GENOTYPE AND ENVIRONMENTAL EFFECTS ON QUALITY TRAITS OF DURUM WHEAT GROWN IN NORTH DAKOTA

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Program:
Cereal Sciences

July 2020

Fargo, North Dakota

North Dakota State University

Graduate School

Title

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ABSTRACT

Variation in grain, semolina, dough strength, and pasta quality traits were evaluated using the effect of genotype and weather factors (air temperature, rainfall, and relative humidity). In addition, size exclusion high performance liquid chromatography (SE-HPLC) was applied to determine the correlation between quality traits and protein molecular weight distribution (MWD) with cooked firmness and cooked weight of fresh pasta made from four durum wheat genotypes. Results indicated that the environment was a great source of variation in the majority of quality traits such as test weight, 1000-kernel weight, grain protein content, vitreous kernel content, falling number, semolina protein content, semolina extraction rate, pasta color, and pasta cooking quality traits. However, grain yellow pigment content, semolina yellowness (b* value), gluten index, and mixogram time-to-peak were mainly affected by genotype.

High air temperature and days with temperature ≥ 30 °C were desirable for high protein content and high pasta cooking quality. Ideal growing locations to achieve the greatest falling number, vitreous kernel content, gluten index, and high pasta color were favored by low relative humidity and low rainfall. Days with temperature ≤ 13 °C favored high 1000-kernel weight and test weight. In addition, damp conditions such as high relative humidity favored 1000-kernel weight and semolina extraction rate.

Protein content and its fractions had a predominant role on the variation of fresh pasta cooked firmness and cooked weight, while gluten index did not relate to cooking quality. The quantitative increase in extractable monomeric protein (gliadins) was associated with a decline in cooked firmness, while it enhanced cooked weight. The possible gel forming properties of some protein fractions, including albumin + globulin during cooking were associated with high cooked firmness, low cooked weight, and low cooking loss in fresh pasta.

Genotypes differed in their genetic potential for quality traits evaluated and in the magnitude of their response to the environment. A trait is defined as stable when it is not greatly affected by the environment. Stable traits are necessary in order to have consistency in crop quality across years and growing locations.

ACKNOWLEDGMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Frank Manthey for providing invaluable advice and guidance throughout my Ph.D. research project. His encouragement has deeply inspired me not only put all my effort into conducting research but also it was a great privilege to apply my knowledge and skills for my future career.

Besides my advisor, my gratitude extends to the rest of my research committee, Dr. Senay Simsek, Dr. Jae-Bom Ohm, Dr. Elias Elias, and Dr. Julie Garden-Robinson for their valuable guidance and support. My special acknowledge expresses to Dr. Senay Simsek and Dr. Jae-Bom Ohm for their constant assistance and guidance and for providing this opportunity to conduct some parts of my research in their laboratories.

I am extending my thanks to the Durum Wheat and Pasta Processing Laboratory technicians for their constant assistance.

Last but not the least, I am extremely grateful to my parents, Abbas Moayedi and Ghamar Pour-Moayedi, and my brother Sam Moayedi for their continuous support and endless love.

DEDICATION

I dedicate this dissertation to my parents and my brother who encouraged me to pursue my dreams in achiving my goals in my life.

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GENERAL INTRODUCTION

Physical and biochemical properties of durum wheat are obtained by assessment of the impact of genotype, environment, and their interactions. Stability in quality traits is essential for both the durum wheat breeder and the durum wheat industry (Letta et al., 2008). According to Becker and Leon (1988), genotypes with high stability maintain their superior quality traits and performance under diverse environmental conditions. Trait stability is defined as maintaining its quality by having a low genotype × environment interaction (Grausgruber et al., 2000). Knowing the effect of genotype, environment, and genotype × environment interaction on quality traits provides useful insight when releasing genotypes with the good quality traits in the grain and final products.

Wheat millers desire grain that results in high semolina/flour extraction. High extraction is generally associated with high grain test weight, 1000-kernel weight, and uniform kernel size distribution (Clarke et al., 2012). Pasta industry desires semolina that has high protein content, strong gluten, and brilliant yellow color as these traits often result in bright yellow pasta that has excellent cooking properties (Clarke et al., 2012).

The environment has a predominant role in determining quality traits in grain such as test weight, 1000-kernel weight, falling number, kernel vitreousness, protein content, semolina extraction rate, semolina ash content, and pasta cooking quality traits; while yellow pigment content, polyphenol oxidase activity, and gluten index, are primarily under genetic control (Rharrabti et al., 2003 a,b; Vida et al., 2014; Ohm et al., 2017). Furthermore, genetic and environment interaction have an impact on gluten protein composition and its size and dough strength (Johansson et al. 2013).

Protein content and kernel vitreousness have been reported to be affected by the environment more than by genetics (Rharrabti et al., 2003b; Fois et al., 2011). The environment includes agronomic practices such as application of nitrogen fertilizer, control of weeds, insects, and diseases, and weather factors such as air temperature, rainfall, and humidity (Robinson et al., 1979; Rharrabti et al., 2003 a,b; Ferreira et al., 2012). Increase in protein content can be a result of enhanced deposition of storage proteins and/or by a decline in photosynthesis and accumulation of less starch due to pest damage to the plant or weather factors such as high air temperature or low soil moisture during grain filling duration (Gooding et al., 2003; Rharrabti et al., 2003b; Ozturk and Aydin, 2004; Ferreira et al., 2012; Pinheiro et al., 2013). Conversely, adequate soil moisture during planting and growing season can result in increased 1000-kernel weight and starch accumulation and decreased protein content (Altenbach et al., 2003). Combination effects of drought stress and high air temperature throughout grain filling (Rharrabti et al., 2003 b; Pinheiro et al., 2013) can cause a considerable decline in grain test weight and consequently yield (Gooding et al., 2003; Ozturk and Aydin, 2004; Grant et al., 2012). Falling number is favored by high air temperature and drought. While, cool and wet weather during grain filling (Lunn et al., 2001; Gooding et al., 2003), and damp conditions during mid to late harvest promote the production of α-amylase, and decrease in falling number (Manthey et al., 2004; Dencic et al., 2013).

Brightness and yellowness of pasta are important color attributes (Elias and Manthey, 2005). Genetic factors, environmental conditions, and technological processes can cause variation in the yellow pigment content of grain and semolina (Ficco et al., 2014). Yellowness in pasta was positively correlated to yellow pigment content which was strongly influenced by

genotype (Borelli et al., 1999), while, the environment had a predominant role in pasta brightness (Matsuo et al., 1982).

Cooking quality refers to cooked firmness, cooked weight, and cooking loss. The protein content is a predominant factor that determines pasta cooked firmness, and high semolina protein concentration makes pasta with superior cooking quality with low cooking loss (Dexter and Matsuo, 1977; Turnbull, 2001; Fois et al., 2011; Ohm et al., 2017). Protein quality is determined by gluten proteins (Hare, 2017; Ohm et al., 2017). Gluten strength reflects the ability of proteins to form a strong network capable of embedding starch granules and confer good cooking quality (Sissons, 2008). Gluten is composed of glutenins and gliadins which together determine dough strength, viscoelastic properties of dough, and technological properties of the final product (Ammar et al., 2000; Wieser and Kieffer, 2001). In fact, glutenins contribute to dough elasticity, while dough extensibility is mainly affected by gliadins (Edwards, et al., 2003). In addition, the relative proportion of glutenin subunits, their structure, and interactions in the polymeric network influence dough rheological properties (Wieser and Kieffer, 2001; Edwards, et al., 2003). Glutenin protein comprises of two subunits including high molecular weight (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). This separation is according to the different mobilities in SDS-PAGE (Edwards et al., 2003; Sissons et al., 2007). Results of correlation analysis indicated that association of HMW-GS and LMW-GS on dough resistance was the same; however, quantity of LMW-GS should be twice as much as HMW-GS to confer the same strength to the dough compared to HMW-GS (Wieser and Kieffer, 2001).

SDS-unextractable HMW-glutenin in total protein (%UPP) is known to correlate significantly with gluten strength, elastic property of gluten, and quality traits of bread in hexaploid wheat genotypes (Gupta et al., 1993; Ohm et al., 2017). The contribution of SDS-

extractable polymeric proteins negatively influenced gluten strength in hard spring wheat. Although Sissons et al. (2007) showed that HMW-GS did not have any impact on cooked pasta firmness, Ohm et al. (2017) highlighted the importance of (%UPP) in the improvement of durum wheat technological properties such as pasta firmness.

Semolina protein quantity and quality and yellow pigment concentration are the most important characteristics necessary to make high-quality pasta. Besides improvement in quality attributes, the development of genotypes with high tolerance to diverse climatic conditions is important to durum breeders when developing new cultivars (Uthayakumaran and Wrigley, 2017) because environmental conditions influence the performance of many quality traits (Rharrabti et al., 2003 b).

The durum wheat breeding program at North Dakota State University has expended much effort in developing durum genotypes that have excellent grain quality and end-use traits. As mentioned above, the environment involves both agronomic practices and weather factors. Most research on genotype and environment does not separate the environment into agronomic practices and weather factors.

The current research was undertaken with the following objectives:

1. To determine genotype and environmental effects on quality traits in durum wheat commercially grown in North Dakota and Montana.
2. To evaluate genotype response to weather factors and their impact on quality traits of nine durum wheat genotypes grown in 24 environments in North Dakota.
3. To determine correlation between quality traits and protein molecular weight distribution with pasta cooking quality of four durum wheat genotypes grown in North Dakota and Montana.

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CHAPTER 1: LITERATURE REVIEW

Durum Grain Structure and Composition

Durum grain (*Triticum turgidum* L. var. durum) contains three important sections such as bran, germ, and endosperm (Posner and Hibbs, 2005). The wheat kernel is surrounded by the bran layer. The bran restricts moisture movement into the kernel and protects the germ and endosperm from biotic pests. Bran is composed of an outer pericarp, inner pericarp, seed coat, and nucellar epidermis. Bran contains starch, protein, ash, lipid, and nonstarch polysaccharides which the latter are associated with dietary fiber (Kunerth and Youngs, 1984). From milling aspect, bran also includes the aleurone layer. Aleurone layer is one cell thick and is botanically part of the endosperm. Aleurone cells contain many hydrolytic enzymes. Aleurone layer contains high amounts of ash, protein, fat, and vitamins, and has high enzymatic activity (Hoseney, 1998a). Germ consists of embryonic axis and scutellum (Posner and Hibbs, 2005), and contains protein, sugar, lipid, ash, vitamins, and many enzymes. Endosperm encompasses the aleurone layer and starchy endosperm. Endosperm functions as storage source of protein and starch which are degraded into amino acids and sugar needed for the growth of developing embryo into a seedling (Hoseney, 1998a; Gruber and Sarkar, 2012). Cells in the endosperm are filled with starch granules surrpounded by a protein matrix (Hoseney, 1998a).

Grain Quality

Physical properties

Physical grain quality involves the determination of test weight, 1000-kernel weight, and kernel vitreousness. Test weight (kilogram per hectoliter) is a grading factor in the US and needs to be at least 78.2 kg/hL (60 lb/bu) to meet the US No. 1 grade requirement (USDA, 2013). Test weight is a combination of grain weight and grain packing efficiency, which are affected by both

grain size and shape (Dziki and Laskowski, 2005; Hare, 2017). Grain with an irregular shape, wrinkled, not fully filled, generally will result in low test weight. Test weight is useful in determining the amount of grain that can be stored or shipped in a given vessel. According to studies conducted by Pinheiro et al. (2013), test weight was positively correlated to semolina yield.

1000-kernel weight is evaluated based on counting 10 g of clean, sound, and unbroken kernel, and is affected by density and size of the grain (Sissons et al., 2012). Durum kernels typically have 1000-kernel weights in the range of 37 to 46 g (Regional Crop Survey, 2019). Grain (harvest) yield is positively correlated to 1000-kernel weight (Ozturk and Aydin, 2004; Posner and Hibbs, 2005). 1000-Kernel weight is an indication of wheat milling value. High extraction of durum semolina which is important from the milling point of view, was positively affected by high grain weight and large kernel size (Pinheiro et al., 2013).

Kernel vitreousness is important to durum wheat and is the basis of its subclassification in the US grading system (USDA, 2013). US grade includes the following subclasses: Hard Amber Durum ($\geq$75% vitreous kernels), Amber Durum (60 to 74% vitreous kernels), and Durum (<60% vitreous kernels). Vitreous kernel content positively correlated to protein content, and lack of vitreousness can be due to low protein content and/or to kernel bleaching due to moisture penetrating the endosperm. Vitreousness is determined by the degree of translucent grain (Pagnotta et al., 2005). The importance of vitreous kernel content is due to the fact that it reflects a kernel's tendency to fracture and produce coarse semolina particles. Nonvitreous kernels tend to be crushed and form fine flour particles during milling. Vitreous kernel content is an important quality characteristic determining superior milling yield and pasta making qualities, while grain

with lower level of vitreousity have less protein content with higher tendency to produce finer semonia particles (Sissons et al., 2012).

Chemical properties

Protein content and composition

Durum grain comprises of protein content (8-20% of dry matter) that has an essential role in pasta making both from a nutritional aspect and technological characteristics. Protein can be fractionated into four groups, prolamin, glutelin, albumin, and globulin, depending on solubility as described by Osborne fractionation. Prolamins are soluble in 70% ethyl alcohol, glutelins are soluble in dilute acid or base solutions, albumin proteins are soluble in water, and globulins in dilute salt solutions (Hoseney, 1998b). Albumins and globulins are non-gluten proteins which consist of structural protein, metabolic enzymes, and enzyme inhibitors (Lafiandra et al., 2012). Prolamins and glutelins are gluten forming proteins that are important for the production of pasta with superior texture. Gluten forming proteins are classified into gliadin and glutenin proteins (Sissons, 2008). The gliadins which account for 50-60% of the gluten proteins (Johansson et al, 2013), composed of monomeric proteins and are separated into α, β, γ, and ω fractions according to their mobility in polyacrylamide electrophoresis gel at acidic pH. Gliadins are involved in dough extensibility (Oak and Dexter, 2006). Glutenins which account for 40-50% of gluten forming proteins, can be fractionated into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (20% and 80%, respectively) (Lafiandra et al., 2012; Johansson et al., 2013). Dough elasticity is affected by glutenins (Sissons, 2008) and gluten network is formed through an exchange of thiol-disulfide reactions.

Starch content and composition

Starch makes up 80% of durum wheat and has an important role in pasta quality (Lafiandra et al., 2012; Uthayakumaran and Wrigley, 2017). Starch contains two polymers such as amylose and amylopectin. Amylose with a linear structure is made up of glucose molecules that are linked through α-1,4 (Hoseney, 1998a) and has a molecular weight of 10^5-10^6 (Sissons, 2008). Amylopectin comprises of α-D-glucose linked by α-1,4 bonds, and is greatly branched and has a molecular weight of 10^7-10^8 (Hoseney, 1998a). Amylopectin structure contains 4-5% of glycosidic bonds connected through α-1,6 linkage (Hoseney, 1998a). Crystalline domains of the starch granules are due to the clustered branches of amylopectin, while amylose determines the amorphous part of starch (Lafiandra et al., 2012).

Damaged starch contributes negatively to pasta quality. Starch granules can be damaged during milling and as a result of α-amylase activity. Falling number test indirectly measures α-amylase activity in grain and the latter associates with kernel sprouting. In fact, excess α-amylase activity hydrolyzes starch content in a grain and due to its negative effects on rheological characteristics of dough, the products' final quality is impaired (Dencic et al., 2013).

Lipid content/composition

Lipids located in the germ and aleurone layers are mainly nonpolar and consists of triacylglycerides (Morrison, 1988). Lipids localized in the endosperm are starch lipids and non-starch lipids. Starch lipids reside inside the helical coil of amylose and have no or little effects on flour functionality or baking (Morrison, 1988). Non-starch lipids are other types of lipids in grain and can be divided further into free (extractable by non-polar solvent) and bound (extractable by polar solvent) lipids. Components of both free lipids and bound lipids include free fatty acids, acylglycerols, hydrocarbon, and free sterols. The quantity of these components in free lipids are

relatively twice as much as their amount compared to that of bound lipids per 100 g, db (Lafiandra et al., 2012).

Pigments are lipid and are important in providing desirable color for pasta. Endosperm yellow pigment comprises three main components such as xanthophylls (lutein), carotenoids (carotene), and flavones (tricin). α-carotene and β-carotene along with β-cryptoxanthin (as part of xanthophylls) are considered as the provitamin- A- active carotenoids (Liu, 2007).

The yellow color of pasta depends on the carotenoid content of semolina and lipoxygenase activity (Pagnotta et al., 2005). Oxidation of yellow pigments can happen due to lipoxygenase activity during pasta processing including hydration, mixing, and extrusion through formation of free fatty acid radicals (Pagnotta et al., 2005). Grain yellow pigment content can be classified into three groups including low pigment (<5.0 ppm), medium pigment (5.0-7.0 ppm), and high pigment (>7.0 ppm) (Pagnotta et al., 2005). For commercial and nutritional purposes, prevention of carotenoid content from bleaching during processing is an important criterion that needs to be considered (Feillet, 2000).

Peroxidase and polyphenol oxidase (PPO) are associated with brown pigment content. PPO activity is formed early in kernel development and deceases as grain matures. During grain maturation, the level of PPO increases in embryo and scutellum with decreasing activity in the endosperm (Kruger, 1976). Since in mature grain most of PPO accumulates in the bran layer, it is least likely to associate with formation of brown color in semolina due to bran removal during the milling process (Kruger, 1976) unless there is bran contamination which would be a possible source of PPO and formation of brown color in semolina or pasta. Moreover, other flour components could contribute to variation in semolina lightness. In fact, an increase in protein and ash contents led to a decline in lightness (L*) after 24 h (Davies and Berzonsky, 2003).

Pasta Quality

Pasta quality includes color and cooking quality traits such as cooked firmness, cooked weight, and cooking loss. Brightness and yellowness of pasta as important color attributes (Elias and Manthey, 2005), are affected by semonlina color as well as durum wheat (*Triticum turgidum* L. var. *durum*) grains (Schulthess et al., 2013). In fact, carotenoid pigments concentration (Borrelli et al., 2008) and processing conditions have an impact on yellowness of semolina and pasta (Borrelli et al., 1999; Feillet, 2000). Formation of reducing sugar and Maillard reaction during very high-temperature drying influences pasta color (Pagani et al., 1992). In addition, an increase in peroxidase and PPO activity makes the brown color to be predominant over the yellow color of pasta (Feillet et al., 2000). Peroxidase enzymes are distributed in various parts of the grain during development, and aleurone layer is rich in peroxidase (Pagnotta et al., 2005). Lipoxygenase activity (LOX) has a significant role in bleaching flour pigment during pasta making (Feillet, 2000), and there is a high correlation between LOX activity and carotenoid loss.

Structural properties of the pasta is under influence of modification in the protein and starch such as protein coagulation and starch gelatinization during cooking (Irie et al., 2004). The principles of good cooking quality in durum wheat is the existense of a strong and elastic gluten protein network with a capability of holding swollen and gelatinized strach granules which limits disintigration of spaghetti surface and furthur leaching of amylose into cooking water during boiling (Feillet, 1988; Aalami, 2006). Low protein content leads to pasta with soft textural properties (Ounane et al., 2006), while high protein content in durum wheat leads to improved cooked firmness and lower cooking loss (Lafiandra et al., 2012). In addition, Hatcher et al. (2009) found that protein content affected resistance to compression and recovery of yellow alkaline noodles. In fact, an increase in protein content improves the formation of polypeptide

chains through increased interaction to make a resistant network (Feillet, 1988; Aalami, 2006). Moreover, high hydrophobicity of gluten proteins restricts water penetration into pasta strands during cooking and results in decreased swelling and surface disintegration (D'Egidio et al., 1979 as cited by Feillet, 1988). Gluten index as an indicator of gluten viscosity and elasticity (Marchylo et al., 2004), was not associated with cooked noodle texture due to nonsignificant correlation between these two traits (Hatcher et al., 2009).

Cooked weight and cooking loss are the other pasta cooking quality traits. Cooked weight was negatively associated with pasta firmness indicating that the level of water absorbed into pasta strands after cooking and expressed as the percentage.

Cooking loss has an effect on surface conditions of pasta such as degree of stickiness and lower cooking loss. Cooking loss is due to leaching of amylose or starch fragments from starch granules and of soluble proteins and other water-soluble compounds found in pasta. Cooking loss negatively correlated with semolina protein content (Ounane et al., 2006). In fact, coagulation of dense and continuous protein networks around starch granules limits the leaching of starch soluble molecules into cooking water during cooking of pasta and reduces cooking loss (Irie et al, 2004). Cooking loss is determined through water evaporation to dryness in a forced-air-oven at 110 °C overnight. The importance of starch in pasta quality is due to protein-starch interaction that has an impact on the viscoelastic behavior of dough. In fact, starch water uptake, gelatinization temperature, swelling, and the role of starch in the maintenance or breakdown of gluten network is a basis of pasta quality (Delcour, et al., 2000a,b).

Dough rheological properties, as well as spaghetti cooking quality, are influenced by amylose content in the starch granule. Dexter and Matsuo (1979) showed that amylose content positively affected pasta firmness. In particular, a high amount of amylose inside the granule

limited gelatinization and finally increased gelatinization temperature. In addition, Soh et al. (2006) found that tightly packed starch granules due to the high amount of amylose showed more resistance to rupture and deformation which explained firmer cooked pasta.

Elevated level of B-type starch granules associated with higher farinograph water absorption, greater pasta cooked firmness, and lower cooking loss as reported by Soh et al. (2006). They stated that the optimum B-type granule content was ~ 32-44%. B-Type granules have a high surface-to-volume ratio that resulted in fast hydration, and consequently efficient swelling. In addition, B-type granules cause more interaction between starch and protein due to their smaller shape and greater surface area, and reduced leaching of amylose during cooking that finally reduces cooking loss in pasta (Soh et al., 2006).

Role of Gluten Composition on Dough Strength and Pasta Quality

Variation in the gluten index could be affected by protein composition such as the percentage of unextractable polymeric protein and glutenin-to-gliadin ratio (Gupta et al., 1993; Saperstein and Fu, 1998). Dough mixing strength and baking quality, also, could be affected by unextractable glutenin proteins (Gupta et al., 1993; Sapirstein and Fu, 1998). In fact, the strong correlation of gluten strength with unextractable HMW-glutenin in total protein (%UPP) is associated with the elastic property of gluten (Gupta et al., 1993; Ohm et al., 2017). Ohm et al. (2017) also highlighted the importance of these components in the improvement of durum wheat technological properties such as pasta firmness. These results were in agreement with Gupta et al. (1993) who reported a strong and significant correlation between SDS-unextractable HMW polymeric protein in total protein with dough strength such as resistance or elasticity, and dough development time measured by both mixograph and farinograph with bread wheat flour. In addition, Edwards et al. (2007) found a strong correlation between %UPP with gluten index,

mixograph dough development time, peak resistance, and bandwidth at the peak, and a moderate correlation between %UPP with alveograph *P* and L values. Alveograph *P*-value is the maximum overpressure needed to blow the dough bubble indicating dough resistance to deformation. L value is the average abscissa (length) at rupture and indicates dough extensibility (Bettgeet al., 1989).

In fact, genotypes with UPP content equal to or less than 30%, had gluten index lower than 10%, whereas, those having UPP higher than 35%, had increased gluten index values. Adding isolated glutenin fractions to the flour led to an increase in dough strength. While, adding more gliadin and LMW-glutenins negatively affected dough strength with no influence on pasta texture (Sissons et al., 2007).

Wieser and Kieffer (2001) stated that glutenin subunits such as HMW-GS and LMW-GS contribute to dough rheological properties such as gluten index. Although the contribution of these two subunits on the improvement of dough resistance was the same (due to the arrangement of disulfide bonds), generally the quantity of LMW-GS should be twice as much as HMW-GS in order to have the same dough resistance compared to HMW-GS. However, dough extensibility was mainly affected by gliadin-to-glutenin ratio, meaning that a rise in gliadin quantity was contributed to the formation of more viscous dough. These results were in accordance with Edwards et al. (2003) who showed a weakening effect of gliadin on dough through a decline in mixing time in mixograph due to gliadin's inability to form a network structure.

The total amount and composition of HMW-GS affect gluten polymer structure (Johansson et al., 2013). In fact, results of Edwards et al. (2007) revealed that an optimum HMW-to-LMW-GS ratio ranging from 0.15 to 0.25 results in desirable dough properties,

however, genotypes having HMW/LMW ratios equal or above 0.30, associated with a particular allelic pattern in HMW-GS such as 7+8, 6+8, 20, and 14+15 that had the weak dough, weak gluten, and low unextractable polymeric protein in total protein (UPP content). Also, those genotypes including γ-gliadin 42 types resulted in lower unextractable polymeric protein in total protein.

Sissons et al. (2007) showed that high level of HMW-GS in semolina dough positively affected dough strength but did not affect cooked pasta firmness. However, Flagella et al. (2010) showed that cultivar Simeto had better pasta-making characteristics due to a higher amount of HMW-GS, higher HMW-GS/LMW-GS ratio, and higher %UPP (Flagella et al., 2010). Conversely, the results of Edwards et al. (2007) illustrated HMW-GS negatively correlated to the gluten index. In fact, LMW-GS played a predominant role in the arrangement of a well-developed gluten network due to the involvement of short-chain and formation of crosslinks, all of which improved dough strength.

Results of Chaudhary et al. (2016) showed that the first peak after fractionation of protein using SE-HPLC represented glutenin-rich components that were positively and strongly correlated to dough stability, dough development time, and dough resistance to extension (R/E) ratio, and led to an improvement in gluten index. Enhancement in dough development time was due to increased glutenin quantity, the formation of more disulfide bonds, and stronger gluten network in the dough. In contrast, their results indicated a negative correlation between gliadins and gluten index which was confirmed by Ohm et al. (2017). Presence of a negative correlation between gluten index and extractable monomeric protein in both total flour and total protein confirmed softening effect of gliadins on dough strength and their contribution to the viscous nature of dough and lower cooked pasta firmness (Edward et al., 2003; Ohm et al., 2017).

In addition, Ohm et al. (2017) reported a negative impact of extractable gliadins on mixogram peak time (MPT). Sissons et al. (2007) evaluated the influence of glutenin and gliadins as well as HMW-glutenin and LMW-glutenin on dough strength and pasta cooking quality by adding these components from two sources (one having good quality and one with poor quality) to base semolina which had a high RBD (percentage resistance breakdown) and short MPT. Results showed that the addition of both high quality and poor quality glutenin improved mixing tolerance. The addition of HMW-glutenin improved some mixograph parameters such as dough strength. Conversely, the addition of LMW-glutenin from stronger wheat source decreased MPT, while increased RBD. LMW-glutenin from a weaker source did not cause any significant change. Addition of gliadin extracted from good quality wheat, had a smoothing effect on mixogram, while gliadin from weaker source reduced width of the band at peak, and width of mixograph eight minutes after mixing (WA8), and increased RBD (Sissons et al., 2007).

Role of Protein Composition on Pasta Firmness

According to Fois et al. (2011), protein quantity favored by high temperature and drought during grain filling positively affected cooked pasta firmness due to possible modification in glutenin polymer aggregation. Similar results were obtained by Jia et al. (1996) who concluded that environmental elements through modification in protein polymerization affect quality characteristics of wheat flour. However, gluten strength was considered the secondary contributor in changing pasta texture which was dried at high temperatures. These findings were explained by Fois et al. (2011) who reported cultivar Trinakria led to the formation of greatest pasta firmness due to high protein content although it had the low gluten index.

In terms of protein composition, Sissons et al. (2007) found that the adding of glutenin and gliadin to semolina as a control sample during a reconstitution process resulted in pasta with lower firmness presumably due to inadequate incorporation of these components with dilution effect on the formation of gluten network. Furthermore, Dexter and Matsuo (1977) concluded that increase in glutenin/gliadin ratio and consequently moderate increase in protein content led to an improvement in dough rheological properties, as well as pasta firmness, and tolerance to overcooking of two Canadian durum cultivars (Wascana and Stewart, $r = 0.962$ and 0.983 at $p < 0.01$).

According to Joubert et al. (2018), mixing of dry semolina into wet agglomerates as well as the extrusion process resulted in segration and depolymerization of the SDS-unextractable glutenin polymers, and a rise in the amount of SDS-extractable protein content. Their results showed that the resting period after mixing and extrusion of semolina dough led to improvement in the percentage of unextractable polymeric glutenins (evolution of %UPP) due to re-assembly of SDS-unextractable glutenin, and a drop in SDS-extractable glutenins. Additionally, drying of pasta at 55 °C (17 h) significantly reduced total SDS soluble proteins. These findings suggested that after extrusion process, unextractable glutenin proteins are able to retrive which affect ultimate rheological properties of cooked pasta.

Genotype and Environment Effects on Quality Traits

Crop quality is determined by the genotypes grown and the environment during the growing and harvest season. Thus, genotype and environment individually and together determine overall grain and end-use quality of durum wheat. Genes that make up each cultivar are collectively referred to as its genotype. Genotype determines the genetic potential for a given trait. The ability to achieve genetic potential is affected by the environment.

Environmental conditions affect the performance of grain quality traits which can result in inconsistent quality for a given genotype (Rharrabti et al., 2003b). The environment consists of abiotic stresses, for example, weather and soil properties; and biotic stresses such as disease, insects, and weeds. Genotype × environment interaction is defined as deviation in the genotypic response while exposed to various environmental situations. Combined effects of genotype × environment interaction leads to greater complexity in choosing of breeding line with superior quality traits in genotypes (Ames et al., 1999). Thus, genotypes are screened for quality characteristics at specific locations and under intense production practices.

Traits that are not greatly affected by the environment are considered stable. A stable genotypic trait is defined as one which maintains its performance under any type of environment (Letta et al., 2008). In fact, quality traits are considered stable when genotype × environment interaction is low (Brancovic et al., 2014). The stability of quality traits is important in terms of producing a consistent and reliable source of high-quality durum wheat for end-users (Letta et al., 2008).

Weather and agronomic practices are important factors that are associated with the environment. Agronomic practices are activities conducted by the grower to manipulate the environment in favor of the crop and subsequent crop quality because crops are grown in regions where the weather generally favors production and not grown in regions where the weather typically is unfavorable. Examples of agronomic practices include soil fertilization, weed control, disease control, insect control, planting date, and planting density. All these activities are performed to protect or enhance crop quality. Agricultural production practices such as control of weeds, nutrient management, and irrigation often aim to manipulate abiotic and biotic stresses to

favor agronomic and end-use quality (Ottman et al., 2000; López-Bellido et al., 2001; Subedi et al., 2007; Abedi et al., 2011; Grant et al., 2012; Uthayakumaran and Wrigley, 2017).

Grain Quality Traits

Test weight, 1000-kernel weight (TKW), kernel vitreousness, falling number, and protein content are grain quality traits that are predominantly affected by the environment (Gooding et al., 2003; Rharrabti et al., 2003b). Among weather parameters, test weight was mainly affected by rainfall and air temperature throughout grain filling (Rharrabti et al., 2003b; Pinheiro et al., 2013). Pinheiro et al. (2013) also showed that high temperature decreased test weight. In addition, test weight negatively correlated with water stress (lack of moisture) due to lower kernel weight (Ozturk and Aydin, 2004).

1000-Kernel weight was negatively affected by high air temperatures during grain filling (Pinheiro et al., 2013). According to Dias and Lidon (2009), high temperature reduced grain weight which arose from its effect on grain filling rate and duration. Regarding moisture availability, results showed that water uptake affected cell expansion; while, in case of drought and lack of moisture, cells no longer expanded resulting in termination of seed growth and maturation (Ellis et al., 1992; Egli, 1998). In addition, drought stress and high temperature negatively affected starch accumulation in grain resulting in a decreased TKW (Gooding et al., 2003). Conversely, TKW is positively affected by grain filling duration (Rharrabti et al., 2001; Rharrabti et al., 2003b; Pinheiro et al., 2013). The positive effect of precipitation on TKW was highlighted by Rharrabti et al. (2003a,b). Their results showed that rainfall caused the production of heavy grains presumably due to starch synthesis and an increase in grain dry mass (Rharrabti et al., 2003a). Lower air temperature prolongs grain filling duration which favors starch deposition and increases TKW (Altenbach et al., 2003; Koga et al., 2015).

Kernel vitreousness is under the influence of both genotype and environment (Hadjichristodoulou, 1979). N application at the average amount (270 kg N/ha) resulted in the formation of yellow berry kernels (non-vitreous) to less than 25% (Robinson et al., 1979). In addition, the application of nitrogen fertilizer as well as rainfed conditions had a positive effect on protein content and kernel vitrouesness (Rharrabti et al. 2003a).

Kernel vitreousness was positively affected by the high air temperature. An indirect effect of high temperature on grain viteousness can be explained by the positive impact of high temperature on protein content, and the positive correlation between protein content and vitreousness. Also, combined impacts of the high temperature and loss of water during grain filling resulted in the formation of compact structure inside the grain, reduction in grain volume, and finally an increase in vitreousness (Ferreira et al., 2012). High temperature and drought stress favor protein deposition over starch deposition. The reduction in starch content results in an increase in percent protein.

Among environmental conditions, when the weather is cool and wet during grain filling, the falling number showed a lower value due to higher α-amylase activity (Gooding et al., 2003). Mechanisms associated with excess production of α-amylase activity in wheat grain in the U.K. were investigated by Lunn et al. (2001). Their results showed that in a controlled environment, a short period of exposure to a high temperature before grain moisture reached 45%, led to an increase in pre-maturity α-amylase production (Lunn et al., 2001). Conversely, the positive effects of moisture stress and high temperature during grain filling on the falling number was identified by Gooding et al. (2003). Expression of late maturity α-amylase is another factor associated with fluctuation in falling numbers. In fact, Mares and Mrva (2008) showed that this enzyme was influenced by temperature so that warm and hot temperature from the middle to the

late stage of grain development resulted in an increase in α-amylase activity and a decline in falling number value.

Fluctuation in protein quantity and its composition can be influenced by agricultural practices, such as nitrogen fertilizer, planting time, and intensity of light during a day; and environmental factors, including air temperature, rainfall, and humidity during grain filling among different genotypes (Feillet, 1988; Rharrabti et al., 2003b; Fois et al., 2011; Uthayakumaran and Wrigley, 2017). Environmental conditions such as drought stress and high temperature (between 30 - 35 °C) during grain filling reduced yield but improved protein quantity (Gooding et al., 2003; Rharrabti et al., 2003a; Flagella et al., 2010; Gooding, 2017). In fact, this positive effect on protein content was due to decreased grain filling duration and increased nitrogen concentration rate (Ferreira et al., 2012; Gooding, 2017). Also, according to Gooding et al. (2003), the rate of starch accumulation had been more affected by drought stress than was nitrogen accumulation.

Besides temperature, there was a negative correlation between precipitation during stem elongation and protein content (Smit and Gooding, 1999 as cited by Gooding, 2017). In fact, early rainfall may reduce nitrogen available in the plant by leaching soil nitrogen out of the root zone (Smit and Gooding, 1999 as cited by Gooding, 2017).

Timing of grain filling and maturation can be influenced by planting date. Delayed planting is another factor has been associated with increased protein concentration (Fois et al., 2011; Gooding, 2017). In fact, delayed planting time forced grain filling to happen when the temperature reaches to its highest point. The number of days when the temperature was above 30 °C was positively correlated with grain protein content (Fois et al., 2011).

Nutrients especially nitrogen play a significant role in grain quality traits particularly protein content and gluten polymer structure (Grant et al., 2012; Malik, 2012 as cited by Johansson et al., 2013; Uthayakumaran and Wrigley, 2017). In fact, differences in grain protein content (GPC) is primarily affected by nitrogen availability, irrigation, and temperature (Bole and Dubetz, 1986; Li et al., 2013). In a study conducted by Ames at al. (2003), nitrogen fertilizer enhanced protein concentration in 10 different durum wheat genotypes with response varying with genotype.

Nitrogen timing and exposure of plant to the temperature during initial development and the grain filling duration have an influence on the variation of gluten structure (Johansson et al., 2005; Malik et el., 2011). In fact, the combined effect of late-maturing cultivars and early nitrogen applications such as those applied at spike formation resulted in an increased proportion of unextractable polymeric protein in total protein (%UPP) (Malik et al., 2011). Early-maturing cultivars along with low temperature and late application of nitrogen (at flowering) were associated with decreased %UPP and improved protein content in the grain (Feillet, 1988).

Gluten index is primarily influenced by genotype (Matsuo et al., 1982; Mariani et al., 1995; Vida et al., 2014); although environment (year) and genotype × environment had a significant but less important effect on this quality trait. Concerning effect of weather conditions, Flagella et al. (2010) showed that combination of high temperature ranging from 30 - 35 °C and water deficiency during grain filling positively affected gluten index due to a decline in gliadin/glutenin ratio, assembly of the glutenin subunits, and increase in the percentage of UPP. Similar results were found in studies conducted by Ferreira at al. (2012) and Koga et al. (2015). Desiccation of kernels favored polymerization of insoluble glutenin polymers through the formation of disulfide and hydrogen bonds (Ferreira et al., 2012). Gooding et al. (2003) showed

that restricting moisture before the end of grain filling significantly decreased high molecular weight glutenin polymer due to a shift from high molecular size polymers toward lower molecular weight glutenins.

According to Fois et al. (2011) findings, late sowing time causing grain filling occurred at a higher temperature which associated with a decrease in gluten index when the temperature was above the threshold of 30 °C. In fact, unextractable polymeric proteins which associate with higher gluten index (Edwards et al., 2007), negatively influenced by heat stress, while the latter increased low molecular weight gliadins, gli/glu ratio, and extractable polymeric proteins (Corbellini et al., 1997; Fois et al., 2011). An increase in gli/glu had a softening effect on gluten and resulted in less elasticity (Fois et al., 2011).

Although gluten index negatively correlated to rainfall, Vida et al. (2014) showed that strong genotypes maintained their gluten structure stable in both humid and dry conditions, while excessive rainfall caused a considerable decline in the gluten index of weak genotypes. Similarly, Koga et al. (2015) reported that genotypes with weak gluten showed less adaptability to variation in temperature. Thus, the intensity of environmental effects on gluten protein fractions is genotype dependent (Koga et al., 2015).

Genetic factors, environmental conditions, and technological processes cause a variation in the pigment of grain (Ficco et al., 2014). Yellow pigment content is a heritable trait in which genotype had a dominant effect on its variation compared to that environment (Borrelli et al., 1999). Results of one study by Clarke et al. (2005) indicated that yellow pigment can be influenced directly or indirectly by environmental effects. Examples of direct effect could be explained by biotic or abiotic stresses, while increase in thousand kernel weight and plumpness have an indirect and diluting effect on yellow pigment concentration. In environmental

conditions with adequate rainfall (ample soil moisture) and cool air temperature during the growing season, an increase in 1000-kernel weight and other grain constituents such as starch in large kernels had a diluting effect on pigment content. In addition, the yellow pigment was weakly and positively correlated to average temperature (Clarke et al., 2005). These results were in agreement with Rharrabti et al. (2003b) who reported that high seasonal temperature greatly increased pigment content in durum wheat presumably due to a decrease in TKW. Davies and Berzonsky (2003) showed that although growing location, year, and genotype × environment interaction significantly affected PPO activity, the contribution of genotype on a variation on this quality trait appeared greater than other factors.

Ash content is controlled by the environment. So, any undesirable environmental situations that cause damage to the grain such as low test weight or 1000-kernel weight due to increase in temperature during grain filling, have an adverse effect on milling quality characteristics (increase in ash content and low endosperm separation index) (Rharrabti et al., 2003b). In addition, growing conditions that increased mineral uptake from the soil also increased ash content and decline semolina extraction rate (Di Fonzo et al., 2005; Pagnotta et al., 2005). In fact, ash content was greater with high than low crop transpiration rates (Clarke et al., 2012).

Agricultural production practices can affect ash concentration in grain. Wozniak and Makarski (2012) determined the effect of different cultivation conditions in ash and other mineral contents in spring wheat cultivar Koksa. Their results showed that ploughless tillage increased the content of total ash content in the grain probably due to insufficient endosperm development or grain lower density which associated with high ash content. In the case of crop

rotation, results indicated that the grain of wheat cultivated after soybean contained more total ash (Wozniak and Makarski, 2012).

Semolina Quality Traits

The relationships between physical and chemical properties of grain with semolina and pasta quality were discussed earlier. The effects of the environment on semolina and pasta quality are indirect. Semolina and pasta quality are the result of changes in protein and starch quantity and quality in the grain. The environment effect on semolina and pasta quality traits manifest itself through changes in grain's protein, starch, or yellow pigment quality during deposition. Various studies have confirmed the positive effect of moderately high air temperature along with drought on dough strength through an increase in the percentage of unextractable polymeric protein (%UPP) and their polymerization (Flagella et al., 2010; Fois et al., 2011; Li et al., 2013). An environment with adequate rainfall and the cool temperature has a diluting effect on pigment content through an increase in kernel size and TKW due to starch accumulation (Clarke et al., 2005). High seasonal temperatures greatly increased pigment content in durum wheat (Rharrabti et al., 2003b; Clarke et al., 2005).

Pasta Cooking Quality Traits

Genotype and environment have relatively a similar impact on pasta cooking quality (Matsuo et al., 1982; Marchylo et al., 2001). However, despite a significant effect of genotype on pasta firmness and cooked weight, environmental factors greatly influenced these quality traits (Fois et al., 2011). Fois et al. (2011) reported that late sowing time was associated with improvement in pasta cooked firmness due to the increase in protein content. In fact, late sowing time caused reduced grain filling duration under the thermal condition which positively enhanced semolina protein content and consequently pasta cooking quality. In fact, their results confirmed

pasta cooking quality was greately affected by superior role of protein quantity. Similarly, Ohm et al. (2017) reported that the environment had a dominant role in variation of pasta cooking quality traits through the impact of weather factors on protein content and composition. This result could be explained by the strong correlation between pasta cooking quality especially cooked firmness with protein content (Mariani et al., 1995; Hatcher et al., 2009; Fois et al., 2011).

Rationale and Significance

The goal of durum breeding program is to improve durum genotypes for good yield, desirable quality parameters, and disease resistance. Limited research has been conducted to evaluate the effect of genotype and weather factors on variation in quality traits in durum grain, semolina, and pasta for durum grown in various locations and years in Northern Great Plains of USA. Thus, importance of this research was to make an improvement in quality traits because it will maximize economic return to producers and provide high quality durum wheat for pasta industry.

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CHAPTER 2: GENOTYPE AND ENVIRONMENT AFFECT QUALITY TRAITS OF DURUM WHEAT COMMERCIALLY GROWN IN THE NORTHERN GREAT PLAINS OF USA

Abstract

The objective of this research was to determine genotype and environment effects on selected grain, semolina, and pasta quality traits of commercially grown durum wheat. Four durum wheat genotypes (Alkabo, Carpio, Divide, and Tioga) were obtained from durum samples collected at seven random locations in North Dakota and Montana during the annual crop survey conducted in 2015, 2016, and 2017. Grain yellow pigment content, semolina yellowness (b*), gluten index, and mixogram peak time were influenced more by genotype than by environmental factors. Conversely, changes in the falling number, protein content, semolina extraction rate, and pasta cooking quality were predominantly affected by the environment. Variation in pasta firmness was associated with changes in protein content due to the greater and positive effect of environmental factors such as high temperature on protein quantity. Genotypes differed in their response to the environment. Of the four genotypes evaluated, Carpio had the least variability for falling number, grain and semolina protein, and the greatest variability for semolina b* and extraction rate across the growing environment. Divide and Tioga showed more stability for both gluten index and mixogram peak time. For pasta cooked firmness Alkabo and Divide had the most variation, while Tioga varied the least. Genotypes differed in their genetic potential for quality traits evaluated and in their response to the environment. These results indicate that for a given quality trait, genotypes differ in the magnitude of their response to the environment and that there is potential for selecting genotypes that have a small response to the environment which will improve the consistency of the crop across locations and years.

Introduction

Crop quality is determined by the genotypes grown and the environment during the growing and harvest season. Thus, genotype and environment individually and together determine overall grain and end-use quality of durum wheat. Genes that make up each cultivar are collectively referred to as its genotype. Genotype determines the genetic potential for a given trait. The ability to achieve genetic potential is affected by the environment. Research has shown that some quality traits are affected more and other traits are affected less by the environment. For example, Mariani et al. (1995) showed that the environment was a great source of variation on protein quantity in durum wheat, while genotype mainely affected protein quality. Ames et al. (1999) reported that the environment (year effect) greatly affected pasta cooking traits. Matsuo et al. (1982) identified genotype predominantly had an impact on dough strength properties.

Environmental conditions affect the performance of grain quality traits which can result in inconsistent quality for a given genotype (Rharrabti et al., 2003b). The environment consists of abiotic stresses, for example, weather and soil properties; and biotic stresses such as disease, insects, and weeds. Genotype × environment interaction is defined as deviation in the genotypic response while exposed to various environmental situations. Combined effects of genotype × environment interaction leads to greater complexity in choosing of breeding line with superior quality traits in genotypes (Ames et al., 1999). Thus, genotypes are screened for quality characteristics at specific locations and under intense production practices. Each location is selected to represent a specific growing environment.

Traits that are not greatly affected by the environment are considered stable. A stable genotypic trait is defined as one which maintains its performance under any type of environment (Letta et al., 2008). In fact, quality traits are considered stable when genotype × environment

interaction is low (Brancovic et al., 2014). The stability of quality traits is important in terms of producing a consistent and reliable source of high-quality durum wheat for end-users (Letta et al., 2008).

Agricultural production practices often aim to manipulate abiotic and biotic stresses to favor agronomic and end-use quality (Ottman et al., 2000; Subedi et al., 2007; Abedi et al., 2011; Grant et al., 2012; Uthayakumaran and Wrigley, 2017). For example, planting date can be adjusted early or late to avoid outbreaks of common diseases and insects or unfavorable weather (Fois et al., 2011). Limited information is available concerning the effect of genotype, environment, and production practices on quality characteristics of different genotypes of durum wheat grown in Montana and North Dakota, USA. Unlike plant breeders who grow plants using optimal production practices, farmers use agricultural practices according to their equipment availability, finances, and farming philosophy. Depth and density of planting, sowing time, seeding rate, tillage practices, crop rotation, control of weeds, diseases and pests, delayed harvest, soil fertility, nutrient management, and irrigation are examples of agricultural production practices (López-Bellido et al., 2001).

Since 2005, the durum breeding program at North Dakota State University has concentrated on improved gluten strength and dough properties along with increased yellow pigment content and subsequent improvement of dry pasta color. As new genotypes replaced old genotypes grown by farmers, it was expected that their improved traits would be reflected in the harvested crop resulting in a steady improvement in overall crop quality. Oddly, this has not been observed (personal observation, Frank Manthey). Considering significant impact of environment and agricultural practices on quality traits, the main objective of this research was to document or quantify the variability of quality for a given genotype grown at different

locations and years by farmers using their own production practices by determining the genotype and environment effects on selected end-use qualities.

Materials and Methods

Durum Wheat Samples

Samples were collected during durum harvest in 2015, 2016, and 2017. Sample collection occurred over the course of the harvest period (late August to early October) and reflected the crop harvested over time. Locations were randomly selected throughout the durum growing region in North Dakota and Montana. Climatic conditions during each growing season are summarized in Table 1. Samples of durum wheat genotypes [Alkabo (Elias and Manthey, 2007), Carpio (Elias et al., 2014), Divide (Elias and Manthey, 2007), and Tioga (Elias and Manthey, 2012)] were obtained from durum samples collected during the annual crop survey. These genotypes were selected as they represented at least 50% of the durum acres planted in North Dakota in 2015-2017. These samples were tested to determine variability (stability) and durum genotype and environment effects on durum grain, semolina, and pasta quality traits.

Table 1. Weather situations during grain growing season in four durum wheat genotypes during years 2015, 2016, and 2017 in North Dakota and Montana [a].

Year	Planting time	Growing season	Harvest time	Meteorological factors
2015	3rd week of April-end of May	Ideal growing condition	Early August-mid September, and early October	Dry soil during planting, dry at the end of the growing season and through harvest, few and no significant harvest rain
2016	Fourth week of April-end of May	Timely precipitation in Northern areas, low moisture in Southern areas	Mid August-end of September	Adequately soil moisture during planting and growing season, dry during harvest, sporadic late harvest rain
2017	3rd week of April-end of May	Minimal precipitation	Early August-3rd week of September	Drought, hot and dry, scattered rain toward the end of the harvest

[a] Four durum genotypes included Alkabo, Carpio, Divide, and Tioga.

Chemical Tests on Durum Grain and Semolina

Grain quality

Grain samples were tested for protein content, falling number, and yellow pigment content. Protein content was determined using the crude protein-combustion method described in the AACCI Approved Method 46-30.01. The falling number was determined using AACCI Approved Method 56-81.03. Yellow pigment content was determined by a modified AACCI Approved Method 14-50.01, where the sample size was reduced from 8 to 4 g of ground whole wheat.

Semolina quality

Tempering to 15.5% moisture was performed to individual durum wheat samples and after that, they were milled to semolina utilizing a Quadramat Jr. Mill, as described by AACCI Approved Method 26-50.01 (Brabender GmbH & Co.KG, Germany). The extraction rate was determined by calculating total weight of semolina divided by sum of weights of bran, shorts and semolina after milling. Semolina samples were kept at 4 ºC until needed for chemical tests. Semolina from these samples was analyzed for protein content, Commission Internationale d' Eclairage (CIE) *b**-value, and rheological tests. *b**-Values vary from blue when negative and yellow when positive. Protein content was evaluated using AACCI Approved Method 46-30.01. Semolina color was evaluated according to AACCI Approved Method 14-22.01 utilizing Minolta colorimeter (model CR410, Japan) configured to determine *b**- value. Mixograph test and gluten index test were run on each sample to determine their rheological quality using AACCI Approved Method 54-40.02 and 38-12.02, respectively.

Fresh Pasta Cooking Quality

Semolina samples were hydrated to 38% moisture with distilled water at 45 ºC. KitchenAid mixer (4.3 L KitchenAid CLASSIC Stand Mixer 5K45SS, Michigan, USA) was adjusted at speed 4 and sample was mixed for 2 min. Laboratory pasta extruder (Model AEX18, Arcobaleno, Lancaster, PA) was run to extrude the mixed dough into spaghetti. Extrusion conditions included: screw speed: 50 rpm, barrel length: 7 cm, screw diameter: 4 cm, and inside width of channels: 2.2 cm. Fresh pasta (approximately 10 g) was extruded and cooked for 2 min. Cooked firmness and cooking loss were determined using AACCI Approved Method 66-50.01. Pasta product weight was measured and converted to percentage of increase in pasta weight after cooking and indicated as cooked weight (Deng, Elias, & Manthey, 2017).

Statistical Analysis

Least square means of durum grain, semolina, dough rheological properties and pasta cooking quality traits (firmness, cooked weight, and cooking loss) for four durum genotypes across years and locations were analyzed using a mixed model (type III), considering year and location as random effects, and genotype was a fixed effect. In general, mixed model is used for analysis of variance for unbalanced data. The mean comparison of quality traits for each genotype and year was performed and LSD values were calculated at the 95% level of confidence. Standard deviation was calculated from the mean values. Pearson correlation coefficient was determined between semolina with other grain and pasta quality traits. All analysis was done using SAS software version 9 (SAS Institute, Cary, NC, U.S.A). Intraclass correlation coefficient was calculated as the proportion of variance attributed to genotype relative to that of variation of genotype × environment interaction and error variance as described by Caffe-Treml et al. (2011). In particular, the variance of each component including genotype,

environment, genotype × environment interaction, and residual on quality traits were obtained using analysis of varience (ANOVA). The proportion of variance for each component was reported as the ratio of the variance estimate for an individual component to the total. Data was analyzed through boxplot utilizing Microsoft Excel 2016. Boxplots are descriptive statistics that provide information about the distribution of data by the arranging of them according to minimum, first quartile, median, third quartile, and maximum. First quartile indicates the median of the lower half of the data, meaning that in a data point, about 25% of the numbers are located below first quartile. Third quartile shows the median of the upper half of data, meaning that in a data point, about 75% of the numbers are located below third quartile (Montis and Peil, n.d.).

Results and Discussion

Estimates of variance components and intraclass correlation coefficients are presented in Table 2. Genotype × environment interaction was confounded with experimental error, which together is explained by residuals as described by Caffe-Treml et al. (2011). Estimates of variance components were calculated to evaluate the effect of environment, genotype, and their interaction on quality traits. In simple terms for an individual cultivar, phenotype = genotype + environment. When comparing cultivars, phenotype = genotype + environment + genotype × environment. In this research, genotype × environment interactions could not be separated from experimental error variance.

Table 2. Estimates of variance components and intraclass correlation coefficient from evaluation of four durum genotypes in 21 environments for grain, semolina, and fresh pasta cooking quality traits [a].

	Relative proportion (%) of variance components			
Quality traits	Genotype	Environment	Residual	Intraclass correlation
Grain				
Falling number	15	78	16	0.49
Protein content	21	61	27	0.44
Yellow pigment content	58	39	6	0.91 [III]
Semolina				
Extraction rate	14	83	6	0.69
Yellowness (b*)	77	10	22	0.78 [II]
Protein content	21	56	35	0.37
Gluten index	60	23	21	0.74
MPT [b]	77	0	31	0.71
Cooking quality				
Cooked firmness	6	92	3	0.69
Cooked weight	0	99	0	0.50
Cooking loss	2	60	62	0.03

[a] Four genotypes (Alkabo, Carpio, Divide, and Tioga) were grown in 2015, 2016, and 2017, in seven locations.
[b] MPT: Mixogram peak time.
[II, III] For intraclass correlation coefficient, parameter with II is moderately high (0.75-0.90); with III is excellent (>0.90).

One method of determining which factor (genotype, environment, or residual) has the greatest influence on a trait is to compare the magnitude of contribution of variance for each component; where the greatest relative proportion of variance of each component has the most influence on that trait. For example, resulst of analysis of varience (ANOVA) indicated that grain characteristics of yellow pigment content were affected most by genotype (58%) than by the environment (39%). However, falling number and protein content were influence most by the environment (78 and 61%, respectively, Table 2), compared to those estimated by genotype (15 and 21%, respectively; Table 2). Similar results were reported by (Rharrabti et al., 2003a; Rharrabti et al., 2003b; Pinheiro et al., 2013). These results indicated greater influence of growing environment either weather parameters, agronomic factors, or diseases on variation of both falling number and protein content.

Grain Quality Traits

Effect of genotype and environment

Falling number

Among genotypes within a row, LS-Mean values for falling numbers did not vary with genotype (Table 3). However, there were differences among years. These results are supported by the high relative proportion of variance associated with the environment (78%) compared to that of genotype (15%) (Table 2). Falling numbers were below 400 sec for all genotypes in 2017, which would reflect scattered rainfall and generally damp conditions during mid to late harvest (Tables 1, 3). Damp conditions promote preharvest sprouting as well as microbial growth; both of which produce α-amylase (Gooding et al., 2003; Manthey et al., 2004; Mares and Mrva, 2008; Dencic et al., 2013). So, the presence of moisture in 2017 toward the end of harvest, presumably favored the production of α-amylase due to growth of microorganisms and/or to preharvest sprouting.

Table 3. LS-Mean and range of grain quality traits for each genotype (Alkabo, Carpio, Divide, and Tioga) and years (2015, 2016, and 2017).

		Genotype			
Quality traits	Year	Alkabo	Carpio	Divide	Tioga
Falling number (sec)	2015	430 ±40 a ‡	436±17 b	411±41 b	422±23 b
	2016	447±29 a ††	486±16 a	474±84 a	469±57 a
	2017	337±85 b	389±24 c	391±35 b	391±32 b
	LS Mean ¶	407 a	429 a	427 a	427 a
	Range	120 - 511	325 - 485	300 - 868	311 - 539
Grain protein content (%)	2015	13.0±1.2 b	13.2±0.4 b	13.8±1.5 b	13.6±0.9 a
	2016	13.2±1.9 b	11.9±0.8 c	13.5±1.5 b	14.6±1.4 a
	2017	14.5±1.4 a	14.2±1.4 a	14.7±1.6 a	14.4±2.1 a
	LS Mean	13.6 a	13.4 a	14.1 a	14.1 a
	Range	10.2 - 17.4	10.5 - 16.9	10.7 - 18.3	10.2 - 16.7
Yellow pigment content (ppm)	2015	11.2±0.8 a	11.9±0.8 a	10.1±1.0 a	11.1±0.8 a
	2016	10.7±1.2 a	10.4±0.9 b	9.8±1.0 a	10.9±0.6 a
	2017	10.3±0.9 a	10.5±1.2 b	8.8±0.9 b	9.7±1.1 b
	LS Mean	10.8 a	11.0 a	9.6 b	10.6 a
	Range	8.9 - 12.2	8.0 - 12.9	7.0 - 12.5	7.9 - 12.6

‡ For each genotype among years means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a *t*-test.
†† Second value represents the standard deviation.
¶ Among genotypes means followed by the same letter within a row, are not significantly different at $P \leq 0.05$ as determined using a *t*-test.

Range in falling numbers varied with genotype (Table 3, Figure 1). Stability is based on size of the range for given trait across the environment and the smaller range represents higher stability. The overall range was smallest with Carpio (160 sec) and greatest with Divide and Alkabo (568 and 391 sec, respectively). Based on boxplots, Divide had greater variability with high falling numbers and Alkabo had greater variability with low falling numbers. Similarly, the interquartile range (IQR) was smallest for Carpio (59 sec) and greatest for Alkabo (69 sec) and Divide (70 sec). The narrowest range in IQR for Carpio indicated less variation within 50% of values. The interquartile range (IQR) is the range in values that make up the second and third

quartiles, which represent 50% of the samples. Falling numbers varied more with the environment with Alkabo and Divide than with Carpio.

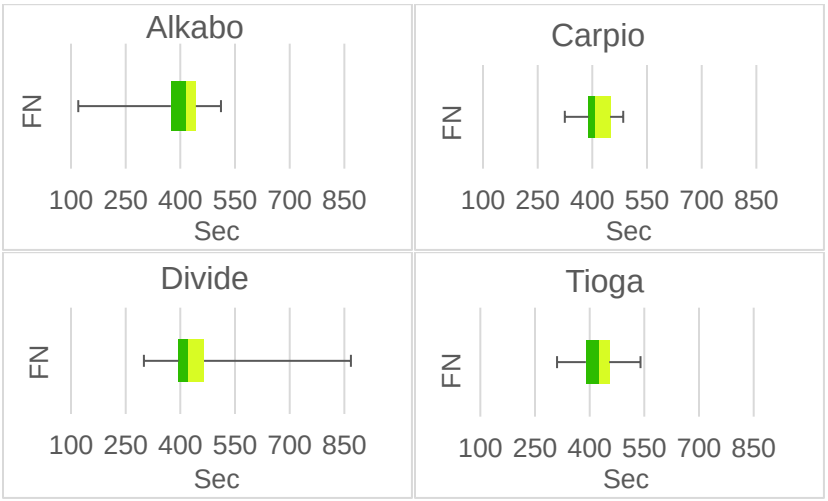

Figure 1. Boxplots of grain falling number (FN). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

Protein content

Among genotypes within a row, LS-Mean values for grain protein content did not differ with genotype (Table 3). However, there were differences among years. According to data in Table 2, environmental effect was greater (61%) than genotype (21%) and residual (27%) on protein quantity. Comparing years within each genotype, Alkabo, Carpio, and Divide showed the highest protein values in 2017 which had drought conditions during the growing season. However, grain protein content was similar for all three growing seasons for Tioga. Drought stress and hot and dry conditions in 2017 during grain filling is attributed to the high protein content compared to those of 2015 and 2016 (Table 1; Rharrabti et al., 2003a,b). Gooding et al. (2003), Rharrabti et al. (2003b), and Ozturk and Aydin (2004) also reported that dry conditions (moisture stress) resulted in increased protein content. This increase in protein content is attributed to the decline in starch formation during grain filling because rate of starch

accumulation had been reported to be more affected by drought stress than that of protein (Gooding et al., 2003). Thus, the percentage of protein in grain increased with the decrease in starch content.

Range in grain protein content varied with genotype (Table 3, Figure 2). The overall range for grain protein content was smaller with Carpio and Tioga (6.4 and 6.5 percentage units) than with Alkabo and Divide (7.2 and 7.6 percentage units). IQR was smallest with Carpio (1.3 percentage units) compared to Tioga, Alkabo, and Divide (1.8, 2.1, and 2.3 percentage units, respectively). Higher stability with Carpio indicates that this genotype was less affected by environment. In all genotypes except Tioga, fourth (high grain protein content) quartile had greater range than first (low grain protein content) quartile indicating favorable environment for higher grain protein content resulted in more variation.

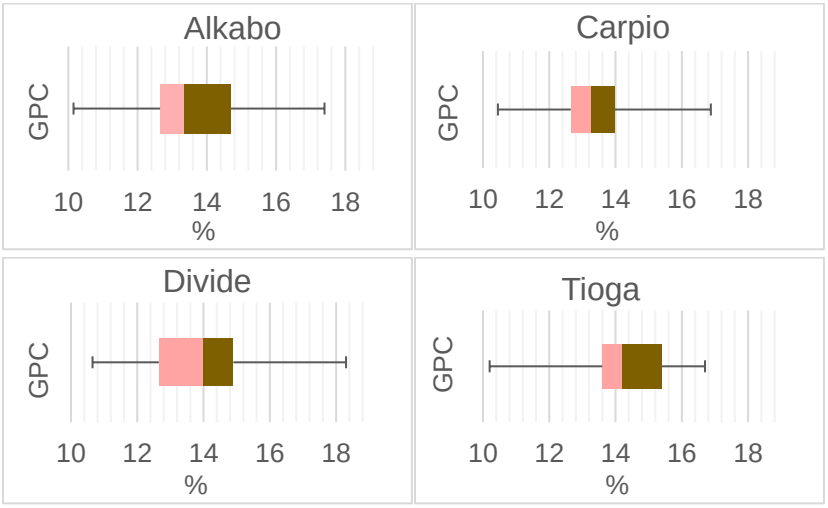

Figure 2. Boxplots of grain protein content (GPC). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

Yellow pigment content

Among genotypes within a row, LS-Mean values for yellow pigment content varied with genotype and growing year. (Table 3). Yellow pigment content was affected more by genotype

than environment as indicated through the proportion of variance for genotype (58%) compared to that for environment (39%) (Table 2). Borrelli et al. (1999) reported that genotype had a bigger determinant effect on yellow pigment content than did the environment. Yellow pigment content was similar for Alkabo, Carpio, and Tioga and all had more yellow pigment content than Divide.

Results in Table 3 indicated that for each genotype among years, those grown in 2015, had the highest yellow pigment value. In 2015, favorable dry conditions existed at the end of the growing season and through harvest. Conversely, yellow pigment content was lowest in 2017. Although 2017 growing season experienced drought conditions, damp conditions prevailed during harvest which probably contributed to a decline in yellow pigment content. Relatively low falling numbers (337- 391 sec) in 2017, reflect the damp conditions during harvest. These results agree with those reported by Cabas-Luhmann (2017) who reported that yellow pigment content was greater with Carpio than Divide and that yellow pigment content generally declined with the delayed harvest which was attributed to prolonged exposure to damp conditions.

Range in yellow pigment content varied with genotype (Table 3, Figure 3). The overall range was smallest with Alkabo (3.3 ppm) and greatest with Divide (5.5 ppm). Comparing the range of the first (low yellow pigment content) and fourth (high yellow pigment content) quartiles, Alkabo, Carpio, and Tioga had greater first than fourth quartiles; while Divide had a smaller first than the fourth quartile. Thus, environments favorable for higher yellow pigment content resulted in more variability with Divide, whereas, environment favorable for low yellow pigment content resulted in more variability with Alkabo, Carpio, and Tioga.

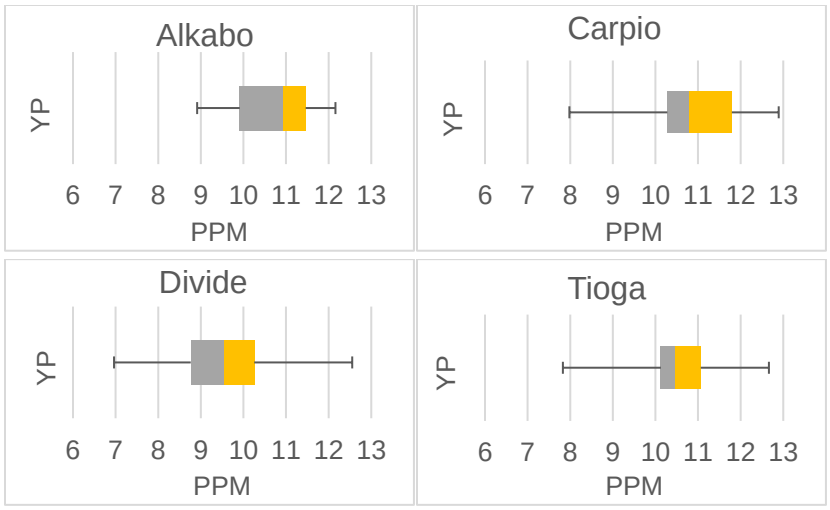

Figure 3. Boxplots of grain yellow pigment (YP). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

The intraclass correlation for grain quality traits

Intraclass correlation coefficients (ICC) are presented in Table 2. The intraclass correlation coefficient is a measure of broad-sense heritability where a high value indicates effective selection within an environment for that trait in a breeding program. In this research, ICC values were low for falling number and protein content but were high for yellow pigment content. These results indicate that selecting genotypes with the high falling number and for protein content would be less effective than selecting for yellow pigment. These results support those reported by Clarke et al. (2005) who reported that the heritability of yellow pigment was in range of 0.88 to 0.95 indicating the greater impact of genotypes for the six inbred crosses within environment where was assessed for the test. Low interclass correlation indicates the complicated role of the environment in determining these quality traits (Caffe-Treml et al., 2011; Eagles et al., 2002). For example, protein content is dependent on soil fertility, rainfall during the vegetative stage and the effect of temperature and rainfall during grain filling on photosynthesis and subsequent starch accumulation (Feillet, 1988; Rharrabti et al., 2003b; Fois et al., 2011;

Uthayakumaran and Wrigley, 2017). Cool environment promotes extended grain filling which often results in greater 1000-kernel weight and large plump, starchy kernels with low percentage of protein (Rharrabti et al., 2003a,b).

Semolina Quality Traits

Effect of genotype and environment

Estimates of variance components for semolina traits indicate that environment had the greatest impact on changing of protein content and extraction rate, while the influence of genotype was more predominant for yellowness (b*), gluten index, and mixograph peak time (Table 2). The relative variance for genotype (21 and 21%) and environment (61 and 56%) were similar for grain protein content and semolina protein content, respectively. This would be expected since they are measures of protein content.

LS-Means of semolina quality traits for each durum genotypes (Alkabo, Carpio, Divide, and Tioga) and year, as well as LS-mean comparison among genotypes across years (2015, 2016, and 2017), are presented in Table 4.

Table 4. LS-Mean and range of semolina quality traits for each genotype (Alkabo, Carpio, Divide, and Tioga) and years (2015, 2016, and 2017).

		Genotype			
Quality traits	Year	Alkabo	Carpio	Divide	Tioga
Extraction rate (%)	2015	58.8±2.5 b ‡	62.0±2.0 b	58.2±2.8 b	59.3±1.7 b
	2016	64.0±2.4 a ††	65.8±1.4 a	63.8±1.5 a	62.7±2.2 a
	2017	59.7±3.8 b	59.8±4.7 b	58.4±2.9 b	57.8±2.5 b
	LS Mean ¶	60.9 a	61.7 a	59.9 b	60.4 ab
	Range	52.0 - 67.6	48.1 - 67.2	51.7 - 66.6	51.9 - 64.9
Yellowness (b*)	2015	30.10±1.3 a	29.49±0.9 a	27.99±1.2 b	29.13±1.0 a
	2016	30.63±1.4 a	29.79±2.2 a	29.23±1.3 a	29.90±2.1 a
	2017	29.96±1.5 a	31.13±3.1 a	28.51±1.4 b	29.50±2.0 a
	LS Mean	30.2 a	30.4 a	28.5 b	29.5 ab
	Range	27.8 - 32.6	26.8 – 39.4	25.5 - 31.9	25.8 - 32.5
Protein (%)	2015	11.6±1.1 a	11.7±0.3 b	12.4±1.4 ab	12.2±0.7 a
	2016	11.5±1.1 a	10.7±0.6 c	11.7±1.3 b	12.9±1.2 a
	2017	12.7±1.1a	12.5±1.1 a	12.8±1.3 a	12.6±1.8 a
	LS Mean	12 a	11.9 a	12.2 a	12.5 a
	Range	9.3 - 14.6	9.7 - 14.4	9.3 - 15.6	8.7 - 14.6
Gluten index (%)	2015	46±22 b	80±14 a	51±13 b	45±18 b
	2016	36±19 b	64±32 a	72±13 a	53±10 b
	2017	67±18 a	84±15 a	68±14 a	70±17 a
	LS Mean	49 b	77 a	64 ab	57 ab
	Range	1 - 93	9 - 97	27 - 95	16 - 95
Mixogram peak time (sec)	2015	177±9 a	195±12 a	162±7 a	166±10 a
	2016	154±10 ab	194±12 a	153±7 a	173±9 a
	2017	150±9 b	181±8 a	171±7 a	181±8 a
	LS Mean	160 c	190 a	162 c	174 b
	Range	120 - 295	123 - 240	123 - 225	122 - 222

‡ For each genotype among years, means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a *t*-test.
†† Second value represents standard deviation.
¶ Among genotypes, means followed by the same letter within a row, are not significantly different at $P \leq 0.05$ as determined using a *t*-test.

Extraction rate

Among genotypes within a row, LS-Mean values for semolina extraction varied with genotype and growing year (Table 4). Semolina extraction was greatest with Carpio (61.7%) and

Alkabo (60.9%), intermediate with Tioga (60.4%), and least with Divide (59.9%). For all genotypes, semolina extraction was greater in 2016 than either 2015 or 2017. These findings support the relatively high proportion of variance for the environment (83%) compared to genotype (14%) on the extraction rate as presented in Table 2. In fact, compared to dry conditions, high soil moisture during planting and growing season in 2016 (Table 1) decreased protein quantity and increased the semolina extraction rate. This result was supported by a negative correlation between the protein quantity and semolina yield (Pinheiro et al., 2013; Ozturk and Aydin, 2004; Table 6).

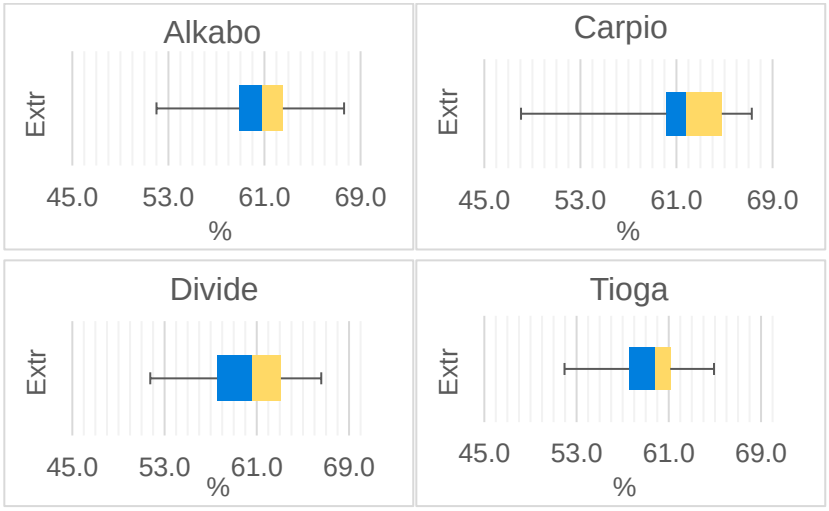

Figure 4. Boxplots of semolina extraction rate (Exrt). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

The range in extraction rate varied with genotypes (Table 4, Figure 4). The overall range was smallest with Tioga (13.0 percentage units) and greatest with Carpio (19.1 percentage units). The first quartile had a greater range than either IQR or fourth quartile for all genotypes, indicating that there was more variability with low than high semolina extraction rates.

Semolina yellowness

Among genotypes within a row, LS-Mean values for semolina yellowness (b*) did not vary with the growing year for Alkabo, Carpio, and Tioga (Table 4). However, semolina from Divide had greater yellowness in 2016 than in 2015 or 2017. Semolina yellowness was mainly affected by genotype as specified through the proportion of variance for genotype (77%) compared to that for the environment (10%) (Table 2). In fact, significant differences were observed between Divide and both Carpio and Alkabo (Table 4). The predominant influence of genotype on yellowness (b*) was also highlighted by Pinheiro et al. (2013). For all genotypes, a positive correlation was found between grain yellow pigment content and semolina b* (data was not presented).

The range in semolina yellowness (b*) varied with genotypes (Table 4, Figure 5). The overall range was smallest with Alkabo (4.8) and greatest with Carpio (12.6). Similarly, IQR was smallest with Alkabo (2.1) compared to Carpio (2.8). Comparing the range of the first (low yellowness) and fourth (high yellowness) quartiles, Tioga had greater first than the fourth quartile; Alkabo and Divide relatively had similar first and fourth quartiles, and the fourth quartile was almost 4 times larger than the first quartile in Carpio. Thus, environments favorable for higher yellowness resulted in more variability with Carpio, while environment favorable for low yellowness resulted in more variability with Tioga.

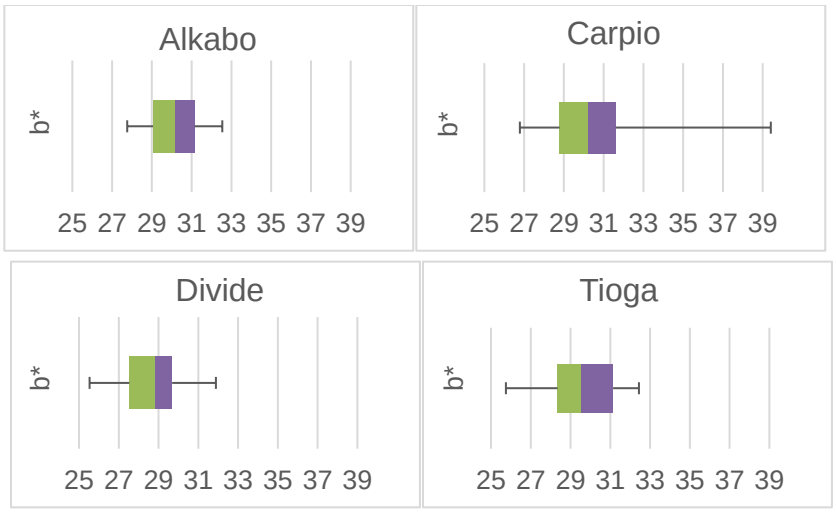

Figure 5. Boxplots of semolina yellowness (b*). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

Protein content

Among genotypes within a row, LS-Mean values for semolina protein content averaged across growing years did not change with genotype (Table 4). For individual genotypes, semolina protein content was similar in 2015, 2016, and 2017 for Alkabo and Tioga. However, both Carpio and Divide had the greatest semolina protein in 2017, intermediate in 2015 and least in 2016. These results support the higher relative proportion of variance associated with the environment (56%) compared to that of genotype (21%) on semolina protein (Table 2). The impact of the environment on protein content was in agreement with findings by Ames et al. (1999) and Mariani et al. (1995). In addition, Ohm et al. (2017) indicated that a growing environment had a dominant impact on the variation of proteins quantity in semolina.

For each genotype and among years, with the exception of Tioga, the highest value for semolina protein was found in 2017 which could be explained by the positive effect of high temperature on protein content during grain filling due to exposure to hot and dry conditions (Rharrabti et al., 2003a; Pinheiro et al., 2013). However, the lowest protein value was assigned in

2016 due to the presence of adequate soil moisture during planting and growing season which probably prolonged grain filling duration and consequently promoted an increase in starch accumulation and 1000-kernel weight, and a decrease in protein content (Altenbach et al., 2003).

The range in semolina protein content varied with genotype (Table 4, Figure 6). Similar to grain protein, the overall range was smallest with Carpio (4.7 percentage units) and highest with Divide and Tioga (6.3 and 5.9 percentage units, respectively). IQR was smaller with Carpio and Tioga (1.4 and 1.5 percentage units, respectively) compared to Divide, and Alkabo, (1.8 and 1.9 percentage units, respectively). The presence of low range for Carpio, along with the narrowest range for IQR, and similarity in first and fourth quartiles as an indication of uniform variability at lower and higher protein content values led to the greatest stability in this genotype. Conversely, unfavorable environment resulted in more variation for Tioga at low protein content. These results showed that Carpio was less affected by the environment compared to those of Divide and Tioga.

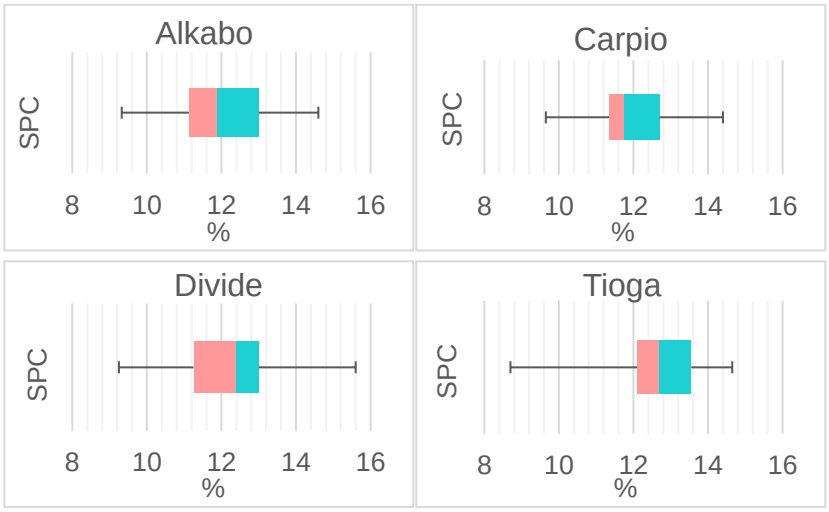

Figure 6. Boxplots of semolina protein content (SPC). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

Gluten index

Among genotypes within a row, LS-Mean values for the gluten index varied with genotype and with year within a genotype (Table 4). Gluten index was greatest with Carpio, intermediate with Divide and Tioga, and least with Alkabo. Within each genotype, the gluten index did not vary with the year for Carpio; however, Alkabo and Tioga had a higher gluten index in 2017 than 2016 or 2015. The gluten index for Divide was greater in 2016 and 2017 than in 2015. Results in Table 2 indicate that genotype had a greater effect on gluten index than did environment with the proportion of variance for genotype (60%) compared to that of the environment (23%) for gluten index. According to Ames et al. (1999), gluten quality parameters particularly gluten index were more influenced by genotype. In addition, results by Vida et al. (2014) showed the primary influence of genotype on gluten index although environment (year) and genotype × environment had a significant but less important effect on this trait. For each genotype among years, 2017 had the highest gluten index with the exception of the Divide that the highest value belonged to 2016; however, it was not significantly different from 2017. Flagella et al. (2010) also showed that presence of both high-temperature and water deficiency during grain filling resulted in improved gluten functionality through assembly of glutenin subunits. Similar results were found in studies conducted by Ferreira at al. (2012) and Koga et al. (2015).

The range in gluten index values varied with genotype and was remarkably high (Table 4, Figure 7). The overall range was smallest with Divide (68 percentage units), intermediate with Tioga (79 percentage units), and greatest with Alkabo and Carpio (92 and 88 percentage units, respectively). The range in IQR values was similar (19-23 percentage units) for genotypes indicating low variability within 50% of values. Comparing the range of the first (low gluten

index) and fourth (high gluten index) quartiles, in all genotypes the first quartile had a greater range than the fourth quartile. In particular, for Carpio, the range of the first quartile was nearly three times as big as IQR. Thus, environments favorable for lower gluten index resulted in more variability. Similarly, weak genotype such as Alkabo, showed greater variation which indicated that they were more prone to be influenced by the environment. However, genotypes with intermediate gluten index including Tioga and Divide resulted in low variation in gluten index. These results were in agreement with those in chapter 4 in this dissertation.

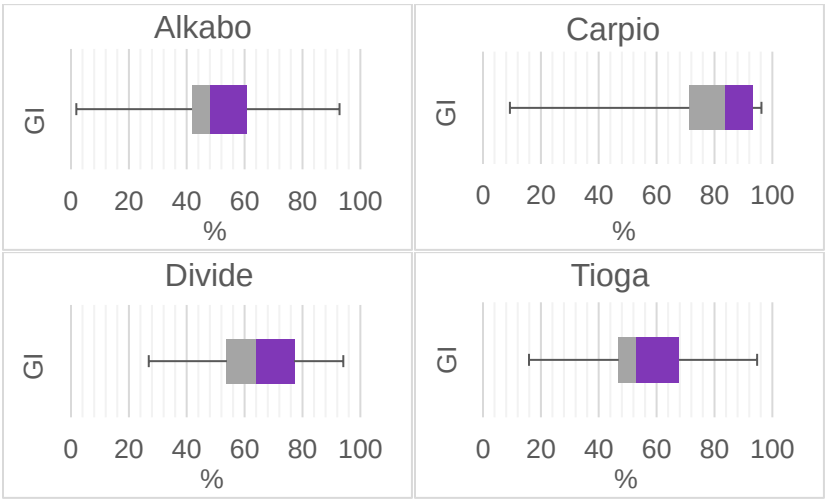

Figure 7. Boxplots of gluten index (GI). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

Mixogram peak time (MPT)

Genotype affected MPT more than did the environment with the proportion of variance for genotype being 77% compared to 0% for the environment (Table 2). Among genotypes within a row, mixogram time-to-peak (MPT) varied with genotypes (Table 4). LS-Mean values for MPT was greatest with Carpio (190 sec), intermediate with Tioga (174 sec), and least with Divide (162 sec) and Alkabo (160 sec). Carpio had the greatest gluten index and MPT indicating that Carpio produced dough with the greatest strength. Fois et al. (2011) also observed a strong

positive correlation between gluten index and dough mixing properties. Within a genotype, MPT did not change with years except for Alkabo where MPT was greatest in 2015, intermediate in 2016, and least in 2017.

Range in MPT varied with genotype (Table 4, Figure 8). MPT range was least with Tioga (100 sec) and Divide (102 sec), intermediate with Carpio (117 sec), and greatest with Alkabo (175 sec). Thus, Tioga and Divide had the greatest stability for MPT while Alkabo had the poorest stability for MPT. Alkabo and Divide had a greater range for the fourth (high MPT) than the first quartile (low MPT), while Carpio had a greater range for the first quartile (low MPT) than the fourth (high MPT) quartile. Alkabo had the poorest stability with the greatest variation occurring with the fourth (high MPT) quartile. Overall, environments favorable for higher MPT resulted in more variability with Alkabo and Divide, while environment favorable for low MPT resulted in more variability with Carpio.

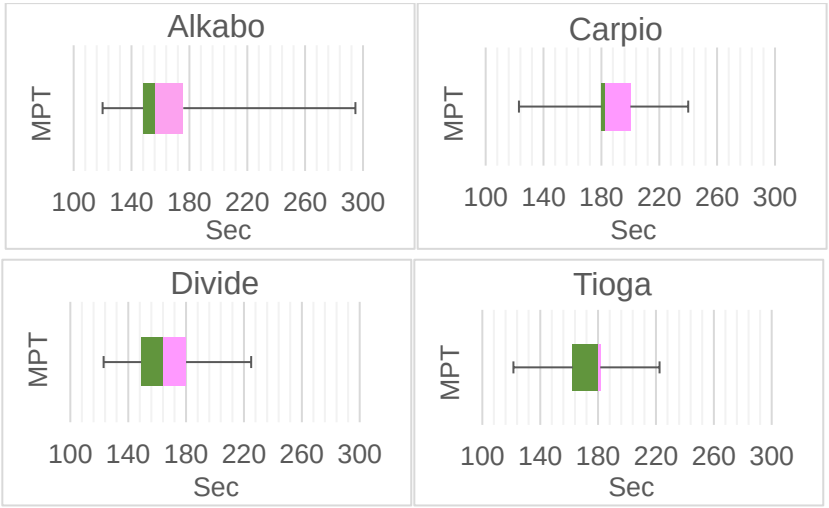

Figure 8. Boxplots of mixograph peak time (MPT). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

Carpio and Alkabo, genotypes with strong and weak gluten properties, respectively, exhibited more variability in gluten index and MPT indicating the importance of environment on

a variation on these quality traits. These results were in agreement with Vide et al. (2014) who reported that even under hot and dry conditions, genotypes with strong gluten lost their superior dough rheological properties.

The intraclass correlation for semolina quality traits

Intraclass correlation coefficients for semolina quality traits are presented in Table 2. The intraclass correlation coefficients and the proportion of variance for genotype were high for semolina yellowness, gluten index, and mixogram time-to-peak. The intraclass correlation coefficient was relatively high for the semolina extraction rate even though the proportion of variance for genotype was low. The relatively high intraclass coefficient for semolina extraction indicates that the selection of this trait within an environment would be effective. Conversely, both the intraclass coefficient and the proportion of variance for genotype were low for semolina protein which indicates that selection for this trait would be less effective, as discussed above.

Cooking Quality Traits

Effect of genotype and environment

Estimates of variance components indicate that environment accounted for the largest source of variation in pasta cooking quality traits. In fact, the relative proportion of variance associated with the environment was 92%, 99%, and 60% for cooked firmness, cooked weight and cooking loss, respectively (Table 2). These results indicate that the development of genotypes with good cooking properties could be region or environment specific.

Cooked firmness

Among genotypes within a row, LS-Means for cooked firmness for each durum genotype and year are presented in Table 5. Cooked firmness varied with genotype, with the greatest firmness occurring with spaghetti made with semolina from Tioga, which was significantly

different from Alkabo with the lowest value. Within a genotype, firmness varied with year. For Alkabo, Carpio, and Divide, cooked firmness was greatest in 2017, intermediate in 2016, and least in 2015; while, cooked firmness for Tioga was greater in 2016 and 2017 than in 2015. Cooked firmness has been correlated with protein content (Table 6; Fois et al., 2011; Ohm et al., 2017). High air temperature and moisture stress probably resulted in increased protein content resulting in high cooked firmness in 2017.

Table 5. LS-Mean and range of fresh pasta cooking qualities for each genotype (Alkabo, Carpio, Divide, and Tioga) and years (2015, 2016, and 2017).

		Genotype			
Quality traits	Year	Alkabo	Carpio	Divide	Tioga
Firmness (g.cm)	2015	3.2±0.3 c ‡	3.3±0.2 c	3.4±0.4 c	3.4±0.1 b
	2016	4.1±0.6 b ††	4.1±0.2 b	4.2±0.5 b	5.0±0.3a
	2017	4.5±0.5 a	4.8±0.5 a	4.8±0.5 a	5.0±0.6 a
	LS Mean ¶	3.9 b	4.1 ab	4.2 ab	4.4 a
	Range	2.6 - 5.5	3.1 - 5.7	2.7- 5.7	3.3 - 5.7
Cooked weight (%)	2015	197.0±0.7 a	199.0±0.1 a	195.0±0.5 a	196.0±0.3 a
	2016	172.0±0.3 b	173.0±0.2 b	172.0±0.3 b	169.0±0.4 b
	2017	168.0±0.3 b	170.0±0.3 b	168.0±0.4 c	171.0±0.4 b
	LS Mean	179.0 a	180.0 a	178.0 a	179.0 a
	Range	163.0 - 207.0	164.0 - 200.0	163.0 - 207.0	160.0 - 202.0
Cooked loss (%)	2015	1.5±0.1 ab	1.6±0.04 a	1.6±0.1 a	1.5±0.1 a
	2016	1.4±0.1 b	1.3±0.2 b	1.4±0.3 b	1.4±0.2 a
	2017	1.6±0.1 a	1.5±0.2 ab	1.4±0.3 b	1.4±0.2 a
	LS Mean	1.5 a	1.5 a	1.5 a	1.4 a
	Range	1.2 - 1.9	1.1 - 1.9	0.3 - 2.0	1 - 1.8

‡ For each genotype among years, means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a *t*-test.
†† Second value represents standard deviation.
¶ Among genotypes, means followed by the same letter within a row, are not significantly different at $P \leq 0.05$ as determined using a *t*-test.

Range in cooked firmness varied with genotypes (Table 5, Figure 9). The overall range for pasta cooked firmness was smaller with Tioga (2.4 gcm), and Carpio (2.6 gcm) than with Alkabo (2.9 gcm) and Divide (3.0 gcm). Comparing the range of the first (low cooked firmness) and fourth (high cooked firmness) quartiles, Alkabo and Divide had greater fourth than first

quartile. Thus, environments favorable for higher cooked firmness resulted in more variability with Alkabo and Divide. Conversely, Tioga and Carpio had similar first and fourth quartiles. So, lower range and uniform variability at lower and higher cooked firmness accounted for high stability in these two genotypes.

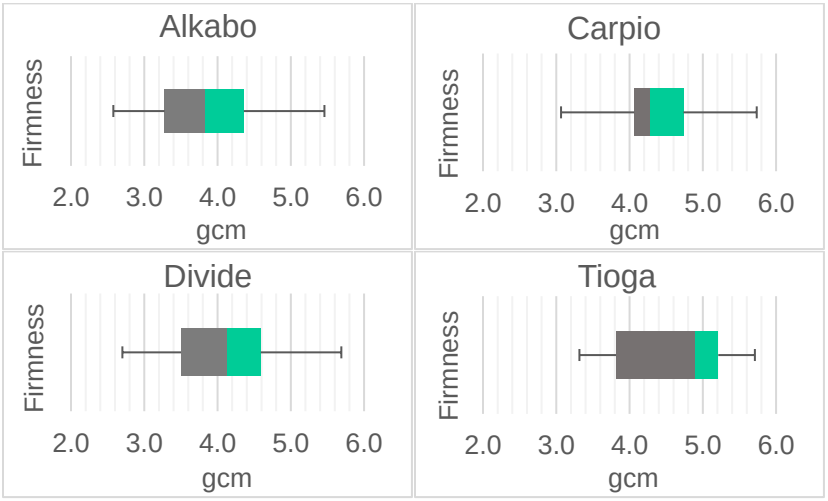

Figure 9. Boxplots of cooked pasta firmness. The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

Cooked weight

Among genotypes within a row, LS-Mean values showed that genotypes did not vary in their cooked weight (Table 5). However, for each genotype among years, cooked weight was greatest in 2015 and lowest in 2017. Cooked weight seemed to be affected by protein content, as cooked weight and protein content were negatively correlated (Table 6; Ohm et al., 2017). It assumed that high protein content results in a dense protein matrix which can reduce water absorption into pasta strands resulting in high firmness and decline in cooked weight (Irie et al., 2004). All genotypes had their highest semolina protein content and their lowest cooked weight in 2017 (Tables 4, 5).

Range in pasta cooked weight varied with genotypes (Table 5, Figure 10). The overall range was smallest with Carpio (36 percentage unit) and greatest with Alkabo and Divide (44 percentage unit). Similarly, the smallest and largest IQR were found in Carpio and Alkabo, respectively. Comparing the range of the first (low cooked weight) and fourth (high cooked weight) quartiles, all genotypes had greater fourth than first quartile. Thus, environments favorable for higher cooked weight resulted in more variability.

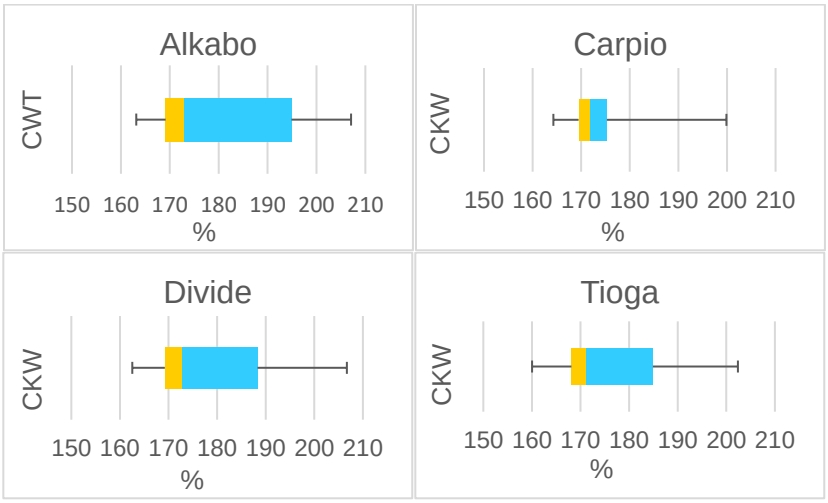

Figure 10. Boxplots of pasta cooked weight (CWT). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

Cooking loss

Among genotypes within a row, LS-Mean values for cooking loss did not vary with genotypes and there were little differences among years within genotypes (Table 5, Figure 11). Except for Tioga with similar cooking loss for all three growing seasons, 2015 had the highest cooking loss with the other genotypes which could be explained by lowest firmness. In fact, higher cooking loss seems to reflect lower cooked firmness due to lower protein content. Although not statistically significant, all genotypes had a negative correlation between the cooking loss and protein content (r= -0.05 to r= -0.43) (Table 6). The low r value indicates that

other factors are important in determining cooking loss. Starch damage has been related to cooking loss in pasta products. In addition, prolonged grain filling duration due to damp condition associated with more starch synthesis, and consequently more starch leaching into cooking water during boiling. Another possible effect of damp conditions could be related to preharvest germination which results in high α-amylase activity as reflected by low falling number and increase in cooking loss.

Table 6. Correlation coefficient between semolina protein with other quality traits in four durum wheat genotypes across years and locations [a].

		r [b]				
Genotype	Variable	Grain protein (%)	Semolina extraction rate (%)	Pasta cooked firmness (g.cm)	Cooked weight (%)	Cooking loss (%)
Alkabo	Semolina protein	0.98 *	-0.48 *	0.79 *	-0.53 *	-0.43 ns
Carpio		0.95 *	-0.60 *	0.73 *	-0.26 ns	-0.39 ns
Divide		0.98 *	-0.46 *	0.85 *	-0.61 *	-0.05 ns
Tioga		0.98 *	-0.53 *	0.85 *	-0.66 *	-0.22 ns

[a] Four genotypes (Alkabo, Carpio, Divide, and Tioga) were grown in 2015, 2016, and 2017, in seven locations.
[b] Correlation coefficient.
* indicates within each genotype among years correlation coefficient is significantly different from zero at $p < 0.05$; ns displays not significantly different from zero ($p \geq 0.05$).

Range in pasta cooking loss varied with genotypes (Table 5, Figure 11). The overall range was smallest with Alkabo (0.7 percentage unit) and greatest with Divide (1.6 percentage unit). Comparing the range for first (low cooking loss) and fourth (high cooking loss) quartile, Divide and Tioga had greater range for first than fourth quartile; Alkabo and Carpio had relatively similar range for first and fourth quartiles indicating uniform variation with low and high cooking loss. Thus, environment favorable high cooking loss resulted in more variation in Divide and Tioga.

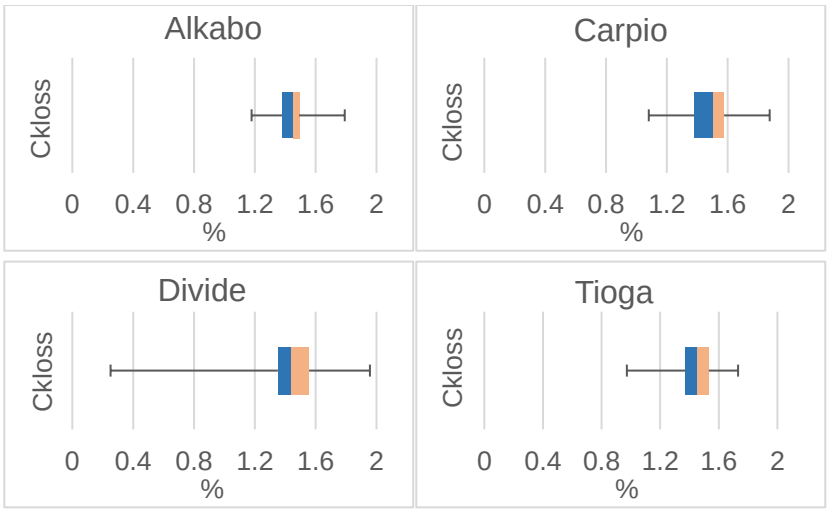

Figure 11. Boxplots of pasta cooking loss (Ckloss). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=21; three years, seven locations).

The Intraclass correlation for pasta cooking traits

Intraclass correlation coefficients for pasta cooking traits are presented in Table 2. The intraclass correlation coefficients and the proportion of variance for genotype were low for cooked weight and cooking loss. The relatively low intraclass correlations for cooked weight and cooking loss indicate the importance of environmental factors and possibly other factors such as processing conditions in determining cooking quality. The intraclass correlation coefficient for cooked firmness was relatively high while the proportion of variance for genotype was low. These results indicate that selecting for cooked firmness would be more effective than selecting for cooked weight or cooking loss.

Conclusion

The environment had a predominant role in changing quality traits especially protein content in both grain and semolina and pasta cooking quality. Variation in pasta firmness was associated with changes in protein content due to the greater and positive effect of environmental factors such as high temperature on protein quantity. However, the most important factor

associates with traits stability among genotypes is differences in the genetic potential of genotypes for quality traits under diverse environment. Each genotype responded differently for a given quality traits. Thus, selection of genotypes should target either their stability with smaller range in order to have consistency under diverse environment or it should be based on desirable quality traits such as improved protein content, gluten index, or pasta cooking quality.

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CHAPTER 3: GENOTYPE RESPONSE TO WEATHER FACTORS AND THEIR IMPACT ON GRAIN QUALITY TRAITS OF DURUM WHEAT GROWN IN NORTH DAKOTA

Abstract

The objective of this research was to determine genotype and weather effects on grain quality traits using nine durum wheat genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) grown in four years (2012-2015) at six locations in North Dakota. The effect of weather factors on grain quality traits was variable for each genotype which resulted in differences in trait stability. The range of variability in protein content and test weight was less for Carpio than for Mountrail, Tioga, and Joppa. However, Carpio had the greatest variability for vitreous kernel content. Across the genotypes (within the environment) grain protein content and falling numbers were favored by growing locations with high maximum air temperature and days with temperature ≥ 30 ºC, while days with temperature ≤ 13 ºC were ideal to have high 1000-kernel weight and test weight. Although damp condition such as high relative humidity favored 1000-kernel weight, low rainfall and low relative humidity positively promoted a high falling number and vitreous kernel content. Overall, grain traits differed in their response to weather factors. The results of this study suggest that trait stability varies with genotype and might be improved by careful selection during the breeding process. Therefore, genotypes could be selected based on their overall quality and their stability across many environments and that trait stability could be an important consideration when growers select genotypes for planting in their fields.

Introduction

Test weight, 1000-kernel weight, kernel vitreousness, protein content, and falling number are important grain quality factors in both grain trading and processing (Gooding et al., 2003; Posner & Hibbs, 2005). These factors affect the final quality of pasta which is the most common end-use product of durum wheat (Ames et al., 1999; Uthayakumaran & Wrigley, 2017). Durum wheat quality traits are under the influence of genetics and environment which includes agricultural production practices and weather conditions (Gooding et al., 2003; Rharrabti et al., 2003a,b; Pinheiro et al., 2013).

The protein content is mainly determined by weather and agronomic factors. Rainfall, relative humidity, and air temperature are examples of weather factors, while, amount of nitrogen fertilizer, nitrogen availability in the soil, and sowing date, are considered agronomic practices (Rharrabti et al., 2003b; Fois et al., 2011; Uthayakumaran & Wrigley, 2017). In fact, the impact of moderately high air temperature and reduced soil water availability has a less detrimental effect on protein synthesis than on starch formation which accounts for a rise in grain protein concentration (Gooding et al., 2003; Rharrabti et al., 2003a; Flagella et al., 2010; Gooding, 2017).

Test weight has been reported to be negatively affected by damp conditions, soil water stress (very low soil moisture), and high air temperature during grain filling period (Rharrabti et al., 2003b; Ozturk & Aydin, 2004; Pinheiro et al., 2013). Conversely, 1000-kernel weight is favored by adequate soil moisture and cool air temperatures that favor photosynthesis and starch accumulation (Rharrabti et al., 2003a) due to longer grain filling duration (Rharrabti et al., 2003b; Pinheiro et al., 2013).

Kernel vitreousness reflects a kernel's tendency to fracture and not be crushed during milling. Fracturing results in coarse particles while crushing results in fine flour production. Semolina is the desired product of durum milling. Semolina is coarsely ground endosperm. Therefore, kernel vitreousness is an important quality parameter of durum wheat. This importance is reflected in the grading system for durum wheat where durum is subclassified, based on vitreous kernel content as hard amber durum (≥75%), amber durum (60 to 74%) and durum (<60%) (USDA, 2013).

Kernel vitreousness is positively affected by protein content (Ferreira et al., 2012; Sieber et al., 2015). Rainfall has been associated with low kernel vitreousness. Excessive rainfall during the vegetative stage can leach nitrogen from the soil resulting in low protein content and low vitreousness. Vitreousness can be reduced by damp conditions prior to harvest where moisture can move into the kernel causing fractures in the endosperm resulting in loss of vitreousness.

Falling number is under the effect of environment, genotype, and genotype × environment interaction (Dencic et al., 2013). In fact, genotype differences and their response to the environment, particularly precipitation and air temperature during preharvest are factors that cause variation in falling numbers among genotypes (MacArthur et al., 1981; Dencic et al., 2013). Some genotypes with strong dormancy are considered sprout-resistant, while genotypes with partial dormancy are intermediately-susceptible, and other genotypes without dormancy are defined as sprout-susceptible (Biddulph et al., 2008). Gooding et al. (2003) also confirmed the positive effects of moisture stress and high temperature on falling numbers during grain filling due to lower α-amylase activity.

Plant breeders generally grow plants using the best production practices that maximize agronomic and end-use quality traits (Uthayakumaran & Wrigley, 2017). However, quality traits

can be highly influenced by growing conditions (Rharrabti et al., 2003b). Knowledge of weather factors on the performance of genotypes could be useful in selecting good quality genotypes to be released to growers as genotypes (Yagdi & Sozen, 2009). Due to significant impact of genotype and weather factors on quality attributes of durum wheat, the objective of this research was to evaluate genotype and environment effects on grain quality traits of nine selected durum wheat genotypes grown in six locations during years (2012-2015) in North Dakota with emphasis on association between quality traits with weather parameters (air temperature and precipitation) during grain filling.

Materials and Methods

Genotypes and Environment

Nine durum wheat genotypes [Alkabo (Elias and Manthey, 2007), Carpio (Elias et al., 2014), Divide (Elias and Manthey, 2007), Grenora (Elias and Manthey, 2007), Joppa (Elias and Manthey, 2016), Maier (Elias and Miller, 2000), Mountrail (Elias and Miller, 2000), Pierce (Elias et al., 2004), and Tioga (Elias and Manthey, 2012)] were harvested from unreplicated drill strip plots (75 × 1.2 m) grown at six locations in four years (2012-2015) (24 environments). Growing locations included Carrington, Dickinson, Hettinger, Langdon, Minot, and Williston, North Dakota. Weather data were obtained from the North Dakota Agricultural Weather Network (NDAWN). NDAWN weather stations are located at the ND Agricultural Research Centers where drill strips samples were grown. Weather factors were recorded daily during grain filling period and included mean of maximum, minimum, and mean air temperature, total rainfall, and dewpoint temperature. The number of days when $T_{max} \geq 30$ ºC, and $T_{min} \leq 13$ ºC, relative humidity, and number of days with relative humidity ≥ 80% were determined from the collected weather data. To calculate relative humidity (RH), the equation described by Alduchov

& Eskridge (1996) based on Magnus equation was used. This formula was obtained from (http://bmcnoldy.rsmas.miami.edu/ Humidity.html).

$$RH = 100\ (EXP((17.625 \times TD)/(243.04+TD))/EXP((17.625 \times T)/(243.04+T)))$$

where EXP is the exponential function in Excel; TD is dew-point temperature (ºC); and T is average temperature (ºC).

Chemical and Physical Tests on Grain

Grain samples were tested for test weight, 1000-kernel weight, vitreous kernel content, protein content, and falling number. Grain test weight was determined utilizing AACCI Approved Method 55-10.01; 1000-kernel weight was determined based on counting 10 g of clean, sound, and unbroken kernels, and adjusting weight to 1000 kernels. Counting was done by using an electronic seed counter. Vitreous kernel content was determined through splitting kernels utilizing a farinator, which cut 50 kernels in half. Endosperm of vitreous kernels appeared translucent, while non-vitreous kernels had partial or entire cross section of endosperm was white. For each sample of grain, the farinator test was performed twice so that a total of 100 kernels from each sample were examined. Protein content was determined using the crude protein-combustion method explained in the AACCI Approved Method 46-30.01; and falling number was determined using AACCI Approved Method 56-81.03.

Statistical Analysis

Least square mean, median, and range for grain quality traits were determined. Least square means of durum grain quality traits for nine durum genotypes across environments were analyzed using mixed model (type III), considering environments as a random effect, and genotype as a fixed effect. In general, mixed model is used for analysis of variance for unbalanced data and in this method, locations were considered as replications. Correlations

between quality traits with weather data were evaluated on a basis of Pearson's correlation coefficient. Stepwise linear regression was applied to determine which weather data explained greatest variation in different grain quality traits. Each of the individual quality trait was considered as a dependent variable.

All analysis was done using SAS software version 9 (SAS Institute, Cary, NC, U.S.A). Effect of environment, genotype, and genotype × environment interaction on different quality traits in grain were determined. Intraclass correlation coefficient was calculated as the proportion of variance attributed to genotype relative to that of variation of genotype × environment interaction and error variance as described by Caffe-Treml et al. (2011). Data was analyzed through boxplot utilizing Microsoft Excel 2016. Boxplots are descriptive statistics that provide information about distribution of data by grouping of them based on minimum, first quartile, median, third quartile, and maximum.

Results and Discussion

Estimates of variance components and intraclass correlation coefficients for each grain quality trait are shown in Table 7. Estimates of variance components were calculated to evaluate the effect of environment, genotype, and their interaction on quality characteristics. The highest relative proportion of each variance component has the greatest impact on that trait (Caffe-Treml et al., 2011; Chapter 4; Chapter 2). Thus, according to the results, all grain quality traits evaluated were predominantly affected by environment. The predominant influence of environment on these quality traits has also been observed by other researchers (Dick et al., 1974; Rharrabti et al., 2003a,b; Biddulph et al., 2008; Pinheiro et al., 2013). The combination of genotype × environment interaction with experimental error was represented as residuals which had very low proportion of variance and so had the least effect on all quality traits in grain.

Table 7. Estimates of variance components and intraclass correlation coefficient from evaluation of nine durum genotypes in 24 environments for grain quality traits [a].

	Relative proportion (%) of variance components			
Quality traits	Genotype	Environment	Residual	Intraclass correlation
Grain				
Test weight	3	97	1	0.71 [I]
TKW [b]	8	92	1	0.88 [II]
Protein content	2	97	1	0.77 [II]
Vitreous kernel content	14	83	4	0.79 [II]
Falling number	2	98	0	0.80 [II]

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.
[b] TKW: Thousand kernel weight.
[I, II] For intraclass correlation coefficient, parameter with I is moderate (0.5-0.75); with II is moderately high (0.75-0.90).

These results are supported by comparing the magnitude of range of response for a genotype across environments (Table 8) and response of genotypes within an environment (Table 9). The average range of response of genotypes within each environment and of environments within each genotype were 109 and 482 sec for falling number, 1.7 and 6.3% for grain protein, 18 and 35% for kernel vitreousness, 6.6 and 21.6 g for 1000 kernel weight, and 3.3 and 9.8 kg/hL for test weight (Tables 8 and 9). Thus, for each parameter tested the magnitude of the range of response of genotypes within an environment (Table 9) was much less than the range of response of environments within each genotype (Table 8), which indicates that environment had a bigger effect than did genotype on grain quality traits tested.

Table 8. Descriptive statistics for quality parameters for each genotype average over 24 environments [a].

	Test weight (kg/hL)			1000 kernel weight (g)			Grain protein (%)			Vitreous Kernel content (%)			Falling Number (sec)		
Genotype	Mean [b]	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Alkabo	80.0 a	80.7	(9.2) 73.7-82.9	41.5 abc	41.3	(19.7) 30.6-50.3	13.9 cde	13.5	(6.2) 11.4-17.6	88 c	92	(41) 59-100	406 d	427	(505) 80-585
Carpio	79.7 abc	80.3	(7.2) 75.5-82.8	42.2 a	41.8	(22.3) 31.2-53.5	13.9 cde	13.4	(5.6) 11.7-17.3	82 d	82	(46) 52-98	469 a	514	(480) 123-603
Divide	79.5 abc	79.9	(8.1) 74.3-82.4	40.8 bcd	40.4	(21.6) 29.2-52.1	14.0 bcd	14.0	(6.3) 11.0-17.3	88 c	91	(36) 63-99	451 ab	488	(488) 102-590
Grenora	78.9 cd	79.5	(10.4) 71.8-82.2	41.7 ab	42.0	(22.9) 29.2-52.1	13.9 cde	13.7	(6.1) 11.4-17.5	93 ab	93	(22) 78-100	431 bc	462	(498) 108-606
Joppa	79.5 abc	80.7	(11.3) 71.0-82.3	40.7 bcd	41.2	(22.7) 25.8-48.5	13.6 e	12.9	(6.6) 11.5-18.1	88 bc	93	(39) 61-100	419 cd	450	(513) 97-610
Maier	79.5 abc	80.1	(9.9) 72.7-82.6	40.4 cd	40.6	(20.7) 29.1-49.8	14.6 a	14.7	(6.1) 12.4-18.5	93 ab	95	(30) 70-100	403 d	447	(473) 83-556
Mountrail	78.6 d	79.4	(10.7) 71.6-82.4	39.8 d	39.6	(22.7) 26.8-49.5	14.1 bc	13.8	(6.9) 11.6-18.5	92 ab	97	(45) 55-100	425 cd	447	(481) 107-588
Pierce	79.9 ab	80.5	(9.9) 72.7-82.7	37.3 e	38.6	(18.9) 26.3-45.2	14.2 b	13.9	(6.2) 11.9-18.1	95 a	96	(17) 83-100	413 cd	433	(425) 122-547
Tioga	79.2 bcd	81.0	(11.1) 71.4-82.5	41.9 ab	41.8	(23.2) 29.4-52.6	13.8 de	13.4	(6.7) 11.5-18.2	88 c	92	(37) 63-100	412 cd	446	(475) 69-544
Mean	79.5	80.2	9.8	40.7	40.8	21.6	14.0	13.7	6.3	90	92	35	425	457	482

[a] Nine genotypes were grown in 2012, 2013, 2014, and 2015 in six locations.

[b] For each quality trait in each column, means followed by the same letter are not significantly different at $P < 0.05$.

Table 9. Descriptive statistics for quality parameters in each location and year average across genotypes [a].

	Test weight (kg/hL)		1000 kernel weight (g)		Grain protein (%)		Vitreous Kernel content (%)		Falling number (sec)		Grain filling duration	
Environment [b]	Mean [c]	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
L-12	76.1	(4.2) 73.8-77.4	43.1	(6.6) 39.5-46.1	13.7	(1.3) 13.1-14.4	74	(26) 60-86	386	(74) 352-426	28	(2) 27-29
L-13	81.7	(2.3) 80.8-82.5	47.6	(6.6) 43.7-50.3	14	(1.5) 13.3-14.8	95	(17) 82-99	413	(94) 367-461	40	(2) 39-41
L-14	81.0	(2.6) 79.7-81.7	47.4	(5.6) 44.4-50.0	12.9	(1.9) 12.0-13.9)	81	(34) 62-97	269	(115) 208-323	32	(3) 30-33
L-15	82.1	(2.0) 81.3-82.7	42.1	(6.3) 39.2-45.5	13.8	(2.1) 12.8-14.9	90	(29) 69-98	481	(88) 442-530	31	(3) 29-32
M-12	81.9	(3.2) 80.3-82.9	44.9	(8.6) 40.7-49.3	14.6	(2.6) 13.3-15.9	93	(15) 81-96	437	(117) 386-503	34	(2) 33-35
M-13	82.3	(1.3) 81.9-82.6	49.4	(10) 43.5-53.5	14.8	(1.1) 14.2-15.3	95	(9) 89-98	419	(152) 365-517	36	(3) 34-37
M-14	75.7	(7.8) 71.4-78.6	42.8	(8.6) 38.3-46.9	13	(1.3) 12.4-13.7	75	(25) 63-88	107	(123) 69-192	36	(3) 34-37
M-15	81.7	(2.7) 80.5-82.5	39.4	(9.9) 33.8-43.7	13.6	(1.7) 12.8-14.5	92	(20) 76-96	498	(128) 434-562	35	(3) 33-36
C-12	75.8	(3.5) 73.9-76.8	39.2	(5.3) 36.5-41.8	16.8	(1) 16.4-17.4	97	(3) 96-99	535	(168) 442-610	29	(3) 27-30
C-13	82.0	(2.0) 81.1-82.5	45.2	(4.5) 42.2-46.7	13.3	(1.2) 12.6-13.8	91	(14) 84-98	512	(82) 473-555	28	(2) 27-29

Table 9. Descriptive statistics for quality parameters in each location and year average across genotypes [a] (Continued).

	Test weight (kg/hL)		1000 kernel weight (g)		Grain protein (%)		Vitreous Kernel content (%)		Falling number (sec)		Grain filling duration	
Environment [b]	Mean [c]	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
C-14	79.1	(3.7) 77.4-80.5	43.2	(6.9) 40.0-46.9	13	(2.6) 11.7-14.3	82	(43) 52-95	360	(119) 296-415	34	(5) 32-37
C-15	77.6	(3.8) 75.9-79.1	33.4	(9.4) 29.1-38.5	14.9	(1.4) 14.4-15.8	94	(6) 91-97	483	(165) 377-542	22	(3) 21-24
W-12	72.9	(5.1) 71.0-75.5	28.8	(5.4) 25.8-31.2	17.9	(1.5) 17.0-18.5	99	(3) 97-100	529	(80) 500-580	27	(11) 25-36
W-13	80.0	(1.9) 79.5-80.5	39.7	(5) 36.8-41.8	13.7	(2) 12.9-14.9	95	(10) 88-98	472	(113) 531-418	34	(5) 31-36
W-14	80.1	(2.7) 78.9-81.0	36.4	(7.3) 31.2-38.5	12.4	(2.5) 11.0-13.5	89	(28) 72-99	456	(62) 423-485	26	(6) 23-29
W-15	80.4	(2.9) 79.0-81.3	35.4	(5) 32.5-37.5	12.9	(0.9) 12.5-13.4	85	(23) 76-99	514	(72) 481-553	24	(4) 22-26
D-12	77.9	(3.3) 76.2-78.8	35.4	(5.6) 31.9-37.5	16.2	(1.1) 15.6-16.7	99	(2) 98-100	428	(46) 400-446	24	(2) 22-24
D-13	81.2	(3.1) 80.0-82.4	35.3	(6.2) 31.4-37.6	11.7	(1) 11.4-12.4	95	(10) 89-99	443	(57) 418-475	30	(3) 29-32
D-14	76.4	(4.6) 73.8-77.7	48.8	(6.3) 45.2-51.5	12.9	(1.9) 11.7-13.6	79	(38) 55-94	130	(100) 82-182	41	(2) 40-42
D-15	77.9	(3.8) 76.3-79.5	34.2	(6.7) 31.0-37.7	16.8	(1.5) 16.1-17.6	97	(6) 93-99	551	(81) 507-588)	25	(3) 23-26

Table 9. Descriptive statistics for quality parameters in each location and year average across genotypes [a] (Continued).

	Test weight (kg/hL)		1000 kernel weight (g)		Grain protein (%)		Vitreous Kernel content (%)		Falling number (sec)		Grain filling duration	
Environment [b]	Mean [c]	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
H-12	79.1	(3.6) 77.4-80.3	36.1	(6.5) 32.1-38.6	14.8	(1.4) 14.0-15.4	100	(1) 99-100	550	(81) 522-603	17	(4) 15-19
H-13	81.4	(2.5) 80.5-82.3	40.4	(8.4) 36.8-45.2	13.7	(2.8) 12.7-15.5	98	(3) 97-100	490	(81) 458-539	27	(4) 26-30
H-14	80.9	(3.6) 79.2-82.1	47.4	(4.6) 44.2-48.8	12.2	(1.8) 11.5-13.3	81	(27) 67-94	270	(293) 136-429	33	(3) 32-35
H-15	81.1	(2.4) 80.3-82.1	41.2	(3.1) 40.2-43.3	12.4	(2.1) 11.4-13.5	74	(29) 59-88	473	(117) 418-535	31	(3) 29-32
Mean	79.4	3.3	40.7	6.6	14.0	1.7	90	18	425	109	30.2	3.5

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

[b] L: Langdon; M: Minot; C: Carrington; D: Dickinson; H: Hettinger.

[c] For each quality trait in each column, means followed by the same letter are not significantly different at $P < 0.05$.

Intraclass correlation coefficients can be used as a measure of broad sense heritability (Caffe-Treml et al., 2011). High intraclass correlation coefficients for all quality traits indicated that selection for these traits should be effective (Caffe-Treml et al., 2011; Eagles et al., 2002). Therefore, although environment had the biggest effect on trait response, genotypes within an environment still differed in response and this difference can be used to select genotypes in a breeding program to improve quality.

Grain Quality Traits

Mean, median, and range of grain quality traits for each durum wheat genotype (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) averaged over 24 environments are presented in Table 8. Similarly, the mean and range of grain quality traits for each environment averaged over nine genotypes are presented in Table 9.

Test weight

Mean values averaged over environment for test weight varied with genotypes (Table 8). Among genotypes, Alkabo had the highest mean test weight (80.0 kg/hL), which was significantly different from Grenora (78.9 kg/hL), Mountrail (78.6 kg/hL), and Tioga (79.2 kg/hL). Mountrail had the lowest mean test weight value (78.6 kg/hL). Test weight varied with environment (Table 9). All but 8 of the 24 environments resulted in mean test weight above 78.2 kg/hL, which is needed for US No. 1 grade (USDA, 2013). The lowest mean test weight occurred at Williston-12 where overall average was 72.9 kg/hL, which would be a US No. 4 grade.

Within a given genotype, the ranges in test weight over the 24 environments (Table 8) were greater than the ranges in response over genotypes within a given environment (Table 9). The overall average range of test weight for environments within a genotype was 9.8 kg/hL

(Table 8) while the overall average range of test weight for genotypes within an environment was 3.3 kg/hL (Table 9). These results support those from Table 7 which indicated that test weight was affected more by environment than by genotype.

Range in test weight varied with genotype (Table 8, Figure12). The overall range was smallest with Carpio (7.2 kg/hL) and greatest with Joppa and Tioga (11.3 and 11.1 kg/hL, respectively). Magnitude of range is an indicator of trait stability. Thus, these results indicate that test weight was more stable (less affected by environment) with Carpio and least stable with Joppa and Tioga. Interquartile range (IQR) can also be used as a measure of variability based on splitting data set into quartiles and represents 50% of the samples' distribution. In all genotypes, first quartile had greater range than fourth quartile indicating more variation with low than high test weight. These results showed that environments favorable for lower test weight resulted in more variability.

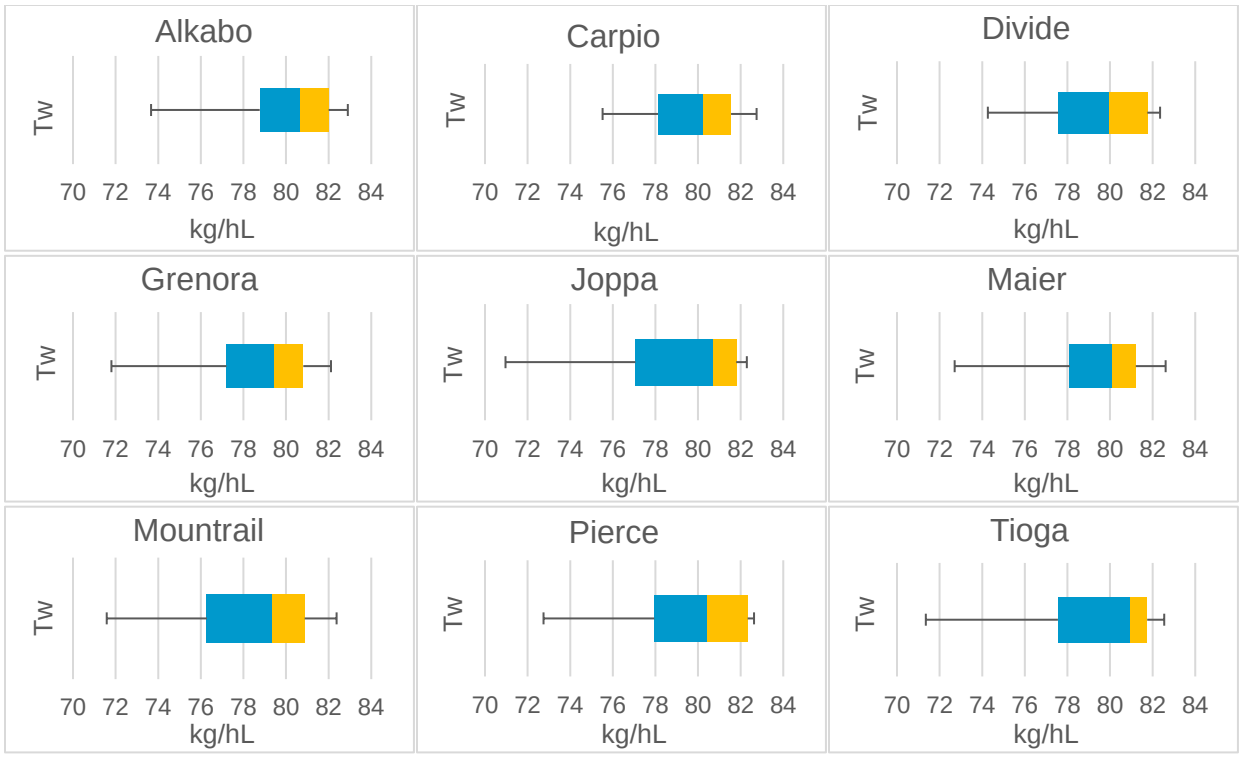

Figure 12. Boxplots of grain test weight (Tw). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Correlation analysis with weather factors indicate that test weight was favored by cool air temperatures. For all genotypes, test weight increased as the number of days with temperature ≤ 13 ºC increased and the daily minimum temperature decreased (Table 10). Similarly, test weight increased as the daily maximum temperature decreased for Carpio, Grenora, Joppa, Mountrail, and Pierce. It is assumed that cooler temperatures resulted in better grain filling, which resulted in increased weight and improved packing efficiency necessary for high test weight (Gooding et al., 2003; Rharrabti et al., 2003b). Interestingly, test weight did not correlate with grain filling duration, total rainfall or high relative humidity.

Table 10. Correlation coefficient between grain quality trait and weather factors with test weight in nine durum wheat genotypes across years and locations [a].

	Test weight								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain trait									
Grain filling duration	-0.03 ns	0.27 ns	0.24 ns	0.28 ns	0.25 ns	-0.10 ns	0.40 ns	0.24 ns	0.09 ns
Weather factors									
T max (ºC)	-0.25ns	-0.45*	-0.21ns	-0.42*	-0.44*	-0.37ns	-0.51*	-0.48*	-0.16 ns
T min (ºC)	-0.53**	-0.56 **	-0.56**	-0.59**	-0.61 **	-0.60**	-0.71***	-0.62**	-0.51*
# days temp ≥ 30 ºC	-0.19 ns	-0.24 ns	-0.06 ns	-0.25 ns	-0.29 ns	-0.27 ns	-0.29 ns	-0.32 ns	-0.04 ns
# days temp ≤ 13 ºC	0.50 *	0.61 **	0.61 **	0.66***	0.65***	0.61 **	0.76***	0.63***	0.51 *
Total rain (mm)	-0.51*	-0.42*	-0.40ns	-0.30ns	-0.40 ns	-0.39ns	-0.30ns	-0.38 ns	-0.58 **
# days RH ≥ 80%	-0.006 ns	-0.04 ns	-0.15 ns	-0.05 ns	-0.04 ns	-0.07 ns	0.003 ns	-0.0003 ns	-0.24 ns
RH	0.07 ns	0.17 ns	-0.02 ns	0.10 ns	0.18 ns	0.08 ns	0.18 ns	0.20 ns	-0.07 ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and grain quality trait with test weight is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Stepwise linear regression indicated that weather factors accounted for 43-58% of variation in test weight among genotypes (Table 11). Test weight was positively affected by number of days with temperature ≤ 13 ºC and negatively affected by total rainfall during grain filling period. Cool temperatures would favor photosynthesis and subsequent starch deposition in the grain. This would increase kernel weight and size, both of which are associated with high test weight. Rainfall near harvest would be associated with bran swelling and decline in test weight. However, rainfall during early to mid-grain filling period would not affect bran which probably explains why there was not a significant correlation between rainfall and test weight as the response would depend on when rain occurred (Table 10; Pinheiro et al., 2013).

Table 11. Stepwise linear regression for weather factors with grain test weight (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
Test weight	Alkabo	T min	(-)	0.28	0.50
		Total rain	(-)	0.22	
	Carpio	# days temp ≤ 13 ºC	(+)	0.38	0.55
		Total rain	(-)	0.18	
	Divide	# days temp ≤ 13 ºC	(+)	0.37	0.53
		Total rain	(-)	0.16	
	Grenora	# days temp ≤ 13 ºC	(+)	0.43	0.43
	Joppa	# days temp ≤ 13 ºC	(+)	0.42	0.54
		Total rain	(-)	0.12	
	Maier	# days temp ≤ 13 ºC	(+)	0.36	0.49
		Total rain	(-)	0.12	
	Mountrail	# days temp ≤ 13 ºC	(+)	0.58	0.58
	Pierce	# days temp ≤ 13 ºC	(+)	0.40	0.51
		Total rain	(-)	0.11	
	Tioga	Total rain	(-)	0.34	0.58
		T min	(-)	0.24	

[a] (+) indicates positive effect on the grain test weight; (-) indicates negative effect on the grain test weight.

1000-Kernel weight

Mean values averaged over environment for 1000-kernel weight varied with genotype (Table 8). Carpio had the highest 1000-kernel weight (42.2 g), although not significantly different from Alkabo (41.5 g), Grenora (41.7 g), and Tioga (41.9 g). However, Pierce had the lowest mean 1000-kernel weight (37.3 g). Pierce is known to have relatively low 1000-kernel weight. Within a given genotype, the range of 1000-kernel weight over the 24 environments was greater (Table 8) than the range in response over genotypes within a given environment (Table 9). The overall average range of 1000-kernel weight for environments within a genotype was 21.6 g (Table 8), while the overall average range for 1000-kernel weight for genotypes within an environment was 6.6 g (Table 9).

Range in 1000-kernel weight varied with genotype (Table 8, Figure 13). The smallest range was found with Pierce (18.9 g) which had the lowest mean 1000-kernel weight, while Carpio and Tioga had intermediate and highest ranges (22.3 and 23.2 g, respectively). Thus, 1000-kernel weight was more stable (less affected by environment) for Pierce than for Tioga. Comparing the range of the first (low 1000-kernel weight) and fourth (high 1000-kernel weight) quartiles, for all genotypes except Carpio, greater variability was found in the first quartiles indicating environment favorable for low 1000-kernel weight resulted in more variability. Carpio had relatively similar first and fourth quartiles meaning uniform variation in both low and high 1000-kernel weight. These results were supported by intermediate overall range for Carpio. IQR had narrowest range with Joppa (8 g) indicating less variability within 50% of values. Conversely, first quartile was almost four times greater than fourth quartile indicating highest variability at low 1000-kernel weight.

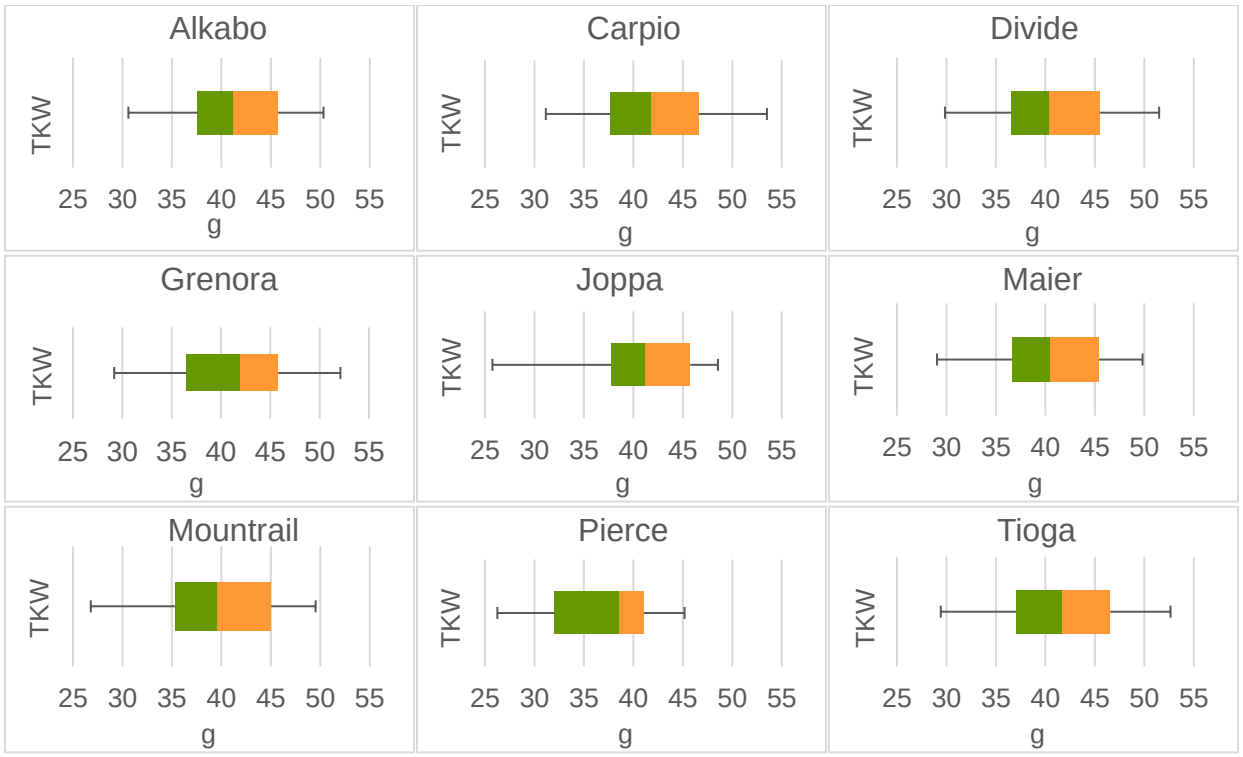

Figure 13. Boxplots of grain thousand kernel weight (TKW). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

1000-Kernel weight also varied with environment (Table 9). Except for Hettinger-12 and Hettinger-13, environments that had short grain filling period (<28 days) produced seed with low 1000-kernel weight (Table 9). 1000-Kernel weight was lowest at Williston-12 (28.8 g) which had 27 days from anthesis to harvest. Lowest mean 1000-kernel weight at Williston-12 could be explained by exposure to more days with temperature ≥ 30 ºC and fewest days with temperature ≤ 13 ºC (data was presented in appendix Table A5). In contrast, 1000-kernel weight was greatest at Minot-13 (49.4 g) and Dickinson-14 (48.8 g) and had 36 and 41 days from anthesis to harvest, respectively.

Correlation analysis between weather factors and 1000-kernel weight indicated that 1000-kernel weight increased with number of days from anthesis to harvest, number of days ≤13 ºC,

and with increased relative humidity, and decreased with increased maximum temperature, high minimum temperature, and number of days ≥30 ºC (Table 12). Increase in 1000-kernel weight when exposed to lower temperature is attributed to prolonged grain filling duration and more starch accumulation inside the granules (Rharrabti et al., 2003b; Pinheiro et al., 2013; Table 12). Negative correlation between 1000-kernel weight with maximum temperature and number of days with temperature ≥ 30 ºC support these results (Table 12; Gooding et al., 2003; Dias and Lidon, 2009; Pinheiro et al., 2013).

Table 12. Correlation coefficient between grain quality trait and weather factors with thousand kernel weight in nine durum wheat genotypes across years and locations [a].

	Thousand kernel weight								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain trait									
Grain filling duration	0.55**	0.76***	0.74***	0.70***	0.63**	0.60**	0.73***	0.67 ***	0.76 ***
Weather factors									
T max (ºC)	-0.57**	-0.63**	-0.55**	-0.62**	-0.67 ***	-0.54 **	-0.71***	-0.63 ***	-0.58 **
T min (ºC)	-0.59**	-0.55**	-0.52**	-0.56**	-0.67 ***	-0.72 ***	-0.68***	-0.67 ***	-0.55 **
# days temp ≥ 30 ºC	-0.48 *	-0.45*	-0.40*	-0.47 ns	-0.54 **	-0.39ns	-0.57**	-0.49*	-0.42*
# days temp ≤ 13 ºC	0.63 ***	0.61**	0.58**	0.61 **	0.64 ***	0.70 ***	0.73***	0.68***	0.58**
Total rain (mm)	0.23ns	0.30ns	0.31ns	0.30ns	0.27ns	0.17ns	0.33ns	0.26ns	0.19ns
# days RH ≥ 80%	0.28 ns	0.35 ns	0.30 ns	0.37 ns	0.39 ns	0.26 ns	0.39 ns	0.39 ns	0.33 ns
RH	0.42 *	0.51*	0.41 *	0.47 *	0.54 **	0.37 ns	0.53 **	0.53**	0.48 *

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and grain quality trait with thousand kernel weight is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Stepwise linear regression showed that for all genotypes except Alkabo, grain filling duration was a significant positive factor that explained 10 to 58% of the variation in 1000-kernel weight (Table 13). Carpio with the greatest average 1000-kernel weight (42.2 g), was mainly affected by grain filling period as it explained 58% of the variation. Positive correlation between the 1000-kernel weight and grain filling period also supported these results (Table 12). Conversely, the lowest 1000-kernel weight was observed in Pierce. Pierce was negatively affected by a number of days ≤ 13 ºC, which accounted for 46% of the variation in 1000-kernel weight.

Table 13. Stepwise linear regression for weather factors with grain thousand kernel weight (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
Thousand kernel weight	Alkabo	# days temp ≤ 13 ºC	(+)	0.39	0.39
	Carpio	Grain filling duration	(+)	0.58	0.58
	Divide	Grain filling duration	(+)	0.55	0.55
	Grenora	Grain filling duration	(+)	0.49	0.49
	Joppa	T max	(-)	0.45	0.56
		Grain filling duration	(+)	0.10	
	Maier	T min	(-)	0.51	0.62
		Grain filling duration	(+)	0.11	
	Mountrail	Grain filling duration	(+)	0.54	0.67
		T min	(-)	0.13	
	Pierce	# days temp ≤ 13 ºC	(-)	0.46	0.57
		Grain filling duration	(+)	0.11	
	Tioga	Grain filling duration	(+)	0.58	0.58

[a] (+) indicates a positive effect on the grain thousand kernel weight; (-) indicates a negative effect on the grain thousand kernel weight.

Protein content

Mean values averaged over the environment for kernel protein content varied with genotype (Table 8). Maier had the highest mean protein (14.6%) and Joppa the lowest mean protein content (13.6%). The overall mean for each genotype was greater than 13.5%, which is the minimum average protein content targeted for genotypes released as genotypes. Having a minimum of 13-13.5% grain protein ensures that semolina protein content will be at least 12.5% which is necessary to make pasta that meets 7 g protein per serving; that is standard for commercial pasta sold in the US. Not all growing environments were favorable for high protein content. Seven environments including Langdon-14, Williston-14, Williston-15, Dickinson-13, Dickinson-14, Hettinger-14, and Hettinger-15 had overall mean protein content below 13%. Of the seven environments, all but one had at least one genotype that had protein content greater than 13%. However, none of the genotypes grown in Dickinson-13 had protein contents above 13% and the genotypes ranged from 11.4-12.4%.

Protein content did not relate to the number of days from anthesis to harvest, as the low protein content environments ranged from 24 (Williston-15) to 41 days (Dickinson-14). A significant negative correlation between grain protein content and days from anthesis to harvest occurred for Carpio and Mountrail; the other genotypes did not have a significant correlation, but they had a trend toward a negative correlation between protein content and the number of days from anthesis to harvest. Generally, long-grain filling period would result in prolonged starch accumulation which would result in a decline in percent protein. A lack of significant negative correlation between protein content and the number of days from anthesis to harvest could be due to a delay in the harvest which while increasing days from anthesis to harvest, it might not favor photosynthesis and subsequent starch accumulation.

For each genotype, the range in protein content over 24 environments was greater (Table 8) than the range in response over genotypes within a given environment (Table 9). The overall average range of protein content for environments within a genotype was 6.3 percentage units (Table 8) while the overall average range for protein content for genotypes within an environment was 1.7 percentage units (Table 9). These results explain why the relative proportion of variance was high for the environment (97%) than the genotype (2%) (Table 7).

Range in kernel protein content varied with genotype (Table 8, Figure 14). The overall range was smallest for Carpio (5.6 percentage units) and was greatest for Mountrail and Tioga (6.9 and 6.7 percentage units, respectively). Comparing range for quartiles, results showed that in all genotypes, the fourth quartile had greater range than either IQR or first quartile which suggests that environment favorable for high protein content resulted in greater variability than did an unfavorable environment for low or intermediate grain protein content. The smaller range for Carpio compared to Mountrail and Tioga indicate that Carpio was less affected by environment.

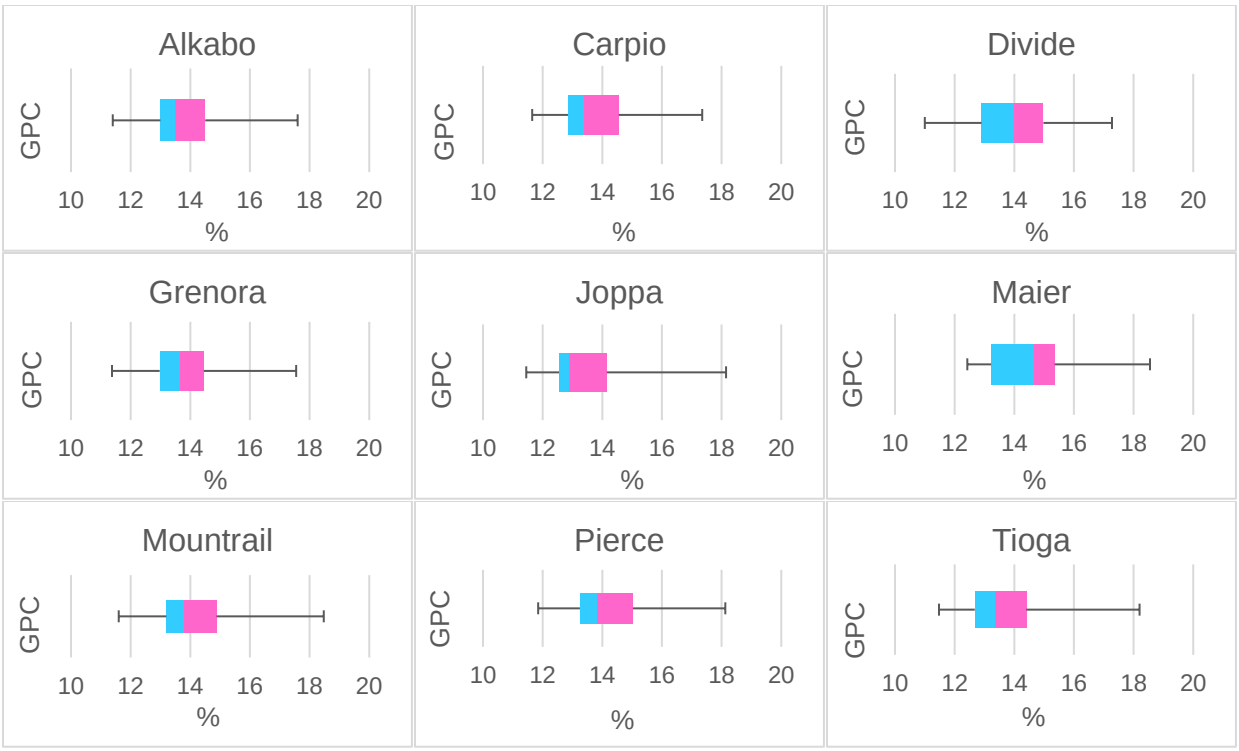

Figure 14. Boxplots of grain protein content (GPC). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Correlation analysis between weather factors and grain protein content indicated that grain protein content was positively correlated with maximum air temperature, high minimum air temperature, and the number of days $\geq$ 30 ºC and negatively correlated to the number of days $\leq$13 ºC (Table 14). While a positive correlation between protein content and maximum air temperature and minimum air temperature seems to be contradictory, high minimum air temperature is warmer than low minimum temperature. Therefore, both high maximum and minimum temperatures indicate warm day and night air temperatures, respectively, which favor protein content. Starch biosynthesis is more negatively affected by high air temperatures than is protein biosynthesis (Triboi et al., 2002). Protein content did not correlate with moisture parameters including total rainfall, relative humidity, or days with relative humidity 80% or

higher. The positive effect of high air temperatures on variation in protein content was also highlighted by Rharrabti et al. (2003a,b). A rise in grain protein content due to high temperature can also be attributed to shorter grain filling duration, and to decline in the starch deposition, kernel weight, test weight, and grain yield (Gooding et al., 2003; Flagella et al., 2010; Ferreira et al., 2012). Fois et al. (2011) and Pinheiro et al. (2013) reported similar results. In fact, any adverse growing conditions such as late sowing alongside increased temperature throughout grain filling resulted in improved grain protein content. Conversely, a negative correlation between days ≤13 ºC with grain protein (Table 14) could be explained by photosynthesis which is favored by cool nights. Koga et al. (2015) also reported that low air temperature resulted in prolonged grain filling, which resulted in a decreased proportion of protein content, and increased grain weight and yield.

Table 14. Correlation coefficient between grain quality traits and weather factors with grain protein in nine durum wheat genotypes across years and locations [a].

	Grain protein								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain trait									
Grain filling duration	-0.15ns	-0.47*	-0.30ns	-0.18ns	-0.38 ns	-0.28 ns	-0.49*	-0.35 ns	-0.40ns
Test weight (kg.hl)	-0.48 **	-0.42*	-0.42*	-0.51*	-0.67 ***	-0.44*	-0.66***	-0.49*	-0.43*
Weather factors									
T max (ºC)	0.41*	0.58**	0.33ns	0.42*	0.54**	0.46*	0.58**	0.57 **	0.54**
T min (ºC)	0.51*	0.56**	0.51**	0.44*	0.60**	0.55**	0.67***	0.58**	0.67***
# days temp ≥ 30 ºC	0.44*	0.51*	0.30ns	0.48*	0.50*	0.51*	0.52**	0.59**	0.49*
# days temp ≤ 13 ºC	-0.42*	-0.53**	-0.50*	-0.38ns	-0.56 **	-0.48*	-0.63***	-0.48 *	-0.60**
Total rain (mm)	-0.08 ns	-0.22ns	-0.01ns	-0.04ns	-0.06 ns	-0.17 ns	-0.26ns	-0.22 ns	-0.09ns
# days RH ≥ 80%	-0.19ns	-0.25ns	0.07ns	-0.07ns	-0.23ns	-0.25ns	-0.21ns	-0.24ns	-0.09ns
RH	-0.31ns	-0.37ns	-0.08ns	-0.29ns	-0.37ns	-0.36ns	-0.37ns	-0.43*	-0.31ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and quality traits with grain protein is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Stepwise linear regression analysis indicated that 23-45% of the variation in protein content among genotypes was explained by weather data. For Alkabo, Divide, Joppa, Maier, Mountrail, and Tioga, high minimum temperature explained the most variation in grain protein content; while with Carpio, maximum temperature, and with Pierce and Grenora, exposure of more days with temperature ≥ 30 ºC caused greatest changes in this quality trait. These results indicate the importance of air temperature on protein content, where some genotypes are more affected by high maximum temperatures as experienced during the day and other genotypes are more affected by high minimum or night-time temperatures. The effect of minimum temperature on the variation of protein content in both Maier and Joppa (highest protein content and lowest protein content, respectively) was almost similar (Table 15). However, different protein content values were due to other environmental factors besides the weather.

Table 15. Stepwise linear regression for weather factors with grain protein content (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
Protein content	Alkabo	T min	(+)	0.26	0.26
	Carpio	T max	(+)	0.34	0.34
	Divide	T min	(+)	0.26	0.26
	Grenora	# days temp ≥ 30 ºC	(+)	0.23	0.23
	Joppa	T min	(+)	0.35	0.35
	Maier	T min	(+)	0.31	0.31
	Mountrail	T min	(+)	0.45	0.45
	Pierce	# days temp ≥ 30 ºC	(+)	0.34	0.34
	Tioga	T min	(+)	0.45	0.45

[a] (+) indicates positive effect on the grain protein content.

Vitreous kernel content

Mean values averaged over environments for grain vitreousness varied with genotypes (Table 8). All genotypes met the criteria for Hard Amber Durum (>75% vitreous kernel content).

In addition, Grenora (93%), Maier (93%), Mountrail (92%), and Pierce (95%) met the vitreous kernel content (≥90%) criteria for Choice Milling durum. Among genotypes, Pierce had the greatest overall average vitreous kernel content (95%) which was not significantly different from Grenora, Maier, and Mountrail, while Carpio had the lowest average kernel vitreous content (82%) (Table 8). Vitreous kernel content varied with the environment; as reflected by durum grown at 15 of the 24 environments having vitreous kernel contents of at least 90% while average vitreous kernel content of durum grown at Langdon-12 and Hettinger-15 was 74%.

Within a genotype, the range in vitreous kernel content over the 24 environments was greater (Table 8) than the range in response over genotypes within a given environment (Table 9). The overall average range of vitreous kernel content for environments within a genotype was 35 percentage units (Table 8) while the overall average range of vitreous kernel content for genotypes within an environment was 18 percentage units (Table 9). These results support those presented in Table 7 where the relative proportion of variance associated with the environment (83%) was larger than that of genotype (14%).

Range in vitreous kernel content varied with genotype (Table 8, Figure 15). The overall range was smallest with Pierce (17 percentage units) and greatest with Carpio (46 percentage units). Thus, Pierce had the greatest stability (smallest range among environments, 17 percentage units) and had the highest vitreous kernel content (95%), while Carpio had the greatest variability (least stability; greatest range among environments, 46 percentage units) and the lowest average vitreous kernel content (82%) (Table 8). IQR also had a wider range for Carpio indicating more variability within 50% of values. When comparing the range of the first (low vitreous kernel content) and fourth (high vitreous kernel content) quartiles, environments

favorable for low vitreous kernel content resulted in more variability due to greater range in the first quartile.

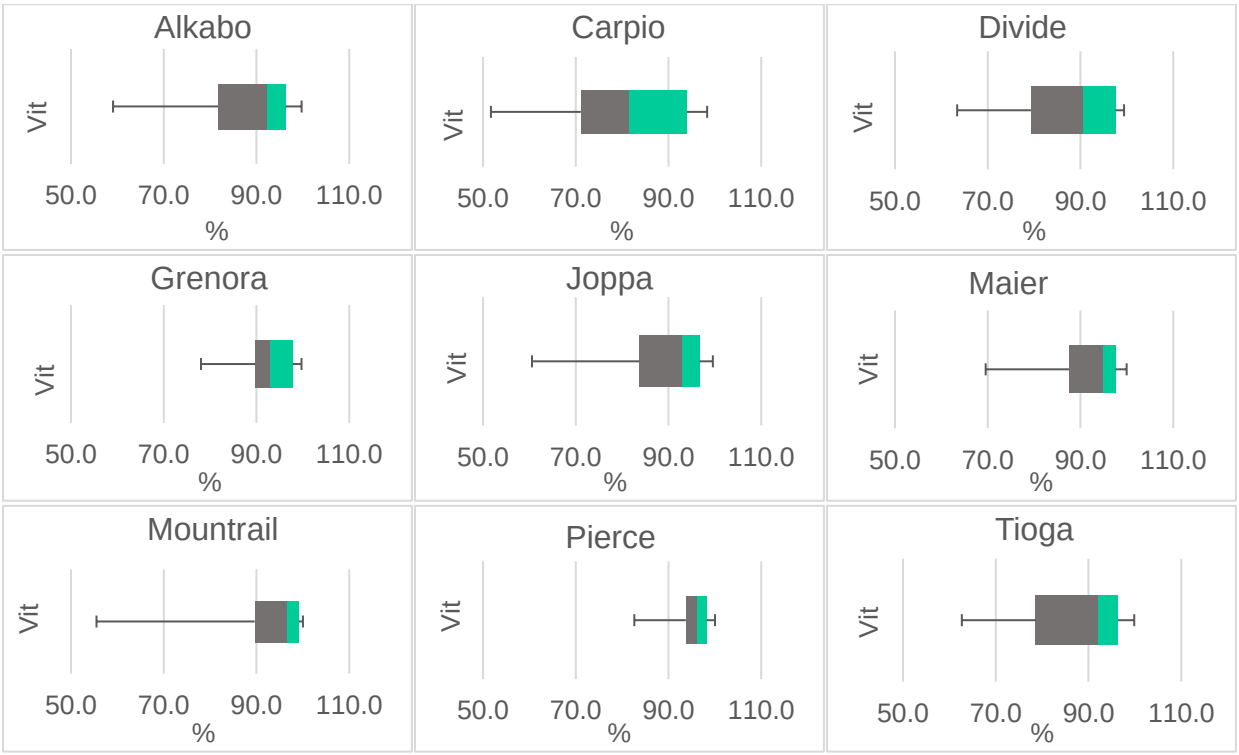

Figure 15. Boxplots of grain vitreousness (vit). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

The impact of weather factors on vitreous kernel content varied with genotype but in general, vitreous kernel content was negatively affected by high rainfall and exposure to the relative humidity of higher than 80% (Table 16; Rharrabti et al., 2003b). Vitreous kernel contents of Alkabo, Maier, Mountrail, and Tioga were significantly and negatively affected by total rainfall. Similarly, high relative humidity significantly and negatively affected the vitreous kernel content of Carpio, Maier, and Pierce.

Table 16. Correlation coefficient between grain quality traits and weather factors with vitreous kernel content in nine durum wheat genotypes across years and locations [a].

	Vitreous Kernel Content								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain traits									
Grain filling duration	-0.20 ns	-0.26 ns	-0.19 ns	-0.02 ns	-0.39 ns	-0.43 *	-0.56 **	-0.28 ns	-0.39 ns
Grain protein (%)	0.62**	0.65***	0.68***	0.58**	0.56**	0.56**	0.54**	0.49*	0.54**
Weather factors									
T max (ºC)	0.10 ns	0.28 ns	0.15 ns	0.13 ns	0.24 ns	0.39 ns	0.27 ns	0.28 ns	0.34 ns
T min (ºC)	0.15 ns	0.25 ns	0.20 ns	0.03 ns	0.18 ns	0.20 ns	0.23 ns	0.07 ns	0.22 ns
# days temp ≥ 30 ºC	0.12 ns	0.34 ns	0.17 ns	0.19 ns	0.21 ns	0.41 ns	0.26 ns	0.31 ns	0.34 ns
# days temp ≤ 13 ºC	-0.08 ns	-0.18 ns	-0.11 ns	0.04 ns	-0.20 ns	-0.14 ns	-0.25 ns	0.05 ns	-0.19 ns
Total rain (mm)	-0.43*	-0.13 ns	-0.16ns	-0.14ns	-0.40ns	-0.47*	-0.72***	-0.40 ns	-0.45*
# days RH ≥ 80%	-0.09 ns	-0.43 *	-0.004 ns	-0.06 ns	-0.35 ns	-0.42 *	-0.32 ns	-0.37 ns	-0.31 ns
RH	-0.18 ns	-0.43 *	-0.16 ns	-0.22 ns	-0.26 ns	-0.47 *	-0.26 ns	-0.45 *	-0.38 ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and quality traits with vitreous kernel content is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

These results are supported by the stepwise linear regression models presented in Table 17. Weather data explained none to 52% of the variation in vitreous kernel content. Vitreous kernel content of Divide, Grenora, and Joppa were not explained by weather data, while Alkabo, Carpio, Maier, Mountrail, Pierce, and Tioga were negatively affected by damp conditions caused by either rainfall or high humidity. Sieber et al. (2015) also reported a decline in vitreousness of durum wheat due to exposure to humidity. Since in most genotypes, weather data accounted for 18-52% of variability, other factors beyond weather, such as agronomic factors, explained why durum grown at Langdon-12 and Hettinger-15 had low kernel vitreousness.

Table 17. Stepwise linear regression for weather factors with vitreous kernel content (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
Vitreous kernel content	Alkabo	Total rain	(-)	0.18	0.18
	Carpio	# days RH ≥ 80%	(-)	0.19	0.19
	Divide	----	----	----	----
	Grenora	----	----	----	----
	Joppa	----	----	----	----
	Maier	Total rain	(-)	0.22	0.22
	Mountrail	Total rain	(-)	0.52	0.52
	Pierce	RH	(-)	0.20	0.20
	Tioga	Total rain	(-)	0.21	0.21

[a] (-) indicates negative effect on the vitreous kernel content.

Relative humidity accounted for similar variation in vitreousness of Carpio and Pierce (lowest and highest vitreous kernel content, respectively). It is assumed that differences in their stability were highly dependent on grain protein content and the ability to maintain kernel vitreousness. There was a positive and significant correlation between protein content and vitreousness in Pierce and Carpio, (r=0.49 at $P < 0.05$ and r= 0.65 at $P < 0.001$, respectively,

Table 16). In fact, Pierce had the second-highest protein content. The high protein content is associated with the formation of a compact protein matrix which leads to kernel vitreousness. Carpio had the fourth-lowest protein quantity which was not significantly different from Joppa (lowest protein content). So, the lowest vitreous kernel content in Carpio was presumably due to its low compact protein structure, more sensitivity of this genotype to damp conditions (more days with RH ≥ 80% (Table 17), and eventually its inability to maintain its vitreousness under humid environment. These results were in agreement with those reported by Sieber et al. (2015).

Falling number

Mean values for falling numbers averaged over the environment varied with genotype (Table 8). Alkabo and Maier had the lowest average falling number (406 and 403 sec, respectively) and Carpio had the highest average falling number (469 sec). The mean for falling numbers for all genotypes were all above 400 sec. However, falling numbers varied with the environment. All environments where the number of days from anthesis to harvest were $\underline{\leq}$ 30, had high falling numbers (386-551 sec). The effect of days from anthesis to harvest >30 on the falling number was variable ranging from 107 to 498 sec. The days to harvest for the four lowest falling number environments (Langdon-14, 269 sec; Hettinger-14, 270 sec; Minot-14, 130 sec; and Dickinson-14, 130 sec) were 32, 33, 36, and 41, respectively. It is not the duration of grain fill that causes the low falling number but the exposure of the grain to damp conditions. The occurrence of low falling numbers with extended days from anthesis to harvest is attributed to the increased probability and increased time of being exposed to damp conditions that are favorable for preharvest germination. Within a given genotype, the ranges in falling number values over the 24 environments were greater (Table 8) than the ranges in response over genotypes within a given environment (Table 9). The overall average range of falling numbers

for environments within a genotype was 482 sec (Table 8) while the overall average range of falling number for genotypes within an environment was 109 sec (Table 9). These results explain why the relative proportion of variance was high for the environment (98%) than the genotype (2%) (Table 7).

Range in falling numbers varied with genotype (Table 8, Figure 16). The overall range was smallest with Pierce (425 sec) and greatest with Joppa (513 sec). For all genotypes, the second, third, and fourth quartiles (75% of values) had a narrower range than the first quartile alone and all had falling number values above 360 sec, which suggests that an unfavorable environment caused bigger variation in falling number than did a favorable environment. The range is an indicator of environmental effect. The smaller range for Pierce compared to Joppa indicates that Pierce was less affected by the environment than was Joppa.

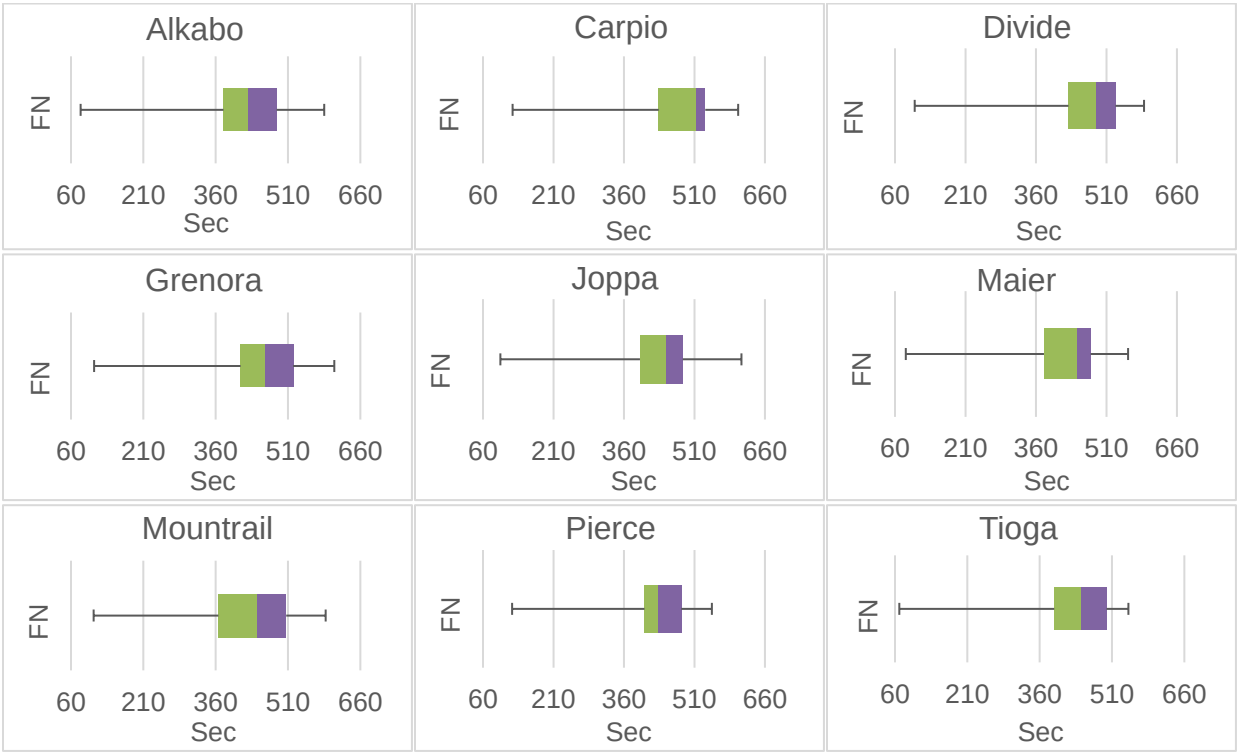

Figure 16. Boxplots of grain falling number (FN). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Correlation analysis between weather factors and the falling number indicated that a high falling number was favored by high maximum air temperature and more days with temperature ≥ 30 ºC. However, cooler air temperature (≤ 13 ºC), total rainfall, and more days with relative humidity above 80% negatively affected the falling number (Table 18). These findings were supported by a positive correlation between warmer days with a falling number, and a negative correlation between the latter with humid and cool environmental conditions (Table 18; Gooding et al., 2003). In fact, the decline in falling number value in the mentioned environment was due to the impact of rainfall on pre-harvest sprouting, and consequently, more activity of α-amylase. Gooding et al. (2003) also reported that wet conditions during grain filling decreased falling numbers. Stepwise linear regression analysis using weather data indicated that for all genotypes total rain and grain filling duration had a negative effect, reduced falling number, and accounted for the greatest variation (44-64%) in falling number (Table 19).

Table 18. Correlation coefficient between grain quality trait and weather factors with falling number in nine durum wheat genotypes across years and locations [a].

	Falling number								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain trait									
Grain filling duration	-0.50*	-0.54**	-0.50*	-0.54**	-0.58**	-0.52*	-0.66***	-0.61**	-0.61 **
Weather factors									
T max (ºC)	0.42*	0.30ns	0.36ns	0.39ns	0.43*	0.40ns	0.50 *	0.43 *	0.42 *
T min (ºC)	0.14ns	0.06ns	0.15ns	0.30ns	0.32ns	0.20ns	0.26ns	0.21ns	0.17ns
# days temp ≥ 30 ºC	0.44*	0.30ns	0.35ns	0.38ns	0.39ns	0.44*	0.49*	0.45*	0.42*
# days temp ≤ 13 ºC	-0.21ns	-0.12ns	-0.20ns	-0.30ns	-0.33ns	-0.23 ns	-0.32ns	-0.26ns	-0.21ns
Total rain (mm)	-0.66***	-0.74 ***	-0.67 ***	-0.55**	-0.59**	-0.57 **	-0.62**	-0.59 **	-0.66 ***
# days RH ≥ 80%	-0.48*	-0.38ns	-0.34ns	-0.35ns	-0.29ns	-0.49*	-0.49*	-0.41*	-0.44*
RH [b]	-0.40ns	-0.20ns	-0.23ns	-0.32ns	-0.26ns	-0.45*	-0.45*	-0.42*	-0.39ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and quality trait with falling number is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

[b] RH: Relative humidity.

Table 19. Stepwise linear regression for weather factors with the falling number (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
Falling number	Alkabo	Total rain	(-)	0.44	0.54
		Grain filling duration	(-)	0.11	
	Carpio	Total rain	(-)	0.55	0.55
	Divide	Total rain	(-)	0.45	0.45
	Grenora	Total rain	(-)	0.31	0.44
		Grain filling duration	(-)	0.13	
	Joppa	Total rain	(-)	0.35	0.51
		Grain filling duration	(-)	0.16	
	Maier	Total rain	(-)	0.33	0.45
		Grain filling duration	(-)	0.12	
	Mountrail	Total rain	(-)	0.20	0.64
		Grain filling duration	(-)	0.44	
	Pierce	Total rain	(-)	0.17	0.54
		Grain filling duration	(-)	0.37	
	Tioga	Total rain	(-)	0.44	0.61
		Grain filling duration	(-)	0.18	

[a] (-) indicates negative effect on the falling number.

Conclusions

Weather factors such as air temperature, rainfall, and relative humidity during grain filling were the most important elements in a variation of grain quality traits for each genotype which resulted in differences in trait stability. High air temperature and more days with temperature ≥ 30 ºC promoted high falling number and grain protein content; ideal growing locations to achieve high vitreous content were those with lower rainfall and relative humidity. However, high 1000-kernel weight and test weight favored by growing locations with cooler air temperature. The results of this research indicate that variability in grain traits as affected by the environment can differ with genotype. Therefore, genotypes could be selected by growers for planting based on their overall quality and their traits stability across many environments.

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CHAPTER 4: GENOTYPE AND WEATHER INFLUENCED VARIATION IN SEMOLINA AND PASTA COOKING QUALITY TRAITS IN DURUM WHEAT GROWN IN NORTH DAKOTA

Abstract

The objective of this research was to evaluate effects of genotype and weather (air temperature, rainfall, and relative humidity) on semolina and pasta quality traits using nine durum wheat genotypes grown in four years (2012-2015) at six locations in North Dakota. Environment had a dominant impact on semolina protein, pasta color, and pasta cooking quality, while, gluten index was affected most by genotype. Stability of genotype or given trait depended on the small size of range in response across 24 environments. Highest stability for semolina protein and gluten index with Carpio, along with greatest stability in cooked firmness and cooked weight (Grenora), and cooking loss (Divide) indicated that these genotypes were less affected by environment. Across the genotypes (within environment), high semolina protein content and high cooking quality were favored by growing locations with high air temperature, while greatest gluten index and high pasta color were favored by growing locations with low relative humidity and low rainfall, respectively. High semolina protein content resulted in pasta with greatest cooking quality. Each genotype differed in their response to environment that resulted in variability in quality traits and stability. Thus, results of this study could assist breeders in selecting genotypes based on their overall quality and on their stability across environments.

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum*) is a required raw ingredient for making pasta with great quality (Mariani et al., 1995). Durum breeding programs emphasize releasing

durum cultivars with high grain protein content, preferably above 13%, as well as high protein quality (gluten strength), and yellow color due to high levels of yellow pigments in the endosperm; all of these quality factors are considered to be key attributes necessary in manufacturing high quality pasta (Hare, 2017; Miskelly, 2017). In fact, protein content is the key quality trait contributes to pasta quality, while gluten strength is considered as a secondary contributor in pasta when dried at high temperatures (Dexter and Matsuo, 1977; D'Egidio et al., 1990).

In effort to select genotypes that produce premium pasta, durum breeding programs concentrate on improved of agronomic and end-use quality traits. Phenotypic response of trait is determined by genes (genotype) and environment. Cultivars have unique set of genes or genotype. The expression of genes is controlled in part by environment. Cultivars (genotypes) often respond differently to environmental conditions. Environment can be described by weather factors such as temperature, humidity, timing and amount of precipitation and agronomic factors such as soil nitrogen and sulphur fertilization, disease control, and insect control (Grausgrubera et al., 2000).

Durum wheat quality traits mainly depend on environment, genotype, and genotype × environment interaction (Rharrabti et al., 2003a,b; Vida et al., 2014), which manifest during grain filling period (Fois et al., 2011). A given trait can be more or less affected by environment. For example, grain protein content has been reported to be influenced primarily by environment, while yellow pigment content and gluten index were predominantly under genetic control (Rharrabti et al., 2003a,b; Vida et al., 2014). Vida et al. (2014) reported that year (environment) and genotype × environment were lesser contributors to gluten index; however, genotype and environment have relatively a similar impact on pasta cooking quality (Marchylo et al., 2001).

Ohm et al. (2017) reported that environment had a dominant role on variation of pasta cooking quality traits through impact of weather factors on protein content.

High air temperature and water deficiency are important weather elements that have been reported to affect durum wheat quality through rise in protein quantity and improved gluten strength (Gooding et al., 2003; Flagella et al., 2010; Fois et al, 2011; Ferreira et al., 2012). Increase in grain protein concentration is due to a decline in duration of grain filling and reduction of starch synthesis (Triboi et al., 2006). Improvement in gluten index is due to an increase in glutenin fractions (Flagella et al., 2010). Fois et al. (2011) reported a decline in gluten index due to exposure to high temperature. In fact, heat stress contributed to changes in gluten functionality through a decline in unextractable polymeric protein and a rise in soluble polymeric protein and low molecular weight gliadins (Fois et al., 2011). These results indicate that there might be a threshold above which temperature has a detrimental effect on gluten index. Besides temperature, there was a negative correlation between precipitation during stem elongation and protein content (Smit and Gooding, 1999 as cited by Gooding, 2017). In fact, early rainfall may reduce nitrogen available to the plant by leaching soil nitrogen out of the root zone (Smit and Gooding, 1999 as cited by Gooding, 2017).

Brightness and yellowness of pasta are important color attributes (Elias and Manthey, 2005). Variation of yellowness in pasta and semolina (Borrelli et al., 2008) depends on carotenoid pigments concentration and their degradation through oxidation. Semolina yellow color is under genetic control (Borrelli et al., 1999); however, environment has a predominant role on brightness (Matsuo et al., 1982). Yellow pigment content is a heritable trait which genotype has a dominant effect on its variation compared to that environment (Borelli et al., 1999). However, high seasonal temperatures can led to an increase in this trait (Rharrabti et al.,

2003b). Results of a study reported by Pinheiro et al. (2013) showed that semolina brightness (L*) was negatively affected by rainfall and maximum temperature, while yellowness (b*) did not show any changes under water input and high temperature.

Improvement of durum wheat quality traits is one of the most important objectives of a durum breeding program. In fact, industry requires stability in quality of the raw material because it supports consistent end-product quality (Grausgruber et al., 2000). Since genotype and weather factors can significantly affect the quality attributes of durum wheat and end-use pasta products, understanding variation in semolina quality traits, would be useful for improving pasta quality (Pinheiro et al., 2013). The objective of this research was to determine the effects of genotype and weather on semolina and pasta quality traits of nine selected durum wheat genotypes grown in six locations during years (2012-2015) in North Dakota. These results will be useful in assisting the breeder in selecting the best genotypes capable of maintaining their quality under diverse environmental situations.

Materials and Methods

Genotypes and Environment

Nine durum wheat genotypes [Alkabo (Elias and Manthey, 2007), Carpio (Elias, Manthey, & AbuHammad, 2014), Divide (Elias and Manthey, 2007), Grenora (Elias and Manthey, 2007), Joppa (Elias and Manthey, 2016), Maier (Elias and Miller, 2000), Mountrail (Elias and Miller, 2000), Pierce (Elias, Manthey, & Miller, 2004), and Tioga (Elias and Manthey, 2012)] were harvested from unreplicated drill strip plots (75 × 1.2 m) grown at six locations in four years (2012-2015). Growing locations included Carrington, Dickinson, Hettinger, Langdon, Minot, and Williston, North Dakota. Weather data was obtained from North Dakota Agricultural Weather Network (NDAWN). NDAWN weather stations located at individual ND Agricultural

Research Centers where drill strips samples were grown. Weather factors that were recorded daily during grain filling period included daily mean of maximum, minimum, and mean air temperature, total rainfall, and dewpoint temperature. The number of days when $T_{max} \geq 30$ ºC, and $T_{min} \leq 13$ ºC, relative humidity, and number of days with relative humidity ≥ 80% were determined from the collected weather data. The equation described by Alduchov & Eskridge (1996) based on Magnus equation was used to calculate relative humidity (RH). This formula was obtained from (http://bmcnoldy.rsmas.miami.edu/Humidity.html).

$$RH = 100\ (EXP((17.625 \times TD)/(243.04+TD))/EXP((17.625 \times T)/(243.04+T)))$$

where EXP is the exponential function in Excel; TD is dew-point temperature (ºC); and T is average temperature (ºC).

Quality Tests on Semolina

Tempering to 15.5% moisture was performed to durum wheat samples and after that, they were milled to semolina utilizing a Quadramat Jr. Mill as described by AACCI Approved Method 26-50.01 (Brabender GmbH & Co.KG, Germany). Semolina samples were stored at 4 ºC for further chemical tests. Crude protein-combustion method was conducted to determine protein content as described in the AACCI Approved Method 46-30.01. Gluten index and wet gluten tests were run in duplicate using AACCI Approved Method 38-12.02. Gluten index and wet gluten were measured using Perten Instruments (Model 2200, Hudding, Sweden).

Quality Tests on Pasta

Semolina samples were hydrated to 32% moisture with warm (45°C) distilled water. Then, Hobart mixer (Model 100, Troy, Ohio, USA) was adjusted at high speed and sample was mixed for 4 min. Pasta extruder (DeMaCo, Brooklyn, N.Y, USA) was run to extrude the mixed dough into spaghetti. Laboratory dryer (Standard Industries, Fargo, ND, USA) was utilized at

low temperature drying cycle as describe by Ohm et al. (2017) with a minor modification (length: 21 h; peak temperature 40°C) to dry extruded spaghetti. The dry spaghetti samples were evaluated for physical quality by determining their color according to AACCI Approved Method 14-22.01 using Minolta colorimeter (Model CR410, Japan). The scores were generated using a color map according to the method of Debbouz (1994). Color map is set on scale from 4.0 to 12.0. A score of 8.0 or higher is considered as good.

Pasta cooking quality was determined by cooking each spaghetti sample (10 g) in boiling water (350 mL) for 12 min. Cooking qualities such as cooked firmness and cooking loss were determined using AACCI Approved Method 66-50.01. Evaluation of firmness was conducted by using texture analyzer (TA-XT2, Texture Technologies, Scarsdale, NY, USA). Cooking loss was determined through water evaporation to dryness in a forced-air-oven at 110 °C overnight. Pasta product weight was measured and converted to percentage of increase in pasta weight after cooking and indicated as cooked weight (Deng et al., 2017).

Statistical Analysis

Least square mean, median, and range for quality traits were determined. Least square means of semolina and pasta quality traits for nine durum genotypes across environment (years and locations) were analyzed using mixed model (type III), considering environment as a random effect and genotype as a fixed effect. In general, mixed model is used for analysis of variance for unbalanced data and in this method, locations were considered as replictions. Correlations between meteorological factors (weather data) with quality traits were evaluated on a basis of Pearson's correlation coefficient. Simple linear stepwise regression was applied to explain which weather data explained greatest variation in different semolina and pasta quality traits. Each of the individual quality trait was considered as a dependent variable.

All analysis was done using SAS software version 9 (SAS Institute, Cary, NC, U.S.A). Effects of environment, genotype, and genotype × environment interaction on different quality traits were determined. Intraclass correlation coefficient was calculated as the proportion of variance attributed to genotype relative to that of variation of genotype × environment interaction and error variance as described by Caffe-Treml et al. (2011). Data was analyzed through boxplot utilizing Microsoft Excel 2016. Boxplots are descriptive statistics that provide information about distribution of data by grouping of them based on minimum, first quartile, median, third quartile, and maximum.

Results and Discussion

Estimates of variance components and intraclass correlation coefficients are presented in Table 20. Effect of genotype, environment, and combination of genotype × environment interaction with experimental error (residuals) were evaluated to determine which source of variation had the greatest impact on each quality traits in semolina and cooked pasta. The greatest relative proportion of variance for each component has the most influence on that trait (Caffe-Treml et al., 2011). The low residual values for all traits evaluated indicated that they were not greatly impacted by G × E interaction. Gluten index was affected most by genotype (75%) while the remaining quality traits were predominately affected by environment (88-97%). These results are in agreement with Vida et al. (2014) and with Chapter 2 where both reported that gluten index was most influenced by genotype. Dominant effect of environment on protein content as well as pasta cooking quality was in agreement with (Rharrabti et al., 2003 a,b; Fois et al., 2011; Ohm et al., 2017; Chapter 2).

Table 20. Estimates of variance components and intraclass correlation coefficient from evaluation of nine durum genotypes in 24 environments for semolina and pasta cooking quality traits [a].

	Relative proportion (%) of variance components			
Quality traits	Genotype	Environment	Residual	Intraclass correlation
Semolina				
Protein content	2	97	1	0.76 [II]
Wet gluten	11	88	1	0.91 [III]
Gluten index	75	24	2	0.98 [III]
Pasta				
Cooked firmness	4	96	1	0.87 [II]
Cooked weight	4	95	2	0.66 [I]
Cooking loss	5	94	2	0.68 [I]
Pasta color	6	93	1	0.81 [II]

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.
[I, II, III] For intraclass correlation coefficient, parameter with I is moderate (0.5-0.75); with II is moderately high (0.75-0.90); with III is excellent (>0.90).

The greater average range in response of environments within each genotype (Table 21) compared to the range of response of genotypes within an environment (Table 22) also indicate the predominant influence of environment on variation of semolina and pasta quality traits. In fact, the average range of response of environments within each genotype and of genotypes within each environment were 5.4 and 1.5% for semolina protein, 2.6 and 0.9 for pasta color, 2.6 and 0.9 g.cm for cooked firmness, 37 and 15% for cooked weight, and 3.2 and 1.3% for cooking loss (Tables 21 and 22). However, gluten index was mainly affected by genotype so that the average range of response of genotype within each environment was greater than that of environments within each genotype 70 and 48.5% (Tables 21 and 22). For wet gluten, although the average range of environments within each genotype (7.3%, data is presented in appendix Table A1) was slightly less than that of genotypes within each environment (8.1%, data is presented in appendix Table A2), results presented in Table 20 indicate the greater effect of environment on variation of this quality trait. While most quality traits evaluated were greatly impacted by environment, the intraclass correlation coefficients for all traits indicate that they

had moderate to high level of broad sense heritability. This suggests that these traits can be improved by plant breeding selection (Caffe-Treml et al., 2011, Eagle et al., 2002).

Table 21. Descriptive statistics for quality parameters for each genotype average over 24 environments [a].

	Semolina protein			Gluten index			Pasta color			Cooked firmness			Cooked weight			Cooking loss		
	(%)			(%)			(---)			(g.cm)			(%)			(%)		
Genotype	Mean [b]	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Alkabo	12.4 e	12.1	(5.5) 10.2-15.7	50.0 e	50.9	(78.4) 2.9-81.4	9.0 ab	9	(2.5) 7.5-10.0	4.1 d	3.8	(3.3) 2.7-6.0	311 abc	314	(34) 291-325	7.0 ab	7	(4.3) 5.4-9.6
Carpio	12.7 bcd	12.4	(4.7) 10.9-15.6	93.0 a	94.1	(19.5) 78.6-98.1	8.9 b	9	(2.0) 8.0-10.0	4.3 ab	4.2	(2.5) 3.4-5.9	310 abcd	311	(32) 294-326	7.0 ab	7.1	(2.7) 5.3-8.1
Divide	12.8 bc	12.7	(5) 10.5-15.5	76.0 c	80	(46.7) 46.8-93.5	8.9 b	9	(2.0) 7.5-9.5	4.2 cd	4.2	(2.4) 3.3-5.7	309 bcd	311	(38) 291-329	6.9 abc	6.8	(2.2) 5.7-7.9
Grenora	12. 6 cde	12.2	(5) 10.6-15.6	65.0 d	71	(52.8) 38.2-91.0	8.9 b	9	(2.5) 7.5-10.0	4.3 bc	4	(2.2) 3.4-5.5	311 abc	313	(30) 293-323	7.0 ab	6.9	(3.4) 5.0-8.4
Joppa	12.3 e	11.9	(5.7) 10.4 - 16.1	83.0b	86.4	(36.0) 62.2-98.2	9.2 a	9.5	(2.0) 8.0-10.0	4.1 d	3.9	(2.4) 3. 0-5.4	312 ab	314	(31) 296-327	7.1 a	7.2	(2.6) 5.7-8.2
Maier	13.1 a	13	(5.2) 11.3-16.5	54.0 e	53.8	(53.1) 29.7-82.7	8.8 b	9	(2.5) 7.5-10.0	4.5 a	4.1	(2.6) 3.6-6.2	307 d	306	(41) 283-324	6.7 cd	6.6	(3.4) 5.4-8.8
Mountrail	12.7 bc	12.3	(5.9) 10.5-16.4	23.0 f	27.7	(52.3) 1.3-53.6	8.4 c	8.5	(3.5) 6.0-9.5	3.8 e	3.5	(2.2) 2.8-5.0	313 a	313	(44) 290-334	6.7 d	6.7	(2.6) 5.5-8.0
Pierce	12.9 ab	12.4	(5.3) 10.8-16.1	62.0 d	61.6	(49.3) 40.8-90.2	8.8 b	9	(2.0) 7.5-9.5	4.4 abc	3.9	(2.7) 3.3-6.0	307 d	309	(44) 282-326	6.8 bcd	6.8	(3.9) 5.4-9.4
Tioga	12.5 de	12.3	(6) 10.2-16.2	77.0bc	80.4	(48.2) 49.5-97.7	8.8 b	9	(4.5) 5.5-10.0	4.4 ab	4.3	(2.9) 3.1-6.0	308 cd	309	(35) 287-322	7.1 a	7.1	(4.1) 5.5-9.6
Mean	12.7	12.4	5.4	65	67.3	48.5	8.9	9.0	2.6	4.2	4.0	2.6	309	311	37	6.9	6.9	3.2

[a] Nine genotypes were grown in 2012, 2013, 2014, and 2015 in six locations.

[b] For each quality trait in each column, means followed by the same letter are not significantly different at $P < 0.05$.

Table 22. Descriptive statistics for semolina and pasta quality parameters in each location and year average across genotypes [a].

	Semolina protein		Gluten index		Pasta color		Cooked firmness		Cooked weight		Cooking loss		Grain filling duration	
	(%)		(%)		(---)		(g.cm)		(%)		(%)		(---)	
Environment [b]	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
L-12	12.4	(1.3) 11.7-13.0	64	(61) 31-92	8.6	(1.0) 8.0-9.0	4.6	(1.5) 3.0-5.0	305	(13) 302-315	7.5	(1.3) 6.9-8.2	28	(2) 27-29
L-13	12.8	(1.1) 12.3-13.4	68	(63) 24-88	8.4	(2.0) 7.5-9.5	5.3	(1.3) 4.7-6.0	312	(11) 307-318	7.0	(1.5) 6.4-7.9	40	(2) 39-41
L-14	11.9	(1.6) 11.0-12.6	58	(89) 3-92	8.1	(1.0) 7.5-8.5	3.7	(0.7) 3.3-4.1	315	(15) 306-321	7.8	(1.9) 6.5-8.5	32	(3) 30-33
L-15	12.3	(2.0) 11.1-13.1	76	(67) 28-96	9.4	(1.0) 9.0-10.0	3.5	(0.7) 3.2-3.9	313	(16) 303-319	7.0	(2.2) 7.6-5.4	31	(3) 29-32
M-12	12.9	(2.1) 11.9-14.0	56	(79) 5-83	9.0	(1.0) 8.5-9.5	4.7	(0.8) 4.2-5.0	306	(18) 299-317	6.8	(1.6) 6.4-8.0	34	(2) 33-35
M-13	13.1	(1.1) 12.6-13.7	77	(63) 34-97	8.5	(1.5) 7.5-9.0	4.9	(1.2) 4.5-5.7	308	(18) 301-319	7.2	(1.3) 6.5-7.7	36	(3) 34-37
M-14	11.8	(1.2) 11.3-12.5	51	(85) 3-87	7.2	(2.5) 5.5-8.0	3.4	(0.8) 3.0-3.8	316	(11) 310-322	8.7	(1.8) 7.9-9.6	36	(3) 34-37
M-15	12.1	(1.6) 11.4-13.0	48	(82) 3-84	9.3	(0.5) 9.0-9.5	3.4	(0.7) 3.2-3.9	314	(11) 309-320	6.9	(2.0) 6.2-8.2	35	(3) 33-36
C-12	15.1	(1.0) 14.7-15.7	63	(52) 40-92	8.7	(0.5) 8.5-9.0	5.7	(1.1) 5.0-6.0	297	(11) 292-303	6.7	(0.5) 6.3-6.8)	29	(3) 27-30

Table 22. Descriptive statistics for semolina and pasta quality parameters in each location and year average across genotypes [a] (Continued).

	Semolina protein		Gluten index		Pasta color		Cooked firmness		Cooked weight		Cooking loss		Grain filling duration	
	(%)		(%)		(---)		(g.cm)		(%)		(%)		(---)	
Environment [b]	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
C-13	11.9	(1.1) 11.3-12.4	57	(64) 27-91	9.4	(0.5) 9.0-9.5	5.0	(0.8) 4.4-5.3	318	(10) 314-323	7.2	(1.0) 6.8-7.8	28	(2) 27-29
C-14	12.0	(2.3) 10.8-13.1	58	(91) 2-93	8.3	(1.0) 7.5-8.5	3.7	(0.7) 3.5-4.2	312	(11) 305-315	7.3	(1.4) 6.7-8.0	34	(5) 32-37
C-15	13.4	(1.6) 12.8-14.4	59	(80) 13-93	9.0	(1.0) 8.5-9.5	4.0	(0.9) 3.5-4.3	305	(17) 295-311	6.9	(0.6) 6.6-7.2	22	(3) 21-24
W-12	16.0	(1.1) 15.4-16.5	82	(44) 54-98	9.0	(1.0) 8.5-9.5	4.3	(1.7) 3.4-5.0	293	(23) 282-305	5.6	(0.7) 5.3-6.0	27	(11) 25-36
W-13	12.2	(1.4) 11.4-12.8	80	(53) 45-98	9.4	(0.5) 9.0-9.5	5.3	(1.6) 4.6-6.2	312	(19) 301-320	5.9	(1.0) 5.6-6.5	34	(5) 31-36
W-14	11.5	(2.2) 10.2-12.4	66	(91) 7-98	9.3	(0.5) 9.0-9.5	3.5	(0.4) 3.3-3.7	318	(25) 302-326	6.5	(1.6) 5.7-7.3	26	(6) 23-29
W-15	11.8	(0.7) 11.5-12.2	72	(57) 39-96	9.8	(0.5) 9.5-10.0	3.8	(0.7) 3.3-4.0	312	(8) 309-317	6.6	(0.7) 6.2-6.9	24	(4) 22-26
D-12	14.6	(1.1) 14.2-15.3	78	(46) 52-98	9.6	(0.5) 9.5-10.0	5.6	(1.0) 4.9-5.9	295	(24) 285-309	6.3	(0.6) 6.1-6.7	24	(2) 22-24

Table 22. Descriptive statistics for semolina and pasta quality parameters in each location and year average across genotypes [a] (Continued).

	Semolina protein		Gluten index		Pasta color		Cooked firmness		Cooked weight		Cooking loss		Grain filling duration	
	(%)		(%)		(---)		(g.cm)		(%)		(%)		(---)	
Environment [b]	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
D-13	10.8	(0.9) 10.5-11.4	64	(62) 35-96	9.4	(0.5) 9.0-9.5	4.0	(0.9) 3.6-4.4	321	(34) 300-334	6.9	(1.1) 6.6-7.6	30	(3) 29-32
D-14	11.8	(1.9) 10.5-12.4	47	(94) 1-95	7.9	(1.0) 7.5-8.5	3.5	(0.9) 2.8-3.8	316	(19) 308-327	7.9	(1.2) 7.3-8.6	41	(2) 40-42
D-15	14.9	(1.1) 14.4-15.5	62	(61) 30-91	9.2	(0.5) 9.0-9.5	4.7	(0.8) 4.4-5.2	296	(8) 293-300	5.8	(1.5) 5.0-6.5	25	(3) 23-26
H-12	13.5	(1.2) 12.8-14.0	81	(62) 36-98	8.9	(1.0) 8.5-9.5	5.1	(0.6) 4.8-5.3	295	(11) 290-301	6.4	(0.7) 6.1-6.7	17	(4) 15-19
H-13	12.4	(2.3) 11.6-13.9	74	(69) 28-97	9.0	(1.0) 8.5-9.5	3.8	(0.7) 3.5-4.2	312	(6) 309-315	6.2	(1.3) 5.8-7.2	27	(4) 26-30
H-14	11.0	(1.5) 10.4-11.9	41	(76) 2-79	7.9	(1.0) 7.5-8.5	3.4	(0.5) 3.1-3.6	318	(13) 314-327	7.7	(1.4) 6.9-8.3	33	(3) 32-35
H-15	11.1	(2.0) 10.2-12.2	61	(95) 1-96	8.9	(1.0) 8.5-9.5	3.2	(1.0) 2.7-3.7	312	(15) 305-320	7.5	(1.4) 6.6-8.0	31	(3) 29-32
Mean	12.6	1.5	64	70	8.8	0.9	5.0	0.9	30.9	15	6.9	1.3	30.2	3.5

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

[b] L: Langdon; M: Minot; C: Carrington; D: Dickinson; H: Hettinger.

Semolina Quality Traits

Effect of genotype and weather

Mean, median, and range of semolina and pasta quality traits for each durum wheat genotype (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) averaged over 24 environments are shown in Table 21. Similarly, the mean and range of semolina and pasta quality traits for each environment averaged over nine genotypes are presented in Table 22.

Semolina protein

Mean values averaged over environment for semolina protein content varied with genotypes (Table 21). Among cultivars, Maier had the highest mean semolina protein (13.1%) while Joppa and Alkabo had the lowest mean protein contents 12.3 and 12.4%, respectively. Except for Joppa and Alkabo, all genotypes met the minimum target semolina protein content of 12.5% (Table 21). Semolina needs to contain at least 12.5% protein in order to meet the 7 g protein per serving indicated on the food label for most dry pasta products.

Semolina protein was mainly affected by environment. Not all growing locations were favorable for high semolina protein (Table 22). Of the 24 environments, 14 environments (L-12, L-14, L-15, M-14, M-15, C-13, C-14, W-13, W-14, W-15, D-13, D-14, H-13, H-14, and H-15) had overall mean protein content below 12.5%. Of these 14 environments, seven environments had at least one genotype that had mean semolina protein content equal or greater than 12.5%.

Within a given genotype, the average range in response of semolina protein content over 24 environments was greater (Table 21) than the average range in response over genotypes within a given environment (Table 22). The overall average range of protein content for environments within a genotype was 5.4 percentage units (Table 21) while the overall average

range for protein content for genotypes within an environment was 1.5 percentage units (Table 22). These results explain why the relative proportion of variance was higher for environment (97%) than for genotype (2%) (Table 20).

Range in semolina protein content varied with genotype (Table 21, Figure 17). The size of the range gives an indication of stability of the trait across environments. The overall range was smallest for Carpio (4.7 percentage unit) and greatest for Mountrail and Tioga (5.9 and 6.0 percentage units, respectively). Thus, semolina protein was more stable (less affected by environment) for Carpio than for Mountrail and Tioga. Interquartile range (IQR) is a measure of variability based on splitting data set into quartiles and represents 50% of the samples' distribution. IQR with a narrow range indicates that there is not a lot of variability within 50% of values. In all genotypes, fourth quartile had greater range, more variability, than either IQR or first quartile indicating that variation was more prevalent with high than with low or medium semolina protein contents.

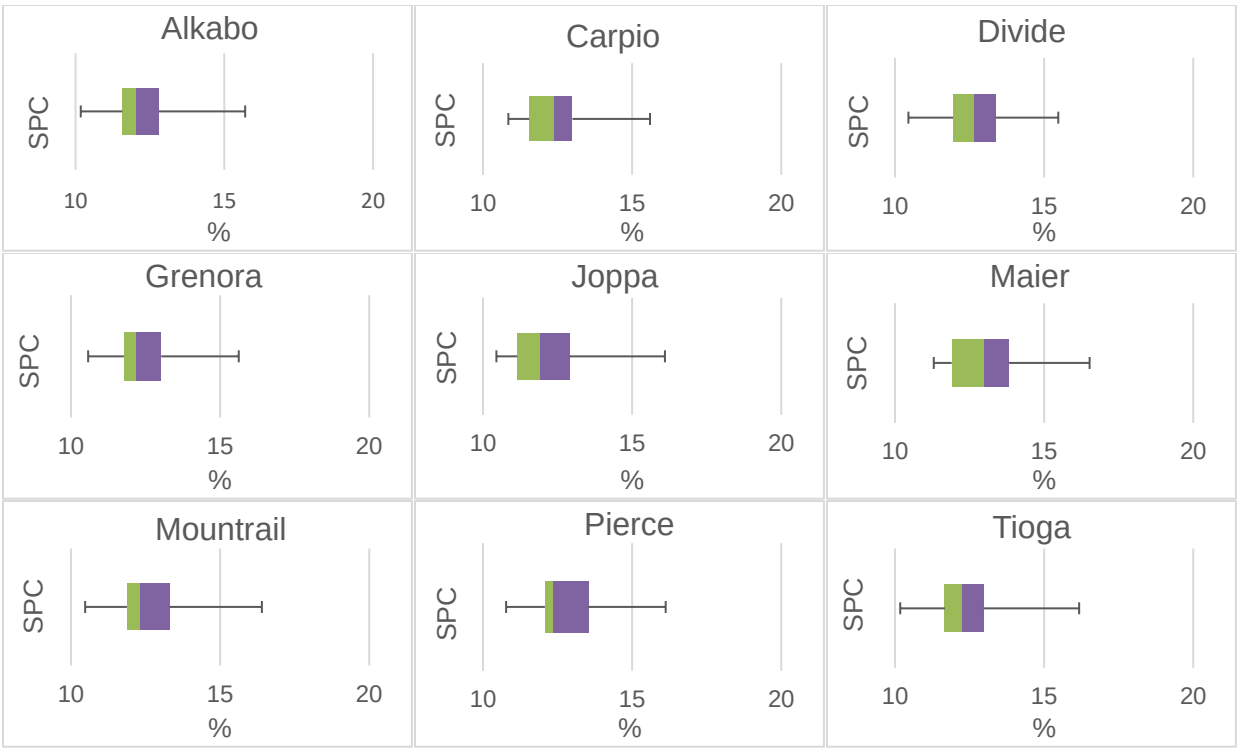

Figure 17. Boxplots of semolina protein content (SPC). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Analysis of weather factors that occurred from anthesis-to-harvest showed that semolina protein content, similar to grain protein content (Chapter 3), was positively correlated to high maximum and high minimum air temperature (which was considered as warm day and night temperatures, respectively), and to exposure to days with temperature ≥ 30 ºC (Table 23). Conversely, semolina protein content was negatively correlated to the number of days with cool temperature (≤ 13 ºC). Semolina protein content did not correlate with rainfall, relative humidity, or days with relative humidity ≥ 80% (Table 23). A significant negative correlation between semolina protein content and days from anthesis-to-harvest was observed for Carpio, Joppa, and Mountrail; however, semolina protein content in other genotypes were negatively but not significantly correlated to grain filling duration. Presence of negative association between grain

filling duration and semolina protein content is attributed to starch accumulation and consequently decline in percent protein with prolonged grain period of grain filling (Chapter 3).

Improvement in semolina protein content due to high temperature is attributed to the negative effect that high temperature has on grain filling duration and consequently a decrease in starch synthesis (Triboi et al., 2006; Fois et al., 2011; Chapter 3). These findings were also highlighted by positive correlation between maximum air temperature during grain filling with protein quantity (Table 23). In addition, environmental factors that affect grain protein had a significant impact on variation of semolina protein due to strong positive correlation between grain protein and semolina protein (r= 0.97 through r=0.99 at $p < 0.001$). Dominant effect of environment on variation of protein content was also found by Fois et al. (2011), Ferreira et al. (2012), Sieber et al. (2015), and Ohm et al. (2017).

Table 23. Correlation coefficient between grain quality trait and weather factors with semolina protein in nine durum wheat genotypes across years and locations [a].

	Semolina protein								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain trait									
Grain filling duration	-0.21ns	-0.42*	-0.31 ns	-0.29 ns	-0.42*	-0.32ns	-0.51*	-0.33 ns	-0.32 ns
Grain protein (%)	0.98***	0.97***	0.99***	0.97***	0.99***	0.98***	0.99***	0.98***	0.98 ***
Weather factors									
T max (ºC)	0.44*	0.59**	0.33 ns	0.50*	0.52*	0.52*	0.57**	0.56**	0.49*
T min (ºC)	0.52**	0.59**	0.53**	0.51*	0.57**	0.61**	0.67***	0.58**	0.64 ***
# days temp ≥ 30 ºC	0.45 *	0.50*	0.30ns	0.52**	0.48*	0.54**	0.49*	0.56**	0.44*
# days temp ≤ 13 ºC	-0.43*	-0.55**	-0.49*	-0.47*	-0.55**	-0.52 *	-0.62***	-0.47*	-0.56 **
Total rain (mm)	-0.14 ns	-0.05 ns	0.01 ns	-0.10 ns	-0.13 ns	-0.14 ns	-0.27 ns	-0.13 ns	-0.01 ns
# days RH ≥ 80%	-0.18 ns	-0.17 ns	0.08 ns	-0.10 ns	-0.27 ns	-0.24 ns	-0.61 ns	-0.20 ns	-0.001 ns
RH (%) [b]	-0.32 ns	-0.34 ns	-0.09 ns	-0. 33 ns	-0.36 ns	-0.40 ns	-0.35 ns	-0.41 *	-0.24 ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and grain quality traits with semolina protein is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

[b] RH: Relative humidity.

Results of stepwise linear regression indicated that high minimum temperature accounted for 27-45% of variation in semolina protein content. For all genotypes, minimum temperature was a predominant contributor except for Grenora where it was more influenced by number of days with temperature ≥ 30 ºC (Table 24). High minimum temperature accounted for more variation in semolina protein content with Mountrail (R^2=0.45) and Tioga (R^2=0.42) compared to that of Alkabo (R^2=0.27) and Divide (R^2=0.28) which indicates that high minimum temperature had a greater impact on Mountrail and Tioga than on Alkabo and Divide. Low stability of semolina protein content with Mountrail and Tioga is reflected by the relatively high sensitivity to high minimum temperatures (Table 24).

Table 24. Stepwise linear regression for weather factors with semolina protein content (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
	Alkabo	T min [b]	(+)	0.27	0.27
	Carpio	T min	(+)	0.34	0.34
	Divide	T min	(+)	0.28	0.28
	Grenora	# days temp ≥ 30 ºC	(+)	0.27	0.27
Protein content	Joppa	T min	(+)	0.32	0.32
	Maier	T min	(+)	0.37	0.37
	Mountrail	T min	(+)	0.45	0.45
	Pierce	T min	(+)	0.33	0.33
	Tioga	T min	(+)	0.42	0.42

[a] (+) indicates positive effect on the semolina protein content.
[b] Minimum temperature represents cool temperature, for all genotypes across years and locations.

Greater effect of environment on wet gluten compared to that of genotype was identical to semolina protein indicating these two quality traits behaved similarly across 24 environments (Table 20). These results were supported by strong positive correlations between wet gluten

content and semolina protein content (r= 0.86 to 0.96 at $p < 0.001$; data is shown in appendix Table A3). Since results for semolina protein and wet gluten content were similar and since most research on pasta quality evaluate semolina protein content than wet gluten content, wet gluten content is not further discussed.

Gluten index

Mean values averaged over environment for gluten index varied with genotype (Table 21). Among genotypes, gluten index was greatest with Carpio, intermediate with Divide, Tioga, and Joppa, and least with Mountrail. Gluten index was mainly affected by genotype, while environment was a secondary contributor. In fact, the average range of response of genotypes within each environment was greater than that of environments within each genotype (70 and 48.5%, respectively) (Tables 21 and 22). These results support the findings in Table 20 where relative proportion of variance associated with environment (24%) was nearly three times less than that of genotype (75%) indicating that variation in gluten index was affected more by genotype than by environment.

Range in gluten index values varied with genotype (Table 21, Figure 18). In general, genotypes with low gluten indexes (Pierce, Maier, Alkabo, and Mountrail) showed more variation in their range for this quality trait than genotypes with high gluten index (Table 21). Carpio had the greatest stability with highest average gluten index (93%) as it had the smallest overall range (19.5 percentage units). Carpio also had the smallest IQR (8.1 percentage units). Alkabo had the greatest variability, low stability, as indicated by the greatest range (78.4 percentage units) indicating that it had more variability with a relatively weak gluten index (50%, Table 21). Comparing the range of the first (low gluten index) and fourth (high gluten index) quartiles, Alkabo, Carpio, Divide, Joppa, and Tioga had greater first than fourth quartiles;

Genora and Maier had similar first and fourth quartiles; and Mountrail and Pierce had smaller first than fourth quartiles. Thus, environments favorable for higher gluten index resulted in more variability with Mountrail and Pierce, while environment favorable for low gluten index resulted in more variability with Alkabo, Carpio, Divide, Joppa, and Tioga.

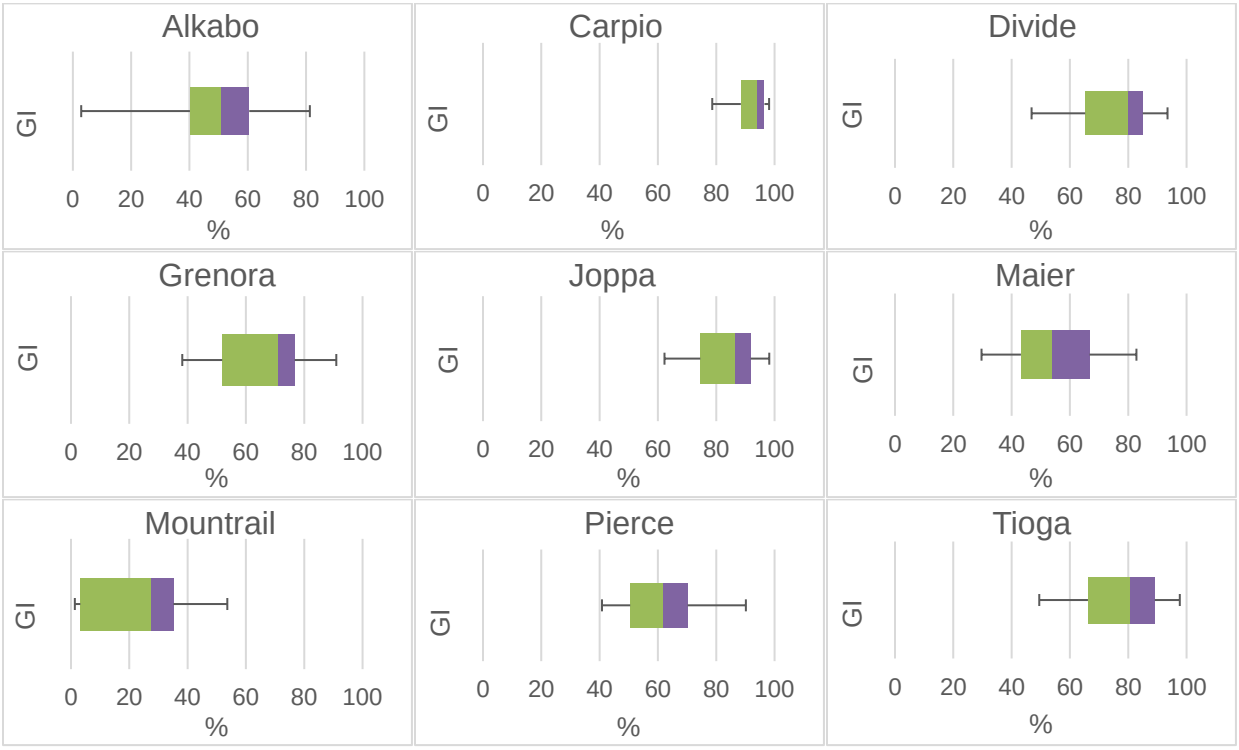

Figure 18. Boxplots of gluten index (GI). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Correlation analysis between weather factors and gluten index indicated that generally there was little to no significant correlation between gluten index and weather factors (Table 25). These results reflect those of relative proportion (%) of variance components in Table 20 which indicated that genotype had a bigger role in determining gluten index than did environment or genotype by environment interaction (residual values).

Table 25. Correlation coefficient between grain quality traits and weather factors with gluten index in nine durum wheat genotypes across years and locations [a].

	Gluten index								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain traits									
Grain filling duration	-0.38 ns	-0.32 ns	-0.34 ns	-0.25 ns	-0.19 ns	-0.30 ns	-0.52 **	-0.42 *	-0.54 **
Semolina protein (%)	0.49 *	0.06 ns	-0.03 ns	0.03 ns	0.47 *	0.42 *	0.62 **	0.57 **	0.13 ns
Weather factors									
T max (ºC)	0.32 ns	0.11 ns	0.13 ns	0.06 ns	0.18 ns	0.41 ns	0.39 ns	0.45 *	0.29 ns
T min (ºC)	0.36 ns	0.05 ns	0.04 ns	0.06 ns	0.08 ns	0.29 ns	0.33 ns	0.35 ns	0.17 ns
# days temp ≥ 30 ºC	-0.31 ns	0.05 ns	0.08 ns	-0.03 ns	0.21 ns	0.41 *	0.34 ns	0.44 *	0.19 ns
# days temp ≤ 13 ºC	-0.35 ns	-0.06 ns	-0.04 ns	-0.04 ns	0.04 ns	-0.18 ns	-0.36 ns	-0.31 ns	-0.18 ns
Total rain (mm)	-0.37 ns	0.14 ns	-0.28 ns	-0.27 ns	-0.13 ns	-023 ns	-0.30 ns	-0.08 ns	-0.21 ns
# days RH ≥ 80%	-0.24 ns	-0.21 ns	-0.25 ns	-0.15 ns	-0.04 ns	-0.28 ns	-0.41 *	-0.27 ns	-0.44 *
RH (%)	-0.32 ns	-0.31 ns	-0.23 ns	-0.17 ns	-0.22 ns	-0.49 *	-0.40 ns	-0.47 *	-0.38 ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,** indicates in each column within each genotype, correlation coefficient between weather factors and grain quality traits with gluten index is significantly different from zero at $P < 0.05$ and 0.01, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Results from stepwise linear regression using weather data to predict gluten index were variable and indicated that weather data accounted for 0 to 24% of variation in gluten index. Variation in gluten index for Alkabo, Carpio, Divide, Genora, and Joppa could not be accounted for by weather data. However, high relative humidity had a significant negative impact on gluten index of Maier, Mountrail, Pierce and Tioga, accounting for R^2 = 0.17 to 0.24% of the variability. In fact, genotypes with weak gluten showed less adaptability to variation in climatic situations such as temperature (Koga et al., 2015) and precipitation (Vida et al., 2014). Lowest gluten index value with Mountrail (23%) showed that for this genotype gluten index was more prone to change depending on weather conditions so that exposure to days with relative humidity ≥ 80% accounted for 17% of variation on gluten index. For Pierce and Maier (with gluten index of 62% and 54%, respectively), relative humidity caused similar variation on gluten index. These findings were also supported by negative and significant correlation between relative humidity and gluten index for Pierce and Maier (r= -0.47 and r= -0.49 at $p < 0.05$, respectively).

Table 26. Stepwise linear regression for weather factors with gluten index (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
	Alkabo	------	------	------	------
	Carpio	------	------	------	------
	Divide	------	------	------	------
	Grenora	------	------	------	------
Gluten index	Joppa	------	------	------	------
	Maier	RH	(-)	0.24	0.24
	Mountrail	# days RH ≥ 80%	(-)	0.17	0.17
	Pierce	RH	(-)	0.22	0.22
	Tioga	# days RH ≥ 80%	(-)	0.19	0.19

[a] (-) indicates negative effect on the gluten index.

Pasta Quality Traits

Effect of genotype and weather

Pasta color

Mean values averaged over environment for pasta color varied with genotype (Table 21). Among genotypes, Joppa and Alkabo had the highest mean pasta color scores of 9.2 and 9.0, respectively, and Mountrail had the lowest mean score of 8.4. The other genotypes had intermediate scores for pasta color (Table 21). In addition, pasta color was greatly influenced by environment. Within a genotype, the ranges in response over the 24 environments were greater (2.6, Table 21) than the ranges in response over genotypes within a given environment (0.9, Table 22). These results support those presented in Table 20 where proportion of variance components associated with environment (93%) was greater than those of genotypes (6%) for pasta color. Nine of 24 environments (Langdon-15, Minot-15, Carrington-13, Williston-13, Williston-14, Williston-15, Dickinson-12, Dickinson-13, and Dickinson-15) had average pasta color scores greater than 9. Williston-15 and Dickinson-12 had the highest pasta color scores (9.8 and 9.6, respectively).

Range in pasta color varied with genotype (Table 21, Figure 19). The overall range was greatest with Tioga (4.5), followed by Mountrail (3.5), while other genotypes had similar range (2-2.5). By comparing the range of the first (low pasta color) and fourth (high pasta color) quartiles, with all genotypes except Carpio, the first quartile had greater range than fourth quartile. Tioga had the greatest range for the first quartile indicating high level of variation at low pasta color score. For Carpio, a wider range for IQR compared to first and fourth quartiles represented more variability within 50% of scores, while, there was uniform distribution of scores in both first and fourth quartiles.

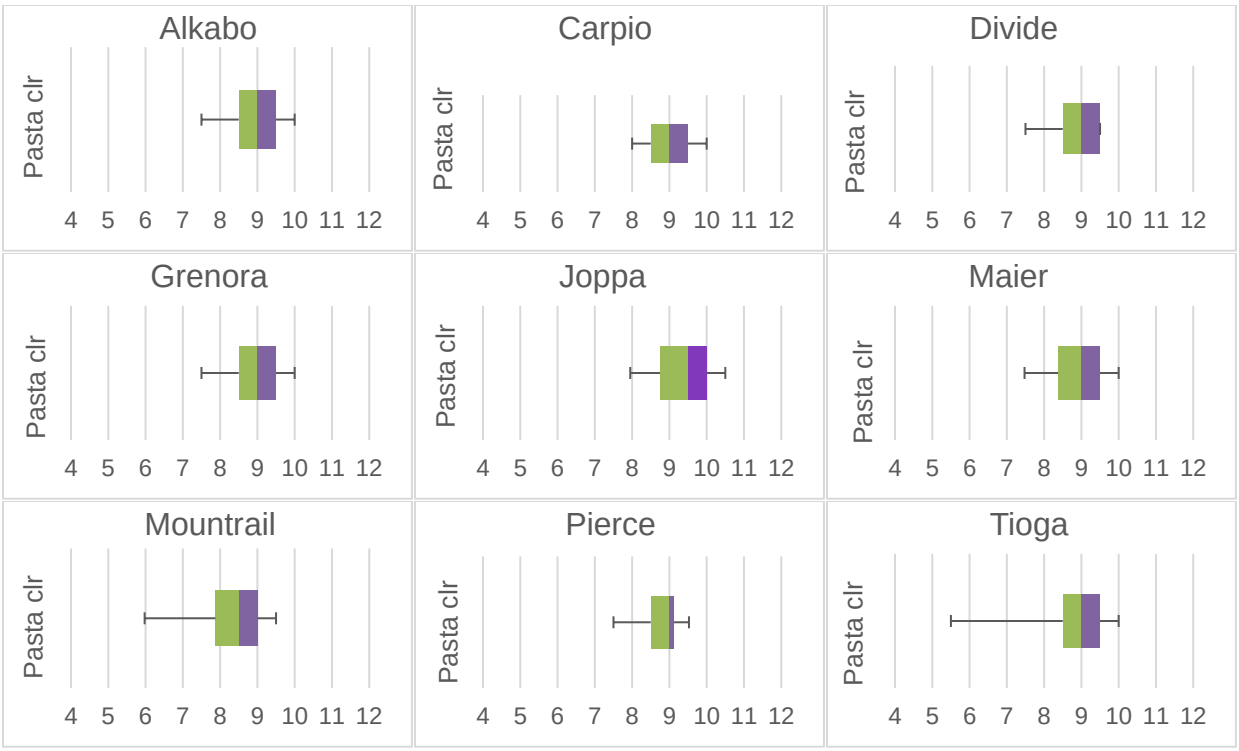

Figure 19. Boxplots of pasta color (pasta clr). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Results of correlation analysis revealed that except for Mountrail and Pierce where pasta color was favored by high air temperature (Table 27), pasta color for the remaining genotypes did not significantly correlate with maximum temperature, minimum temperature, or number of days with temperature ≥ 30 ºC or ≤ 13 ºC. However, total rainfall, relative humidity, and number of days with relative humidity ≥ 80% negatively affected pasta color (Table 27). In fact, highest pasta color scores in growing locations such as Williston-15 and Dickinson-12 (Table 22) were due to less exposure to relative humidity ≥ 80% (data is presented in appendix Table A5). Pasta color score is determined by L-value (brightness) and *b*-value (yellowness) (AACCI). Pasta color score increases as brightness and yellowness increase. Positive impact of high temperature on pasta color was probably through an increase in yellow pigment content (Rharrabti et al., 2003b).

Conversely, Pinheiro et al. (2013) revealed negative correlations between precipitation and maximum air temperature with semolina brightness. According to their findings, semolina L* was favored by long grain filling duration presumably due to cooler air temperature and its positive effect on 1000-kernel weight (Pinheiro et al., 2013). This result was in contrast with our findings where there was a negative correlation between 1000-kernel weight and pasta color (Table 27). Negative impact of 1000-kernel weight on pasta color was in agreement with a study conducted by Clarke et al. (2005). Their results showed indirect impact of environment on yellow pigment by dilution effects through an increase in the proportion of starch and other constituents, and a decline in the relative proportion of yellow pigments. Presence of negative correlation between 1000-kernel weight and pigment color was also reported by Rharrabti et al. (2003b) and Schulthess et al. (2013).

Table 27. Correlation coefficient between grain quality traits and weather factors with pasta color in nine durum wheat genotypes across years and locations [a].

	Pasta color								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain traits									
Grain filling duration	-0.44 *	-0.53 **	-0.44 *	-0.56 **	-0.36 ns	-0.42 *	-0.51 *	-0.48 *	-0.44 *
TKW (g)	-0.44 *	-0.51 *	-0.47 *	-0.62 **	-0.37 ns	-0.53 **	-0.67 ***	-0.73 ***	-0.37 ns
Falling number (sec)	0.81 ***	0.61 **	0.66 ***	0.82 ***	0.67 ***	0.71 ***	0.82 ***	0.75 ***	0.78 ***
Weather factors									
T max (ºC)	0.34 ns	0.25 ns	0.29 ns	0.35 ns	0.27 ns	0.38 ns	0.52 **	0.41 *	0.39 ns
T min (ºC)	0.21 ns	0.20 ns	0.08 ns	0.25 ns	0.11 ns	0.31 ns	0.21 ns	0.41 *	-0.005 ns
# days temp ≥ 30 ºC	0.36 ns	0.22 ns	0.30 ns	0.30 ns	0.28 ns	0.40 ns	0.53 **	0.38 ns	0.38 ns
# days temp ≤ 13ºC	-0.33 ns	-0.28 ns	-0.11 ns	-0.26 ns	-0.02 ns	-0.34 ns	-0.27 ns	-0.41 *	-0.05 ns
Total rain (mm)	-0.55 **	-0.52 *	-0.63 ***	-0.54 **	-0.49 *	-0.57 **	-0.62 **	-0.44 *	-0.54 **
# days RH ≥ 80%	-0.46 *	-0.45 *	-0.42 *	-0.42 *	-0.31 ns	-0.54 **	-0.57 **	-0.39 ns	-0.47 *
RH (%)	-0.33ns	-0.38 ns	-0.33 ns	-0.41 *	-0.32 ns	-0.43 *	-0.51 *	-0.45 *	-0.39 ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and grain quality traits with pasta color is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Stepwise linear regression analysis indicated that weather data accounted for 20-55% of variation in pasta color. For all genotypes, variation in this quality trait was mainly affected by rainfall (24-40%) except for Pierce where RH explained 20% of variation in pasta color (Table 28). Although total rainfall accounted for greater and similar variation in pasta color for Mountrail and Tioga, low stability of pasta color with Tioga is reflected by the high response to total rainfall (Table 28). For Mountrail, variation in pasta color was explained by total rainfall and maximum temperature. Greater response to precipitation and high temperature negatively affected its pasta color. These results were in agreement with data in Table 27 and Pinheiro et al. (2013). For Joppa, 24% of variation in pasta color was explained by rainfall. Although this genotype had the highest pasta color score, it is assumed that other factors besides weather factors are associated with improvement in pasta color (Table 28). The same trend was observed for Alkabo (second highest pasta color), where 55% of variation in pasta color was explained by rainfall and number of days ≤ 13 ºC.

Table 28. Stepwise linear regression for weather factors with pasta color (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
Pasta color	Alkabo	Total rain	(-)	0.31	0.55
		# days temp ≤ 13 ºC	(-)	0.15	
		T min	(-)	0.09	
	Carpio	Total rain	(-)	0.27	0.27
	Divide	Total rain	(-)	0.40	0.40
	Grenora	Total rain	(-)	0.30	0.30
	Joppa	Total rain	(-)	0.24	0.24
	Maier	Total rain	(-)	0.32	0.46
		# days temp ≤ 13 ºC	(-)	0.14	
	Mountrail	Total rain	(-)	0.39	0.53
		T max	(+)	0.14	
	Pierce	RH	(-)	0.20	0.20
	Tioga	Total rain	(-)	0.30	0.30

[a] (+) indicates positive effect on the pasta color; (-) indicates negative effect on the pasta color.

Pasta cooked firmness

Significant differences in mean values averaged over environment for cooked firmness were observed among genotypes (Table 21). Maier (highest protein content) had the greatest cooked firmness (4.5 g.cm) and Mountrail had the lowest cooked firmness (3.8 g.cm). Joppa and Alkabo (lowest protein content) had the second lowest cooked firmness (both 4.1 g.cm) (Table 21). Cooked firmness was mainly affected by environment as indicated by higher proportion of variance components associated with environment (96%) compared to that of genotype (4%) (Table 20). The importance of environment in determining cooked firmness is supported by the results where for each genotype the overall average range in response over the 24 environments was 2.6 g.cm (Table 21) compared to the overall average range in response for genotypes within an environment of 0.9 g.cm (Table 22).

Range in cooked firmness varied with genotype (Table 21, Figure 20). The overall range was smallest with Grenora and Mountrail (2.2 g.cm) and greatest with Alkabo (3.3 g.cm). Greatest stability assigned to Grenora where it had the smallest range. The lowest range for IQR was also assigned to Grenora (1.1 g.cm) indicating less variability within 50% of values. Therefore, presence of lowest range for Grenora, along with narrowest range for IQR, and relative similarity in first and fourth quartiles as indication of uniform variability at lower and higher cooked firmness values contribute to its high stability for cooked firmness. Conversely, except for Grenora and Mountrail, the remaining genotypes had a greater range in the fourth quartile than first quartile indicating greater variability with high than low cooked firmness.

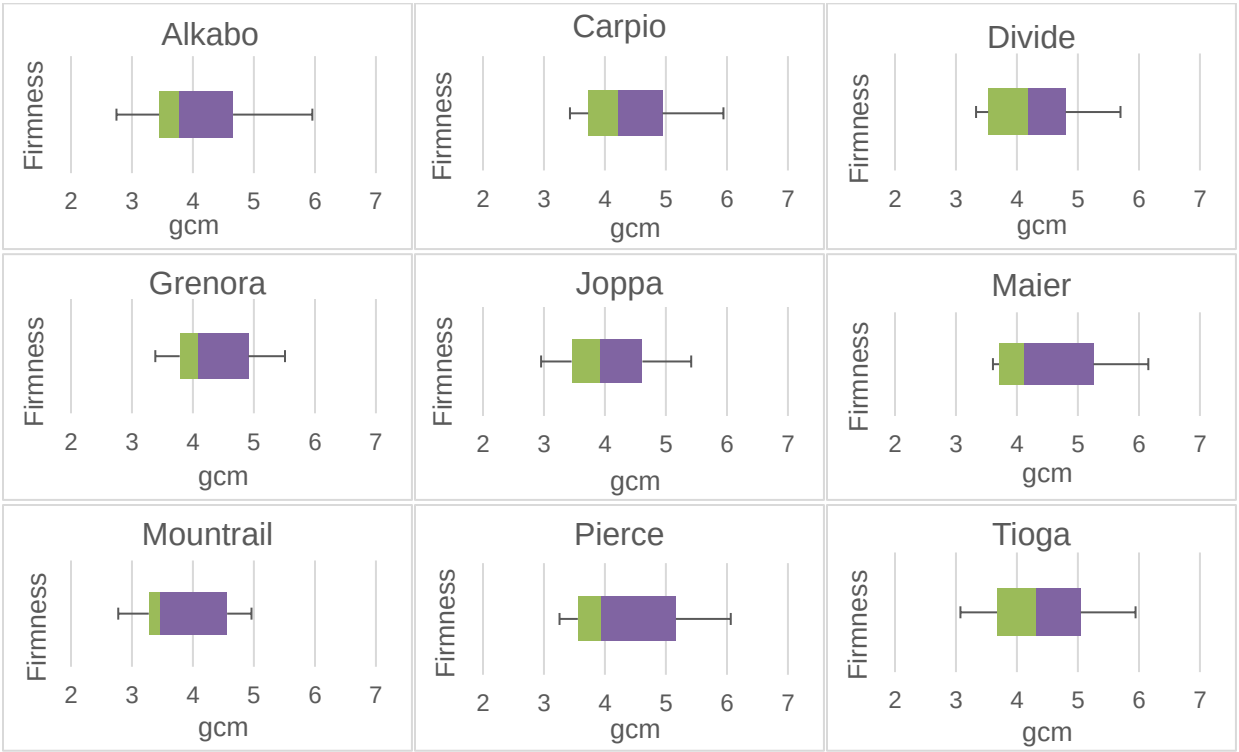

Figure 20. Boxplots of cooked pasta firmness. The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Correlation analysis indicated that no significant association was found between weather factors and pasta firmness. Although not statistically different, cooked firmness for all genotypes had a positive correlation with high maximum and minimum air temperatures, and with days with temperature ≥ 30 ºC, and a negative correlation with number of days with cool temperatures (≤ 13 ºC), rainfall, relative humidity, and more days with RH ≥ 80% (Table 29). These results could be explained by indirect effects of weather conditions on protein content and the presence of a positive significant correlation between semolina protein content and cooked pasta firmness (Table 29; Chapter 2; Ohm et al., 2017). In fact, impact of high temperatures on increased protein content could improve cooked firmness (Fois et al., 2011; Ferreira et al., 2012). These findings were in agreement with stepwise linear regression's results where variation in cooked firmness could not be accounted for by any weather factors (data was not shown). Overall, variation in cooked firmness is attributed to variation in semolina protein content.

Although significant correlation between gluten index and cooked firmness was observed in genotypes with low gluten index values such as Mountrail, Alkabo, Maier, and Pierce (Tables 21 and 29), gluten index was not necessarily associated with variation in cooked firmness indicating predominant role of protein content. In particular with Maier, Pierce, and Alkabo, high and low semolina protein content resulted in high and low cooked firmness, respectively (Table 21), while with Mountrail, semolina protein content did not explain variation in cooked firmness. So, it is assumed that gluten index could be a possible reason resulted in change of cooked firmness with Mountrail.

Table 29. Correlation coefficient between grain quality traits and weather factors with cooked firmness in nine durum wheat genotypes across years and locations [a].

	Cooked firmness								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain/Semolina traits									
Grain filling Duration	-0.23 ns	-0.11 ns	-0.30 ns	-0.15 ns	-0.16 ns	-0.13 ns	-0.25 ns	-0.12 ns	-0.24 ns
Semolina protein (%)	0.60 **	0.54 **	0.66 ***	0.47 *	0.68 ***	0.53 **	0.54 **	0.69 ***	0.59 **
Gluten index (%)	0.45 *	-0.03 ns	0.17 ns	0.18 ns	0.34 ns	0.51 **	0.60 **	0.51 *	0.35 ns
Weather factors									
T max (ºC)	0.20 ns	0.06 ns	0.23 ns	0.08 ns	0.11 ns	0.21 ns	0.17 ns	0.19 ns	0.19 ns
T min (ºC)	0.17 ns	0.06 ns	0.25 ns	-0.01 ns	0.12 ns	0.15 ns	0.05 ns	0.16 ns	0.20 ns
# days temp ≥ 30 ºC	0.18 ns	0.12 ns	0.24 ns	0.10 ns	0.11 ns	0.35 ns	0.20 ns	0.28 ns	0.20 ns
# days temp ≤ 13 ºC	-0.18 ns	-0.05 ns	-0.27 ns	-0.26 ns	-0.17 ns	-0.11 ns	-0.05 ns	-0.10 ns	-0.21 ns
Total rain (mm)	-0.29 ns	-0.25 ns	-0.26 ns	-0.32 ns	-0.16 ns	-0.28 ns	-0.38 ns	-0.25 ns	-0.28 ns
# days RH ≥ 80%	-0.31 ns	-0.22 ns	-0.15 ns	-0.15 ns	-0.18 ns	-0.36 ns	-0.26 ns	-0.22 ns	-0.19 ns
RH (%)	-0.17 ns	-0.14 ns	-0.14 ns	-0.07 ns	-0.4 ns	-0.33 ns	-0.23 ns	-0.18 ns	-0.13 ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and other quality traits with cooked firmness is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Pasta cooked weight

Mean values averaged over environment for pasta cooked weight varied with genotype. Mountrail had the greatest cooked weight (313%), which was significantly different from Divide, Pierce, Maier, and Tioga with the lowest cooked weight values of 309%, 307%, 307%, and 308%, and 308%, respectively (Table 21). Also, cooked weight was influenced by environment. In fact, for each genotype, the range in response over 24 environments was greater (Table 21) than the range in response over genotypes within a given environment (Table 22). The overall average range of cooked weight for environments within a genotype was 37 percentage units (Table 21) while the overall average range for cooked weight for genotypes within an environment was 15 percentage units (Table 22). These results explain why the relative proportion of variance was high for environment (95%) than genotype (4%) (Table 20).

Range in pasta cooked weight varied with genotype (Table 21, Figure 21). The overall range was smallest with Grenora (30 percentage unit) and greatest with Mountrail and Pierce (44 percentage units). Smallest range with Grenora (Table 21) indicated more stability, while genotypes with highest and lowest cooked weight values (Mountrail and Pierce, Table 21) had more variation. Therefore, cooked weight was more stable (less affected by environment) for Grenora than for Mountrail and Pierce. Comparing the range of the first (low cooked weight) and fourth (high cooked weight) quartiles, Alkabo, Grenora, Joppa, Mountrail, Tioga, Pierce, and Maier had greater first than fourth quartiles; Carpio had similar first and fourth quartiles; and Divide had smaller first than fourth quartiles. Thus, environments favorable for high cooked weight resulted in more variability for Divide, while environments favorable for low cooked weight resulted in more variability with all genotypes except Carpio and Divide.

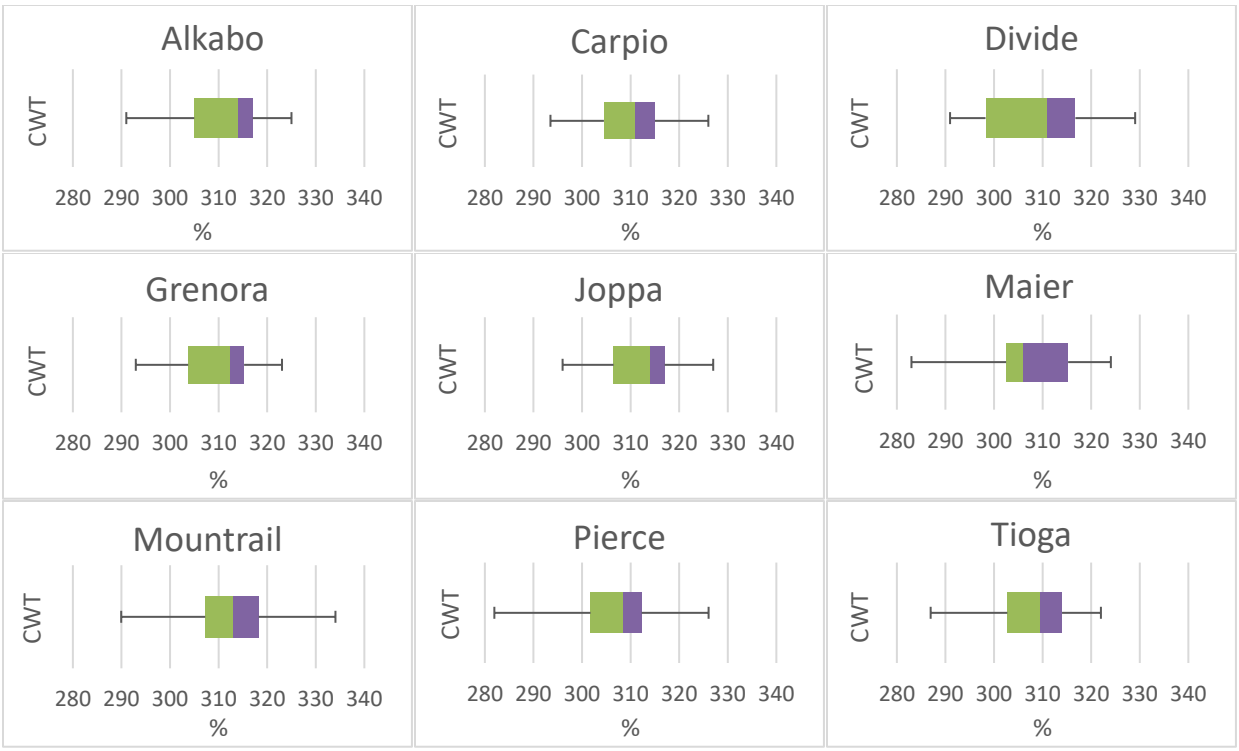

Figure 21. Boxplots of pasta cooked weight (CWT). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Correlation analysis between weather factors and pasta cooked weight indicated that the latter was negatively influenced by high maximum and minimum air temperatures, and days with temperature ≥ 30 ºC, whereas exposure to cooler temperature (≤ 13 ºC) had a positive effect on cooked weight. These results were supported mainly by strong negative correlation between cooked weight and semolina protein content (Table 30). High protein content would result in formation of compact protein structure, a decline in water absorption rate, an improvement in firmness, and finally low cooked weight. In addition, negative correlation between cooked weight and protein content also explained differences in cooked weight in different environments. In fact, Carrington-12, Dickinson-12, Dickinson-15, and Williston-12 had the highest protein content which resulted in lowest cooked weight (Table 22). Besides role of

protein content, cooked weight was negatively affected by cooked firmness (Table 30). Maier and Mountrail had the highest and lowest cooked firmness, respectively, which resulted in the lowest and highest cooked weight (Table 21). Depending on the genotype, there was a correlation between cooked weight and gluten index (Table 30). Cooked weight with Alkabo, Maier, Pierce, and Joppa significantly correlated to gluten index. These results indicated that although there was a moderate and negative correlation between gluten index and cooked weight with Maier (r= -0.63 at $p < 0.001$, Table 30), this genotype by having the low gluten index value had the second lowest cooked weight indicating role of other factors such as semolina protein content on variation of cooked weight. The same trend was observed with Joppa. Despite cooked weight negatively and significantly correlated to gluten index with Joppa (gluten index value of 83%); this genotype had the highest cooked weight. These results indicated that gluten index is not a primary factor determining cooked weight and that protein content had a significant role. In addition, the presence of both low semolina protein content and weak gluten index was associated with high cooked weight. This result was supported by high cooked weight value (311%) with Alkabo (Table 21). Thus, if genotypes have high protein content and low gluten index, variation in cooked weight depended on protein content.

Table 30. Correlation coefficient between quality traits and weather factors with pasta cooked weight in nine durum wheat genotypes across years and locations [a].

	Pasta cooked weight								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain/Semolina/Pasta traits									
Grain filling duration	0.29 ns	0.30 ns	0.49 *	0.42 *	0.34 ns	0.44 *	0.62 **	0.44 *	0.41 *
Semolina protein (%)	-0.86 ***	-0.71 ***	-0.81 ***	-0.78***	-0.84 ***	-0.79 ***	-0.74 ***	-0.89 ***	-0.90 ***
Gluten index (%)	-0.51 *	-0.05 ns	0.02 ns	0.02 ns	-0.46 *	-0.63 ***	-0.37 ns	-0.58 **	-0.35 ns
Cooked firmness (g.cm)	-0.58 **	-0.37 ns	-0.62 **	-0.43 *	-0.80 ***	-0.62 **	-0.47 *	-0.62 **	-0.64 ***
Weather factors									
T max (ºC)	-0.67 ***	-0.41 *	-0.61 ***	-0.61 **	-0.29 ns	-0.69 ***	-0.78 ***	-0.70 ***	-0.55 **
T min (ºC)	-0.72 ***	-0.42 *	-0.68 ***	-0.65 ***	-0.35 ns	-0.65 ***	-0.61 **	-0.71 ***	-0.60 **
# days temp ≥ 30 ºC	-0.59 **	-0.39 ns	-0.52 **	-0.50 *	-0.23 ns	-0.71 ***	-0.67 ***	-0.63 ***	-0.48 *
# days temp ≤ 13 ºC	0.64 ***	0.45 *	0.64 ***	0.64 ***	0.39 ns	0.60 **	0.59 **	0.66 ***	0.53 **
Total rain (mm)	0.06 ns	0.06 ns	-0.04 ns	0.82 ns	-0.03 ns	0.29 ns	0.31 ns	0.04 ns	-0.004 ns
# days RH ≥ 80%	0.29 ns	0.23 ns	0.06 ns	0.05 ns	0.09 ns	0.33 ns	0.29 ns	0.28 ns	0.16 ns
RH (%)	0.41 *	0.25 ns	0.33 ns	0.31 ns	0.10 ns	0.58 **	0.52 **	0.47 *	0.35 ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and quality traits with pasta cooked weight is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Results of stepwise linear regression indicated that temperature factors, high maximum or high minimum temperatures accounted for 21-60% of variation in pasta cooked weight among genotypes (Table 31). In general, results indicate that high air temperatures at night (high minimum temperature) or high daytime temperature (high maximum temperature and high number of days ≥ 30 ºC) were associated with low cooked weight. Carpio had improved cooked weight with increased number of days ≤ 13 ºC, indicating the cool temperatures might favor improved cooked weight. These results were supported by strong negative correlation between semolina protein with cooked weight (r= -0.71 to r= -0.90, $p < 0.001$) (Table 30). High temperatures associated with low cooked weight are associated with high protein content.

Table 31. Stepwise linear regression for weather factors with pasta cooked weight (n= 24).

Quality trait	Genotypes	Weather data	Effect[a]	Partial R^2	R^2
Pasta cooked weight	Alkabo	T min	(-)	0.52	0.52
	Carpio	# days temp ≤ 13 ºC	(+)	0.21	0.21
	Divide	T min	(-)	0.46	0.46
	Grenora	T min	(-)	0.41	0.41
	Joppa	------	------	------	------
	Maier	# days temp ≥ 30 ºC	(-)	0.51	0.51
	Mountrail	T max	(-)	0.60	0.60
	Pierce	T min	(-)	0.51	0.51
	Tioga	T min	(-)	0.37	0.37

[a] (+) indicates positive effect on the pasta cooked weight; (-) indicates negative effect on the pasta cooked weight.

Pasta cooking loss

Mean values averaged over environment for cooking loss varied with genotype (Table 21). Tioga and Joppa had the greatest mean cooking loss (7.1 and 7.1%, respectively), while Mountrail and Maier had the lowest mean cooking loss (6.7 and 6.7%, respectively). Environment greatly affected variation in cooking loss. For each genotype, the range in response

over 24 environments was greater (3.2 percentage units, Table 21) than the range in response over genotypes within a given environment (1.3 percentage units, Table 22). These results reflect the relative proportion of variance which was higher for environment (94%) than genotype (5%) (Table 20).

Range in cooking loss varied with genotype (Table 21, Figure 22). The overall range was smallest with Divide (2.2 percentage units) and greatest with Alkabo and Tioga (4.3 and 4.1 percentage units, respectively). Lower range for Divide indicates more stability for cooking loss compared to those of Alkabo and Tioga. Comparing the range of the first (low cooking loss) and fourth (high cooking loss) quartiles, Carpio, Divide, Grenora, and Joppa had greater first than fourth quartile; and Alkabo, Mountrail, Tioga, Pierce, and Maier had smaller first than fourth quartiles. Thus, environments favorable for high cooking loss resulted in more variability for Alkabo, Mountrail, Tioga, Pierce, and Maier, while environments favorable for low cooking loss resulted in more variability for Carpio, Divide, Grenora, and Joppa (Table 21, Figure 6).

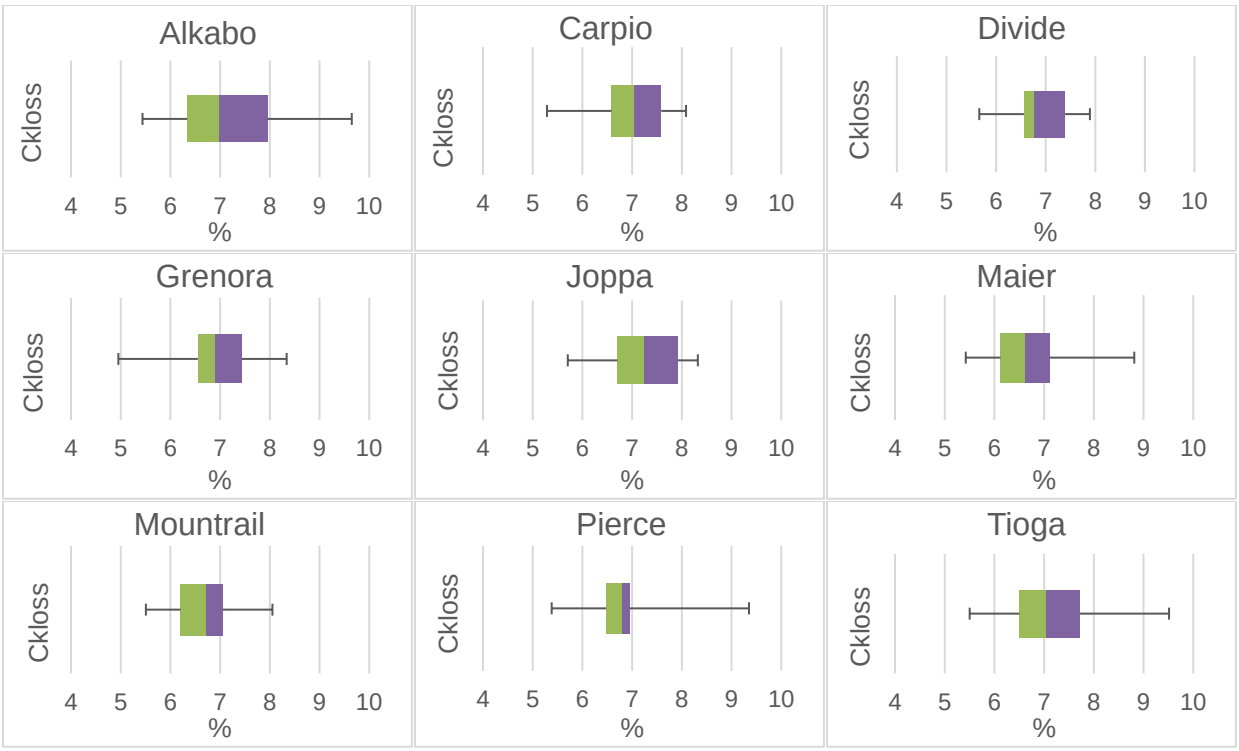

Figure 22. Boxplots of pasta cooking loss (Ckloss). The minimum and maximum values are shown on horizontal line (at the extremes), and median, first quartile, and third quartile values are displayed on the middle, left, and right side of lines on the box, respectively (n=24; four years, six locations).

Correlation analysis between weather factors and cooking loss indicated that cooking loss was negatively related to high maximum temperature and number of days ≥ 30 ºC, and positively correlated with relative humidity (Table 32). In addition, cooking loss was negatively correlated with semolina protein content and gluten index. In fact, Carrington-12, Dickinson-12, Dickinson-15, and Williston-12 resulted in low cooking loss also had high protein content (Table 22). These findings confirm that weather factors that cause improved semolina protein content also resulted in low cooking loss (Table 32). Positive effect of damp conditions on cooking loss probably due to its effect on grain filling duration, resulting in more starch synthesis, and consequently more starch leaching into cooking water during boiling. These results were confirmed by positive correlation between grain filling and cooking loss (Table 32).

Mountrail, Maier, and Pierce by having weak gluten index resulted in lowest cooking loss although there was a negative and significant correlation. Same trend was observed with Tioga which resulted in highest cooking loss by having the second highest gluten index (Tables 21 and 32). Conversely, Alkabo (second lowest gluten index) had the highest cooking loss (Table 21). These findings indicate that gluten index was not a main contributor to the variation in cooking loss, as the latter (cooking loss) was mainly explained by semolina protein content (Table 32). In fact, low cooking loss values for Mountrail, Maier, and Pierce could be explained by their high semolina protein content; while for Tioga, its low protein content resulted in the highest cooking loss (Table 21). These results were supported by negative and significant correlation between protein content and cooking loss (r= -0.48 at $p < 0.05$ for Tioga through r= -0.71 at $p < 0.001$ for Maier, Table 21). Thus, high protein content dominated low gluten index, and consequently decreased cooking loss. However, with Alkabo association of both low protein and low gluten index resulted in high cooking loss (Table 21).

Table 32. Correlation coefficient between grain quality traits and weather factors with pasta cooking loss in nine durum wheat genotypes across years and locations [a].

	Pasta cooking loss								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain/Semolina/Pasta traits									
Grain filling duration	0.43 *	0.43 *	0.38 ns	0.33 ns	0.47 *	0.40 ns	0.48 *	0.46 *	0.55 **
Semolina protein (%)	-0.55 **	-0.59 **	-0.57 **	-0.55 **	-0.68 ***	-0.53 **	-0.53 **	-0.50 *	-0.45 *
Gluten index (%)	-0.60 **	-0.33 ns	-0.20 ns	-0.13 ns	-0.40 ns	-0.71 ***	-0.57 **	-0.60 **	-0.48 *
Cooked firmness (g.cm)	-0.43 *	-0.32 ns	-0.42 *	-0.46 *	-0.58 **	-0.47 *	-0.44 *	-0.36 ns	-0.41 *
Weather factors									
T max (ºC)	-0.51 *	-0.48 *	-0.41 *	-0.45 *	-0.32 ns	-0.38 ns	-0.53 **	-0.44 *	-0.49 *
T min (ºC)	-0.35 ns	-0.46 *	-0.30 ns	-0.26 ns	-0.20 ns	-0.28 ns	-0.24 ns	-0.25 ns	-0.33 ns
# days temp ≥ 30 ºC	-0.54 **	-0.47 *	-0.45 *	-0.50 *	-0.30 ns	-0.43 *	-0.59 **	-0.42 *	-0.47 *
# days temp ≤ 13 ºC	0.39 ns	0.45 *	0.27 ns	0.24 ns	0.22 ns	0.26 ns	0.21 ns	0.23 ns	0.38 ns
Total rain (mm)	0.51 *	0.23 ns	0.32 ns	0.46 *	0.29 ns	0.42 *	0.67 ***	0.36 ns	0.44 *
# days RH ≥ 80%	0.54 **	0.49 *	0.41 *	0.40 ns	0.38 ns	0.33 ns	0.53 **	0.41 *	0.45 *
RH (%)	0.51 *	0.53 **	0.46 *	0.48 *	0.38 ns	0.42 *	0.60 **	0.45 *	0.48 *

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and grain quality traits with pasta cooking loss is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Stepwise linear regression analysis indicated that 19-63% of variation in cooking loss was accounted for by weather factors. Cooking loss from spaghetti made with Alkabo, Carpio, Divide, Mountrail, and Pierce was increased with increased relative humidity and rainfall. Damp conditions are associated with kernel bleaching and preharvest germination. Preharvest germination results in high α-amylase activity as reflected by low falling number. Cooking loss is due to leaching of amylose or starch fragments from starch granules and soluble proteins and other water soluble compounds found in pasta. Grenora and Maier had less cooking loss with high air temperatures which would favor increased protein content and subsequent greater gluten matrix surrounding and protecting starch granules from rupture during cooking. None of the weather factors affected cooking loss with Joppa (Tables 32 and 33). It is assumed that other factors such as protein content indirectly affected variation in cooking loss with this genotype. Joppa having the lowest semolina protein content and second lowest cooked firmness resulted in second highest cooking loss (Tables 21, 32, 33).

Table 33. Stepwise linear regression for weather factors with pasta cooking loss (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
Pasta cooking loss	Alkabo	# days RH ≥ 80%	(+)	0.30	0.30
	Carpio	RH	(+)	0.28	028
	Divide	RH	(+)	0.21	0.21
	Grenora	# days temp ≥ 30 ºC	(-)	0.25	0.25
	Joppa	------	------	------	------
	Maier	# days temp ≥ 30 ºC	(-)	0.19	0.19
	Mountrail	Total rain RH	(+) (+)	0.44 0.18	0.63
	Pierce	RH	(+)	0.20	0.20
	Tioga	T max	(-)	0.24	0.24

[a] (+) indicates positive effect on cooking loss; (-) indicates negative effect on cooking loss.

Conclusions

Weather with high maximum and high minimum air temperature, along with exposure to more days with temperature ≥ 30 ºC promotes high protein content and high pasta cooked firmness. In addition, ideal growing locations to achieve high gluten index and greatest pasta color scores are those with low relative humidity, and low rainfall, respectively. Results of this research indicated that genotypes differed in the magnitude of their response to environment that resulted in variation in stability of semolina and pasta quality traits. The fact that some genotypes are more affected by environment while, some of them are less affected, strongly depends on their genetic potential. Thus, different performance of genotypes and their quality traits due to various weather factors, justifies selection of durum wheat for either genotypes with high stability or desirable improved quality traits.

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CHAPTER 5: RELATIONSHIP BETWEEN QUALITY TRAITS AND PROTEIN MOLECULAR WEIGHT DISTRIBUTION WITH COOKED FIRMNESS AND COOKED WEIGHT OF FRESH PASTA MADE FROM DURUM WHEAT

Abstract

Although storage protein fractions are known to have a dominant impact on pasta cooking quality, detailed information is still lacking on associations between these fractions and the cooking quality of fresh pasta. The objective of this research was to assess associations of grain, semolina, and pasta quality traits and protein molecular weight distribution (MWD) parameters with cooking quality of fresh pasta made from four durum wheat genotypes (Alkabo, Carpio, Divide, and Tioga). Protein contents were greater with samples that produced fresh pasta with high cooked firmness, low cooked weight, and low cooking loss. However, gluten index and mixogram peak time did not correlate with cooked firmness. Extractable polymeric (glutenin) proteins in total flour and protein negatively influenced cooked firmness, while positively affecting cooked weight without any significant effect on cooking loss in fresh pasta. However, effect of unextractable polymeric (glutenin) proteins (UF1 and UP1) and extractable monomeric (gliadin) proteins (EP2) on cooked firmness and cooked weight varied with genotype. Quantitative increase in extractable monomeric protein was associated with a declined in cooked firmness, while it enhanced cooked weight. Variation in cooked firmness was positively associated with unextractable monomeric proteins along with combination of extractable and unextractable albumin + globulin proteins. Overall, cooked weight was positively affected by extractable polymeric and monomeric protein fractions, while impact of unextractable proteins was negatively correlated with cooked weight across all genotypes. Variation in cooking loss was predominantly affected by protein content. Protein content and its

fractions had a predominant role on variation of cooked firmness and cooked weight of fresh pasta, while gluten index and mixogram time-to-peak did not seem to relate with cooking quality. These results showed non-significant correlations between gluten index and cooked firmness with all genotypes. The size-exclusion HPLC of protein was useful in identifying protein components that had dominant roles in determining pasta cooking quality of fresh pasta.

Introduction

Unique yellow color and protein quantity and quality of durum wheat (*Triticum turgidum* L. var. *durum*) make it a required raw ingredient for pasta with great quality (Dexter and Matsuo, 1977; Sissons et al., 2007). Cooking quality is the main characteristic from consumers' aspects (Elias and Manthey, 2005). Cooking quality includes pasta cooked firmness, cooked weight, cooking loss, and surface stickiness (Lafiandra et al., 2012).

Dough rheological properties and pasta cooking quality depend on protein content and particularly endosperm storage proteins (Sissons et al., 2007). Pasta with low protein content generally results in low cooked firmness (Ounane et al., 2006) and a high level of stickiness on its surface due to leaching of starch components into cooking water (Aalami, 2006). High protein content not only surrounds and protects starch granules during cooking, but also retains firmness with overcooking and improves cooked pasta firmness (Ounane et al., 2006).

Gliadins and glutenins are two major classes of wheat storage protein. Glutenins are polymeric proteins that can have high molecular weight subunits (HMW-GS) and low molecular weight subunits (LMW-GS) (Edwards et al., 2003; Johansson et al., 2013). Gliadins are monomeric proteins. There are four types of gliadins classified according to their mobility in acid polyacrylamide gel electrophoresis (α, β, γ, ω) (Sissons, 2008). The ratio of gliadins and glutenins in gluten affects dough rheological properties (Wieser and Kieffer, 2001). In fact,

Edwards et al. (2003) reported that increased glutenin content correlated with high gluten strength in durum wheat. Furthermore, increased glutenin-to-gliadin ratio in semolina as a control sample, enhanced dough strength through increasing the percentage of UPP but its impact on pasta quality traits were different (cooked firmness and stickiness) depending on source of gluten in reconstituted formulation (Sissons et al., 2005). For example, gluten that was extracted from wheat Glenda, resulted in firm cooked pasta, while waxy wheat gluten made soft cooked pasta with high degree of stickiness (Sissons et al., 2007).

Variations in both dough mixing properties and baking quality of different wheat genotypes were under influence of unextractable glutenin proteins (Gupta et al., 1993; Sapirstein and Fu, 1998). In fact, strong positive correlation of dough strength (mixograph properties) and gluten strength (gluten index) with %UPP in total flour and total protein has been associated with elastic property of gluten (Gupta et al., 1993; Ohm et al., 2017). Importance of these components in improvement of durum wheat technological properties such as cooked pasta firmness has also been highlighted by Ohm et al. (2017) and Lamacchia et al. (2007). Protein content is a primary contributor to pasta cooked firmness, while gluten strength has less influence when applying high and ultra-high drying temperature. However, gluten strength may be considered an important factor in cooking quality of pasta dried at low temperature (Fois et al., 2011).

High drying temperature of pasta causes profound changes in structure of gluten forming proteins. Lamacchia et al. (2007) found that as drying temperature increased from 60 °C toward 75 °C and 90 °C, there was a tendency toward a decline in large and small monomeric proteins and a growth in size of HMW-GS proteins. These changes happened through polymerization along with increase in size and percentage of total UPP. Unextractable polymeric proteins associated with dough strength and improved pasta cooking quality, including cooked firmness

and cooking loss. In particular, making of large and unextractable polymeric proteins limited water absorption by starch granules and reduced amylose leaching into cooking water which resulted in a decline in cooking loss and surface stickiness (Lamacchia et al., 2007).

Besides role of gluten, Sissons, et al. (2005) in a reconstitution formulation compared combined effect of gluten from different sources on changing of pasta firmness at two different protein levels. At 12% protein, they found that some samples made pasta with high cooked firmness. However, at 9% protein, low firmness was observed in majority of samples. Their results indicated primary role of protein content on changing of cooked pasta firmness. Thus, the objective of this research was to determine the correlation between quality traits and protein molecular weight distribution with cooking quality of fresh pasta made from semolina of four durum wheat genotypes.

Materials and Methods

Durum Wheat Samples

Previous research identified samples of durum wheat genotypes (1kg) [Alkabo (Elias and Manthey, 2007), Carpio (Elias et al., 2014), Divide (Elias and Manthey, 2007), and Tioga (Elias and Manthey, 2012) that varied in cooked firmness (Chapter 2). Each genotype cohort consisted of twenty samples and data were sorted based on cooked firmness. For each genotype, three samples of each cooked firmness level, high (top 20%), medium (middle 20%) and low (bottom 20%) were selected and used in this research.

Tests on Grain and Semolina

UDY mill was used to mill durum grain to whole wheat flour. Semolina was also made from milling of durum wheat utilizing Quadrumat Jr. Mill (Brabender GmbH & Co.KG, Germany) according to AACCI Approved Method 26-50.01. Whole durum wheat grain and

semolina were tested for protein content using the crude protein-combustion method described in the AACCI Approved Method 46-30.01.

Semolina was evaluated for gluten index and wet gluten content in duplicate using AACCI Approved Method 38-12.02. Gluten index and wet gluten were measured using a Glutomatic (Model 2200 Perten Instruments, Hudding, Sweden). Mixograph test was run on each sample to determine their dough quality using AACCI Approved Method 54-40.02.

Fresh Pasta Cooking Tests

Semolina samples were hydrated to 38% moisture with distilled water at 45 °C and mixed for 2 min at speed 4 using a KitchenAid mixer (4.3 L KitchenAid CLASSIC Stand Mixer 5K45SS, Michigan, USA). Extrusion process was applied to the hydrated semolina to make a spaghetti using a laboratory pasta extruder (Model AEX18, Arcobaleno, Lancaster, PA). Extrusion conditions included: screw speed: 50 rpm, chamber's length: 7 cm, screw diameter: 4 cm, and inside width of channels: 2.2 cm. Fresh spaghetti (10 g) was cooked for 2 min in 300 mL boiling distilled water. Cooked firmness and cooking loss were evaluated through AACCI Approved Method 66-50.01. Measurement of firmness was determined by using a texture analyzer (TA-XT2, Texture Technologies, Scarsdale, NY, U.S.A). Cooking loss was determined through water evaporation to dryness in a forced-air-oven at 110 °C overnight. Pasta product weight was measured and converted to percentage of increase in pasta weight after cooking and indicated as cooked weight (Deng et al., 2017).

Protein Extraction

Extractable and unextractable proteins in durum whole wheat flour were obtained based on the procedure explained by Gupta et al. (1993) with minor modification (Ohm et al., 2009). First, durum whole flour (10 mg, 14% moisture basis) was suspended in 1 mL of 0.5% SDS and

0.05 mol sodium phosphate buffer (pH 6.9) and was stirred for 5 min at 2,500 rpm utilizing a pulsing vortex mixer (Fisher Scientific, U.S.A). Centrifugation process was applied on the mixture for 15 min at 20,000 × *g* (Centrifuge 5224, Eppendorf AG, Hamburg, Germany). After that, supernatant including the dissolved extractable protein was filtered via a membrane filter (0.45 µm PVDF Membrane, Sun Sri, Rockwood, TN). The sample was transferred to the water bath and it was heated for 2 min at 80 °C immediately after filtering (Larroque et al., 2000). Then, 1 mL of extraction buffer (Sonic Dismembrator 100, Fisher Scientific, U.S.A) was added to residue containing the unextractable protein, and for 30 sec the residues were sonicated. Finally, centrifugation process was applied on the mixture for 15 min at 20,000 × *g*, and the filtration and heating were used on the supernatant before SE-HPLC analysis.

Size Exclusion-HPLC

The protein extracts were analyzed by SE-HPLC (Batey et al., 1991). SE-HPLC was conducted utilizing Agilent 1100 Series (Agilent Technologies, Waldbroann, Germany), and a Phenomenex BIOSEP SECS4000 narrow bore column (300 x 4.6 mm, Phenomenex, Torrance, CA) with an in-line filter (0.2 µm x 0.125" dia., Analytical Scientific Instruments, Richmond, CA) and a guard cartridge (Ohm et al., 2009). Injection was conducted on 10 µL of supernatant, which was filtered earlier. Then, it was eluted utilizing 50% acetonitrile (HPLC grade) and 50% water with 0.1% trifluroacetic acid. Elution process was adjusted with a flow rate of 0.5 mL/min for 10 min. At 214 nm, solutes were detected by utilizing Agilent 1200 Photodiode Array Detector (Agilent Technologies, Waldbroann, Germany) (Zhang et al., 2011).

Statistical Analysis

Mean values for quality traits along with mean values for extractable and unextractable protein fractions in total flour and total protein were determined. Means were calculated using

the three samples within each firmness group for each genotype as described above. For each genotype, the three high, three medium, and three low firmness values were considered replications. Analysis of variance (ANOVA) was performed to calculated mean values at three levels of cooked firmness for each quality traits. The mean comparison of quality traits for each genotype across years and locations was performed and LSD values were calculated at the 95% level of confidence. Correlation coefficient on a basis of Pearson's correlation coefficient was implemented between quality traits and protein molecular weight distribution with pasta cooking quality traits, such as firmness and cooked weight. All analysis was done using SAS software version 9 (SAS Institute, Cary, NC, U.S.A).

Results and Discussion

Quality Traits of Individual Genotypes

Mean values of quality traits for four durum wheat genotypes (Alkabo, Carpio, Divide, and Tioga) each having three different cooked firmness values (high, medium, and low) are summarized in Table 34. For Alkabo, Divide, and Tioga, grain and semolina protein contents were or tended to be greater with samples that produced fresh spaghetti with high than medium or low cooked firmness. These results were confirmed by the positive correlation between cooked firmness and protein content (Table 35). Although less pronounced, wet gluten content was or tended to be greatest with samples having high cooked firmness. Results for Carpio were more variable; although samples with high cooked firmness had greater protein content than did samples with medium or low cooked firmness. This variability for cooked firmness resulted in a lack of significant correlation between protein content with cooked firmness in Carpio (Table 35). High protein content has been associated with enhanced cooked firmness (Sissons et al., 2005; Fois et al., 2011). High protein content results in a dense protein matrix which can reduce

or restrict water absorption into pasta strands resulting in high firmness (Irie et al., 2004; Chapters 2 and 4).

Protein strength was indirectly measured by gluten index and dough strength was measured by mixogram peak time. Mean values for both gluten index and mixogram peak time did not vary with cooked firmness or with protein content (Table 34).

Table 34. Mean of each quality traits in different cooked firmness values for fresh pasta made from four durum wheat genotypes.

		Quality traits							
	Cooked firmness	Grain protein	Semolina protein	Gluten index	Wet gluten	Mix peak time [a]	Cooked firmness	Cooked weight	Cooking loss
Genotype	Category	(%)	(%)	(%)	(%)	(sec)	(g.cm)	(%)	(%)
Alkabo	High	15.4 a	13.6 a	56 a	37.3 a	154 a	4.9 a	168 b	1.4 a
	Medium	13.3 b	11.7 b	46 a	32.4 a	162 a	3.9 b	173 b	1.4 a
	Low	12.9 b	11.5 b	39 a	32.6 a	162 a	2.9 c	192 a	1.4 a
Carpio	High	14.3 a	13.0 a	66 a	34.7 a	143 b	4.9 a	168 c	1.4 b
	Medium	12.9 a	11.5 b	85 a	29.4 b	182 ab	4.2 b	173 b	1.6 a
	Low	13.4 a	11.9 ab	73 a	31.6 ab	192 a	3.2 c	198 a	1.6 a
Divide	High	16.8 a	14.5 a	72 a	38.5 a	177 a	5.5 a	165 b	1.5 a
	Medium	15.3 ab	13.3 ab	65 a	36.4 a	143 a	4.1 b	175 b	1.4 a
	Low	12.8 b	11.6 b	51 a	30.9 a	165 a	3.0 c	197 a	1.7 a
Tioga	High	16.0 a	14.3 a	57 a	38.1 a	162 a	5.6 a	168 b	1.3 a
	Medium	13.1 b	11.6 b	55 a	31.3 b	185 a	4.6 b	172 b	1.3 a
	Low	13.1 b	11.7 b	45 a	31.5 b	158 a	3.3 c	196 a	1.6 a
Across genotypes	High	15.7 a	13.8 a	63 a	37.1 a	159 a	5.2 a	167 c	1.4 a
	Medium	13.7 b	12.0 a	63 a	32.4 b	168 a	4.2 b	173 b	1.4 a
	Low	13.0 b	11.7 b	52 a	31.6 b	169 a	3.1 c	196 a	1.5 a

[a] Mixogram peak time.

Table 35. Correlation coefficient between quality traits and protein fractions with cooked firmness values of fresh pasta made from four durum wheat genotypes.

Quality traits/ Protein fractions	Cooked firmness				
Quality traits	Alkabo	Carpio	Divide	Tioga	Across genotypes
Grain protein (%)	0.80 **	0.49 ns	0.88 **	0.76 *	0.72 ***
Semolina protein (%)	0.75 *	0.63 ns	0.80 **	0.73 *	0.71 ***
Gluten index (%)	0.60 ns	-0.20 ns	0.55 ns	0.35 ns	0.29 ns
Wet gluten (%)	0.58 ns	0.49 ns	0.60 ns	0.67 *	0.55 ***
Mix peak time [a] (sec)	-0.13 ns	-0.78 *	0.27 ns	0.10 ns	-0.09 ns
Cooked weight (%)	-0.87 **	-0.95 ***	-0.85 **	-0.91 ***	-0.85 ***
Cooking loss (%)	0.30 ns	-0.80 **	-0.32 ns	-0.43 ns	-0.29 ns
Protein fractions [b]					
EF1	-0.71 *	-0.89 **	-0.64 ns	-0.83 **	-0.65 ***
EF2	-0.03 ns	0.55 ns	0.37 ns	0.30 ns	0.27 ns
EF3	0.56 ns	0.86 **	0.93 ***	0.81 **	0.70 ***
EP1	-0.90 ***	-0.92 ***	-0.83 **	-0.93 ***	-0.80 ***
EP2	-0.74 *	-0.09 ns	-0.76 *	-0.49 ns	-0.54 ***
EP3	-0.33 ns	0.68 *	0.82 **	0.65 ns	0.37 *
UF1	0.76 *	-0.64 ns	-0.05 ns	0.16 ns	0.08 ns
UF2	0.94 ***	0.94 ***	0.90 ***	0.95 ***	0.86 ***
UF3	0.78 *	0.71 *	0.93 ***	0.82 **	0.71 ***
UP1	0.43 ns	-0.86 **	-0.63 ns	-0.56 ns	-0.43 **
UP2	0.87 **	0.90 ***	0.77 *	0.81 **	0.75 ***
UP3	0.62 ns	0.77 *	0.77 *	0.79 **	0.60 ***

[a] Mixogram peak time.
[b] E: extractable fraction; U: unextractable fraction; F: based on flour weight; P: total protein; 1,2,3: polymeric (glutenin proteins), monomeric gliadins, and albumin and globulin, respectively.

Gluten index did not correlate with cooked firmness (Table 35). Similarly, cooked firmness did not correlate with mixogram peak time for Alkabo, Divide and Tioga, but did have a negative correlation with Carpio (Table 35). A negative correlation suggests that cooked firmness increased as mixogram peak time decreased. A low peak time indicates that it takes less time to develop the dough and subsequently the gluten matrix. This pasta press had a short extrusion barrel which means short time to develop the dough. More research is needed to determine if a semolina with low than high mixogram peak time would be more desirable when extruding using a machine that had a short barrel. However, the importance of this is uncertain

since the other three genotypes had no significant correlation and both Divide with medium cooked firmness and Carpio with high cooked firmness had identical mixogram peak times and similar semolina protein content (Table 34). These results suggest that cooked firmness was influenced by factors other than those tested, maybe gluten protein composition. Sissons et al. (2007) indicated that variability due to a wide range in polymeric protein affected pasta quality. In addition, results of Flagella et al. (2010) showed better pasta making characteristics due to higher amount of HMW-GS, greater HMWGS/LMW-GS ratio, and greater %UPP in cultivar Simeto (Lamacchia et al., 2007; Flagella et al., 2010; Ohm et al., 2017).

For all four genotypes, cooked weight was higher for fresh pasta with low than with intermediate or high cooked firmness (Table 34). Cooked weight did not correlate consistently with any of the quality traits tested (Table 36). Cooked weight did have a significant negative correlation with grain protein content for both Alkabo and Divide; the correlation while negative was not significant for Carpio and Tioga. Other researchers have reported greater cooked weight with pasta having low than high protein content (Grzybowski and Donnelly, 1979). Negative effect of high protein content on cooked weight was presumably due to formation of dense protein matrix which reduced water absorption and decreased cooked weight (Irie et al., 2004; Sissons et al., 2005).

Gluten strength factors including gluten index and mixogram peak time did not have significant influence on cooked weight except with Divide where gluten index showed a negative and significant correlation with cooked weight ($r= -0.73$ at $p < 0.05$, Table 36). Presence of lowest cooked weight in Divide could be supported by both high protein content and second highest gluten index and negative correlation between these factors with cooked weight (Chapter

2; Table 36). In addition, our previous results in Chapter 4 showed that protein content had a significant role on variation of cooked weight, while gluten index was not a factor.

Table 36. Correlation coefficient between quality traits and protein fractions with cooked weight values of fresh pasta made from four durum wheat genotypes.

Quality traits/ Protein fractions	Cooked weight				
Quality traits	Alkabo	Carpio	Divide	Tioga	Across genotypes
Grain protein (%)	-0.73 *	-0.28 ns	-0.73 *	-0.53 ns	-0.56 ***
Semolina protein (%)	-0.68 *	-0.38 ns	-0.60 ns	-0.52 ns	-0.52 **
Gluten index (%)	-0.32 ns	0.01 ns	-0.73 *	-0.25 ns	-0.25 ns
Wet gluten (%)	-0.53 ns	-0.22 ns	-0.31 ns	-0.42 ns	-0.35 *
Mix peak time [a] (sec)	0.36 ns	0.63 ns	0.17 ns	-0.23 ns	0.24 ns
Cooking loss (%)	-0.06 ns	0.63 ns	0.48 ns	0.27 ns	0.31 ns
Protein fractions [b]					
EF1	0.57 ns	0.96 ***	0.79 *	0.75 *	0.72 ***
EF2	-0.01 ns	-0.47 ns	-0.23 ns	-0.52 ns	-0.28 ns
EF3	-0.46 ns	-0.72 *	-0.83 **	-0.66 ns	-0.62 ***
EP1	0.77 *	0.95 ***	0.93 ***	0.81 **	0.82 ***
EP2	0.65 ns	-0.05 ns	0.74 *	0.15 ns	0.36 *
EP3	0.42 ns	-0.72 *	-0.86 **	-0.74 *	-0.49 **
UF1	-0.53 ns	0.74 *	0.32 ns	0.04 ns	0.20 ns
UF2	-0.93 ***	-0.94 ***	-0.95 ***	-0.87 **	-0.89 ***
UF3	-0.65 ns	-0.55 ns	-0.84 **	-0.58 ns	-0.62 ***
UP1	-0.12 ns	0.88 **	0.81 **	0.60 ns	0.63 ***
UP2	-0.89 **	-0.97 ***	-0.90 ***	-0.84 ***	-0.85 ***
UP3	-0.50 ns	-0.63 ns	-0.73 *	-0.60 ns	-0.56 ***

[a] Mixogram peak time.
[b] E: extractable fraction; U: unextractable fraction; F: based on flour weight; P: total protein; 1,2,3: polymeric (glutenin proteins), monomeric gliadins, and albumin and globulin, respectively.

For Carpio, cooking loss was lower for fresh pasta with high than with medium or low cooked firmness (Table 34). Cooking loss did not vary significantly with cooked firmness with Alkabo, Divide, or Tioga; although for Divide and Tioga, there was a trend for lower cooking loss with high compared to low cooked firmness. Cooking loss of fresh pasta made from Alkabo was similar for all samples regardless of cooked firmness. Lower cooking loss could be explained by short cooking time for 2 minutes (Grzybowski and Donnelly, 1979). In addition, cooking loss seemed to be less with high protein content.

These findings were in agreement with Ohm et al. (2017) and Ounane et al. (2006). In fact, more protein results in greater gluten matrix which would protect starch from swelling and fracturing during cooking which would reduce the amount of amylose leached from starch (Irie et al, 2004). Similarly, wet gluten was negatively correlated to cooking loss (data was not shown) indicating the importance of gluten matrix in reducing leaching of starch and other molecules into cooking water. Sissons et al. (2005) reported that even by changing in gluten composition, protein content was still a main contributor affecting pasta cooking loss.

Quality Traits Across Genotypes

Mean values of quality traits averaged across genotypes are shown in Table 34. Grain and semolina protein contents and wet gluten content were greater with fresh pasta that had high than medium or low cooked firmness. Cooked firmness was positively correlated with grain and semolina protein content, r=0.72 and r= 0.71 at $p < 0.001$, respectively (Table 35). Gluten index and mixogram peak time did not differ with cooked firmness of fresh pasta. Cooked weight was greatest with low, intermediate with medium, and least with high cooked firmness as indicated by strong negative correlation between cooked weight and cooked firmness, r= -0.85 at $p < 0.001$. Cooking loss did not differ with cooked firmness. There are two possible reasons that can be attributed to non-significant correlation between cooking loss and cooked firmness. First is the nature of fresh pasta. During drying with high temperature, lower cooking loss is associated with formation of coagulated and dense protein network around starch granules that restrict excessive swelling, and results in reduced leaching of amylose or starch fragments into cooking water (Chapters 2 and 4; Irie et al, 2004; Brennan and Tudorica, 2007; Bruneel et al., 2010). In addition, cooking loss positively and significantly affected by cooking time (Lemlioglu and Jackson, 2013). In fact, increase in cooking time, resulted in starch granules swelling, starch

pasting, starch gelatinization, and consequently disintegration of starch granules, leaching of amylose molecules into cooking water, along with disruption of protein matrix and decrease in pasta firmness (Lemlioglu and Jackson, 2013). Conversely, short cooking time caused many starch granules to remained intact and resulted in high cooked firmness (Lemlioglu and Jackson, 2013). Presence of non-significant correlation reported in this research was possibly due to very short cooking time. Cooking fresh pasta only 2 minutes probably was not enough to complete gelatinization of starch granules (Lemlioglu and Jackson, 2013). The latter reduced further diffusion of amylose molecules into the cooking water and finally decreased surface stickiness.

SE-HPLC Protein Fractions of Individual Genotypes

Chromatograms were separated into three main protein fractions, including peak (1): 3.3-5.3 min; peak (2): 5.3-6.3 min; and peak (3): 6.3-7.5 min (Figure 23). Main components of Peak 1 was polymeric proteins (glutenin), components of peak 2 included monomeric proteins (gliadins), and peak 3 composed of other monomeric proteins such as combination of albumins + globulins (Ohm et al., 2017).

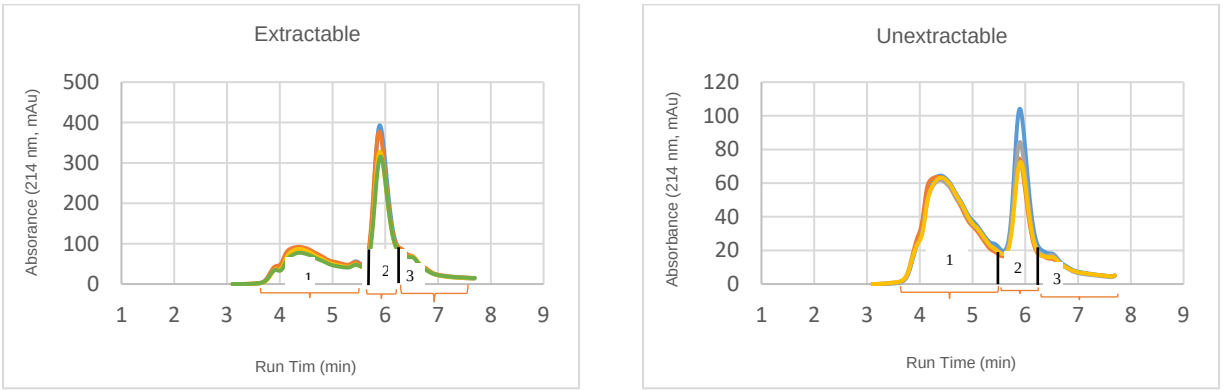

Figure 23. Size exclusion HPLC profiles of extractable and unextractable proteins obtained from durum whole wheat flour samples. Peaks 1,2,3: polymeric (glutenin proteins), monomeric gliadins, and albumin and globulin, respectively.

Mean values of SE-HPLC protein fractions for four durum wheat genotypes (Alkabo, Carpio, Divide, and Tioga) each having three different cooked firmness values (high, medium,

and low) are summarized in Table 37. For all four genotypes, extractable polymeric protein (glutenins) in total flour and total protein (EF1 and EP1, respectively) was greater with samples that produced fresh spaghetti with low compared to high cooked firmness (Table 37). This is reflected by the strong negative correlations between cooked firmness and EF1 (r= -0.64 to -0.89 at $p < 0.05$) and EP1 (r= -0.83 to -0.93 at $p < 0.05$) (Table 35). However, Ohm et al. (2017) reported a positive correlation between cooked firmness with extractable polymeric proteins in total flour (EF1). Results from Ohm et al. (2017) are with dried spaghetti while fresh pasta was evaluated in this research. The difference could be due to genotypic correlation (Ohm et al., 2017) and drying of pasta and effect of drying temperature on polymerization of high molecular weight polymeric proteins and formation of firmer pasta after cooking (Lamacchia et al., 2007).

Table 37. Mean of each protein fraction in different cooked firmness values of fresh pasta made from four durum wheat genotypes.

		Protein fractions [a]											
Genotype	Cooked firmness	EF1	EF2	EF3	UF1	UF2	UF3	EP1	EP2	EP3	UP1	UP2	UP3
	Category	(%)											
Alkabo	High	2.1 a	0.6 a	4.9 a	2.5 a	0.6 a	1.6 a	13.9 b	4.1 a	32.3 a	16.1 a	4.2 a	10.5 a
	Medium	2.1 a	0.6 a	4.3 a	1.9 b	0.5 a	1.3 a	16.1 b	4.7 a	32.3 a	14.1 a	4.0 a	9.7 a
	Low	2.9 a	0.6 a	4.4 a	1.9 b	0.3 b	1.0 a	22.1 a	4.9 a	33.8 a	14.8 a	2.2 b	7.9 a
Carpio	High	1.5 b	0.6 a	4.8 a	1.9 b	0.8 a	1.7 a	10.3 b	4.4 a	33.7 a	13.4 b	5.4 a	11.8 a
	Medium	1.5 b	0.6 ab	4.3 ab	1.9 b	0.7 a	1.2 a	11.9 b	4.6 a	33.1 a	14.8 b	5.2 a	9.2 a
	Low	2.7 a	0.6 b	4.0 b	2.5 a	0.3 b	1.1 a	20.4 a	4.4 a	30.1 b	18.8 a	2.5 b	8.3 a
Divide	High	1.5 a	0.7 a	6.1 a	2.1 a	0.8 a	2.1 a	8.7 b	4.0 b	36.4 a	16.3 a	4.8 a	12.6 a
	Medium	2.1 a	0.7 a	5.2 a	2.3 a	0.6 a	1.6 b	13.4 ab	4.5 ab	34.0 ab	14.6 a	4.0 ab	10.9 a
	Low	2.6 a	0.6 a	4.1 b	2.1 a	0.3 b	1.1 c	20.3 a	4.8 a	32.3 b	12.2 a	2.4 b	8.6 a
Tioga	High	2.1 b	0.6 a	5.2 a	2.4 a	0.6 a	1.6 a	13.1 b	4.0 a	32.8 a	15.0 a	4.0 a	9.9 a
	Medium	2.2 b	0.6 a	4.1 b	1.9 a	0.6 a	1.1 b	16.6 b	4.9 a	31.5 a	14.9 a	4.3 a	8.1 ab
	Low	3.0 a	0.6 a	4.0 b	2.2 a	0.3 b	0.9 b	22.8 a	4.7 a	30.5 a	17.0 a	2.2 b	7.1 b
Across genotypes	High	1.8 b	0.6 a	5.3 a	2.2 a	0.7 a	1.8 a	11.5 c	4.2 b	33.8 a	14.2 b	4.6 a	11.2 a
	Medium	2.0 b	0.6 a	4.5 b	2.0 a	0.6 b	1.3 b	14.5 b	4.7 a	32.7 ab	14.6 b	4.4 a	9.5 b
	Low	2.8 a	0.6 a	4.1 c	2.2 a	0.3 c	1.0 c	21.4 a	4.7 a	31.7 b	16.8 a	2.3 b	8.0 c

[a] E: extractable fraction; U: unextractable fraction; F: based on flour weight; P: total protein; 1,2,3: polymeric (glutenin proteins), monomeric gliadins, and albumin and globulin, respectively.

Extractable monomeric proteins (gliadins) in total flour and total protein, EF2 and EP2, respectively, did not differ with cooked firmness (Table 37). There was no correlation between EF2 and cooked firmness for genotypes tested. However, increase in amount of EP2 tended to decrease cooked firmness in both Alkabo and Divide (Table 37). This result was supported by negative, strong, and significant correlation between EP2 and cooked firmness with Alkabo and Divide (r= -0.74 and -0.76 at $p < 0.05$, respectively) but not with Carpio and Tioga (Table 35). The effect of EP2 on variation of cooked firmness seems to be genotype dependent. Negative effect of extractable monomeric protein in total protein on cooked firmness was also observed in research conducted by Ohm et al. (2017). In fact, extractable monomeric gliadins are associated with viscous properties of gluten which resulted in decreased pasta firmness.

For each genotype, the amount of extractable albumin + globulin protein in total flour and total protein (EF3 and EP3, respectively) were greater in samples that produced fresh pasta with high than low cooked firmness (Table 37). There was a positive correlation between EF3 and cooked firmness for Carpio, Divide, and Tioga, but not for Alkabo (Table 35). Similarly, EP3 was positively correlated with cooked firmness for Carpio and Divide but not for Alkabo or Tioga (Table 35). EP3 for Alkabo and Tioga did not statistically differ with cooked firmness (Table 37). Positive correlation between albumin + globulin with cooked firmness was in contrast with Ohm et al. (2017) findings who reported a negative correlation. The results reported here could reflect the differences between fresh cooked pasta and dried cooked pasta. A possible explanation is that albumin and globulin are involved in gel formation (Ahmenda et al., 1999; Alzuwaid et al., 2020) which resulted in improvement in fresh pasta firmness. One of the functional properties of these proteins is their high water absorption capacity (Alzuwaid et al., 2020). In fact, cooking of pasta resulted in unfolding of these proteins, followed by further

aggregation, formation of intermolecular aggregates, and final formation of gel (Tobitani and Ross-Murphy, 1997). According to Brennan and Tudorica (2007), it was assumed that these proteins have a role in structural properties of cooked pasta and confer strength to it. In particular, they formed a network around starch granules which led to strong interaction between starch and protein matrix, and to an improvement in cooked pasta firmness (Brennan and Tudorica, 2007). In addition, formation of this network was associated with decline in cooking loss which was attributed to the formation of a network that restricted water diffusion into and swelling of starch granules, ultimately reducing the leaching of amylose into cooking water (Brennan and Tudorica, 2007). Further research should be conducted to support these findings.

The amount of unextractable polymeric protein in total flour (UF1) of samples that had different levels of cooked firmness did not differ with Divide or Tioga; and the amount of unextractable polymeric protein in total protein (UP1) did not differ with Alkabo, Divide or Tioga (Table 37). However, in Alkabo, high quantity of UF1 occurred with high cooked firmness. Conversely, high quantity of UF1 occurred with low cooked firmness of fresh pasta made from Carpio. In Carpio, high proportion of both UF1 and UP1 significantly decreased cooked firmness. These results indicate genotype differences affect final pasta cooking quality (Table 37). Conversely, Ohm et al. (2017) showed that UP1 associated with elasticity of gluten which consequently had a positive effect on cooked firmness. According to Lamacchia et al. (2007), applying high air temperature drying resulted in formation of large unextractable polymeric protein that positively affected gluten network and pasta cooked firmness.

For all four genotypes, UF2 and UP2 (unextractable gliadin) contents were lowest with low cooked firmness but their contents were similar with medium and high firmness (Table 37).

Both UF2 and UP2 were positively correlated with cooked firmness, which is in agreement with Ohm et al. (2017) findings.

Unextractable albumin + globulin in total flour (UF3) was greater with high than with low cooked firmness for fresh pasta made from Divide and Tioga (Table 37). While not statistically significant, UF3 content tended to be greater with high than low cooked firmness for both Alkabo and Carpio. Similar trends were found for UP3 (unextractable albumin + globulin in protein). UF3 and UP3 were both positively correlated with cooked firmness for all genotypes (Table 35). These results suggest possible importance of albumin and globulins in cooked firmness of fresh pasta.

Overall, extractable polymeric proteins negatively influenced cooked firmness (Table 37). Extractable monomeric proteins in both total flour and total proteins did not have any significant effect on cooked firmness. However, high proportion of extractable albumin + globulin proteins increased cooked firmness. In addition, cooked firmness was favored by increased amount of unextractable monomeric proteins along with combination of unextractable albumin + globulin proteins. It seemed that effect of UF1 and UP1 on cooked firmness was genotype dependent. This result was supported by negative effect of UP1 content on cooked firmness fresh pasta made with Carpio. Similarly, Ohm et al. (2017) reported on the importance of genotype on content of UP1 and the strong correlation between UP1 and cooked firmness.

SE-HPLC Protein Fractions Averaged Across Genotypes

When averaged over genotypes, extractable polymeric protein in total flour and total protein (EF1 and EP1) were associated with decreased cooked firmness. EF1 and EP1 were greater with fresh spaghetti that had low than high cooked firmness (Table 37). UF1 content was

similar regardless of cooked firmness, while high amount of unextractable polymeric protein in total protein (UP1) was associated with decreased cooked firmness.

Extractable monomeric protein (EF2) did not have a significant effect on cooked firmness. However, cooked firmness was higher with fresh pasta made from samples with low amounts of EP2. High levels of unextractable monomeric protein (UF2 and UP2) and high levels of extractable albumin + globulin (EF3 and EP3) and unextractable albumin + globulin (UF3 and UP3) favored cooked firmness.

Overall, cooked firmness was positively correlated with unextractable monomeric protein (gliadin) and extractable and unextractable albumin + globulin; and was negatively correlated with extractable and unextractable polymeric protein (glutenins) and extractable monomeric protein (gliadin).

Correlation of Protein Molecular Weight Distribution with Cooked Weight

Results presented in Table 36 show that extractable polymeric protein in total flour (EF1) positively affected cooked weight, and this impact was significant for all genotypes (r=0.75 to r=0.96) except Alkabo. Extractable monomeric protein in total flour (EF2) did not have any effect on cooked weight. Extractable albumin + globulin (EF3) had a negative and significant impact on cooking weight for Carpio and Divide but was not significant for Alkabo and Tioga. Overall, an increase in quantity of EF1 increased cooked weight, while high amount of extractable albumin + globulin significantly reduced cooked weight. This result also was supported by positive impact of extractable albumin + globulin in total flour (EF3) on cooked firmness and presence of negative correlation between cooked firmness and cooked weight (Table 35).

Extractable polymeric protein in total protein (EP1) had a strong, positive, and significant correlation with cooked weight with all genotypes. Similarly, with all genotypes, extractable monomeric protein in total protein (EP2) affected cooked weight positively. In fact, extractable monomeric proteins are associated with viscous properties of dough and had a softening effect of on it, while decreasing cooked firmness and increasing cooked weight (Edward et al., 2003; Ohm et al., 2017). Depending on the genotype, there was a correlation between cooked weight and extractable monomeric protein in total protein (EP2) (Table 36). In fact, cooked weight in Divide was significantly correlated with this protein fraction (r=0.74 at $p < 0.05$). Cooked weight was negatively affected by extractable albumin + globulin (EF3 and EP3) (Table 36) while these protein fractions improved cooked firmness.

Although not significantly different, UF1 (unextractable polymeric proteins in total flour) had a positive correlation with cooked weight (Table 36). Similarly, there was a positive associated between UP1 (unextractable polymeric proteins in total protein) with cooked weight (r=0.81 to 0.88 at $p < 0.01$) indicating low content of unextractable polymeric proteins was associated with low cooked weight. However, UP1 negatively affected cooked firmness (Table 35). Lack of drying step in processing of fresh pasta, might be associated with fewer large and insoluble protein aggregates along with high amount of small and large monomeric protein. The greater amount of both extractable and unextractable albumin + globulin and their potential contribution in gel formation, improved cooked pasta firmness, while probably limited water diffusion into pasta stands and decreased cooked weight. This result was in agreement with Walsh and Gilles (1971) who showed a negative correlation between albumin and cooked weight. This finding was supported by negative correlation between both extractable and unextractable albumin + globulin in both total flour and total protein (EF3, EP3, UF3 and UP3)

with cooked weight (Table 36). Unlike cooked firmness that was positively influenced by unextractable monomeric protein in total flour and total protein, cooked weight had a negative and significant correlation with both UF2 and UP2. High unextractable monomeric protein content appeared to be related to low cooked weight. Also, there is a possibility that peak 3 which corresponds to monomeric proteins (albumin + globulin) had been incorporated into the peak 2, and resulted in formation of large aggregate, which improved firmness and reduced cooked weight. This result could explain the negative correlation between cooked weight with these protein (UF2 and UP2).

Overall, cooked weight was positively affected by extractable polymeric and monomeric protein fractions (glutenin and gliadins), while the impact of unextractable proteins (except UP1) on cooked weight were negative across all genotypes. These results reflect the negative correlation between cooked firmness and cooked weight, where protein fractions that improved firmness had a negative influence on cooked weight (Tables 35 and 36).

Conclusion

Results of this study indicated that protein content rather than gluten strength (as measured by gluten index) was responsible for making fresh pasta with good cooking qualities such as high cooked firmness, low cooked weight and low cooking loss. The firmness of cooked pasta was favored by high protein content, while cooked weight and cooking loss were negatively affected by high protein content due to formation of dense protein matrix that surrounds starch granules and restricts water absorption. In addition, protein composition positively affected pasta cooking quality. In fact, possible gel forming properties of some protein fractions, including albumin + globulin during cooking were associated with high cooked firmness, low cooked weight, and low cooking loss in fresh pasta.

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CHAPTER 6: OVERALL CONCLUSIONS

The results of this research indicate that quality parameters vary with genotype. In fact, variation in quality traits was due to genotype differences and the effect of weather data on changing of quality traits. Besides genotype differences, the environment had a dominant impact on the majority of quality traits tested including grain test weight, 1000-kernel weight, grain protein content, vitreous kernel content, falling number, semolina protein content, semolina extraction rate, pasta color, and pasta cooking quality; while, grain yellow pigment, semolina yellowness (b*), gluten index, and mixogram peak time were affected most by genotype.

Across the genotypes (within the environment), high grain and semolina protein content favored by growing locations with high air temperature and more days with temperature ≥ 30 ºC, while, greatest falling number, vitreous kernel content, gluten index and high pasta color favored by growing locations with low relative humidity and low rainfall, respectively. Prolonged exposure to days with temperature ≤ 13 ºC enhanced high 1000-kernel weight and test weight. In addition, both 1000-kernel weight and semolina extraction rate favored by high relative humidity.

Semolina protein content strongly correlated to all pasta cooking quality parameters. So, ideal growing locations to achieve pasta with high cooked firmness and low cooked weight were those with higher air temperature. In addition, the lowest cooking loss favored by growing locations with high air temperature and low rainfall.

Utilizing SE-HPLC identified variation in pasta cooked firmness was positively associated with both extractable and unextractable albumin + globulin proteins. Improvement in firmness was possibly due to gel-forming properties of these protein fractions, and formation of the network around starch granules which led to the strong interaction between starch and protein

matrix. While the impact of unextractable proteins were negatively correlated with cooked weight across all genotypes.

For durum breeder in the US Northern Plains, the question is whether the development of new genotypes should be target either stable genotypes or genotypes with an improved quality trait such as high protein content, high gluten index, or great pasta cooked firmness. Not all genotypes are superior for all purposes. Thus, the selection of genotypes should be based on the most important quality parameters.

CHAPTER 7: INDUSTRIAL APPLICATIONS

Demands of the durum wheat industry depend on the sector which includes the seed industry, farmer, milling company, processor (pasta industry), consumer, and exporter. The principle aspect of quality for each sector is different. Farmers deal with grain quality and grain yield. The milling industry considers grain test weight, grain uniformity, semolina yield, and ash content. Grain yellow pigment concentration, semolina protein content, and gluten strength are major quality characteristics necessary to make high-quality pasta. High cooked firmness and yellow color are quality parameters that both pasta industry and consumers attribute the most importance. Quality of durum grain depends on environmental conditions, genotypes, and their interaction which determines the quality of final pasta products. Thus, the importance of these results is beneficial for durum breeders, growers, and pasta manufacturing companies. During the development of new cultivars, not only should breeders improve quality traits, but also, they should be aware of various weather factors on the performance of genotypes and consider the most stable genotypes under diverse weather conditions. Thus, the selection of genotypes by farmers in different growing locations should be based on the most important quality of interest.

CHAPTER 8: FUTURE RESEARCH

Further research is needed to determine the effect of weather factors under a controlled environment (irrigation, rainfed, heat stress, low air temperature) during grain development (early grin development, middle to later stage of grain development) on protein composition. The determination of glutenin-to-gliadin ratio could be used to evaluate variation in gluten index in different genotypes.

Using electrophoresis technique to distinguish the type of possible glutenin subunits (HMW-GS and LMW-GS) and gliadins subunits (α, β,γ, and ω) is required. In addition, reverse-phase-HPLC can be an alternative method for the extraction and quantification of HMW-glutenin and LMW-glutenin. In this method, extraction of glutenin proteins followed by extraction of gliadins and oligomers from the flour. This process will be continued by centrifuging of supernatant, filtering and injecting in a reverse-phase-HPLC inorder to determine quantity of glutenin components.

Comparison between protein molecular weight distribution involved in the variation of firmness in fresh pasta with those are involved in the firmness of pasta dried at different drying temperatures (low, high, and ultra-high) can be applied. Based on my results improvement in firmness of fresh pasta was due to gel forming properties of albumin + globulin proteins. SE-HPLC would be effective to quantify these protein components as well as quantification of proteins of spaghetti dried at three different temperatures. Quantity of unextrctable polymeric protein in total protein could be also compared with those made from fresh pasta.

Utilizing scanning electron microscopy (SEM) to evaluate changes in dough structure of fresh pasta due to gel-forming properties of non-gluten forming proteins is required. In addition, SEM can be applied to evaluate microstructural changes in interior part and exterior surface of

cooked fresh pasta. Determination of protein matrix, starch granules' structure, their gelatinization, and existence of possible networks associated with leaching of amylose, and the interaction between starch and protein is also required.

APPENDIX

Table A1. Descriptive statistics for wet gluten for each genotype average over 24 environments [a].

	Wet gluten (%)		
Genotype	Mean [b]	Median	Range
Alkabo	34.7 d	33.6	(18.6) 29.7- 46.5
Carpio	32.7 f	32.8	(4.4) 26.1- 42.2
Divide	34.2 de	34.2	(6.0) 25.4- 44.1
Grenora	35 cd	34.4	(5.1) 22.7- 45.6
Joppa	32.7 f	32.2	(5.6) 26.0- 43.5
Maier	38 a	35.6	(7.1) 30.4- 50.4
Mountrail	37.2 ab	36.5	(8.0) 28.2-52.6
Pierce	36.3 bc	37.6	(6.1) 30.2- 47.6
Tioga	33.4 ef	32.9	(4.6) 26.1- 46.0
Mean	34.9	34.4	7.3

[a] Nine genotypes were grown in 2012, 2013, 2014, and 2015 in six locations.
[b] For each quality trait in each column, means followed by the same letter are not significantly different at $P < 0.05$.

Table A2. Descriptive statistics for wet gluten in each location and year average across genotypes [a].

Environment [b]	Wet gluten (%)	
	Mean	Range
L-12	33.9	(5.5) 32.1-37.5
L-13	36.2	(6.4) 33.5-39.9
L-14	30.0	(8.5) 26.0-34.5
L-15	34.2	(6.9) 30.6-37.5
M-12	37.5	(10.5) 32.3-42.8
M-13	37.3	(8.5) 34.3-42.8
M-14	30.5	(4.5) 28.5-33.0
M-15	34.3	(9.5) 30.4-39.9
C-12	42.6	(10.8) 38.3-49.1
C-13	35.0	(6.2) 31.6-37.8
C-14	30.8	(6.9) 26.8-33.7
C-15	38.3	(8.0) 34.2-42.2
W-12	46.3	(10.4) 42.2-52.6
W-13	35.7	(8.9) 31.5-40.3
W-14	29.2	(11.6) 22.7-34.2
W-15	33.1	(7.1) 30.8-37.9
D-12	39.5	(11.2) 35.1-46.3
D-13	30.4	(4.6) 29.2-33.8
D-14	31.7	(5.6) 28.5-34.1
D-15	42.3	(8.5) 38.9-47.4
H-12	35.3	(9.8) 29.8-39.7
H-13	34.7	(12) 30.2-42.2
H-14	29.7	(5.1) 27.8-32.9
H-15	29.9	(7.2) 26.8-34.0
Mean	34.9	8.1

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.
[b] L: Langdon; M: Minot; C: Carrington; D: Dickinson; H: Hettinger.

Table A3. Correlation coefficient between quality traits and weather factors with wet gluten in nine durum wheat genotypes across years and locations [a].

	Wet gluten								
	Alkabo	Carpio	Divide	Grenora	Joppa	Maier	Mountrail	Pierce	Tioga
Grain/Semolina traits									
Grain filling duration	-0.15 ns	-0.31 ns	-0.26 ns	-0.13 ns	-0.33 ns	-0.21 ns	-0.53 **	-0.20 ns	-0.19 ns
Grain protein (%)	0.95 ***	0.94 ***	0.97 ***	0.94 ***	0.93 ***	0.95 ***	0.96 ***	0.94 ***	0.90 ***
Semolina protein (%)	0.93 ***	0.94 ***	0.96 ***	0.86 ***	0.94 ***	0.92 ***	0.95 ***	0.92 ***	0.88 ***
Weather factors									
T max (ºC)	0.35 ns	0.43 *	0.26 ns	0.32 ns	0.41 *	0.35 ns	0.53 **	0.39 ns	0.35 ns
T min (ºC)	0.42 *	0.42 *	0.44 *	0.33 ns	0.49 *	0.43 *	0.62 **	0.43 *	0.52 **
# days temp ≥ 30 ºC	0.44 *	0.42 ns	0.27 ns	0.41 *	0.43 *	0.48 *	0.48 *	0.49 *	0.40 ns
# days temp ≤ 13 ºC	-0.36 ns	-0.9 ns	-0.40 ns	-0.28 ns	-0.48*	-0.36 ns	-0.59**	-0.33 ns	-0.48*
Total rain (mm)	-0.20 ns	-0.15 ns	-0.04 ns	-0.10 ns	-0.12 ns	-0.20 ns	-0.30 ns	-0.18 ns	-0.12 ns
# days RH ≥ 80%	-0.28 ns	-0.23 ns	0.05 ns	-0.06 ns	-0.31 ns	-0.37 ns	-0.27 ns	-0.24 ns	-0.04 ns
RH (%)	-0.31 ns	-0.29 ns	-0.06 ns	-0.21 ns	-0.33 ns	-0.37 ns	-0.37 ns	-0.32 ns	-0.18 ns

[a] Nine genotypes (Alkabo, Carpio, Divide, Grenora, Joppa, Maier, Mountrail, Pierce, and Tioga) were grown in 2012, 2013, 2014, and 2015 in six locations.

*,**,*** indicates in each column within each genotype, correlation coefficient between weather factors and quality traits with wet gluten is significantly different from zero at $P < 0.05$, 0.01, and 0.001, respectively; ns displays not significantly different from zero ($P \geq 0.05$).

Table A4. Stepwise linear regression for weather factors with wet gluten (n= 24).

Quality trait	Genotypes	Weather data	Effect [a]	Partial R^2	R^2
	Alkabo	# days temp ≥ 30 ^oC	(+)	0.20	0.20
	Carpio	T max	(+)	0.18	0.18
	Divide	T min	(+)	0.19	0.19
	Grenora	# days temp ≥ 30 ^oC	(+)	0.17	0.17
Wet gluten	Joppa	T min	(+)	0.24	0.24
	Maier	# days temp ≥ 30 ^oC	(+)	0.23	0.23
	Mountrail	T min	(+)	0.38	0.38
	Pierce	# days temp ≥ 30 ^oC	(+)	0.24	0.24
	Tioga	T min	(+)	0.28	0.28

[a] (+) indicates positive effect on the wet gluten.

Table A5. Means for weather data averaged across genotypes during grain filling period in each location and year (n=24).

	Weather data						
Environment	T max	T min	# days temp ≥ 30 ºC	# days temp ≤ 13 ºC	Total rain	# days RH ≥ 80%	RH
	(ºC)	(ºC)	(---)	(---)	(mm)	(---)	(%)
Langdon-12	27	14	5	6	96	5	75
Langdon-13	24	11	6	32	40	12	73
Langdon-14	26	13	1	22	34	9	75
Langdon-15	26	13	2	19	50	16	77
Mean [a]	**26**	**13**	**4**	**20**	**64**	**11**	**75**
Minot-12	28	15	14	10	34	4	66
Minot-13	25	13	8	19	104	2	66
Minot-14	25	14	1	13	128	12	75
Minot-15	28	15	14	12	47	2	66
Mean	**27**	**14**	**12**	**14**	**78**	**5**	**68**
Carrington-12	29	16	13	7	65	9	75
Carrington-13	24	11	1	24	15	4	72
Carrington-14	25	13	2	16	84	19	79
Carrington-15	26	15	1	8	113	14	78
Mean	**26**	**14**	**4**	**14**	**70**	**12**	**76**
Williston-12	31	17	23	3	57	1	54
Williston-13	26	13	6	18	61	1	67
Williston-14	28	14	9	11	46	2	63
Williston-15	29	15	18	8	58	1	61
Mean	**29**	**15**	**14**	**10**	**56**	**1**	**61**
Dickinson-12	32	17	23	4	34	0	52
Dickinson-13	26	13	5	19	49	2	66
Dickinson-14	26	13	7	18	208	10	68
Dickinson-15	29	14	17	13	52	0	57
Mean	**28**	**14**	**13**	**14**	**86**	**3**	**61**
Hettinger-12	32	16	17	6	70	1	52
Hettinger-13	26	12	6	21	70	3	69
Hettinger-14	27	12	10	21	56	6	68
Hettinger-15	29	13	21	19	47	0	57
Mean	**29**	**13**	**14**	**17**	**61**	**3**	**62**

[a] In each location, mean is averaged across years.