

Mechanistic Insight of Water Stress Induced Aggregation in Wheat (*Triticum aestivum* L.) Quality: The Protein Paradigm Shift

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Abstract

Vertical and horizontal expansion of agriculture to provide food, feed, fibre and fuel to escalating populations has affected the availability of wheat in terms of quantity and quality. Irrigation is the most important factor influencing yield and grain quality. To achieve sustainable and quality wheat production, strategic measures should be adopted. Seven water stress-tolerant wheat varieties/strains were crossed with drought-susceptible lines using a line × tester design to evaluate the effect of water stress on genetic variability and heritability of wheat grains. As might be expected, plant traits like moisture, ash, fat, protein and gluten content showed different responses under normal irrigated and water-stress environments. In particular, the quality of wheat grains was found to be highly significant, indicating the presence of high variability in plant attributes like moisture percentage, ash content, crude fat, crude protein percentage and gluten content under both normal irrigation and water stress conditions. Water stress played a key role in reducing the moisture and fat content, whereas correspondingly, it increased protein, ash and gluten contents. The paradigm shifts in the deleterious effects of water stress have been elucidated. The broad-sense heritability estimate was significant for each of these characters under both conditions, with water stress in some measurements altering the heritabilities of all quality characters.

Keywords: heritability, protein, quality, variability, water stress

Introduction

Climate change and global warming has the potential to damage all natural resources and agriculture. Most of the world economy is predominantly agrarian with wheat being in prime position among all crops due to its staple food status. According to the FAO (2009), increasing food production to meet the Millennium Development Goal (MDG), by which hunger has to be defeated by 2015, is going out of reach. On a nutritive note, wheat per capita availability for 170 million people of Pakistan is 140.88 kg per annum (GOP, 2010). Water stress seriously hurts wheat yield potential that might well decline half as much, compared to irrigated areas (GOP, 2010). The position of wheat is crucial in daily food consumption due to its absolute baking performance in contrast to all other cereals (Dewettinck *et al.*, 2008) and is the best source for feeding humans (Mesbah, 2009).

Most of the Pakistani population lives in rural areas and mainly consumes wheat in the form of flat breads (*chapatti*, *naan*, *roti*) which sufficiently fulfill their dietary needs in this region; the same can be said for most of the Indian sub-continent and parts of Africa (Dhingra and Jood, 2001; Nurul-Islam and Johansen 1987). Wheat is the cheapest source of daily caloric and nutritional requirements but declining water availability represents a

threat to food supply around the world (Zwart and Bastiaansen, 2004). Wheat breeder's prime objective is to improve wheat yield under contrasting conditions of water availability. With changing food habits, breeders are paying greater attention to improving quality characteristics of this food staple. The quality of wheat products depends upon the quality of the grain (Al-Karaki, 2012; Finney *et al.*, 1987), thus wheat quality axiomatically affects the quality of flat breads (Rehman *et al.*, 2006). Irrigation water and fertilizer play an elementary role in the viscoelastic properties of gluten (Rehman *et al.*, 1997). Protein quality and quantity both have a role in *chapatti* making and in end production (Prabhasankar, 2002). Global climatic changes and a rise in temperature is a threat for phenology, growth and yield of wheat (Hossain and Teixeira da Silva, 2012). Ultimately, increasing water shortages have diverted the world's attention to evolving water stress-tolerant crop varieties. Due to water stress, wheat yield, as well as the quality of wheat, is affected (Ahmad and Arain, 1999; Mahboob *et al.*, 2005; Mohararam and Habib, 2011). So, the time has come to improve water availability on one hand, and on the other to evolve wheat varieties that can withstand water stress without compromising quality. Wheat genotypes are now classified on quality traits so that traders can sell wheat according to the needs of millers, bakers and other end users

(Morris, 2002). A pre-requisite for selection is to create types of combinations such that genetic variability can be ensured. Noorka and Khaliq (2007) reported genotypic variability in wheat under water stress conditions. Similarly, others (Chowdhry *et al.*, 1999; Hakim *et al.*, 2012; Marry *et al.*, 2001; Noorka *et al.*, 2009) studied genetic variability of wheat genotypes in relation to water stress.

Heritability is the ability and power of a character to transmit its behavior from one generation to the next. The knowledge of heritability helps the plant breeder in predicting the behavior of succeeding generations. The higher the heritability, the simpler will be the selection process, and thus the greater the response to selection. Heritability does not depend only upon genetic factors but also on environmental circumstances. Of course, environment-related performance is not heritable.

The present study attempts to assess to what extent a wheat breeder and agronomist might succeed in developing new stress-tolerant strains of wheat with the genetic materials in hand.

Materials and methods

The study was conducted at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, during the 2006-2007 and 2007-2008 seasons. Seven water stress-tolerant wheat genotypes, including two exotics viz., 'Nesser' and 'Dharwar Dry', and five local varieties viz. 'GA-2002' 'Bakhar-2002', 'Chakwal', 'Inqilab-91' and 'Kohistan-97' were crossed with University drought susceptible lines viz. 9244, 9247, 9252, 9258, 9267, 9316 and 9021. The line \times tester mating design, as described by Kempthorne (1957), was used. F_1 seeds along with 14 parents were sown in two experiments using a randomized complete block design (RCBD) with three replications. As part of the planned treatment, normal irrigation was applied to one plot throughout the reproductive phase of the crop, while the other plot was subjected to water stress. At maturity, the data were collected and subjected to analysis of variance (ANOVA) (Steel *et al.*, 1997). Yield was determined in the best yielding genotypes, namely, 'Nesser', 'Dharwar Dry', 'Nesser' \times 9244, 'Nesser' \times 9316, 'Nesser' \times 9252, 'Dharwar Dry' \times 9267, 'Dharwar Dry' \times 9316, 'Dharwar Dry' \times 9252, 'GA-2002' \times 9252 and 'Inqilab-91' \times 9316 from normal as well as water stressed conditions. The strength grade of flour of each genotype was evaluated for their proximate composition such as moisture contents (AACC Method, 44-15A), ash content, (AACC 08-01) crude fat (AACC 30-25), crude protein (AACC Method 46-13) and nitrogen percentage in the grains was determined using the micro Kjeldahl method as described by Peter and Young (1980) while gluten content (AACC 38-10) was determined according to the methods described in AACC (2000). The estimates of broad-sense heritability (h^2_{BS}) were calculated for both normal irrigation and water stress conditions with the formula:

$$h^2_{BS} = \sigma^2_g / \sigma^2_p$$

and standard errors (SE) of broad-sense heritability were calculated by using the formula as given by Lothrop *et al.* (1985):

$$SE(h^2_{BS}) = \frac{S.E. \sigma^2_g}{\sigma^2_p}$$

Results and discussion

Drought is a complex phenomenon which involves dynamic interactions of soil, plant, and atmosphere continuum. In the present study variation exhibited by five quality traits under both normal irrigation and water stress conditions played an effective role in developing drought-tolerant genotypes. Most of the traits under study such as moisture percentage, ash content, crude fat, crude protein and gluten content showed significant variability among parents (lines and testers) and their hybrids, which suggested the possibility of selecting a promising best genotype or a cross combination to further improve the population under study. The global water crisis is a severe threat for sustainable agriculture, particularly in most of the Asian countries (Huaqi *et al.*, 2002). Farmers and researchers are striving hard to find out appropriate means to dwindling water consumption in wheat production systems. To improve some of the problems currently facing Pakistani crop improvement programs, including limited water at the seedling stage, mid-season water stress, terminal stress or any combination of these. Ten high-yielding wheat genotypes were selected from normal irrigation conditions and their respective crosses under water stressed conditions. Under water stress condition, the trait moisture content decreased significantly while other traits predominantly protein contents increased in the entire crosses showing a great variation in quality traits (Noorka *et al.*, 2009).

Paradigm shift in wheat quality traits due to water stress

In the present study, water stress played a pivotal role in altering normal growth of wheat. Furthermore, water stress reduced most of the characters, ultimately reducing economic yield. Plant traits are, in general, reduced in response to water stress (Moharram and Habib, 2011; Nabipour *et al.* 2002).

In dealing with quality parameters, the wheat breeder is faced with many complex factors. A wheat variety suitable for one purpose may be unsatisfactory for another (Halverson and Zeleny, 1988). Wheat flour is extensively used for production of flat breads such as chapatti a source of nutrients and is a staple diet common to Pakistan, India and some parts of Africa (Nandini and Salimath, 2001). In this study, water stress decisively changed the normal quality measures of wheat. Furthermore, water stress reduced economic yield, percentage moisture and crude fat while increasing ash content, crude protein and gluten contents. These results are a stark contrast to what is usually found,

i.e. a reduction in most quality-related traits and represents showing a shift in paradigm shift in the normal in which it is conventionally thought that water stress always has a deleterious effect on crop production, exemplified by the increase in quality traits like ash content (8.33 to 27.09%), crude protein (1.49 to 16.00%) and gluten content (5.40 to 12.11%) (Tab. 1), although variance was high (Tab. 2, 3). Ahmad and Arain (1999) and Guttien *et al.* (2001) also showed a contradictory simultaneous reduction and addition of plant traits in response to water stress. A detailed discussion based on quality traits follows next.

Moisture content

Moisture content is a very important quality character in terms of storage of wheat grains for definite periods before it is rendered fit for use; lower flour moisture implies better storage quality and stability and wheat grains with high moisture content due to normal irrigation are difficult to store and vulnerable to pest and diseases (Pomeranaz and Williams, 1990). An alternative form of storage is oven drying which is un-natural and may damage the proteins (Gooding and Davies, 1997). Under normal irrigation condition the moisture contents ranged from 11.82-11.39% while under water stress condition it ranged from 11.63-10.90% showing a significant decrease in each genotype. Due to water stress, moisture content percentage decrease ranged from 4.72% ('Nesser') to 0.34% ('Dharwar Dry' × 9252) (Tab. 1) which would be beneficial for wheat grain long-term storage. Other researchers reported a range of moisture content in wheat grain from 12.09-11.78% (Din *et al.*, 2007) and 13.45% (Rehman *et al.*, 2007) under normal irrigation conditions.

Fat content

The fat content in wheat flour plays an important role during the baking process. It has a significant role in the holding capacity of gas during fermentation and is also an excellent source of energy (Pasha, 2006). Under normal irrigation condition the fat contents ranged from 2.33-2.15 while under water stress condition fat content ranged from 1.92-1.63 showing a significant decrease in each genotype. Crude fat decreased under water stress, ranging from 9.00% in 'Nesser' to 25.22% in the cross combination 'Inqilab-91' × 9316 (Tab. 1). A wide range of fat content in wheat has been reported earlier under normal irrigation conditions ranging from 2.93 to 1.74 (Taneja *et al.*, 1983), 1.2 (Raymond, 1993), and 1.35 (Rehman *et al.*, 2007).

Ash content

Ash is the mineral residue remaining after a sample has been completely oxidized in a manner such that all organic volatile material is driven off, while preventing any mineral from being lost (Ponser, 1991). Whole wheat flour is rich in mineral elements since 80% of the total amount of minerals is concentrated in the aleuron layer of the pericarp (bran) while about 20% is present in the endosperm. Rao *et al.* (1986) recommended flour containing 10.6% protein, 8% moisture and 1.45% ash content for making *chappati*. Ash content is a measure of purity and quality of wheat flour. It does not affect the baking quality but shows different levels of bran depending on the variety. Under normal irrigation condition the ash contents ranged from 1.83-1.55 while under water stress condition ash content ranged from 2.06-1.88 showing a significant increase in each genotype. The ash content increased maximum up

Tab. 1. Percent decrease in wheat quality traits due to water stress conditions

Genotypes	Percent decrease in moisture stress	Percent decrease in fat (%)	Percent increase in ash (%)	Percent increase in protein (%)	Percent increase in dry gluten (%)
'Nesser'	4.72	9.00	8.99	11.14	7.90
'Dharwar Dry'	1.49	17.64	11.11	16.00	8.19
'Nesser' × 9244	3.80	19.45	14.44	14.75	5.40
'Nesser' × 9316	4.27	17.60	8.33	3.90	8.60
'Nesser' × 9252	2.04	16.21	27.09	5.47	7.89
'Dharwar Dry' × 9267	3.91	19.53	18.49	4.41	6.85
'Dharwar Dry' × 9316	3.13	23.21	22.07	9.10	6.21
'Dharwar Dry' × 9252	0.34	21.05	7.65	5.25	12.11
'GA-2002' × 9252	0.86	18.77	14.68	1.49	6.16
'Inqilab-91' × 9316	3.41	25.22	16.57	9.33	8.88

Tab. 2. Analysis of variance of quality traits in ten wheat genotypes under normal irrigation conditions

Source of variation	d.f.	Moisture	Crude protein	Crude fat	Ash content	Gluten content
Replication	2	0.001	0.060	0.004	0.000	0.140NS
Genotypes	9	0.061**	0.986**	0.014**	0.032**	0.814**
Error	18	0.002	0.048	0.003	0.000	0.018

Significant at $p < 0.05$ **; Significant $p < 0.01$; NS = Non significant

Tab. 3. Analysis of variance of quality traits in ten wheat genotypes under water stress conditions

Source of variation	d.f.	Moisture	Crude protein	Crude fat	Ash content	Gluten content
Replication	2	0.002	0.047	0.001	0.000	0.142NS
Genotypes	9	0.132**	0.428**	0.026**	0.012**	1.135**
Error	18	0.001	0.025	0.002	0.003	0.022

Significant at $p < 0.05$ ** Significant $p < 0.01$ NS = Non significant

to 27.09% in case of cross between 'Nesser' \times 9252 and minimum 7.65% in 'Dharwar Dry' \times 9252 although there was scarcity of water (Tab. 1). Other researchers reported a range of ash contents in different wheat varieties under normal irrigation conditions from 0.50-0.45 (Raymond, 1993), 0.48 (Nadeem *et al.*, 2004) and 0.80 (Rehman *et al.*, 2007). However, Seleiman *et al.* (2011) revealed that ash content increased under water stress conditions from 1.78 to 1.87.

Protein content

Protein content is of prime importance in wheat quality and wheat flour. Dry and sunny weather as well as water stress favor maximum production of protein in wheat grain. Kent and Evers (1994) reported that protein contents in wheat are affected by genetic as well as non-genetic factors e.g., soil, climate and fertilizer application. Under normal irrigation condition the protein contents ranged from 11.2-13.78% while under water stress condition it ranged from 12.47-13.92% showing a significant increase in each genotype. Water shortage depicted a paradigm shift by showing a significant increase in protein content percentage ranging from 16.00 to 1.49% (Tab. 1) in genotype 'Dharwar Dry' and a cross combination 'GA-2002' \times 9252, respectively. Similar results have been reported by Singh *et al.* (2008) and Naseri *et al.* (2010) who revealed that with the effect of water stress the protein content in wheat increased protein contents by 10.3-13.2% while Seleiman *et al.* (2011) reported that less irrigation and water stress increased protein content by 11.20%-13.40% in first year while 13.00%-14.10% in second year. Under normal irrigation conditions 10.82% protein content was reported by Butt *et al.* (1997) while Randhawa *et al.* (2002) reported food crude protein content as 11.82%. Similarly, 10.2% protein content was reported by Nadeem *et al.* (2004) and 11.71% by Din *et al.* (2007) under normal irrigation. Ozturk and Aydin (2004) also reported that water stress increased grain pro-

tein content by 18.1%, sedimentation volume by 16.5%, and wet gluten content by 21.9%.

Gluten content

Gluten content is a very important trait for the assessment of flour quality. The unique properties of endospermic protein are called gluten. Gluten does not exist as an entity in whole wheat grain or in flour; it is only produced when flour is physically and vigorously mixed with water (Rehman, 1987). Strong dough is suitable for bread making while weak dough is suitable for cookies (Gaines, 1990). The visco-elastic properties of gluten proteins affect the quality of bread gluten (Veraverbeke and Delcour, 2002). Under normal irrigation condition the gluten contents ranged from 15.24-13.67 while under water stress condition it ranged from 16.18-14.75 showing a significant increase in each genotype. Water stress played a positive role once again and increased gluten content percentage, ranging from 12.11 to 5.40% in the cross combinations 'Dharwar Dry' \times 9252 and 'Nesser' \times 9244, respectively (Tab. 1). These results are in line with earlier research by Seleiman *et al.* (2011), who reported that due to six irrigation gluten content was 10.40 and 11.9 in two consecutive years while under water stress the gluten content has been increased up to 13.10 in the first year and 13.5 in the second year. Similarly, Lin *et al.* (2003) reported 7.0 and 16.77 and Pasha *et al.* (2007) 4.46 and 14.55 while Curic (2001) reported 8.44 and 11.77 and Din *et al.* (2007) 8.72 and 10.69 under normal irrigation conditions.

Heritability

Heritability clarifies whether the differences among variables are due to genetic make up or due to environmental variations and serves as the principal tool to estimate the latent value for an assortment of desired traits. Farshadfar *et al.* (2000) reported that high broad-sense heritability estimates indicated a preponderance of the additive variation in the total genetic variability while medium to low

Tab. 4. Estimates of phenotypic variance (σ^2_p), genotypic variance (σ^2_g) and broad sense heritability under normal irrigation conditions

Characters	σ^2_p	σ^2_g	σ^2_e	Heritability
Moisture % age	0.021	0.019	0.002	0.907
Ash content	0.010	0.010	0.000	1.000
Crude fat	0.006	0.036	0.003	0.550
Crude protein % age	0.360	0.312	0.048	0.866
Gluten content	0.283	0.265	0.018	0.936

Tab. 5. Estimates of phenotypic variance (σ^2_p), genotypic variance (σ^2_g) and broad sense heritability under water stress conditions

Characters	σ^2_p	σ^2_g	σ^2_e	Heritability
Moisture % age	0.040	0.040	0.001	0.980
Ash content	0.006	0.003	0.003	0.500
Crude fat	0.010	0.008	0.002	0.800
Crude protein % age	0.150	0.130	0.030	0.840
Gluten content	0.390	0.370	0.020	0.940

heritability suggests that environmental effects accounted for a major portion of total phenotypic variation.

Estimates of genetic variance for quality traits under normal irrigation and water stress conditions

The present study revealed that the values for phenotypic variance were greater than those for genotypic variance for all five traits under both normal irrigation (Tab. 4) and water stress conditions (Tab. 5) except for crude fat under normal irrigation conditions. The broad-sense heritability was significant for all characters under both normal irrigation and water stress.

Under normal irrigation condition, the broad-sense heritability estimates for moisture percentage (90.7%), ash content (100%), crude fat (55%), crude protein (86.6%) and gluten content (93.6%) (Tab. 4) while under water stress condition the broad-sense heritability values fluctuated, depicting a paradigm shift in heritable frequency of traits. As is evident from Tab. 5, the heritability values for moisture percentage remained (98%), ash content (50%), crude fat (80%), crude protein (84%) and gluten content (94%). In this sense, the higher the heritability value, the easier the selection process since if the heritability value of a trait is low than the selection of that phenotype will not be fruitful and the breeder should rather consider progeny testing; however, when values of heritability are high, phenotypic selection will be more effective.

The aforementioned estimates exercised significant effect on the physio-chemical properties as well as genetic heritabilities of the genotypes under study. Significant differences in quality traits, under both normal irrigation and water stress conditions are in accordance with the findings of Farshadfar *et al.* (2000), who reported a range of high, medium and low heritabilities in wheat under normal irrigation conditions; however, Cox *et al.* (1989) were of the view opinion that any decline and deterioration in the quality of wheat might be due to non-genetic factors such as changes in the environment. The water stress seemed to may have played a significant role in reducing the moisture and fat content of grain while concurrently increasing protein, ash and gluten contents as compared under to normal irrigation conditions. As for heritability, Rana *et al.* (1999) and Farshadfar *et al.* (2000) reported that high value of broad-sense heritability estimates denoted a preponderance of additive variation in the total genetic variability while medium to low heritability suggests that environmental effects accounted for the major

part of total phenotypic variation. It is therefore suggested that potential genotypes and their crosses identified in this study should be studied further to assess the stability of characters under a wider range of environmental stresses for the future breeding programs in order to meet both the quantitative and qualitative needs of wheat production throughout the world.

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