Review Paper The Role of Genetic, Agronomic and Environmental Factors on Grain Protein Content of Tetraploid Wheat (Triticum turgidum L.) Abstract For commercial production of tetraploid wheat, grain protein content is considered very important. As the grain received great market attention due to protein premium price paid for farmers, mainly above 13% that will give about 12% of protein in the milled semolina. However, this review paper stated that grain protein content of tetraploid wheat is sensitive to environmental conditions pertaining before and during grain filling, crop genetics and cultural practices. This Comment [A1]: Please add the objectives of this and associated problems universally calls agronomic based alternative solution to ameliorate protein concentration in durum wheat grain. This could be modified through manipulating seeding rates, selection crop varieties, adjusting nitrogen amount and fertilization time and sowing date. The decision of time of nitrogen application however 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. Keywords: seeding rate, tillage, nitrogen application, temperature, Genotype, Protein 

#### 1. Introduction

The tetraploid or "durum wheat" (Triticum turgidum L.) is the second most important Triticum species being cultivated throughout the world next to bread wheat for human consumption and commercial production as well (Pena et al., 2002). The commercial value and quality of durum wheat for pasta and macaroni manufacturing is directly related with its grain protein and gluten content. In recent years grain protein content becomes important issue for durum wheat 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 milled semolina. These means that lower the grain protein content can cause significant economic lose to producers, as protein content is a desired criteria in durum wheat market. 

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

In addition to grain protein content reduction due to agronomic factors, it is also varied agroecology to agro-ecology. It has been reported that, under high rainfall area and wet growing season the protein content was significantly lower, conversely, under drier season and hot area the protein content was higher (Anteneh et al., 2018). The reduction in protein content at potential growing area could be due to leaching of the applied nitrogen, as farmers are applied the recommended nitrogen fertilizer twice during the season, which may aggravate leaching of the element early in the season. This is also an indicator for the peoples who basically living in such 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 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 grain
- protein content to ensuring both food and nutritional security in such group.

**Comment [A2]:** Please add the objectives of this review after this sentences

# 2. Current demand of durum wheat grain in Ethiopia

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

#### 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 75 dough properties that made from durum wheat. High protein content and strong gluten are the most desired parameter to process semolina and suitable end products. The flour with high protein 76 77 content has high water absorption, high loaf volume potential and produces loaves with good 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 80 wheat universally seems to account for 30 to 40% of the variability in pasta cooking quality (Feillet, 1988). The accepted normal values of protein in durum wheat semolina range between 11 81 to 16% are the optimal that are determined by product desired and producers (Turnbull, 2001). 82

#### 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

limiting factor for yield and grain protein content in wheat (Hamid et al., 2002; Anteneh et al., 2018). Storage grain protein content has an inverse relationship with seeding rate. It was stated that, the protein content was lower at higher seeding rate (175 kg ha<sup>-1</sup>) and vice versa under lower seeding rate (100 kg ha<sup>-1</sup>) (Anteneh et al., 2018; Qingwu et al., 2011; Hamid et al., 2002). Higher seeding rate means increased the interplant competition for available moisture, 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 producers used seeding rate above the optimum level and resulting lower the grain protein content (Geleta et al., 2002; Hamid et al., 2002; Gooding et al., 2002; Qingwu et al., 2011; Anteneh et al., 2018). However, the seeding rate effect on grain protein content varied depending upon the climatic conditions of the growing season. Where the cropping season has enough soil moisture, grain protein content cannot affected by the increased seeding rate, but increasing seeding rate during dry season significant quality reduction was occurred (Chen et al., 2008).

# 4.2. Sowing date

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

# 4.3. Tillage practices

It has been reported that, the grain protein content of durum wheat was higher under not tillage condition than under conventional tillage (De Vita 2007; Colecchia et al., 2015). It could be due to high organic matter content of the soil. However, the magnitude of this 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 based cropping (Pringas and Koch 2004). The grain protein content tends to decrease under conventional tillage as compared with no tillage condition (Di Fonzo et al., 2001; De Vita et al., 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 al., 1998; Lopez-Bellido et al., 2001).

#### 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.

#### 4.5. Temperature and Rainfall

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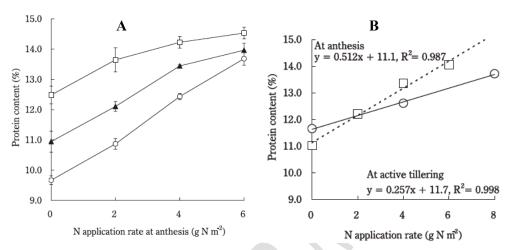
High temperature occurrence at grain filling stage in wheat showed to increase grain protein composition (Gooding et al., 2003; Castro et al., 2007). This increment is mainly through reduction in grain starch deposition which influences the protein concentration through allowing more nitrogen per unit 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.

# 5. How to ameliorate grain protein content?

# 5.1. Managing Nitrogen fertilization

In agricultural crop production, nitrogen might be applied in different forms like compost, manures and urea. Optimally supply in multiple doses and timed to supply of nitrogen fertilizer at different developmental stages of a crop is important. Late season nitrogen application made 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 higher when nitrogen was applied before or during flowering than after flowering (Woodard, 2003; Clain and Kathrin, 2012). This could be partially explained through the fact late season 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 protein content, but also increased bread volume made from wheat flour (Xue et al., 2016).



**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).

With the application of 4 g N m<sup>-2</sup> at active tillering, grain protein content increased linearly at a rate of about 0.5% per 1 g N m<sup>-2</sup> [from 10.9% to 14.0%] with increasing N application rate [from 0 to 6 g N m<sup>-2</sup>] at anthesis (Hiroshi et al., 2008). The other important novel practice is splitting application of nitrogen during the crop growth period (Figure 2B). This approach minimizes the risk of applying single, high rates of nitrogen lose early in the season, especially in wetland wheat production. However, time of application determinate the success of the approach used. The impact of adding more nitrogen at anthesis stage is illustrated in Figure 2. The figure showed that, 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).

# 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 171 172 nutrient use efficacy in crops. Fertilization of sulfur also plays an important role in the formation 173 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).

#### 5.3. Foliar or soil based application of micronutrients

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Foliar or soil application of zinc sulfate greatly enhances the grain protein and gluten content in bread and durum wheat varieties (Ebrahim and Aly, 2005, Nesa et al., 2012; Ali, 2012; Mitra et al., 2015; Anteneh et al., 2018). Similarly, foliar application of iron fertilizer enhances the grain yield and grain quality traits of wheat compared with non-application of iron fertilizer (Zeidan et al., 2010). The foliar application of iron fertilizer could not only improves the grain yield, but also improves the grain protein content and gluten content which are the most important required grain quality traits in durum wheat market (Nesa et al., 2012; Mitra et al., 2015). However, the effectiveness of mineral fertilizers in amelioration of grain protein content could be affected by its application dose, application method and crop developmental stage. For instance, the finding of Seadh et al. (2009) indicated that the increasing of iron application does up to 500 ppm was increase grain protein content in wheat. Similarly, Abbas et al. (2009) stated that the increasing of iron fertilizer application does up to 12 kg ha<sup>-1</sup> increase the grain yield and yield components.

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.

## Conclusion

This review clearly demonstrated that the grain protein content is greatly influenced by the genetic difference, tillage practices, seeding rate and sowing date. Increasing the seeding rate beyond the optimum level it reduces the grain protein level. 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.,

# **Conflict of Interest**

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

**Comment [A3]:** The conclusion must match with the objectives of this review.

204	References	Comment [A4]: Please check the author guideline for the references style and rewrite correctly all of the references.
205	Abbas, G., Khan, M., Jamil, M., Tahir, M., and Hussain, F., (2009) Nutrient uptake, growth	correctly all of the references.
206	and yield of wheat (Triticum aestivum) as affected by zinc application rates. International	
207	Journal Agriculture and Biology, 11(4): 389-396.	
208	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	Comment [A5]: Please insert "and" before the last author.
209 210	Composition of Triticale Grains in Egyptian New Reclaimed Sandy Soils. American-	
211	Eurasian Journal of Sustainable Agriculture 8(16), November, Pages: 1-3	
212	Abeba, T., (2015) Flour milling, pasta and biscuits. Ethiopian millers association. Bayne	
213	building.	Comment [A6]: What kind of the reference? Is t paper or text book? Please rewrite correctly
214	Blanco A.G. Mangini A. Giancaspro S. Giove P. Colasuonno R. Simeone A. Signorile P.	
215	De Vita A. M. Mastrangelo L. Cattivelli A. Gadaleta (2011) Relationships between	Comment [A7]: Please insert "and" before the
216	grain protein content and grain yield components through quantitative trait locus analyses	last author
217	in a recombinant inbred line population derived from two elite durum wheat cultivars	Comment [A8]: What is the name of journal, Vol.No.and page?
218	Bly AG, Woodard HJ (2003) Foliar nitrogen application timing influence on grain yield and	
219	protein concentration of hard red winter and spring wheat. Agron J, 95: 335-338.	
220	Cakmak, I., Pfeiffer, W. and McClafferty, B., (2010) Biofortification of Durum Wheat with	
221	Zinc and Iron. Cereal Chemistry, 87, 10-20. http://dx.doi.org/10.1094/CCHEM-87-1-0010	Comment [A9]: Please check the correct style of references
222	Colecchia, S.A., P. De Vita and M. Rinaldi (2015) Effects of Tillage Systems in Durum Wheat	
223	under Rainfed Mediterranean Conditions. Cereal Research Communications 43(4), pp.	
224	704–716.	Comment [A10]: Please check the correct style of references
225	D'Egidio, M., (2012) From seed to pasta in Ethiopia: Opportunities and challenges to overcome	
226	for small- holder farmers in Bale area. CRA Consiglio Perla Ricercae la Sperimentazione	
227	in Agric., IAO.	Comment [A11]: Please insert how many pages?
228	De Vita P. Di Paolo E., Fecondo G., Di Fonzo N., Pisante M., (2007) No-tillage and	
229	conventional tillage effects on durum wheat yield, grain quality and soil moisture content	
230	in southern Italy. Soil and tillage research, pp.69-78	Comment [A12]: Please rewrite correctly.
231		
232	De Vita, P., Di Paolo, E., Fecondo, G., Di Fonzo, N., Pisante, M. (2007) No-tillage and	
233	conventional tillage effects on durum wheat yield, grain quality and soil moisture content	
234	in southern Italy. Soil Tillage Res. 92:69–78.	Comment [A13]: Please correct it. It is a same reference with previously
		reference with previously

235	Di Fonzo, N., De Vita, P., Gallo, A., Fares, C., Padalino, O., Troccoli, A., (2001) Crop		
236	Management Efficiency as a Tool to Improve Durum Wheat Quality in Mediterranean		
237	Areas. Durum Wheat, Semolina and Pasta Quality, Montpellier (France), Ed. INRA, Paris		
238	(Les Coloques, no: 99). Paris, France. pp. 67–82.		
239	Hiroshi N., Satoshi M., and Osamu K., (2008) Effect of nitrogen application rate and timing on		
240	grain yield and protein content of the bread wheat cultivars 'Minaminokaori' in		
241	southwestern Japan. Plant Prod.Sci.11 (1): 151-157.		
242	Lopez-Bellido, L., Fuentes, M., Castillo, J.E., Lopez-Garrido, F.J., (1998) Effects of tillage,		
243	crop rotation and nitrogen fertilization on wheat-grain quality grown under rainfed		
244	Mediterranean conditions. Field Crops Res. <b>57</b> :265–276.		
245	Lopez-Bellido, L., Lopez-Bellido, R.J., Castillo, J.E., Lopez-Bellido, F.J., (2001) Effects of		
246	long-term tillage, crop rotation and nitrogen fertilization on bread-making quality of hard		
247	red spring wheat. Field Crops Res. <b>72</b> :197–210.		
248	Mitra, B., Payam, M., and Behzad, S., (2015) The Effect of iron nano-particles spraying time		
249	and concentration on wheat. Biological Forum an International Journal, 7(1): 679-683.		
250	Nesan, S., Ali, K., Hadis, N., and Shirani-Rad, A., (2012) The Effect of Iron Sulfate Spraying		
251	on Yield and Revitalizing Health and Agriculture Nuffield Australia Project No. 0911.		

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

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,

development and germination in wheat. Physiology Plantarum, 128 (1):144-152.

1831-1847. http://dx.doi.org/10.1081/PLN-100107315

252

253

254

- 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.

**Comment [A14]:** Plese rewrite completely, the name of journal, vol., No., and page

266	Seadh, S., El-Abady, M., El-Ghamry, A., and Farouk, S., (2009) Influence of micronutrients				
267	foliar application and nitrogen fertilization on wheat yield and quality of grain and seed.				
268	Journal of Biological Science, 9: 851-858.				
269	Sowers, K.E., Miller, B.C., and Pan, W.L., (1994) Optimizing yield and grain protein in s	oft			
270	white winter wheat with split nitrogen applications. Agron.J. 86, 102	:0–			
271	1025.doi:10.2134/agronj1994.00021962008600060017x				
272	Xue C, Schulte auf'm Erley G, Rossmann A, Schuster R, Koehler P and Mühling K-	Н.,			
273	(2016). Split Nitrogen Application Improves Wheat Baking Quality by Influencing Prot	ein			
274	Composition Rather Than Concentration. Front. Plant Sci. 7:738. d	loi:			
275	10.3389/fpls.2016.00738				
276					
277					