The Role of, Genetic, Agronomic and Environmental Factors on Grain Protein Content of Tetraploid Wheat (*Triticum turgidum L.*)

4

1

5

6 Abstract

For commercial production of tetraploid wheat, grain protein content is considered very 7 important. As the grain received great market attention due to protein premium price paid for 8 farmers, mainly above 13% that will give about 12% of protein in the milled semolina. However, 9 grain protein content of tetraploid wheat is sensitive to environmental conditions pertaining 10 before and during grain filling, crop genetics and cultural practices. This and associated problems 11 universally calls agronomic based alternative solution to ameliorate protein concentration in 12 durum wheat grain. This could be modified through manipulating seeding rates, selection crop 13 varieties, adjusting nitrogen amount and fertilization time and sowing date. The decision of time 14 of nitrogen application however should be made based on the interest of the farmers. If the 15 interest gears towards grain yield, apply nitrogen early in the season and apply the fertilizer later 16 i.e. heading for better protein concentration. 17

18 Keywords: Agronomic practices, Environmental factors, Genotype, Protein

19	
20	
21	
22	
23	
24	
25	
26	
27	
28	
29	

30 **1. Introduction**

The tetraploid or "durum wheat" (Triticum turgidum L.) is the second most important Triticum 31 32 species being cultivated throughout the world next to bread wheat for human consumption and commercial production as well (Peńa et al., 2002). The commercial value and quality of durum 33 wheat for pasta and macaroni manufacturing is directly related with its grain protein and gluten 34 content. In recent years grain protein content becomes important issue for durum wheat 35 36 producers, as important as grain yield. The price that producers are received for durum wheat grain is determined by grain protein content, mainly above 13% that will give about 12% in 37 milled semolina. These means that lower the grain protein content can cause significant economic 38 lose to producers, as protein content is a desired criteria in durum wheat market. 39

In spite of its premium importance grain protein content of tetraploid wheat is sensitive to 40 environmental conditions pertaining before and during grain filling, crop genetics and cultural 41 practices. The farming practices could tremendously affect the stored grain protein content. Even, 42 the way that the crop responds to agronomic inputs depends on range of factors including time 43 and amount of nitrogen fertilization, methods and form of application, planting date, seeding rate, 44 irrigation practices and seasonal conditions, which in turn decreased the grain protein composition 45 (Geleta et al., 2002). Of these factors, nitrogen application is very important aspect when grain 46 47 protein improvement is considered and can be easily adjusted by producers as compared with climatic factors. 48

In addition to grain protein content reduction due to agronomic factors, it is also varied agro-49 ecology to agro-ecology. It has been reported that, under high rainfall area and wet growing 50 season the protein content was significantly lower, conversely, under drier season and hot area the 51 protein content was higher (Anteneh et al., 2018). The reduction in protein content at potential 52 growing area could be due to leaching of the applied nitrogen, as farmers are applied the 53 recommended nitrogen fertilizer twice during the season, which may aggravate leaching of the 54 element early in the season. This is also an indicator for the peoples who basically living in such 55 56 area have poorer intake of protein from the daily meal as a result of complex interaction between soils, crop management practices and other environmental factors, as well as social and economic 57 58 circumstance. Hence, agronomic based grain nutritional composition improvement is needed to

improve their dietary intake which could be the best and sustainable way of enhancing grainprotein content to ensuring both food and nutritional security in such group.

61 **2.** Current demand of durum wheat grain in Ethiopia

Ethiopia is considered as a primary center of genetic diversity for durum wheat (Hailu, 1991) and 62 this crop contributes about 40% of the total wheat production (Badebo et al., 2009). This crop 63 64 plays a vital role for industrial purpose for making pasta, macaroni and other end use products. The demand for pasta and macaroni in Ethiopia has shown gradual increase probably due to 65 66 globalization, population growth and change in food habit, which in turn increased the demand for durum wheat grain (D'Egidio, 2012). Nevertheless, the low volumes and undeclared grain 67 quality of the national wheat production compel Ethiopian pasta industries to import the required 68 raw materials from abroad (D'Egidio, 2012). The annual imported wheat and pasta to Ethiopia 69 70 reaches about 1.3 million tons which costs the country millions of dollars of its foreign exchange 71 reserve (Abeba, 2015). This implies that there is huge gap between durum wheat supply and its demand despite the fact that Ethiopia is the center of diversity for durum wheat. 72

73 **3.** The role of protein on end use products

Protein content is not only having direct nutritional value to humans, but also it influences the 74 dough properties that made from durum wheat. High protein content and strong gluten are the 75 most desired parameter to process semolina and suitable end products. The flour with high protein 76 content has high water absorption, high loaf volume potential and produces loaves with good 77 keeping quality in baking industries (Tipples et al., 1994). This implies that, the end use products 78 and its quality are strongly depending upon the stored protein in the grain. The protein content of 79 wheat universally seems to account for 30 to 40% of the variability in pasta cooking quality 80 81 (Feillet, 1988). The accepted normal values of protein in durum wheat semolina range between 11 to 16% are the optimal that are determined by product desired and producers (Turnbull, 2001). 82

83 4. Factors affecting storage grain protein content

84 4.1. Seeding rate

The seeding rate is amount of seeds which falls into the ground to ensure adequate plant stand establishment and grain yield. The use of seeding rate too low or too high is a frequent report as a

87 limiting factor for yield and grain protein content in wheat (Hamid et al., 2002; Anteneh et al., 2018). Higher seeding rate means increased the interplant competition for available moisture, 88 89 light and nutrient; especially for the applied nitrogen which in turn downgrades the grain protein content when these vital resources are limited (Anteneh et al., 2018). These is often notice when 90 producers used seeding rate above the optimum level and resulting lower the grain protein content 91 (Geleta et al., 2002; Hamid et al., 2002; Gooding et al., 2002; Qingwu et al., 2011; Anteneh et al., 92 2018). However, the seeding rate effect on grain protein content varied depending upon the 93 climatic conditions of the growing season. Where the cropping season has enough soil moisture, 94 grain protein content cannot affected by the increased seeding rate, but increasing seeding rate 95 during dry season significant quality reduction was occurred (Chen et al., 2008). 96

97 4.2. Sowing date

98 The optimum sowing date allows the crops to take full advantage of the available growth resource 99 during the growing season. It has been reported that, the grain protein content and dough quality 100 were increase, as the planting date delayed beyond the optimum windows (Rosella et al., 2007). 101 Similarly, Abdel-Salam et al., (2014) stated that grain protein concentration was significantly 102 higher for the late sowing date than for the normal sowing date.

103 4.3. Tillage practices

It has been reported that, the grain protein content of durum wheat was higher under conventional 104 tillage than under not tillage condition (Colecchia et al., 2015). However, the magnitude of this 105 106 effect varied according to the cultivation environment such as soil type, soil moisture status and the cropping season. Under not tillage system, the protein content slightly decreased than tillage 107 based cropping (Pringas and Koch 2004). The grain protein content tends to decrease under 108 conventional tillage as compared with no tillage condition (Di Fonzo et al., 2001; De Vita et al., 109 110 2007). However, in rainfed condition where soil moisture status is enough, the grain protein content was found higher under conventional tillage than no-tillage practices (Lopez-Bellido et 111 al., 1998; Lopez-Bellido et al., 2001). 112

113 4.4. The genetic potential: deviation between grain protein and grain yield

In many durum wheat genotypes, an inverse relationship between yield and grain protein is apparent (Blanco et al., 2011). High yielder wheat varieties have low storage protein and low yielder variety tends to show high grain protein content, probably due to their capacity to convert soil nitrogen into grain protein (Ross et al., 2008). Hence, an inevitable consequence of increased yields appears to be decreased grain protein concentration; even it varies according to a given variety. This could be apparently occurred, if the genes that ameliorate the grain protein content linked with the genes that have a deleterious effect on.

121 **4.5.** *Temperature and Rainfall*

High temperature occurrence at grain filling stage in wheat showed to increase grain protein composition (Gooding et al., 2003; Castro et al., 2007). This increment is mainly through reduction in grain starch deposition which influences the protein concentration through allowing more nitrogen per unit of starch (Stone and Nicolas, 1998). Corbellini et al., (1998) verified that increasing of temperature and reduced rainfall amount at grain filling stage caused to increase nitrogen content in the grain.

128 5. How to ameliorate grain protein content?

129 5.1. Managing Nitrogen fertilization

In agricultural crop production, nitrogen might be applied in different forms like compost, 130 manures and urea. Optimally supply in multiple doses and timed to supply of nitrogen fertilizer at 131 132 different developmental stages of a crop is important. Late season nitrogen application made 133 between booting and early milky stage has proven effective to increase grain protein content (Clain and Kathrin, 2012). In dryland condition, protein content was increased by about two folds 134 higher when nitrogen was applied before or during flowering than after flowering (Woodard, 135 2003; Clain and Kathrin, 2012). This could be partially explained through the fact late season 136 137 nitrogen application mainly benefits protein buildup than grain starch deposition (Sowers et al., 1994). The benefit of late season nitrogen application have not limited by only improving the 138 protein content, but also increased bread volume made from wheat flour (Xue et al., 2016). 139



Figure 2: The relationship between increasing nitrogen application rate and grain protein content of wheat. **O** stands for 0 gram N application m^2 , \blacktriangle 4 gram N application m^2 , \Box 8 gram N application m^2 (Figure 2A). Figure 2B illustrates, **O**: N applied at active tillering, \Box : N applied at anthesis (Hiroshi et al., 2008).

140

With the application of 4 g N m^{-2} at active tillering, grain protein content increased linearly at a 145 rate of about 0.5% per 1 g N m⁻² [from 10.9% to 14.0%] with increasing N application rate [from 146 0 to 6 g N m^{-2}] at anthesis (Hiroshi et al., 2008). The other important novel practice is splitting 147 148 application of nitrogen during the crop growth period. This approach minimizes the risk of applying single, high rates of nitrogen lose early in the season, especially in wetland wheat 149 production. However, time of application determinate the success of the approach used. The 150 impact of adding more nitrogen at anthesis stage is illustrated in Figure 2. The figure showed that, 151 152 as far as protein content is considered an application of nitrogen fertilizer during anthesis stage is more effective than active tillering stage (Hiroshi et al 2008). 153

154 5.2. Identify specific traits for potential protein improvement: Nutrient Use Efficiency (NUE)

The nitrogen utilization involves several processes such as uptake, assimilation, translocation and remobilization (Masclaux-Daubresse et al., 2008). Improvement in NUE through plant breeding and agronomic practices has a potential to improve yield and grain protein content in field crops. The routes to improve NUE include exploiting synergy of the applied nutrients *(i.e. when* *combined fertilizers are used*) and use of efficient varieties. Clain and Kathrin (2012) indicated that the NUE was increase when nitrogen was combined with sulfur fertilizer. This emphasized the need for precision application of sulfur fertilizer. The late season split application nitrogen fertilizer has been also reported to improve nitrogen use efficiency, resulted in higher plant N uptake in turn better grain protein accumulation (Woolfolk et al., 2002; Ercoli et al., 2013). Nitrogen taken up by plants after boot stage has been showed and increase the protein accumulation in a greater extent than grain yield.

Manipulating or adjusting amount of nitrogen fertilization is also other strategy to improve nutrient use efficacy in crops. Fertilization of sulfur also plays an important role in the formation of baking quality due to its effect on stability and quality number of dough, loaf volume and specific volume (Ryant and Hřivna, 2004; Jarvan et al., 2008).

170 **5.3.** Foliar or soil based application of micronutrients

Foliar or soil application of zinc sulfate greatly enhances the grain protein and gluten content in 171 bread and durum wheat varieties (Ebrahim and Aly, 2005, Nesa et al., 2012; Ali, 2012; Mitra et 172 al., 2015; Anteneh et al., 2018). However, the effectiveness of mineral fertilizers in amelioration 173 of grain protein content could be affected by its application dose, application method and crop 174 developmental stage. For instance, the finding of Seadh et al. (2009) indicated that the increasing 175 of iron application does up to 500 ppm was increase grain protein content in wheat. Similarly, 176 Abbas et al. (2009) stated that the increasing of iron fertilizer application does up to 12 kg ha⁻¹ 177 increase the grain yield and yield components. 178

During micronutrient fertilization considering the developmental stage of the crop is also very important. Foliar application of zinc in reproductive stage of the crop at heading and early milky stage was found effective to accumulate more grain zinc than early growth stage at booting and stem elongation stage (Cakmak *et al.*, 2010). Similarly, Ozturk *et al.* (2001) observed that maximum concentration of zinc in wheat grains was found at milky stage.

184 Conclusion

The grain protein content is greatly influenced by the genetic difference and agronomic practices.Improving grain protein content has special advantage, due to its premium price. The route to

improve grain protein contents includes, adjusting seeding rate, sowing date, and application of nitrogen fertilizer in multiple dose and timed to supply. However, the decision of time of nitrogen application should be made based on the interest of the farmers. If the interest gears towards grain yield, apply nitrogen early in the season and apply the fertilizer later i.e. heading for better protein concentration.

192 Conflict of Interest

193 The author declares that there is no conflict of interest.

194 **References**

- Abbas, G., Khan, M., Jamil, M., Tahir, M., and Hussain, F., (2009) Nutrient uptake, growth
 and yield of wheat (*Triticum aestivum*) as affected by zinc application rates. International
 Journal Agriculture and Biology, 11(4): 389-396.
- Abdel-Salam A., Mohamed M., El-Metwally A., Mahmoud H., Hany K., (2014) Influence
 of Sowing Dates and Combined Fertilizers (NPK) on Growth and Chemical
 Composition of Triticale Grains in Egyptian New Reclaimed Sandy Soils. American Eurasian Journal of Sustainable Agriculture 8(16), November, Pages: 1-3
- Abeba, T., (2015) Flour milling, pasta and biscuits. Ethiopian millers association. Bayne
 building.
- Blanco A.G. Mangini A. Giancaspro S. Giove P. Colasuonno R. Simeone A. Signorile P.
 De Vita A. M. Mastrangelo L. Cattivelli A. Gadaleta (2011) Relationships between
 grain protein content and grain yield components through quantitative trait locus analyses
 in a recombinant inbred line population derived from two elite durum wheat cultivars
- Bly AG, Woodard HJ (2003) Foliar nitrogen application timing influence on grain yield and
 protein concentration of hard red winter and spring wheat. Agron J 95: 335–338.
- Cakmak, I., Pfeiffer, W. and McClafferty, B., (2010) Biofortification of Durum Wheat with
 Zinc and Iron. Cereal Chemistry, 87, 10-20. <u>http://dx.doi.org/10.1094/CCHEM-87-1-0010</u>
- Colecchia, S.A., P. De Vita and M. Rinaldi (2015) Effects of Tillage Systems in Durum Wheat
 under Rainfed Mediterranean Conditions. *Cereal Research Communications* 43(4), pp.
 704–716.
- D'Egidio, M., (2012) From seed to pasta in Ethiopia: Opportunities and challenges to overcome
 for small- holder farmers in Bale area. CRA Consiglio Perla Ricercae la Sperimentazione
 in Agric., IAO.
- De Vita, P., Di Paolo, E., Fecondo, G., Di Fonzo, N., Pisante, M. (2007) No-tillage and
 conventional tillage effects on durum wheat yield, grain quality and soil moisture content
 in southern Italy. Soil Tillage Res. 92:69–78.
- Di Fonzo, N., De Vita, P., Gallo, A., Fares, C., Padalino, O., Troccoli, A., (2001) Crop
 Management Efficiency as a Tool to Improve Durum Wheat Quality in Mediterranean
 Areas. Durum Wheat, Semolina and Pasta Quality, Montpellier (France), Ed. INRA, Paris
 (Les Coloques, no: 99). Paris, France. pp. 67–82.

- Hiroshi N., Satoshi M., and Osamu K., (2008) Effect of nitrogen application rate and timing on
 grain yield and protein content of the bread wheat cultivars 'Minaminokaori' in
 southwestern Japan. Plant Prod.Sci.11 (1): 151-157.
- Lopez-Bellido, L., Fuentes, M., Castillo, J.E., Lopez-Garrido, F.J., (1998) Effects of tillage,
 crop rotation and nitrogen fertilization on wheat-grain quality grown under rainfed
 Mediterranean conditions. Field Crops Res. 57:265–276.
- Lopez-Bellido, L., Lopez-Bellido, R.J., Castillo, J.E., Lopez-Bellido, F.J., (2001) Effects of
 long-term tillage, crop rotation and nitrogen fertilization on bread-making quality of hard
 red spring wheat. Field Crops Res. 72:197–210.
- Ozturk, L., Torun, B., Ozkan, H., Kaya, Z. and Cakmak, I. (2001) Tolerance of 65 Durum
 Wheat Genotypes to Zinc Deficiency in a Calcareous Soil. Journal of Plant Nutrition, 24, 1831-1847. <u>http://dx.doi.org/10.1081/PLN-100107315</u>
- Ozturk, L., Yazici, M.A., Yucel, C., Tourn, A., Cekic, C., Bagci, A., Ozkan, H., Braun, H.J.,
 Sayers, Z., and Cakmak, I., (2006) Concentration and localization of zinc during seed
 development and germination in wheat. Physiology Plantarum, 128 (1):144-152.
- Pringas, C., Koch, H.J., (2004) Effects of long term minimum tillage on yield and quality of
 winter wheat as affected by previous crop results from 9 years of on-farm research.
 Pflanzenbauwissenschaften 8:24–33.
- Rosella M., Simonetta F., and Francesco G., (2007) Protein content and gluten quality of
 durum wheat (Triticum turgidumsubsp. durum) as affected by sowing date. J Sci Food
 Agric87:1480–1488
- Ross M., Roger, A., and Murray (2008) High protein wheat production. Practical information
 for Alberta agriculture industry.
- Seadh, S., El-Abady, M., El-Ghamry, A., and Farouk, S., (2009) Influence of micronutrients
 foliar application and nitrogen fertilization on wheat yield and quality of grain and seed.
 Journal of Biological Science, 9: 851-858.
- Sowers, K.E., Miller, B.C., and Pan, W.L., (1994) Optimizing yield and grain protein in soft
 white winter wheat with split nitrogen applications. *Agron.J.* 86, 1020–
 1025.doi:10.2134/agronj1994.00021962008600060017x
- Xue C, Schulte auf'm Erley G, Rossmann A, Schuster R, Koehler P and Mühling K-H.,
 (2016). Split Nitrogen Application Improves Wheat Baking Quality by Influencing Protein

256 Composition Rather Than Concentration. Front. Plant Sci. 7:738. doi:
257 10.3389/fpls.2016.00738

258