2
	25 DAA (2w)	30	39	13.7
LE 2290	**Control**	61	39	16.9
	25 DAA [++]	47 **	35	17.7
	25 DAA (1w)	55	35	17.5
	25 DAA (2w)	39 **	34	17.8
LE 2294	**Control**	57	33	16.4
	25 DAA [++]	43 **	34	16.0
	25 DAA (1w)	46	36	15.5
	25 DAA (2w)	39	31	16.5
Pavón 76	**Control**	54	41	15.5
	25 DAA [++]	41 **	38	16.4
	25 DAA (1w)	45	37	16.5
	25 DAA (2w)	36 †	39	16.2
Trigo 3	**Control**	57	36	17.2
	25 DAA [++]	46 **	35	17.6
	25 DAA (1w)	52	36	17.9
	25 DAA (2w)	39 *	34	17.3
Ventnor	**Control**	38	40	11.5
	25 DAA [++]	31 †	31 **	13.1 *
	25 DAA (1w)	30	33	13.1
	25 DAA (2w)	31	28	13.1
General mean	**Control**	**47**	**40**	**15.2**
	25 DAA [++]	**38 ** **	**37 ***	**16.2 ***
	25 DAA (1w)	**41**	**37**	**16.2**
	25 DAA (2w)	**34 ** **	**37**	**16.2**

[A] See Table 2

25 DAA [++] = Average of heat treatments: 25 DAA (1w) and 25 DAA (2w)

Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 25 DAA [++], and 25DAA (1w) vs 25DAA (2w).

which typically produces very large spikes and heavy grains in field and controlled environment conditions. A significant genotype x treatment interaction was not detected for TKW. However, when analyzing the genotypes individually, four of them showed a significant reduction with heat stress: Estanzuela Cardenal, INIA Boyero ($P<0.10$), LE 2262 and Ventnor ($P<0.05$).

Grain protein concentration values were high compared with typical values for field conditions because the plants were grown in nutrient rich media. The average for GP without heat stress was 15.2 %, with Trigo 3 having the highest GP (17.2 %), and LE 2265, the lowest (12.2 %). The latter is a synthetic derived line, with contributions of *Triticum turgidum* L. ssp. *durum* (Desf.) Husn. (CHEN) and *Aegilops squarrosa* (224) in its pedigree (Table 1.1). Its unique genetic background could account for the low protein level achieved in this experiment. A non-significant treatment x genotype interaction for GP was reported in Table 1.3. However, some genotypes showed a significant increase in GP with heat stress (Table 1.4a).

Influence of heat stress on grain protein composition of a subset of six wheat genotypes is presented in Table 1.4b. Under control conditions, the average values across genotypes were: 32.3 % soluble polymeric protein (SPP), 57.9 % monomeric protein (MP), 9.8 % low molecular weight albumins and globulins (LMWAG), and 0.564 ratio of SPP/MP. Debeira had the highest SPP percentage (37.0 %), and the lowest MP percentage (52.4 %), thus attaining the highest SPP/MP ratio. The contrary occurred with Estanzuela Pelón 90, which had the lowest levels of SPP (28.3 %), and the highest MP (63 %), resulting in the lowest SPP/MP ratio (0.449) of the six genotypes reported. Pavón

Table 1.4b. Experiment 1. Least square means for soluble polymeric protein (SP) (%), monomeric protein (MP) (%), low molecular weight albumins and globulins, and SPP/MP ratio according the response to the duration of heat stress (one or two weeks) starting 25 DAA, in a subset of selected wheat genotypes.

Genotypes	Treatment [A]	SPP (%)	MP (%)	LMWAG (%)	SPP / MP
Debeira	**Control**	37.0	52.4	10.3	0.704
	25 DAA [++]	36.7	52.8	10.6	0.696
	25 DAA (1w)	35.3	54.7	10.0	0.645
	25 DAA (2w)	38.0	50.9 †	11.1	0.747 *
Estanzuela Pelón 90	**Control**	28.3	63.0	8.7	0.449
	25 DAA [++]	27.7	63.2	9.1	0.439
	25 DAA (1w)	27.3	63.9	8.7	0.427
	25 DAA (2w)	28.1	62.4	9.4	0.451
INIA Churrinche	**Control**	36.5	55.0	8.5	0.665
	25 DAA [++]	34.3	59.0 *	6.8 *	0.587 †
	25 DAA (1w)	36.6	56.2	7.2	0.654
	25 DAA (2w)	31.9 *	61.8 **	6.3	0.519 *
LE 2294	**Control**	30.9	58.8	10.3	0.525
	25 DAA [++]	28.4	60.9	10.7	0.468
	25 DAA (1w)	30.0	59.6	10.4	0.504
	25 DAA (2w)	26.8	62.2	11.0	0.431
Pavón 76	**Control**	30.0	59.0	11.0	0.512
	25 DAA [++]	35.1 **	55.0 *	10.0	0.640 *
	25 DAA (1w)	34.9	55.5	9.6	0.630
	25 DAA (2w)	35.3	54.4	10.3	0.649
Trigo 3	**Control**	31.1	58.8	10.0	0.528
	25 DAA [++]	31.9	58.2	10.0	0.548
	25 DAA (1w)	31.3	59.3	9.4	0.528
	25 DAA (2w)	32.4	57.1	10.5	0.568
General mean	**Control**	**32.3**	**57.9**	**9.8**	**0.564**
	25 DAA [++]	**32.4**	**58.2**	**9.5**	**0.563**
	25 DAA (1w)	**32.6**	**58.2**	**9.2**	**0.565**
	25 DAA (2w)	**32.1**	**58.1**	**9.8**	**0.561**

[A] See Table 2

25 DAA [++] = Average of heat treatments: 25 DAA (1w) and 25 DAA (2w)

Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 25 DAA [++], and 25DAA (1w) vs 25DAA (2w).

76 showed the highest value for LMWAG (11.0 %), and INIA Churrinche, the lowest (8.5 %).

A significant response to heat stress for SPP, MP, and SPP/MP was observed in Pavón 76 and I. Churrinche, while Debeira showed a response only for MP and SPP/MP (Table 1.4b). Duration of heat stress affected INIA Churrinche and Debeira, while Pavón 76 was similarly affected by heat stress at both one or two weeks of stress. With two weeks of heat stress, INIA Churrinche showed a decrease in SPP and SPP/MP ratio, while MP increased, compared with one week of stress. Debeira, on the contrary, showed a decrease in MP and an increase in SPP/MP with additional stress. Pavón 76 also showed a similar heat stress response to Debeira, increasing SPP, SPP/MP, and decreasing MP. The SPP/MP ratio is believed to have the greatest influence on dough strength among the protein composition variables.

Low molecular weight albumins and globulins did not show a significant treatment effect, nor a significant treatment x genotype interaction (Table 1.3). It appears that, for this set of genotypes, heat stress had little effect on LMWAG relative to other proteins. However, in the individual genotype comparisons, I. Churrinche showed a decrease in LMWAG with heat stress, without any difference in duration. There were significant genotypic differences for all grain protein composition variables. Data for cultivars are presented in Table 1.4b.

Experiment 2: Timing of heat stress

Response to timing of heat stress

In this experiment, heat stress was applied at two different development stages [15 DAA (1w), and 25 DAA (1w), (Table 1.2)], and each heat treatment had the same duration. Analysis of variance for agronomic, grain quality, and grain protein composition variables are presented in Table 1.5.

Significant differences were observed between the Control and stress treatments for GF and TKW. GF was reduced by four days ($P<0.01$) when wheat genotypes were exposed to one week of heat stress, regardless of timing of the stress. TKW was reduced by an average of 7.3 % ($P<0.01$) with 15 or 25 DAA heat stress treatments. GP was affected by heat stress, but mainly by the timing when the stress occurred. A significant increase in protein concentration ($P<0.05$) was observed when heat stress was applied at 15 DAA (18.4 % GP). Exposure to stress later in GF did not have a significant effect on protein concentration as compared with the Control (17.7 % GP).

There was no evidence of any effect of heat treatments on grain protein composition variables, nor there was a significant treatment x genotype interaction for any of them (Table 1.5).

Table 1.5. Experiment 2. Analysis of variance and observed least square means for grain filling period (GF), thousand kernel weight (TKW), grain protein concentration (GP), soluble polymeric protein (SPP), monomeric protein (MP), low molecular weight albumins and globulins (LMWAG), and ratio SPP/MP.

Source of variation	df	GF (days)	TKW (grs)	GP (%)	Source of variation	df	SPP (%)	MP (%)	LMWAG (%)	SPP/MP
		Mean squares					*Mean squares*			
Rep	2	74.0 **	12.64	0.21	Rep	1	112.5 *	40.1 †	18.23 †	0.0386 *
Treatment (T)	2	272.3 **	159.82 *	8.14 †	Treatment (T)	2	18.5	29.7	3.05	0.0109
Control vs 15, 25 DAA^{++}	1	540.3 **	319.28 **	2.36	Control vs 15, 25 DAA^{++}	1	21.2	48.6	5.62	0.0129
15 DAA vs 25 DAA	1	4.3	0.37	13.92 *	15 DAA vs 25 DAA	1	15.8	10.7	0.48	0.0088
Error a	4	10.7	14.89	1.21	Error a	2	46.3	27.2	3.55	0.0182
Genotype (G)	13	146.4 **	107.17 **	8.85 **	Genotype (G)	5	111.1 **	73.4 **	19.88 *	0.0455 **
T x G	26	16.2	14.11 *	1.13	T x G	10	12.0	8.0	1.83	0.0049
Error b	78	13.7	7.30	1.04	Error b	15	20.0	11.6	5.05	0.0073
Total	125				Total	35				
		Least square means					*Least square means*			
Treatment [A]					Treatment [A]					
Control		37	41	17.7	Control		24.7	66.6	8.7	0.377
15, 25 DAA $^{++}$		33	38	18.0	15, 25 DAA $^{++}$		26.3	64.2	9.6	0.418
25 DAA (1w)		33	38	17.6	25 DAA (1w)		27.1	63.5	9.4	0.437
15 DAA (1w)		32	38	18.4	15 DAA (1w)		25.5	64.8	9.7	0.398

15, 25 DAA $^{++}$ = Average of heat treatments: 15 DAA (1w) and 25 DAA (1w)

[A] See Table 2

Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$.

Response of cultivars to timing of heat stress

Significant genotypic variation was observed for all the variables measured (Table 1.5). Least square means for fourteen genotypes are presented in Tables 1.6a. The subset of six genotypes used for the protein analysis are presented in Table 1.6b. GF averaged 37 days in experiment 2. Debeira and Ventnor had relatively longer GF (46 days); and Estanzuela Pelón 90, INIA Caburé, INIA Mirlo, LE 2262, and LE 2265, had shorter GF (33 days).

Grain protein concentration averaged 17.7 %. Estanzuela Cardenal showed the highest level of protein (19.8 %), while LE 2265, the synthetic derived line, showed the lowest (15.8 %), as it did in experiment 1. Neither GF nor GP variables had a significant treatment x genotype interaction (Table 1.5). In general, the genotypes studied tend to decrease GF and increase GP with the heat stress. However, nine genotypes showed a significant interaction in GF, and three did in GP (Table 1.6a).

Average thousand kernel weight for all genotypes was 41 g. As in experiment 1, LE 2262 had the highest TKW (51 g), and INIA Churrinche, the lowest (32 g). A significant treatment x genotype interaction was observed for TKW ($P<0.05$) (Table 5). Estanzuela Pelón 90, INIA Boyero, INIA Mirlo, LE 2262, LE 2265, and Trigo 3, showed a significant decrease in TKW exposed to heat stress (Table 1.6a). The only genotype with significant response to timing of stress was LE 2262. As was seen before, it had the greater TKW of all genotypes in control conditions, and when growing conditions are stressful, the reduction in TKW is more dramatic than in other genotypes.

Table 1.6a. Experiment 2. Least square means for grain filling period (GF) (days), thousand kernel weight (TKW) (grams), and grain protein concentration (GP) (%) for the response to timing of heat stress starting 15 DAA or 25 DAA, in selected wheat genotypes.

Genotypes	Treatment [A]	GF (days)	TKW (grs)	GP (%)
Debeira	**Control**	46	41	16.3
	15, 25 DAA [++]	38 **	41	16.5
	25 DAA (1w)	36	41	15.9
	15 DAA (1w)	39	41	17.1
Estanzuela Cardenal	**Control**	35	39	19.8
	15, 25 DAA [++]	32	37	19.0
	25 DAA (1w)	30	37	18.6
	15 DAA (1w)	33	36	19.4
Estanzuela Pelón 90	**Control**	33	40	16.3
	15, 25 DAA [++]	31	35 *	17.2
	25 DAA (1w)	30	36	16.7
	15 DAA (1w)	32	34	17.6
INIA Boyero	**Control**	36	41	17.6
	15, 25 DAA [++]	31 †	37 *	18.7
	25 DAA (1w)	33	37	18.3
	15 DAA (1w)	29	37	19.0
INIA Caburé	**Control**	33	39	17.8
	15, 25 DAA [++]	26 *	36	18.0
	25 DAA (1w)	28	36	17.7
	15 DAA (1w)	24	36	18.7
INIA Churrinche	**Control**	35	32	17.1
	15, 25 DAA [++]	30 †	33	17.9
	25 DAA (1w)	30	31	17.8
	15 DAA (1w)	30	35	18.0
INIA Mirlo	**Control**	33	38	19.1
	15, 25 DAA [++]	29	34 *	19.6
	25 DAA (1w)	29	33	18.3
	15 DAA (1w)	29	34	20.8 **

[A] See Table 2

15, 25 DAA [++] = Average of heat treatments: 15 DAA (1w) and 25 DAA (1w)

Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 15, 25 DAA [++], and 15DAA (1w) vs 25DAA (1w).

Table 1.6a. (Continued)

Genotypes	Treatment [A]	GF (days)	TKW (grs)	GP (%)
LE 2262	**Control**	33	51	16.8
	15, 25 DAA [++]	31	43 **	17.6
	25 DAA (1w)	29	47	16.6
	15 DAA (1w)	32	39 **	18.6 *
LE 2265	**Control**	33	48	15.8
	15, 25 DAA [++]	29 †	40 **	16.7
	25 DAA (1w)	30	39	16.9
	15 DAA (1w)	27	41	16.5
LE 2290	**Control**	41	42	19.7
	15, 25 DAA [++]	39 **	39	18.4 †
	25 DAA (1w)	32	40	17.7
	15 DAA (1w)	32	38	19.1
LE 2294	**Control**	37	38	17.2
	15, 25 DAA [++]	35	40	17.4
	25 DAA (1w)	36	38	16.8
	15 DAA (1w)	34	41	18.0
Pavón 76	**Control**	39	43	17.4
	15, 25 DAA [++]	33 *	41	17.7
	25 DAA (1w)	34	39	18.2
	15 DAA (1w)	31	42	17.1
Trigo 3	**Control**	36	44	19.2
	15, 25 DAA [++]	38	39 **	18.8
	25 DAA (1w)	35	38	18.2
	15 DAA (1w)	40	39	19.4
Ventnor	**Control**	46	36	17.4
	15, 25 DAA [++]	42 †	35	18.0
	25 DAA (1w)	45	33	18.0
	15 DAA (1w)	38 *	36	17.9
General mean	**Control**	**37**	**41**	**17.7**
	15, 25 DAA [++]	**33 ******	**38 ******	**18.0**
	25 DAA (1w)	**33**	**38**	**17.6**
	15 DAA (1w)	**32**	**38**	**18.4 ***

[A] See Table 2

15, 25 DAA [++] = Average of heat treatments: 15 DAA (1w) and 25 DAA (1w)

Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 15, 25 DAA [++], and 15DAA (1w) vs 25DAA (1w).

The genetic variation observed in protein composition variables is presented in Table 1.6b. Average least square means for SPP were 24.7 %; for MP, 66.6 %; for LMWAG, 8.7 %, and for SPP/MP ratio, 0.377. As in Experiment 1, Debeira had the highest SPP (32.8 %), and the lowest MP (58.4 %), thus attaining the highest SPP/MP ratio (0.562). The contrary occurred with Estanzuela Pelón 90, which had the lowest SPP (16.7 %), and the highest MP (72.2 %), having as a result the lowest SPP/MP ratio (0.232). This genotype showed the highest value for LMWAG (11.1 %); and LE 2294, the lowest (6.0 %).

Table 1.6b. Experiment 2. Least square means for soluble polymeric protein (SP) (%), monomeric protein (MP) (%), and SPP/MP ratio according to the response to timing of heat stress starting 15 DAA or 25 DAA, in selected wheat genotypes.

Genotypes	Treatment [A]	SPP (%)	MP (%)	LMWAG (%)	SPP / MP
Debeira	**Control**	32.8	58.4	8.8	0.562
	15, 25 DAA [++]	31.7	59.2	9.2	0.537
	25 DAA (1w)	33.5	58.1	8.4	0.576
	15 DAA (1w)	29.9	60.2	9.9	0.497
Estanzuela Pelón 90	**Control**	16.7	72.2	11.1	0.232
	15, 25 DAA [++]	19.5	67.9	12.7	0.290
	25 DAA (1w)	18.5	67.5	14.0	0.280
	15 DAA (1w)	20.4	68.2	11.4	0.300
INIA Churrinche	**Control**	24.1	68.2	7.7	0.354
	15, 25 DAA [++]	26.7	64.4	9.0	0.419
	25 DAA (1w)	24.3	66.4	9.3	0.373
	15 DAA (1w)	29.0	62.4	8.7	0.465
LE 2294	**Control**	27.7	67.0	6.0	0.406
	15, 25 DAA [++]	25.8	67.2	7.1	0.397
	25 DAA (1w)	28.7	65.5	5.8	0.457
	15 DAA (1w)	22.8	68.9	8.3	0.336
Pavón 76	**Control**	23.1	68.0	8.9	0.339
	15, 25 DAA [++]	28.2	62.7	9.2	0.452
	25 DAA (1w)	30.2	61.2	8.6	0.494
	15 DAA (1w)	26.1	64.1	9.8	0.409
Trigo 3	**Control**	24.2	65.9	9.9	0.370
	15, 25 DAA [++]	26.0	63.7	10.4	0.411
	25 DAA (1w)	27.3	62.2	10.5	0.439
	15 DAA (1w)	24.7	65.1	10.2	0.383
General mean	**Control**	**24.7**	**66.6**	**8.7**	**0.377**
	15, 25 DAA [++]	**26.3**	**64.2**	**9.6**	**0.418**
	25 DAA (1w)	**27.1**	**63.5**	**9.4**	**0.437**
	15 DAA (1w)	**25.5**	**64.8**	**9.7**	**0.398**

[A] See Table 2

15, 25 DAA [++] = Average of heat treatments: 15 DAA (1w) and 25 DAA (1w)

Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 15, 25 DAA [++], and 15DAA (1w) vs 25DAA (1w).

Discussion

General response patterns

Grain fill duration was reduced by an average of six days when stress lasted one week, and thirteen days when it lasted two weeks. This is almost one day reduction of GF for each day of heat stress, when the stress was applied starting at 25 DAA. Stone and Nicolas (1995b) have reported that the relationship between temperature and duration of grain growth may change with timing of the heat stress. In this study, however, heat stress imposed at different stages (15 DAA or 25 DAA) had similar effect on GF, averaging four days less than the Control. This decrease in the GF could be associated to a shortening of the cell enlargement and dry matter accumulation phase in the grain, which occurs from 16 to 37 DAA (Wang and Gifford 1995).

Heat stress caused a reduction in thousand kernel weight in both experiments. Experiment 1 showed a 9.8 % reduction in TKW, and Experiment 2, a 7.3 % reduction. Stone et al (1995), working in a higher range of temperatures than the present study [brief heat shock treatments from 15-19 DAA (40/16°C day/night) with susceptible wheat cultivar Oxley], found a reduction in kernel mass of 17 %. Also, Stone and Nicolas (1998b) reported that reduction of kernel mass increased linearly with increased duration of heat treatment, at a rate -1.6 % day $^{-1}$.

Heat stress increased grain protein concentration in both experiments. While the duration of heat stress did not show a differential increase in GP in Experiment 1, timing of the stress showed a significant effect in Experiment 2. Stress during early stages of grain filling (15 DAA) resulted in the highest GP concentration (increase of 0.7 %), as compared with the Control and late stress. Stone and Nicolas (1996), working with short

periods of very high temperature (40°C) reported similar results about effect of timing of heat stress on GP. For the two wheat varieties they studied, early heat treatments increased grain protein percentage more than later heat treatments. Bhullar and Jenner (1985), working with four wheat cultivars exposed to short episodes of heat stress, demonstrated that increase in grain nitrogen was due to a reduction in starch content of the grain rather than to a change in the quantity of nitrogen. Lower activity of the enzyme soluble starch synthase, possibly due to thermal denaturation, may be responsible for the reduced starch deposition in temperatures above 30°C (Jenner 1994). Panozzo (1997), working with wheat in field conditions in Australia, found that grain dry matter accumulation, was more sensitive to high temperatures than nitrogen accumulation. Under field conditions, smaller grains resulted from reduction in grain filling duration, rather than a reduction in grain filling rate. This explanation may account for the reduction in GF, TKW and the concomitant increase in GP after heat stress in the present study.

Grain protein synthesis has been reported to be asynchronous during grain development (Panozzo 1997). The gliadins are the first protein group to be synthesized, starting at 7 DAA or even earlier, followed by an initial rapid phase of synthesis. A rapid phase of glutenin synthesis begins at approximately 14 DAA, when less than 5 % of glutenins have already been synthesized. Protein polymerization occurs in late grain filling. Timing and duration of heat stress are expected to alter protein synthesis, which is evidenced by changes in the glutenin / gliadin ratio (Blumenthal et al. 1994, Stone and Nicolas 1995a). In this study, grain protein molecular weight distribution did not show significant changes due to heat stress. However, significant treatment x genotype

interaction was detected where duration of heat stress was assessed. Surprisingly, timing of heat stress in Experiment 2 did not alter grain protein molecular weight distribution, and there was no evidence of treatment x genotype interaction. In this experiment GF was sixteen days shorter than Experiment 1 for the subset of six genotypes, which could mean that heat stress could have been applied too late in grain development stage, such that protein synthesis was not affected.

Individual genotype responses

Table 1.7a summarizes the response to heat stress of the fourteen genotypes used in this study with regard to TKW, one of the yield component variables. Table 1.7b summarizes the response to heat stress of TKW and SPP/MP ratio for the subset of six genotypes used to asses grain protein composition. As reported by Stone and Nicolas (1995a), these variables appear to be independent. Some genotypes may have one trait affected by heat stress, while the other is unaffected.

From an agronomic point of view, genotypes that show a stable TKW after heat stress could be considered relatively tolerant to this stress (Table 1.7a). When considering both TKW and SPP/MP ratio, INIA Churrinche appears as a genotype that could be producing dough weaker than expected if subjected to heat stress during grain filling, because there is a decrease in the SPP/MP ratio, even if there is no decrease in TKW. Estanzuela Pelón 90 and Trigo 3 may show decreased TKW when exposed to heat stress, but quality attributes , as indicated by SPP/MP ratio, seem to be more stable. Finally, LE 2294, Debeira, and Pavón 76 are likely to be the best choices for growing wheats tolerants to heat stress from both an agronomic and quality point of view.

Table 1.7a. Genotypes notable for their tolerance or sensitivity of TKW to heat stress in Experiments 1 and 2.

Response	Genotype
Decreased TKW	E. Cardenal
	E. Pelón 90
	I. Boyero
	I. Mirlo
	LE 2262
	LE 2265
	Trigo 3
	Ventnor
Stable TKW	Debeira
	I. Caburé
	I. Churrinche
	LE 2290
	LE 2294
	Pavón 76

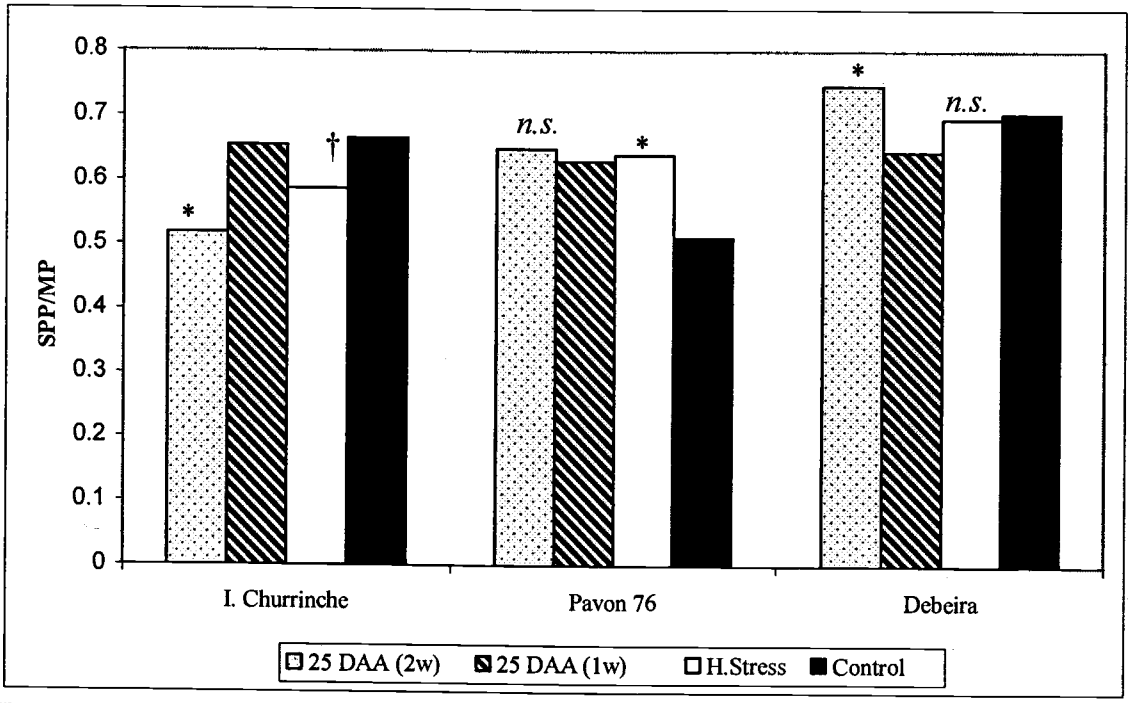
Table 1.7b. Genotypes (subset of six) notable for their tolerance or sensitivity of SPP/MP ratio and TKW to exposure to heat stress, for experiments where each variable had a significant treatment effect and/or significant treatment x genotype interaction.

Response	Genotype
Decreased SPP/MP ratio and stable TKW	I. Churrinche
Decreased SPP/MP ratio and decreased TKW	-
Increased SPP/MP ratio and stable TKW	Debeira, Pavón 76
Increased SPP/MP ratio and decreased TKW	-
Stable SPP/MP ratio and decreased TKW	E. Pelón 90, Trigo 3
Stable SPP/MP ratio and stable TKW	LE 2294

There have been reports of genotypes that have as a tolerant response increased SPP/MP ratio when subjected to heat stress, as Debeira and Pavón 76 did (Blumenthal et al. 1994). In this study, these are promising parent lines for further genetic improvement in heat tolerance. Figure 1.1 shows the comparison of SPP/MP ratio for the heat sensitive line (I. Churrinche), compared with these two genotypes. Responses of Debeira, Pavón 76, and Trigo 3 in this study confirm the previous research that suggests they possess heat tolerance, showing an increase (the first two), or a stable SPP/MP ratio under heat stress.

There was no relationship established between the heat tolerant response of the wheat genotypes tested and the presence of 5+10 subunits of *Glu-D1* locus, as reported by Blumenthal et al (1995b). We have found stable and unstable genotypes (increase or decrease of SPP/MP ratio with heat stress) showing both 5 + 10 HMW-GS.

Figure 1.1. Experiment 1. The response of soluble polymeric protein/monomeric protein ratio (SPP/MP) to the duration of heat stress (one or two weeks) starting 25 DAA, in a susceptible (I. Churrinche) and two resistant (Pavón 76 and Debeira) wheat genotypes.



H. Stress = Average of heat treatments: 25 DAA (1w) and 25 DAA (2w)
Level of significance: †, $P < 0.10$; *, $P < 0.05$; *n.s.*, non significant; for orthogonal contrasts of Control vs. H. Stress, and 25DAA (1w) vs 25DAA (2w).

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CHAPTER 2

Influence of Heat Stress During Grain Fill on Yield, Grain Characteristics, Rheological Properties, and Protein Composition under Field Conditions

Introduction

The increasing world population necessitates expanding agriculture frontiers to produce more food. Wheat (*Triticum aestivum* L.) is increasingly being planted outside optimal region temperatures, which has increased interest in response to supra-optimal temperature for normal growth (Shpiler and Blum 1986). There are 36 million ha (40% of temperate environments) of wheat around the world that suffer terminal heat stress in the growing season (Reynolds et al 2001). Forecasts of general global warming suggest the importance of high temperature stress is likely to increase in future years (Adams et al. 1990).

Low temperatures during kernel maturation contribute to high individual kernel weight, as well as a higher grain fertility, which are important components of yield. Chowdhury and Wardlaw (1978) reported that the optimal temperature regimen for maximizing kernel dry weight was between 15/10°C (day/night) and 18/13°C. When wheat is grown under higher temperatures, the duration of all developmental stages is reduced, and yield is decreased. Kernel dry weight is also affected by heat stress causing grain yield decrease (Chowdhury and Wardlaw 1978, Wiegand and Cuellar 1981, Wardlaw et al. 1989, Calderini et al 1999, Wardlaw et al. 2002).

One strategy that could be used is simply to avoid heat stress. It would be desirable to develop genotypes with high grain filling rates with short to medium grain filling duration. Bruckner and Frohberg (1987), working with 20 spring wheat genotypes in the

Northern Great Plains, US, found only one (ND584 sib) out of the 20 genotypes tested that had both characteristics. Shpiler and Blum (1986) identified heat tolerant varieties, which were able to maintain a larger interval between double ridge and anthesis, producing the higher number of grain per spike.

Wheat grain is composed mainly of starch and protein, and to a lesser extent, by other polysaccharides, minerals, lipids, and vitamins. Within the proteins, four main groups can be categorized according to their solubilization pattern (Osborne classification): albumins (water-soluble), globulins (soluble in salt solution), gliadins (70% aqueous ethanol soluble), and glutenins (insoluble in either salt or 70% ethanol; soluble in alkaline solution). The last two types are gluten-forming proteins, which confer the unique quality attributes to wheat flour. Glutenins, especially high molecular weight glutenins subunits (HMW-GS), are key factors in the process of bread making. They determine gluten elasticity by promoting the formation of large polymers (MacRitchie 1992). Gliadins are monomeric proteins which contribute to increase dough extensibility (Daniel and Triboi, 2001). The balance among gluten proteins is fundamental to determining wheat flour functional properties.

Many factors affect consistency of dough properties in a wheat cultivar. The timing and duration of heat stress is an important source of variation in dough properties (Blumenthal et al. 1993; Stone and Nicolas 1995, 1996, Corbellini et al 1997, Wardlaw et al. 2002). Protein quality, as measured by SDS sedimentation volumes and size-exclusion high-performance liquid chromatography, is highly influenced by the frequency of high temperatures and relative humidity during grain filling (Graybosch et al. 1995). Correll et al. (1994), studying wheat from 109 silos in South Australia from the years 1971-1991,

found that the number of days in October above 30°C were positively correlated with grain-protein content. Randall and Moss (1990), Stone et al. (1997), and Corbellini et al. (1998) have found that grain protein content and dough strength increases as temperatures increase to about 30°C. Very high temperatures (e.g. short episodes of a few days at > 35°C) produced grain with weaker-than-expected dough properties (Randall and Moss 1990, Blumenthal et al. 1991, 1993; Wrigley et al. 1994; Borghi et al 1995, Stone et al. 1997; Corbellini et al. 1998). According to Blumenthal et al. (1990, 1991, 1993, 1994, 1995), and Stone et al. (1997), there is an increased gliadin:glutenin ratio after heat stress, either because of a higher rate of monomeric protein synthesis, or decrease in the glutenin synthesis. Guedira et al. (2002) found that reduction in dough strength and mixing tolerance after heat stress was associated with a reduction in unextractable polymeric proteins. Ciaffi et al (1996a) found that heat stress during grain filling was responsible for modifications in the polymeric fraction composition (soluble/insoluble polymers protein ratio).

Inconsistencies in grain chemical composition due to environmental stresses create difficulties in the marketing and processing of grain (Peterson et al. 1992, 1998; Panozzo, 1997). There are reports (Blumenthal et al. 1994, 1995; Wrigley et al. 1994; Stone and Nicolas 1995, 1998a, 1998b) of variation in wheat in the response to heat stress, with some genotypes showing either a very small change or a decrease, in the gliadin:glutenin ratio. Such lines could be useful for further examination for genetic improvement of heat tolerance.

Uruguay is situated between 30° – 35° South latitude; thus the grain filling stage of wheat crops can be subjected to fairly high temperatures. Heat stress during grain

filling usually occurs in the Northern region of Uruguay, producing seasonal variation in grain quality. The purpose of this study was to assess changes in grain yield, grain quality characteristics, and grain protein composition induced by timing of heat stress in Uruguayan field conditions.

Materials and Methods

Plant material

Six spring wheat (*Triticum aestivum* L.) cultivars and four elite experimental lines from National Institute for Agriculture Research (INIA) La Estanzuela Wheat Breeding Program, Uruguay, were chosen to carry out this study, which represent a range of genetic variability for gain yield and bread-making quality. Pedigree and high molecular weight glutenin subunits (HMW-GS) of the wheat genotypes are listed in Table 2.1.

Field experiments

Two experiments were conducted at the experimental field located in Young, northwest of Uruguay, South America, during the wheat growing seasons of years 2001 (Experiment 2001) and 2002 (Experiment 2002), respectively. Each experiment consisted of two planting dates: an early planting date (a), and a late planting date (b), to increase the probabilities of exposing the wheat genotypes to heat stress during grain fill. The soil type at Young experimental field corresponds to a Typic Argiudoll, fine, (smectitic), termic (USDA-Soil Taxonomy, 1999), and it is representative of the wheat-producing area in Uruguay. Soil tests were performed each year prior to sowing, and in developmental stage Zadoks 22 (Z 22) (Zadoks 1974). In Z 30, plant nitrogen (N) was measured. Nitrogen (N) and phosphorus (P) were added as fertilizers when needed, either at planting, or a split application, to ensure adequate nutrient availability for the growing

Table 2.1. Wheat genotypes studied, their progenitors and HMW-GS.

Genotypes	Pedigree	HMW-GS		
		Glu-A1	*Glu-B1*	*Glu-D1*
Estanzuela Cardenal	KVZ/BUHO'S'//KAL/BB	1	7+9	5+10
Estanzuela Pelón 90	KVZ/TRM	1	13+16	5+10
INIA Boyero	MN72131/BOBWHITE	2*	7+9	5+10
INIA Caburé	EFED//BUCK6/MR74507		7+8	5+10
INIA Churrinche	EFED/ECOL	2*	7+8	5+10
INIA Mirlo	CAR853/COC//VEE5/3/URES	1	7+9	5+10
LE 2262	IA558/4/KAL/BB//CJ71/3/ALD/5/BOW	2*	17+18	5+10
LE 2265	PGO//CHEN/AE. SQUARROSA (224) /3/WEAVER	1	7+8	2+12
LE 2290	LE 2169/BUN'S'	1	7+8	2+12
LE 2294	IBOY//CLEO/INIA66	2*	7+9	5+10

wheat.

The experimental design used for all trials was a Randomized Complete Block design (RCBD) with three replications. The plots had six rows spaced 17 cm apart, trimmed to uniform length at mid-tillering. Final plot dimensions were 102 by 550 cm. The plots were sown at 300 viable seeds m^{-2}, according to the recommended rate for Uruguay. Experiment 2001, was planted on 6th July (Ia), and 1st August (Ib), in year 2001. Experiment 2002 was planted on 14th June (IIa), and 22nd July (IIb), year 2002. Chemicals were used for preventative control of weeds, insects, and diseases. Herbicides used to control weeds were 2-4D amina (1 l ha^{-1}) plus Tordon (0.1 l ha^{-1}), and Hussar (90 g ha^{-1}) plus Glean (20 g ha^{-1}). Insecticides used were Alsistyn (0.05 l ha^{-1}) plus Pirimor (150 g ha^{-1}), and Karate (0.1 l ha^{-1}). To control foliar and spike diseases in Experiment 2001, Swing (1 l ha^{-1}) (a.i. epoxiconazole + carbendazim), and Amistar (0.5 l ha^{-1}) (a.i. azoxystrobine) were applied three and two times during the crop cycle, respectively. In 2001, Fusarium head blight (FHB) reached epidemic levels in the country. In 2002, FHB was expected to be a problem because of the remaining inoculum. In Experiment 2002, Swing was replaced by Caramba (1 l ha^{-1}) (a. i. metconazole), which in previous experiments had shown a higher efficiency for FHB control. However, FHB was not effectively controlled in either of the two years. Early planting dates were the ones more affected. To address this concern, a covariance analysis was performed to remove the confounding effect of this variable over the other dependent variables of interest.

The rainfall during the months of wheat grain filling was abundant, and exceeded the historical records in both years. It can be assumed that there was no drought effect in these experiments.

Collection of data

Plots were harvested with a combine machine. Resulting grain was pre-cleaned (5.5mm x 5.5 mm screen) and air ventilated before further measurements were done. Grain yield values were obtained from all replications of each experiment. Two complete replications were used for measuring test weight, thousand kernel weight, grain protein concentration, Fusarium kernel damage, and for milling.

Grain yield (Y): weight of the grain at field maturity, reported as kg ha^{-1}.

Thousand-kernel weight (TKW): three samples of 100 random kernels from each plot were weighed, and results were reported in grams/1000.

Test weight (TWT): weight of one liter of grain from each plot. Results were reported in kg hl^{-1}

Grain protein concentration (GP): N was determined by Kjeldahl (AACC Method 46-11, modified), with a Nitrogen:Protein factor of 5.7. Results are reported in percentage, at 13.5 % moisture basis.

Fusarium kernel damage (FKD): Kernels with any sign of being colonized by *Fusarium* (not only the mummy-type kernels) were counted from three samples of 100 random kernels from each plot. The average number of FKD of the three samples was reported, and expressed as a percentage of total kernels analyzed.

Milling conditions

Samples of wheat grains from two replications of each experiment were used for milling. Grain samples were tempered overnight at 15 % moisture and milled in a Buhler

mill MLU 202 (Bühler AG, Uzwil, Switzerland). Subsequent rheological analyses were performed using the flour obtained from this process.

Rheological properties

Rheological properties were evaluated using a 35 gram bowl mixograph (National Manufacturing Division, TMCO, Lincoln, NB, USA), performed using AACC Method 54-40A. Destilled water was added to obtain an optimum dough absorption, according to the equation $Y = 1.5X + 43.6$, where X = percent flour content (14 % moisture basis), and Y = percent absorption of water. The mixing parameters measured were mixogram maximum peak height (MPH) (cm) and mixing time (MT) (min). Alveographic analysis was performed according to AACC Method 54-30A, on a Chopin Alveograph (Tripette & Renaud Chopin, Villeneuve-la-Garenne, France). The test was performed for flour samples at a constant 50 % water addition (on a 15 % moisture basis) on the volume of sodium chloride solution. The resulting alveograms were used to determine gluten strength (W) (joules/10000), tenacity (P) (mm), extensibility (L) (mm), and P/L ratio. This P/L ratio was used as an indication of the rheological balance of the dough.

Ground whole wheat protein molecular weight distribution

The pre-cleaned grain, was further cleaned by hand, removing all grain with any sign of *Fusarium* colonization. This was done in order to minimize the effect of hydrolytic enzymes produced by the invasive fungus on proteins. Grain samples from

two replications were milled into ground whole wheat using an UDY-Cyclone Sample Mill (model MS, UD Corporation, Boulder, Colorado, USA) equipped with a 1 mm opening size screen. A 150 μm nitrex sieve was used with the ground samples to obtain uniform particle size.

Ground whole wheat protein molecular weight distribution was measured by size-exclusion high-performance liquid chromatography (SE-HPLC) (Dalla Rizza et al. 2005). Protein extraction was carried out as described by Larroque et al. (2000), with modifications. Proteins from flour (100 mg) were extracted with 10 ml of 0.5% SDS-0.05*M* sodium phosphate buffer, pH 6.9, and sonicated at 20 W for 15 sec, using an Ohtake Works sonicator (Tokyo, Japan) with a 9-mm diameter probe. The supernatant obtained by centrifugation of the samples at 16,000 x g for 10 min was filtered through a 0.45 μm polyvinylidene diflouride filter membranes (Durapore, Millipore Corp., Ireland) and heated for 2 min at 80°C in a water bath.

The SE-HPLC was conducted using an HPLC Äkta Purifier System (Amersham Pharmacia Biotech, Uppsala, Sweden). A Superdex 200 HR 10/30 size exclusion analytical column (10 x 300 nm) that has dextran covalent bonding to highly cross-linked porous agarose beads was used. Protein extract (20 μL) was injected into the column. The same extraction buffer was used for sample elution, which was done at a flow rate of 0.5 mL/min at room temperature. Absorbance was measured at 214 nm. All HPLC measurements were run in duplicate and means values were used. The elution profiles were integrated by the HPLC software.

Proportions of protein fractions were calculated as described by MacRitchie and Gupta (1993). The percentages in protein of soluble polymeric protein (SPP) (mostly

glutenin), monomeric protein (MP) (gliadin), and low molecular weight albumins and globulins (LMWAG) were determined from the total protein profile:

$$\% \text{ SPP} = (\text{area of peak 1/total area}) \times 100 \quad (1)$$

$$\% \text{ MP} = (\text{area of peak 2/total area}) \times 100 \quad (2)$$

$$\% \text{ LMWAG} = (\text{area of peak 3/total area}) \times 100 \quad (3)$$

Statistical analysis

Statistical analyses were performed using SAS computer software (SAS Institute, Cary, NC). Analysis of variance for all traits was performed with the General Linear Model (GLM) procedure, using data for each experiment separately. Analysis of covariance was performed for those variables which could have been influenced by the level of Fusarium kernel damage. A combined analysis for Experiments 2001 and 2002 was not performed, because the timing and intensity of the heat stress during the grain filling period was different for each year.

Associations between traits of interest were further investigated by computing Pearson's correlation coefficients for least square means from each experiment and each planting date separately.

Results

Severe Fusarium head blight (FHB) epidemics took place in Uruguay in years 2001 and 2002, affecting most of the wheat producing area. Grain samples obtained from both experiments were pre-cleaned as described in materials and methods, to minimize influence of FHB on grain and quality characteristics. However, this could not completely eliminate the influence of FHB. Fusarium kernel damage for the early planting date was on average 27 % and 23 % in years 2001 and 2002, respectively, and for the late planting date, 9 % and 14 % for the same years, respectively. Analysis of covariance is presented for variables that are influenced by the presence of FHB, to adjust their mean values to a comparable level of *Fusarium* infection within each experiment. Analysis of variance is presented for the ground whole wheat protein molecular weight distribution variables, as the samples used were extra cleaned by hand, and grains with any sign of *Fusarium* were discarded.

Experiment 2001.

High temperature conditions

Table 2.2 shows the number of days with maximum temperatures above 28°C to which the early (Ia) and late (Ib) planting dates were exposed. Development stage of genotypes at the moment of heat stress is presented by the range in Zadoks' scale (Z) and days after anthesis (DAA).

Table 2.2. Experiment 2001. Consecutive days with maximum temperatures in the range 28-36° C (T 28-36) which occurred during wheat grain filling stage, reported for each planting date in year 2001.

Planting date	Range of grain filling stage at heat stress occurrence		Temperature (°C)	T 28–36
	Zadoks' scale	DAA[1]		
Early (Ia)	65-75	4 – 20	29.0, 32.6	2
	71-77	16 – 32	29.5	1
	83-87	29 - 45	31.0, 32.4, 34.0, 28.3, 31.8, 35.5	6
Late (Ib)	65-73	5 – 17	29.5	1
	73-77	18 - 30	31.0, 32.4, 34.0, 28.3, 31.8, 35.5	6

[1] Days after anthesis

The early planting date had maximum temperatures in the range 28 – 36 °C in three stages of grain filling. Six out of total nine days of high temperatures were above 30°C, and only one of those had a temperature higher than 35°C. The first period consisted in two days of high temperature in the early stages of grain filling. Only one day of high temperature occurred in the second period, with genotypes ranging from Z 71-77, or 16-32 DAA. The last period of heat stress took place in medium to late grain filling. In this period, the plants were subjected to a more intense heat stress due to the number of consecutive days (6) with high temperatures.

Five out of seven days of high temperatures in the late planting date were higher than 30°C, and only one of those, was over 35°C. The period of two days of heat stress seen in the first planting date occurred before grain filling period had started in the second planting date. Thus, there were only two periods with high temperatures during grain filling. The first one was a brief exposure of one day to high temperature at Z 65 – 73, or 5 – 17 DAA. The second, and the most important, was the period with six consecutive days of high temperatures. The genotypes were in mid-grain filling stage.

Agronomic characteristics and grain protein concentration

Experiment 2001 had a mean grain yield (Y) of 4104 kg/ha, thousand kernel weight (TKW) of 30 g, test weight (TWT) of 75.2 kg/hl, and grain protein concentration (GP) of 12.4 %. TKW and TWT values are rather low for what would be considered a good wheat growing year, where TKW would be around 35 g and TWT >78 kg/hl, thus showing the disease problems that year 2001 had. There were differences between two

Table 2.3. Experiment 2001. Analysis of covariance (Fusarium kernel damage as covariate) and observed least square means for grain yield (Y) (kg/ha), thousand kernel weight (TKW) (grams), test weight (TWT) (kg/hl), and grain protein concentration (GP) (%), computed for ten selected wheat genotypes grown in field conditions.

Source of variation	df	Y (kg/ha)	TKW (grams)	df	TWT (kg/hl)	GP (%)
		Mean squares			*Mean squares*	
Rep	**2**	237898.0	0.38	**1**	1.00	0.021
Planting date (PD)	**1**	1765654.2 **	16.03 **	**1**	24.43 **	0.579 *
Genotype (G)	**9**	1413568.9 **	27.32 **	**9**	7.26 **	3.255 **
PD x G	**9**	154883.9	5.52 **	**9**	3.81 **	0.474 **
FKD	**1**	1789594.4 **	1.58	**1**	0.81	0.161
Error	**27**	174835.6	1.43	**16**	0.55	0.076
Total	**49**			**37**		
		Least square means			*Least square means*	
Planting date [A]						
Ia		3512	28		71.9	12.9
Ib		4696	32		78.5	11.9

[A] See Table 2
Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$.
FKD = Fusarium kernel damage, covariate.

planting dates for Y, TKW, TWT, and GP, as shown in the analysis of covariance Table 2.3. Of all these variables, the covariate was significant ($P<0.01$) only for Y, and means were adjusted. For the other variables there was no significant covariate, thus little correction in means resulted. The late planting date resulted in significantly higher average means ($P<0.01$) for all variables but GP. Grain protein concentration showed the lowest level in the late planting date ($P<0.05$), likely from dilution effect which occurs with high Y. Genotypic differences were significant for all variables ($P<0.01$). Three of the variables had a significant planting date x genotype interaction ($P<0.01$): TKW, TWT, and GP. However, none resulted in a significant crossing-over type interaction (Table 2.3a), meaning that the genotypes responded all in the same way between planting dates, but with differences in magnitude. Although the pooled genotype x planting date interaction was not significant for Y (Table 2.3), some significant differences in genotypes between planting dates were found (Table 2.3a).

Least square means for genotypes in each of the two planting dates, and the average for Y, TKW, TWT, and GP are presented in Table 2.3a. Taking into account the two planting dates, INIA Caburé showed the highest Y and lowest GP, while INIA Boyero had the lowest Y, and almost the highest GP. The highest GP level was observed in INIA Mirlo. The latter, which resulted from an introduction from CIMMYT with 'Veery' in its pedigree, is recognized for having high Y and GP. It was the second high grain yielding cultivar in this Experiment. Estanzuela Cardenal, a selection from the widely known 'Veery' cross, also showed high yield, but had the lowest TWT. The highest TWT and TKW were shown by LE 2262. This genotype also had higher TKW in

Table 2.3a. Experiment I. Least square means for grain yield (Y) (kg/ha), thousand kernel weight (TKW) (grams), test weight (TWT) (kg/hl), and grain protein concentration (GP) (%) for experiments with different timing of heat stress, in ten selected wheat genotypes.

Genotypes	Planting date[A]	Y (kg/ha)	TKW (g)	TWT (kg/hl)	GP (%)
E. Cardenal	**Ia**	3613 †	26 **	67.6 **	13.7 **
	Ib	5006	32	78.6	11.7
	average	***4309***	***29***	***73.1***	***12.7***
E. Pelón 90	**Ia**	3300 †	27 **	73.1 *	11.6
	Ib	4334	31	76.5	12.0
	average	***3817***	***29***	***74.8***	***11.8***
I. Boyero	**Ia**	2728 *	28	71.6 **	14.0 **
	Ib	3986	31	78.2	12.7
	average	***3357***	***29***	***74.9***	***13.4***
I. Caburé	**Ia**	4557 **	29	74.0 **	10.4
	Ib	5900	29	77.3	10.0
	average	***5228***	***29***	***75.6***	***10.2***
I. Churrinche	**Ia**	3512 *	26	73.5 **	12.7 †
	Ib	4551	27	77.9	12.1
	average	***4032***	***26***	***75.7***	***12.4***
I. Mirlo	**Ia**	4255	25 **	69.2 **	14.3 *
	Ib	5350	31	80.1	12.8
	average	***4803***	***28***	***74.7***	***13.5***
LE 2262	**Ia**	3575	30 **	74.5 **	13.8 *
	Ib	4064	39	83.6	12.5
	average	***3820***	***34***	***79.0***	***13.1***
LE 2265	**Ia**	3293 *	32	71.9 **	11.9
	Ib	4357	34	77.9	11.7
	average	***3825***	***33***	***74.9***	***11.8***
LE 2290	**Ia**	2995 **	29	70.9 **	13.3 **
	Ib	4945	31	77.5	11.0
	average	***3970***	***30***	***74.2***	***12.2***
LE 2294	**Ia**	3292 *	29	73.1 **	13.1 *
	Ib	4467	31	77.5	12.2
	average	***3879***	***30***	***75.3***	***12.6***
General mean	**Ia**	**3512** **	**28** **	**71.9** **	**12.9** *
	Ib	**4696**	**32**	**78.5**	**11.9**
	average	***4104***	***30***	***75.2***	***12.4***

[A] See Table 2

Level of significance: †, $P < 0.10$; *, $P<0.05$; **, $P<0.01$, for comparisons of planting dates within genotypes.

the controlled environment experiments (Chapter I). Finally, INIA Churrinche had the lowest TKW.

Only two genotypes did not show a significant change in Y between the two planting dates (Table 2.3a): LE 2262 and INIA Mirlo. All the others showed a significant higher Y in the late planting date, compared with the early one. Four out of the ten genotypes tested had significant changes in TKW: Estanzuela Cardenal, Estanzuela Pelón 90, INIA Mirlo, and LE 2262. All genotypes had significant higher values for TWT in the late planting date. There were three genotypes that did not change their GP values: Estanzuela Pelón 90, LE 2265, and INIA Caburé. For the other ones, early planting date significantly increased grain protein concentration.

Rheological properties

In experiment 2001, the average values for each rheological variables were (Table 2.4a): 3.5 min for MT, and 6.7 cm for MPH of the mixogram, and 173 joules/10000 for W, 62 mm for P, 74 mm for L, and 1.0 for P/L ratio of the alveogram. Although P/L ratio showed a desirable equilibrium between tenacity and extensibility of the dough, the W value appears to be low, which again suggests that disease affected the overall quality characteristics of the samples tested.

Table 2.4 presents analysis of covariance for mixogram and alveogram variables, which both measure rheological properties of the dough. The covariate was significant ($P<0.05$) only for maximum peak height, thus little correction in means resulted for the other variables. From the mixogram, only MPH showed a significant increase with late planting date ($P<0.01$).

Table 2.4. Experiment 2001. Analysis of covariance and observed least square means for mixograms parameters [mixing time (MT) (min), and maximum peak height (MPH) (cm)], and alveogram parameters [gluten strength (W) (joules/10000), tenacity (P) (mm), extensibility (L) (mm), and P/L ratio], computed for ten selected wheat genotypes grown in field conditions.

Source of variation	df	MT (min)	MPH (cm)	df	W (joules/10000)	P (mm)	L (mm)	P/L
		Mean squares			*Mean squares*			
Rep	**1**	0.142	0.235 *	**1**	7.4	3.6	99.1	0.00024
Planting date (PD)	**1**	0.074	0.593 **	**1**	2631.7 **	15.2	519.7 †	0.06271
Genotype (G)	**9**	1.511 **	0.814 **	**9**	5312.9 **	98.9 *	732.7 **	0.31724 **
PD x G	**9**	0.780 **	0.150 **	**9**	3059.4 **	121.5 *	323.4 *	0.27509 **
FKD	**1**	0.132	0.185 *	**1**	254.9	10.4	64.0	0.00061
Error	**16**	0.168	0.037	**15**	291.8	31.5	121.5	0.03721
Total	**37**			**36**				
		Least square means			*Least square means*			
Planting date [A]								
Ia		3.7	6.2		133	59	56	1.1
Ib		3.3	7.2		212	65	91	0.8

[A] See Table 2

Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$.

FKD = Fusarium kernel damage, covariate

Table 2.4a. Experiment 2001. Least square means for mixograms parameters [mixing time (MT) (min), and maximum peak height (MPH) (cm)], and alveogram parameters [gluten strength (W) (joules/10000), tenacity (P) (mm), extensibility (L) (mm), and P/L ratio], for experiments with different timing of heat stress, in ten selected wheat genotypes.

Genotypes	Planting date[A]	MT (min)	MPH (cm)	W (joules/10000)	P (mm)	L (mm)	P/L
E. Cardenal	**Ia**	3.5	5.7 *	100 †	54	47 †	1.0
	Ib	2.8	6.9	188	64	96	0.7
	average	***3.1***	***6.3***	***144***	***59***	***71***	***0.8***
E. Pelón 90	**Ia**	3.5	6.0	71 *	75	17 *	2.8 **
	Ib	3.0	6.6	169	56	83	0.8
	average	***3.3***	***6.3***	***120***	***65***	***50***	***1.8***
I. Boyero	**Ia**	2.8	6.5 **	115 **	50	54 *	0.8
	Ib	2.9	7.4	220	63	102	0.7
	average	***2.9***	***7.0***	***167***	***57***	***78***	***0.7***
I. Caburé	**Ia**	4.4	5.6	165 †	54	84	0.7
	Ib	4.6	5.8	201	60	88	0.7
	average	***4.5***	***5.7***	***183***	***57***	***86***	***0.7***
I. Churrinche	**Ia**	4.3	6.5 *	192 **	58	83	0.7
	Ib	4.7	7.0	268	68	108	0.6
	average	***4.5***	***6.7***	***230***	***63***	***95***	***0.7***
I. Mirlo	**Ia**	3.0	6.2 **	78 *	52	31 †	1.3
	Ib	1.9	8.0	196	56	89	0.6
	average	***2.4***	***7.1***	***137***	***54***	***60***	***0.9***
LE 2262	**Ia**	3.6	6.3 **	111 **	57 †	45	1.2
	Ib	3.4	7.6	231	78	80	1.1
	average	***3.5***	***6.9***	***171***	***67***	***63***	***1.1***
LE 2265	**Ia**	4.3 **	6.7 **	195 **	65	82	0.7
	Ib	1.8	7.6	120	54	72	0.8
	average	***3.0***	***7.2***	***157***	***59***	***77***	***0.8***
LE 2290	**Ia**	3.2	5.8 **	79 **	61	30 **	1.8 *
	Ib	4.0	7.5	233	74	85	0.9
	average	***3.6***	***6.6***	***156***	***67***	***58***	***1.3***
LE 2294	**Ia**	4.4	6.5 **	224 *	62	89	0.6
	Ib	4.1	7.5	291	76	109	0.8
	average	***4.3***	***7.0***	***258***	***69***	***99***	***0.7***
General mean	**Ia**	**3.7**	**6.2 ** **	**133 ** **	**59**	**56 †**	**1.1**
	Ib	**3.3**	**7.2**	**212**	**65**	**91**	**0.8**
	average	***3.5***	***6.7***	***173***	***62***	***74***	***1.0***

[A] See Table 2

Level of significance: †, $P < 0.10$; *, $P<0.05$; **, $P<0.01$, for comparisons of planting dates within genotypes.

Among the alveogram parameters, W and L also showed increases in the late planting date ($P<0.05$, and $P<0.10$, respectively). Genotypic differences and planting date x genotype interaction were significant for all rheological properties measuring variables.

Least square means for each genotype in the two planting dates and average over planting dates for mixogram and alveogram variables are displayed in Table 2.4a. Considering the mixogram parameters from both planting dates (Table 2.4a), INIA Churrinche and INIA Caburé had the longest mixing times. INIA Caburé is the only soft wheat used in this study, and it also had the lower MPH. The low MPH is due to low water absorption, while the long mixing time indicates some gluten strength. This combination is unusual in soft wheat cultivars, but explains why INIA Caburé can be used for both French-type bread and soft wheat products in Uruguay. INIA Mirlo showed the lowest MT and one of the highest MPH. This wheat is known for having high water absorption, but weak gluten, which coincides with the mixogram results. Although this cultivar had the highest GP (Table 2.3a), protein quantity does not compensate for lower protein quality. INIA Mirlo has the 1BL/1RS translocation, which has been previously shown to be a detrimental factor for baking quality (Lee at al. 1995, Castro 2001). LE 2265 had the highest MPH, and was the only genotype that showed a significant change in MT between planting dates ($P<0.01$), having a longer MT in the early one. The late planting date had the highest MPH values for all but two cultivars, which did not significantly change between planting dates: Estanzuela Pelón 90, and INIA Caburé.

Based on the average through the two planting dates (Table 2.4a), LE 2294 had the highest W, P, and L , and a P/L ratio of 0.7, a little bit under the optimum 1.0 value. This experimental line is well known for its good quality attributes, and was released

recently in Argentina. Estanzuela Pelón 90, an introduction from CIMMYT released in 1990, shows the lowest W and L, and high P/L ratio, denoting high dough tenacity. These CIMMYT materials are known for their high yielding but poor quality characteristics. INIA Mirlo showed the lowest P. All genotypes had a significant change in W, with the highest values occurring in the late planting date, but for LE 2265, which had the highest value in the early planting date. Only one genotype, LE 2262, had significant change in P ($P<0.10$). There were five genotypes with significant increase in L value in the late planting date: LE 2290 ($P<0.01$), INIA Boyero, Estanzuela Pelón 90 (both at $P<0.05$), Estanzuela Cardenal and INIA Mirlo (both at $P<0.10$). P/L decreased in the late planting date for only two genotypes: Estanzuela Pelón 90 ($P<0.01$), and LE 2290 ($P<0.05$).

Ground whole wheat protein molecular weight distribution

Analysis of variance and least square means for grain protein molecular weight distribution is presented in Table 2.5. Planting date had a significant effect ($P<0.10$) on MP and SPP/MP. MP showed the highest mean value in the early planting date. As SPP/MP is a ratio between the former variable and SPP, it is not unexpected that SPP/MP would decrease in the early planting date. There were significant differences among genotypes for all variables ($P<0.01$). The pooled planting date x genotype interaction was significant only for LMWAG ($P<0.05$). However, some genotypes did show significant differences between planting dates for SPP and SPP/MP (Table 2.5a).

Table 2.5. Experiment 2001. Analysis of variance and observed least square means for protein molecular weight distribution: soluble polymeric protein (SPP) (%), monomeric protein (%), low molecular weight albumins and globulins (LMWAG), and SPP/MP ratio, computed for ten selected wheat genotypes grown in field conditions.

Source of variation	df	SPP (%)	MP (%)	LMWAG (%)	SPP/MP
		Mean squares			
Rep	1	4.4	0.53	1.87	0.0018
Planting date (PD)	1	11.4	19.68 †	1.13	0.0094 †
Genotype (G)	9	60.6 **	81.24 **	6.36 **	0.0422 **
PD x G	9	3.6	2.04	2.73 *	0.0016
Error	19	5.0	5.19	0.82	0.0031
Total	39				
		Least square means			
Planting date [A]					
Ia		29.1	61.5	9.4	0.481
Ib		30.2	60.1	9.7	0.511

[A] See Table 2

Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$.

Least square means for grain protein molecular weight distribution for each genotype in the two planting dates and the average are shown in Table 2.5a. On average, SPP was 29.7 %, MP was 60.8 %, LMWAG was 9.6 % and SPP/MP ratio was 0.496. LE 2265 was the genotype that had the largest SPP and LMWAG, and the smallest MP. Thus, it also had the largest SPP/MP ratio. Estanzuela Cardenal showed the smallest SPP and the largest MP, resulting in the lowest SPP/MP ratio. The lowest LMWAG was shown by INIA Mirlo.

LE 2290 was the only genotype showing significant increase in SPP and SPP/MP ($P<0.10$) in the late planting date (Table 2.5a). For LMWAG variable, three genotypes showed differences between planting dates: Estanzuela Cardenal, INIA Boyero (both at $P<0.01$), and INIA Churrinche ($P<0.10$). The first two had higher LMWAG values in the late planting date, while the contrary occurred with the last genotype.

Table 2.5a. Experiment 2001. Least square means for protein composition: soluble polymeric protein (SPP) (%), monomeric protein (%), low molecular weight albumins and globulins (LMWAG), and SPP/MP ratio, for experiments with different timing of heat stress, in ten selected wheat genotypes.

Genotypes	Planting date [A]	SPP (%)	MP (%)	LMWAG (%)	SPP/MP
E. Cardenal	**Ia**	25.1	67.8	7.1 **	0.370
	Ib	23.2	66.7	10.1	0.352
	average	***24.2***	***67.3***	***8.6***	***0.361***
E. Pelón 90	**Ia**	27.1	63.0	9.9	0.430
	Ib	27.9	61.6	10.5	0.454
	average	***27.5***	***62.3***	***10.2***	***0.442***
I. Boyero	**Ia**	27.7	63.6	8.8 **	0.436
	Ib	27.7	60.3	12.0	0.460
	average	***27.7***	***62.0***	***10.4***	***0.448***
I. Caburé	**Ia**	31.8	57.1	11.1	0.561
	Ib	32.1	57.1	10.8	0.567
	average	***32.0***	***57.1***	***11.0***	***0.564***
I. Churrinche	**Ia**	32.6	57.9	9.5 †	0.563
	Ib	34.0	58.1	7.9	0.586
	average	***33.3***	***58.0***	***8.7***	***0.575***
I. Mirlo	**Ia**	26.0	66.0	8.0	0.394
	Ib	26.9	66.0	7.1	0.408
	average	***26.5***	***66.0***	***7.6***	***0.401***
LE 2262	**Ia**	26.4	64.9	8.7	0.408
	Ib	25.5	64.7	9.7	0.395
	average	***26.0***	***64.8***	***9.2***	***0.401***
LE 2265	**Ia**	35.3	53.5	11.1	0.660
	Ib	37.2	51.7	11.0	0.720
	average	***36.3***	***52.6***	***11.1***	***0.690***
LE 2290	**Ia**	30.7 †	60.7	8.6	0.510 †
	Ib	35.2	57.3	7.5	0.614
	average	***32.9***	***59.0***	***8.1***	***0.562***
LE 2294	**Ia**	28.7	60.6	10.8	0.473
	Ib	32.2	57.6	10.3	0.559
	average	***30.4***	***59.1***	***10.5***	***0.516***
General mean	**Ia**	**29.1**	**61.5** †	**9.4**	**0.481** †
	Ib	**30.2**	**60.1**	**9.7**	**0.511**
	average	***29.7***	***60.8***	***9.6***	***0.496***

[A] See Table 2

Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$, for comparisons of planting dates within genotypes.

Experiment 2002.

High temperature conditions

Table 2.6 shows the number of days with maximum temperatures above 28°C to which the early (IIa) and late (IIb) planting dates were exposed. Development stage of genotypes at the moment of heat stress is presented by the range in 'Zadoks' scale (Z) and days after anthesis (DAA).

The early planting date had maximum temperatures in the range 28 – 32°C in two periods of grain filling. One out of total six days of high temperatures was superior to 30°C, and none had a temperature higher than 35°C. The first period of four consecutive days of high temperatures coincided with the beginning of the grain filling period. The second period was a brief exposure of two days of high temperatures, and the genotypes were at the end of grain filling stage.

The late planting date had maximum temperatures in a wider range than the early one, 28 - 35°C, in four stages throughout almost all the grain filling period of the genotypes. Half of the days out of total twelve days of high temperatures were above 30°C, and none had a temperature higher than 35°C. In this planting date the plants had a higher heat load than in the early one. The first period with high temperatures was the same that caught the early planting date at the end of grain filling. For this planting date however, genotypes were at the initial to middle stage of grain filling. The second period consisted of three days, and contained the highest temperature to which this planting date was subjected (34.5°C). Genotypes were in mid-grain filling stage. The third period

Table 2.6. Experiment 2002. Consecutive days with maximum temperatures in the range 28-35° C (T 28-35) which occurred during wheat grain filling stage, reported for each planting date in year 2002.

Planting date	Range of grain filling stage at heat stress occurrence		Temperature (°C)	T 28–35
	Zadoks' scale	DAA[1]		
Early (IIa)	65-70	8 – 18	29.8, 31.9, 29.6, 29.2	4
	85-90	35 – 44	28.5, 29.5	2
Late (IIb)	69-75	14 – 26	28.5, 29.5	2
	73-83	19 – 31	29.2, 30.9, 34.5	3
	75-85	23 – 35	32.5, 28.4, 29.2, 30.0	4
	83-90	30 - 42	32.0, 33.4, (22.0), 29.8	3

[1] Days after anthesis

(22.0) = Temperature not considered to be in the heat stress range.

occurred close to the second one, with four consecutive days of high temperatures, while the genotypes were still around the mid-filling stage. The last period, with two consecutive days of high temperatures, a third day with normal growing temperatures (22°C), and a fourth one again with high temperature, occurred in the mid to late grain filling stage.

Agronomic characteristics and grain protein concentration

Experiment 2002 had a mean grain yield (Y) of 3721 kg/ha, thousand kernel weight (TKW) of 30 g, test weight (TWT) of 73.3 kg/hl, and grain protein concentration (GP) of 13.8 %. Comparing with Experiment 2001, Experiment 2002 resulted in lower grain yield and higher grain protein concentration, while thousand kernel weight and test weight remained almost the same. Table 2.7 displays analysis of covariance and least square means for Y, TKW, GP, and TWT. The covariate variable FKD, was not significant for any of the variables. Late planting date showed a significant increase for GP ($P<0.05$), and TWT ($P<0.01$). Genotypic differences were significant ($P<0.01$) for all the variables. Planting date x genotype interaction was significant ($P<0.01$) for almost all the variables. The pooled planting date x genotype interaction was not significant for TKW. However, some significant differences in genotypes between planting dates were found for this variable.

Table 2.7a shows least square means for genotypes in each planting dates, and the average. INIA Caburé was the genotype with the highest Y and lowest GP, while in INIA Boyero the contrary occurred. INIA Mirlo had one of the highest GP. These results

Table 2.7. Experiment 2002. Analysis of covariance and observed least square means for grain yield (Y) (kg/ha), thousand kernel weight (TKW) (grams), test weight (TWT) (kg/hl), and grain protein concentration (GP) (%), computed for ten selected wheat genotypes grown in field conditions.

Source of variation	df	Y (kg/ha)	TKW (grams)	GP (%)	df	TWT (kg/hl)
		Mean squares (MS)				*MS*
Rep	**1**	29502.1	3.3	0.2744 *	**1**	0.904
Planting date (PD)	**1**	63028.6	6.4	0.2068 *	**1**	174.136 **
Genotype (G)	**9**	943247.9 **	22.2 **	0.8976 **	**9**	18.044 **
PD x G	**9**	840466.7 **	7.7	0.5493 **	**9**	4.632 **
FKD	**1**	40638.1	6.2	0.0095	**1**	0.022
Error	**18**	77435.9	4.4	0.0391	**17**	0.350
Total	**39**				**37**	
		Least square means (LSM)				*LSM*
Planting date [A]						
IIa		3779	29	13.7		70.4
IIb		3662	30	13.9		76.2

[A] See table 6

Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$.

FKD = Fusarium kernel damage, covariate

Table 2.7a. Experiment 2002. Least square means for grain yield (Y) (kg/ha), thousand kernel weight (TKW) (grams), test weight (TWT) (kg/hl), and grain protein concentration (GP) (%) for experiments with different timing of heat stress, in ten selected wheat genotypes.

Genotypes	Planting date[A]	Y (kg/ha)	TKW (g)	TWT (kg/hl)	GP (%)
E. Cardenal	**IIa**	3926 **	31	69.6 **	13.9 *
	IIb	2928	30	74.3	14.4
	average	***3427***	***31***	***71.9***	***14.1***
E. Pelón 90	**IIa**	2980	28	67.6 **	13.5
	IIb	3462	32	76.9	13.9
	average	***3221***	***30***	***72.2***	***13.7***
I. Boyero	**IIa**	3803 **	30	71.8 **	13.8 **
	IIb	2490	27	75.9	15.4
	average	***3146***	***29***	***73.8***	***14.6***
I. Caburé	**IIa**	4239 *	27	68.3 **	13.6 **
	IIb	5170	26	74.6	12.1
	average	***4705***	***27***	***71.4***	***12.9***
I. Churrinche	**IIa**	3841*	24 **	70.9 **	13.9
	IIb	4573	32	77.4	13.7
	average	***4207***	***28***	***74.2***	***13.8***
I. Mirlo	**IIa**	4497 **	31	72.8 **	14.1 **
	IIb	3261	30	76.2	14.8
	average	***3879***	***30***	***74.5***	***14.5***
LE 2262	**IIa**	5294 **	35	77.5 **	13.5
	IIb	3433	34	79.7	13.8
	average	***4363***	***35***	***78.6***	***13.7***
LE 2265	**IIa**	3250	30 †	64.8 **	13.7
	IIb	3409	34	75.0	13.5
	average	***3330***	***32***	***69.9***	***13.6***
LE 2290	**IIa**	2490 **	26	*m.d.*	13.7
	IIb	4493	28	76.2	13.5
	average	***3492***	***27***	-	***13.6***
LE 2294	**IIa**	3466	29	70.1 **	13.6 *
	IIb	3400	31	76.2	14.1
	average	***3433***	***30***	***73.1***	***13.8***
General mean	**IIa**	**3779**	**29**	**70.4 ** **	**13.7 * **
	IIb	**3662**	**30**	**76.2**	**13.9**
	average	***3721***	***30***	***73.3***	***13.8***

[A] See Table 6.

Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$, for comparisons of planting dates within genotypes. *(m.d.)* = missing data (-) no average data available due to *m.d.*

coincide with the ones reported in Experiment 2001. As was seen throughout all this study (Chapter I and II), LE 2262 ('Tinamou' cross) again had the highest TKW and TWT, denoting how remarkable this genotype is in grain physical quality characteristics. In this experiment, INIA Caburé showed the lowest TKW, together with LE 2290. LE 2265 had the lowest TWT.

Rheological properties

In Experiment 2002, mixogram variables had a mean mixing time (MT) of 3.5 min, maximum peak height (MPH) of 4.8 cm; and alveogram parameters mean gluten strength (W) of 138 joules/10000, tenacity (P) of 59 mm, extensibility (L) of 58 mm, and P/L ratio of 1.1. Comparing with Experiment 2001, P/L ratio was maintained, while W and MPH were lower. Table 2.8 shows analysis of covariance and least square means for mixogram and alveogram parameters. The covariate Fusarium kernel damage (FKD) was not significant for any of these variables. The late planting date had a significant increase for MPH, W and P variables ($P<0.01$). Genotypic differences were significant for MT, MPH ($P<0.01$), and W ($P<0.05$). A significant planting date x genotype interaction was found for W, P, MT ($P<0.01$), and MPH ($P<0.05$). Although the pooled planting date x genotype interaction was not significant for L and P/L, some significant differences in genotypes between planting dates were detected.

The genotypes response in rheological parameters to the different planting dates and the average are shown in Table 2.8a. INIA Mirlo had the lowest MT and the highest MPH, while the contrary occurred with INIA Caburé, in mixogram variables. These results are in agreement with those found in Experiment 2001. Like in Experiment 2001,

Table 2.8. Experiment 2002. Analysis of covariance and observed least square means for mixograms parameters [mixing time (MT) (min), and maximum peak height (MPH) (cm)], and alveogram parameters [gluten strength (W) (joules/10000), tenacity (P) (mm), extensibility (L) (mm), and P/L ratio], computed for ten selected wheat genotypes grown in field conditions.

Source of variation	df	MT (min)	MPH (cm)	df	W (joules/10000)	P (mm)	L (mm)	P/L
		Mean squares			*Mean squares*			
Rep	1	0.268 *	0.086	1	153.2	20.6	131.9	0.052
Planting date (PD)	1	0.031	0.585 **	1	9989.8 **	896.4 **	46.4	0.163
Genotype (G)	9	3.994 **	0.873 **	9	2097.6 *	54.4	97.4	0.020
PD x G	9	0.313 **	0.104 *	9	6378.4 **	354.5 **	312.3	0.190
FKD	1	0.034	0.027	1	681.4	15.1	24.1	0.015
Error	18	0.054	0.042	17	668.8	50.7	169.9	0.099
Total	39			38				
		Least square means			*Least square means*			
Planting date [A]								
IIa		3.5	4.6		115	53	57	1.0
IIb		3.4	5.0		161	66	60	1.2

[A] See table 6

Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$.

FKD = Fusarium kernel damage, covariate.

Table 2.8a. Experiment 2002. Least square means for mixograms parameters [mixing time (MT) (min), and maximum peak height (MPH) (cm)], and alveogram parameters [gluten strength (W) (joules/10000), tenacity (P) (mm), extensibility (L) (mm), and P/L ratio], for experiments with different timing of heat stress, in ten selected wheat genotypes.

Genotypes	Planting date[A]	MT (min)	MPH (cm)	W (joules/10000)	P (mm)	L (mm)	P/L
E. Cardenal	**IIa**	2.7	4.8	207 *	65	78 †	0.8
	IIb	2.8	4.7	125	66	46	1.5
	average	***2.7***	***4.8***	***166***	***65***	***62***	***1.2***
E. Pelón 90	**IIa**	2.9	4.2	80	48	53	1.0
	IIb	2.6	4.5	110	57	51	1.1
	average	***2.7***	***4.3***	***95***	***53***	***52***	***1.0***
I. Boyero	**IIa**	2.8	5.0 †	117 *	47 **	73	0.7 *
	IIb	3.0	5.5	176	74	57	1.3
	average	***2.9***	***5.3***	***147***	***61***	***65***	***1.0***
I. Caburé	**IIa**	4.4 **	3.7	85 **	42 **	57	0.8
	IIb	5.5	4.1	186	70	61	1.1
	average	***5.0***	***3.9***	***136***	***56***	***59***	***1.0***
I. Churrinche	**IIa**	5.0 *	4.3 *	98 **	48 *	46 *	1.1
	IIb	4.4	4.9	217	66	78	0.8
	average	***4.7***	***4.6***	***157***	***57***	***62***	***1.0***
I. Mirlo	**IIa**	2.5 †	5.4	180 *	65	66	1.0
	IIb	2.0	5.4	123	65	57	1.3
	average	***2.2***	***5.4***	***152***	***65***	***62***	***1.1***
LE 2262	**IIa**	4.6 **	4.9	114	55	56	1.0
	IIb	3.4	4.8	136	64	52	1.3
	average	***4.0***	***4.9***	***125***	***59***	***54***	***1.1***
LE 2265	**IIa**	2.3	4.3	139 *	70 **	46	1.5 *
	IIb	2.0	4.7	73	45	58	0.7
	average	***2.2***	***4.5***	***106***	***57***	***52***	***1.1***
LE 2290	**IIa**	3.9	4.6 **	35 **	32 **	46	0.8
	IIb	4.0	5.7	216	82	61	1.3
	average	***4.0***	***5.1***	***125***	***57***	***53***	***1.0***
LE 2294	**IIa**	3.6	5.0 *	91 **	52 **	48 *	1.1
	IIb	4.0	5.6	251	76	81	1.0
	average	***3.8***	***5.3***	***171***	***64***	***64***	***1.0***
General mean	**IIa**	**3.5**	**4.6 ** **	**115 ** **	**52 ** **	**57**	**1.0**
	IIb	**3.4**	**5.0**	**161**	**66**	**60**	**1.2**
	average	***3.5***	***4.8***	***138***	***59***	***58***	***1.1***

[A] See Table 6.
Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$, for comparisons of planting dates within genotypes.

Estanzuela Pelón 90 had the lowest W, P and L, and LE 2294, had the highest W and almost the highest P. All values for P/L ratio were around the optimum 1.0. Significant changes between planting dates for MT occurred in INIA Mirlo ($P<0.10$), INIA Churrinche ($P<0.05$), LE 2262 and INIA Caburé ($P<0.01$) (Table 2.8a). The first three showed a higher mean value in the early planting date, while the last one had the larger mean value in the late planting date. Late planting date had a significant increase for MPH in four genotypes: INIA Boyero ($P<0.10$), INIA Churrinche, LE 2294 ($P<0.05$), and LE 2290 ($P<0.01$). For W (Table 2.8a), all genotypes but two (Estanzuela Pelón 90 and LE 2262) showed significant changes between planting dates. Crossing-over interaction was found among the genotypes. Half of them had a larger W in the early planting date, while the other half had a larger W in the late one. Six genotypes showed significant changes in P (Table 2.8a). Five of them increased P in the late planting date, while one (LE 2265), had a larger P in the early planting date ($P<0.05$). In the same table, it can be seen that L showed significant changes between planting dates for Estanzuela Cardenal ($P<0.10$), INIA Churrinche and LE 2294 ($P<0.05$). While the first one had a higher L in the early planting date, the contrary occurred with the other two. P/L ratio changed significantly only for two genotypes: INIA Boyero and LE 2265 ($P<0.05$). The first one had the largest mean value in the late planting date, while the second showed it in the early planting date.

Ground whole wheat protein molecular weight distribution

Experiment 2002 had a mean of soluble polymeric protein (SPP) of 30.3 %, monomeric protein (MP) of 59.8 %, low molecular weight albumins and globulins (LMWAG) of 9.9 %, and a SPP/MP ratio of 0.516. These values are similar to those in Experiment 2001. Analysis of variance and least square means for grain protein molecular weight distribution is presented in Table 2.9. Significant planting date effects were detected for SPP and LMWAG ($P<0.05$). The first variable showed a higher mean in the early planting date, while the second one showed it in the late planting date. Genotypic effects were significant for all variables. A significant planting date x genotype interaction was found for SPP, MP, and SPP/MP ($P<0.01$). Although the pooled genotype x planting date interaction was not significant for LMWAG, significant differences in genotypes between planting have been found (Table 2.9a).

Least square means for genotypes in each planting date and the average, are shown in Table 2.9a. As in Experiment 2001, LE 2265 had one of the highest SPP and lowest MP, thus having the larger SPP/MP ratio. Estanzuela Pelón 90 showed the lowest SPP and SPP/MP ratio. INIA Mirlo had the highest MP mean and the lowest LMWAG. The highest mean value for this last variable corresponded to INIA Caburé.

There were four genotypes that did not show any changes between planting dates in any variable: LE 2262, LE 2265, LE 2290, and LE 2294. With the exception of LE 2290, these genotypes did not show changes in these variables in Experiment 2001 either. Many genotypes showed cross over type interaction. While late planting date had a significant decrease for SPP in Estanzuela Pelón 90, INIA Boyero, INIA Caburé and

Table 2.9. Experiment 2002. Analysis of variance and observed least square means for protein molecular weight distribution: soluble polymeric protein (SPP) (%), monomeric protein (%), low molecular weight albumins and globulins (LMWAG), and SPP/MP ratio, computed for ten selected wheat genotypes grown in field conditions.

Source of variation	df	SPP (%)	MP (%)	LMWAG (%)	SPP/MP
		Mean squares			
Rep	**1**	2.1	2.00	0.0024	0.0015
Planting date (PD)	**1**	13.7 *	0.59	19.8670 *	0.0035
Genotype (G)	**9**	69.8 **	86.00 **	6.3110 *	0.0462 **
PD x G	**9**	23.9 **	18.21 **	2.0498	0.0133 **
Error	**19**	3.0	2.58	9.9067	0.0016
Total	**39**				
		Least square means			
Planting date [A]					
IIa		30.9	59.9	9.2	0.525
IIb		29.7	59.7	10.6	0.506

[A] See table 6

Level of significance: †, $P< 0.10$; *, $P<0.05$; **, $P<0.01$.

Table 2.9a. Experiment 2002. Least square means for protein molecular weight distribution: soluble polymeric protein (SPP) (%), monomeric protein (%), low molecular weight albumins and globulins (LMWAG), and SPP/MP ratio, for experiments with different timing of heat stress, in ten selected wheat genotypes.

Genotypes	Planting date[A]	SPP (%)	MP (%)	LMWAG (%)	SPP/MP
E. Cardenal	**IIa**	26.0	65.1	8.8	0.400
	IIb	25.3	65.6	9.2	0.385
	average	***25.6***	***65.3***	***9.0***	***0.393***
E. Pelón 90	**IIa**	21.5 **	67.5 **	11.0	0.318 **
	IIb	26.9	61.3	11.8	0.439
	average	***24.2***	***64.4***	***11.4***	***0.379***
I. Boyero	**IIa**	37.0 **	54.8 **	8.2 †	0.676 **
	IIb	24.7	64.8	10.5	0.385
	average	***30.9***	***59.8***	***9.3***	***0.530***
I. Caburé	**IIa**	35.0 †	55.4	9.6 **	0.636
	IIb	31.9	54.5	13.6	0.586
	average	***33.5***	***54.9***	***11.6***	***0.611***
I. Churrinche	**IIa**	30.7 *	59.9 **	9.4	0.512 **
	IIb	35.0	55.0	9.9	0.637
	average	***32.9***	***57.5***	***9.7***	***0.575***
I. Mirlo	**IIa**	28.0 *	66.3	5.6 *	0.422
	IIb	24.2	66.8	9.0	0.363
	average	***26.1***	***66.6***	***7.3***	***0.392***
LE 2262	**IIa**	26.5	63.4	10.1	0.419
	IIb	27.0	62.3	10.7	0.434
	average	***26.8***	***62.8***	***10.4***	***0.426***
LE 2265	**IIa**	34.6	54.4	11.0	0.637
	IIb	35.4	54.1	10.5	0.655
	average	***35.0***	***54.2***	***10.8***	***0.646***
LE 2290	**IIa**	35.6	55.6	8.8	0.640
	IIb	34.7	55.2	10.0	0.629
	average	***35.2***	***55.4***	***9.4***	***0.635***
LE 2294	**IIa**	33.6	56.9	9.6	0.591
	IIb	31.7	57.4	10.9	0.552
	average	***32.6***	***57.2***	***10.2***	***0.572***
General mean	**IIa**	**30.9 ***	**59.9**	**9.2 ****	**0.525**
	IIb	**29.7**	**59.7**	**10.6**	**0.506**
	average	***30.3***	***59.8***	***9.9***	***0.516***

[A] See Table 6.

Level of significance: †, $P < 0.10$; *, $P<0.05$; **, $P<0.01$, for comparisons of planting dates within genotypes.

INIA Mirlo, it showed a significant increase in INIA Churrinche. Late planting date showed a significant increase for MP in INIA Boyero, and a significant decrease in Estanzuela Pelón 90 and INIA Churrinche. For the ratio of SPP/MP, late planting date had a significant increase in Estanzuela Pelón 90 and INIA Churrinche, while in INIA Boyero, had a significant decrease. For LMWAG, late planting date had a significant increase in INIA Boyero, INIA Caburé and INIA Mirlo.

Correlations among rheological properties and gluten protein molecular weight distribution variables.

The association between rheological properties and gluten protein molecular weight distribution variables was investigated using Pearson's correlation coefficients on the parameters means over replications for the ten spring wheats included in Experiments 2001 and 2002 (Table 2.10). Significant correlations were found in the early planting date (Ia) of Experiment 2001, and in the late planting date (IIb) of Experiment 2002. From the alveogram parameters, P did not show significant correlation with any of the gluten protein molecular weight distribution variables. In Ia, W and L were positively correlated with SPP and SPP/MP ratio, and negatively correlated with MP. P/L ratio was negatively correlated with SPP. In IIb, P/L ratio was the only parameter of the alveogram that showed significant correlations. It was negatively correlated with SPP and SPP/MP, and positively correlated with MP. For the mixogram parameters, only MT showed significant correlations. Both in Ia and IIb, it was positively correlated with SPP and SPP/MP ratio, and negatively correlated with MP.

Table 2.10. Phenotypic correlation coefficients among means of rheological parameters and gluten protein molecular weight distribution variables, for ten spring wheat genotypes grown in Young, Uruguay in 2001 and 2002 (early and late planting dates, Experiments 2001 and 2002, respectively).

	SPP				MP				SPP/MP			
	Experiment 2001		Experiment 2002		Experiment 2001		Experiment 2002		Experiment 2001		Experiment 2002	
	Ia	Ib	IIa	IIb	Ia	Ib	IIa	IIb	Ia	Ib	IIa	IIb
MT	0.72 **	0.28	0.14	0.45 *	-0.76 **	-0.20	-0.21	-0.55 *	0.72 **	0.22	0.15	0.47 *
MPH	0.04	0.06	-0.10	-0.08	-0.03	0.04	0.25	0.22	0.04	0.04	-0.17	-0.12
W	0.66 **	0.06	-0.26	0.30	-0.70 **	0.04	0.36	-0.30	0.66 **	-0.03	-0.30	0.30
P	0.02	0.06	-0.26	-0.07	-0.09	0.01	0.22	0.07	0.05	0.01	-0.25	-0.07
L	0.65 **	-0.02	-0.06	0.41	-0.69 **	0.05	0.24	-0.39	0.65 **	-0.08	-0.12	0.40
P/L	- 0.49 *	0.07	-0.12	-0.44 *	0.47	-0.05	-0.03	0.45 *	-0.45	0.09	-0.07	-0.45 *

Level of significance: *, $P<0.05$; **, $P<0.01$

Discussion

The following discussion for the response of the different variables to planting dates has been done assuming the covariate analysis removed the confounding effects of *Fusarium*. However, it cannot be forgotten that some differential effects on grain quality characteristics could have happened, for which the statistical analysis could not have accounted.

General response patterns

Temperatures during grain fill did not exceed the threshold temperature of > 35°C that has been reported as being detrimental to wheat dough rheological characteristics (Randall and Moss 1990, Blumenthal et al. 1991, 1993; Wrigley et al. 1994; Borghi et al 1995, Stone et al. 1997; Corbellini et al. 1998). Heat stress did not occur at exactly the same development stages and intensities in Experiment 2001 and 2002. However, there were similarities comparing early and late planting dates, which enable discussion of common results. In Experiment 2001, the early planting date had significant high heat load at the end of grain fill, when protein synthesis is mostly complete (Carceller and Aussenac 2001). The late planting date was exposed to heat stress at the middle of grain fill, when some influence of stress on grain composition would be expected. In Experiment 2002, the early planting date experienced heat stress both early and very late in grain fill. The situation resembled the one in Experiment 2001 for this same planting date. The late planting date for Experiment 2002 was exposed to heat stress throughout

the grain fill. Mid-grain fill stage had the highest number of consecutive days of stress, as did in this planting date in Experiment 2001.

Planting dates affected grain yield (Y), thousand kernel weight (TKW), test weight (TWT), and grain protein concentration (GP) because of different growing conditions and stresses during grain fill. Late planting date in Experiment 2001 resulted in 33 % more Y and 14 % more TKW than in early planting date. A dilution effect seemed to occur with GP in this experiment. While Y increased in the late planting date, GP decreased significantly from 12.9 % to 11.9 %. The late planting date had increases in TWT of 9% and 8% in Experiments 2001 and 2002, respectively. Y and TKW were not affected by planting dates in Experiment 2002. A significant increase in GP from 13.7 % to 13.9 % in the late planting date, could be attributed to the effect of high temperatures throughout the grain filling period. Similar results of GP increases were found by Correll et al. (1994), and Guedira et al (2002) with temperature stresses up to 30°C.

Rheological properties were affected by planting dates in both experiments. Mixogram MPH increased in both years with the late planting date (increases of 16 % and 9 %, respectively), as did alveogram W, (increases of 59 % and 40 %, respectively). For Experiment 2001, L was increased in late planting date by 63 % compared with early planting date. This indicates higher dough extensibility. Reduced P/L ratio (not significant) and increased L indicates a dough weakening effect of the high temperatures, even though temperatures did not exceed the 35°C threshold temperature. In Experiment 2002, P showed a 25 % increase in the late planting date, which indicates a higher tenacity of the dough. This particular increase, and increased P/L ratio (not significant), suggests a dough strengthening effect of high temperatures. These temperatures were

relatively lower than the ones experienced for the late planting date in Experiment 2001. Similar findings in the range of temperature up to 30°C have been reported by Randall and Moss (1990), Stone et al. (1997), and Corbellini et al. (1998). These studies and previous research suggest that the dough rheological characteristics have a curvilinear response to heat stress. In this study, dough properties have strengthened with temperatures up to 30°C, and weakened when temperatures were more extreme (34.5°C), similar to the results reported by Blumenthal et al. (1993).

As grain protein molecular weight distribution was assessed in samples that were further cleaned than the ones used to assess rheological properties, association among variables was investigated to ensure the representativeness of the former samples. Correlations with the alveogram parameters were not consistent throughout the two years of experiments. The alveograms were performed with fixed amount of water in genotypes that were, all but one, hard wheats. This could have been a problem for this rheological test because these hard wheats have a greater water absorption resulting from higher levels of starch damage occurring upon milling (Ross, personal communication). Ciaffi et al. (1996b) found that W and P of the alveogram were strongly correlated with insoluble polymeric proteins, which are the largest glutenin polymers. Unfortunately, this glutenin fraction was not measured by the method used in this study. Mixograms MT parameter showed a consistent relationship with gluten protein molecular weight distribution. Even in the cases where the correlations were not significant, they showed the same trend (Table 2.10). MT was positively correlated with SPP and SPP/MP, and negatively correlated with MP, as was expected. Singh et al. (1990) found this same relationship with dough development time and percent glutenin in total protein ($r=0.84$).

Changes in grain protein molecular weight distribution were related to differences in growing conditions between planting dates. However, there was no obvious trend that could link changes to response of the rheological variables. In Experiment 2001, MP had a 2% decrease from the early planting date to the late one. Monomeric proteins, mostly gliadins, have a positive influence on dough extensibility (L) (Ciaffi et al. 1996b). According to this, it would be expected that increase in MP would increase L, as seen in the late planting date. This did not occur. SPP/MP ratio increased from the early planting date (0.481) to the late planting date (0.511). This result supports studies that report that heat stress can enhance protein polymerization (Corbellini et al. 1997, Panozzo, 1997). Experiment 2002 showed a 4% decrease for SPP from the early to late planting date. Ciaffi et al (1996b) reported either a non significant or a significantly negative correlation of dough strength and tenacity with levels of soluble polymeric proteins. A lower SPP in the late planting date could contribute to the increase in W of the alveogram, but this is not the expected association. An increase of 15 % of LMWAG also was observed in the late planting date. Stone and Nicolas (1998a) reported that heat stress increases LMWAG, and also found strong and negative correlation (r=-0.91, P<0.0001) between albumin/globulin and polymeric protein. This could explain results observed in this experiment.

Individual genotype response

Table 2.11 summarizes significant changes in least square means between planting dates for the wheat genotypes in Experiments 2001 and 2002. Most of the changes in variables show increased values in the late planting date. Few changes were observed in the grain protein molecular weight distribution (MWD). It could be that the level of temperatures to which experiments were exposed was not sufficient to cause changes in these variables.

Genotypes that are more stable over environments would be expected to have fewer differences in grain characteristics between planting dates. In contrast, unstable genotypes should have more changes, either increases or decreases, in values. A tentative grouping of genotypes was done according to the number of variables that show changes between planting dates, in the two experiments (Table 2.11). INIA Churrinche, with 13 changes out of 14 variables (13/14) would be considered the most unstable genotype. In this same group, INIA Boyero, LE 2290, and INIA Mirlo, could be considered as least stable genotypes, with ten or more changes between planting dates. On the other hand, LE 2265, INIA Caburé, LE 2262, Estanzuela Cardenal, and LE 2294 would be considered as more stable genotypes with eight or fewer changes between planting dates. The last two genotypes would also be considered stable ones regarding quality characteristics, because they did not show changes in key traits like mixogram mixing time, alveograph P/L ratio and SPP/MP ratio. Estanzuela Pelón 90 (9/14) could be considered as an intermediate genotype. There is no obvious link among genotypes in each group, either in genetic background, or maturity. In both groups there are genotypes

Table 2.11. Significant changes in experiments 2001 (I) and 2002 (II) between planting dates in the fourteen variables and for the ten wheat genotypes tested in this study.

Genotypes	Y		TKW		TWT		GP		MT		MPH		W		P		L		P/L		SPP		MP		LMWAG		SPP/MP	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
E. Cardenal	+	-	+		+	+	-	+			+		+	-			+	-							+			
E. Pelón 90	+		+		+	+							+				+		-			+		-				+
I. Boyero	+	-			+	+	-	+			+	+	+	+		+	+			+		-		+	+	+		-
I. Caburé	+	+			+	+		-		+			+	+		+						-				+		
I. Churrinche	+	+		+	+	+	-			-	+	+	+	+		+		+				+		-	-			+
I. Mirlo		-	+		+	+	-	+		-	+		+	-			+					-				+		
LE 2262		-	+		+	+	-			-	+		+		+													
LE 2265	+			+	+	+			-		+		-	-		-				-								
LE 2290	+	+			+	*md*	-				+	+	+	+		+	+		-		+						+	
LE 2294	+				+	+	-	+			+	+	+	+		+		+										

(+) = significant least square mean increase from early to late planting date
(-) = significant least square mean decrease from early to late planting date
(*md*) = missing data

derived from CIMMYT's introductions and local crosses. There was no clear pattern of the HMW-GS subunits between groups. However, interestingly enough, INIA Churrinche was found to be a relatively unstable cultivar, and LE 2294 relatively stable, in both studies as reported in Chapter 1 and 2.

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CONCLUSIONS

Heat stress response varied depending on the range of temperature to which genotypes were exposed. A curvilinear response with increasing temperature has been suggested for many of these traits. Moderate to high heat stress at mid-grain filling stage increased test weight (8.5%) and thousand kernel weight (14%) in field conditions. With higher heat stress, as under controlled environment conditions, thousand kernel weight decreased 8.6 %, showing no difference in relation to duration or timing of stress.

Grain protein concentration increased with moderate to high heat stress in field and controlled environment conditions. Grain protein concentration was more affected by timing than by duration of the stress. Heat stress applied at 15 DAA (1w), or mid-grain fill, showed the highest grain protein concentration (18.4%), compared with the control and late stress treatments (17.7 and 17.6 %, respectively).

Rheological properties, were affected by heat stress under field conditions. Moderate to high heat stress at mid-grain filling stage, produced a weaker dough, and moderate heat stress during almost all the grain filling period, caused a strengthening of dough.

Impact of heat stress was inconsistent on protein molecular weight distribution in field conditions. Moderate to high heat stress at mid-grain filling stage lowered levels of monomeric proteins, while increased the ratio of soluble polymeric proteins/monomeric proteins. Moderate heat stress extended through all the grain fill, decreased the

percentage of soluble polymeric proteins, and increased the percentage of low molecular weight albumins and globulins. We were unable to detect effects of the stress on wheat protein molecular weight distribution in the controlled environment studies. However, with longer duration of heat stress applied, significant genotype x treatment interaction was detected in these conditions.

There were differences in relative stability of genotypes, in terms of changes in agronomic and quality related variables over environments. INIA Churrinche was found to be the least stable genotype in both types of studies. LE 2294 was found to be relatively more stable genotype, particularly with regard to stable quality characteristics, as measured by mixogram mixing time, alveograph P/L ratio, and SPP/MP ratio. Debeira and Pavón 76 had a tolerant response to heat stress, showing an increase of SPP/MP ratio with heat stress. These last three genotypes could be beneficial for overcoming the instability that heat stress produces in wheat production, or could be used as genetic sources for improving tolerance to this abiotic stress.

Further research is needed to understand the interaction of heat stress with other abiotic and biotic factors. Ability to predict crop quality before harvest, taking into account environmental factors and management practices, would be of great benefit to the milling industry. Such information would help the industry to identify problems and find solutions well in advance of wheat processing.

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