

**Factors Affecting the Storage Grain Protein Content of Tetraploid Wheat (*Triticum turgidum* L.) and their Management**

**Abstract**

This review work aims to evaluate the factors affecting the storage grain protein content of tetraploid Wheat (*Triticum turgidum* L.) and their management. 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 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

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## 32 1. Introduction

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

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

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

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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. This review work aims to evaluate the factors affecting the storage grain protein content of tetraploid Wheat (*Triticum turgidum* L.) and their management.

## 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 this crop contributes about 40% of the total wheat production (Badebo *et al.*, 2009). This crop 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 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 poor grain quality in terms of (protein) of the national wheat production compel Ethiopian pasta industries to import the required raw materials from abroad (D'Egidio, 2012). The annual imported wheat and pasta to Ethiopia reaches about 1.3 million tons which costs the country millions of dollars of its foreign exchange 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.

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## 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 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 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 and its quality are strongly depending upon the stored protein in the grain. The protein content of 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 to 16% are the optimal that are determined by product desired and producers (Turnbull, 2001).

## 4. Factors affecting storage grain protein content

#### 88 4.1. Seeding rate

89 The seeding rate is amount of seeds which falls into the ground to ensure adequate plant stand  
90 establishment and grain yield. The use of seeding rate too low or too high is a frequent report as a  
91 limiting factor for yield and grain protein content in wheat (Hamid et al., 2002; Anteneh et al.,  
92 2018). Storage grain protein content has an inverse relationship with seeding rate. It was stated  
93 that, the protein content was lower at higher seeding rate (175 kg ha<sup>-1</sup>) and vice versa under lower  
94 seeding rate (100 kg ha<sup>-1</sup>) (Anteneh et al., 2018; Qingwu et al., 2011; Hamid et al., 2002). Higher  
95 seeding rate means increased the interplant competition for available moisture, light and nutrient;  
96 especially for the applied nitrogen which in turn downgrades the grain protein content when these  
97 vital resources are limited (Anteneh et al., 2018). These is often notice when producers used  
98 seeding rate above the optimum level and resulting lower the grain protein content (Geleta et al.,  
99 2002; Hamid et al., 2002; Gooding et al., 2002; Qingwu et al., 2011; Anteneh et al., 2018).  
100 However, the seeding rate effect on grain protein content varied depending upon the climatic  
101 conditions of the growing season. Where the cropping season has enough soil moisture, grain  
102 protein content cannot affected by the increased seeding rate, but increasing seeding rate during  
103 dry season significant quality reduction was occurred (Chen et al., 2008). Increasing of seeding  
104 rate up to optimum level can increase both grain and biomass yield, but decrease storage protein  
105 content in the harvested grain (Anteneh et al., 2018). Hence, determination of optimum seeding  
106 rate is varied on the required product. If the interest is geared is towards the grain yield, higher  
107 seeding rate is important and vice versa if the interest is on improving storage protein content,  
108 lower seeding rate is favoured. Generally this implies that location and product specific seeding  
109 rate is required to achieve maximum profitability yield and acceptable grain protein content.

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#### 110 4.2. Sowing date

111 The optimum sowing date allows the crops to take full advantage of the available growth resource  
112 during the growing season. It has been reported that, the grain protein content and dough quality  
113 were increase, as the planting date delayed beyond the optimum windows (Rosella et al., 2007).  
114 Similarly, Abdel-Salam et al., (2014) stated that grain protein concentration was significantly  
115 higher for the late sowing date than for the normal sowing date. It could be associated with  
116 terminal moisture stress occurred at flowering period. The more the sowing date is delayed the

more the crop is exposed to end season moisture stress. Mukhtar and Fayyaz-un (2015) verified that, effects of extreme climatic conditions including water stress and high temperature are beneficial for quality traits like proline, grain ash and grain protein but on the expense of grain yield. It can be suggested that, as sowing date is the main determinant factor for crop quality traits therefore, it shall be recommended according to prevailing weather conditions using long-term weather forecasting data

#### 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). This means that, the effect of tillage practices on grain protein content is varied according to the climatic condition of the growing season.

#### 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.

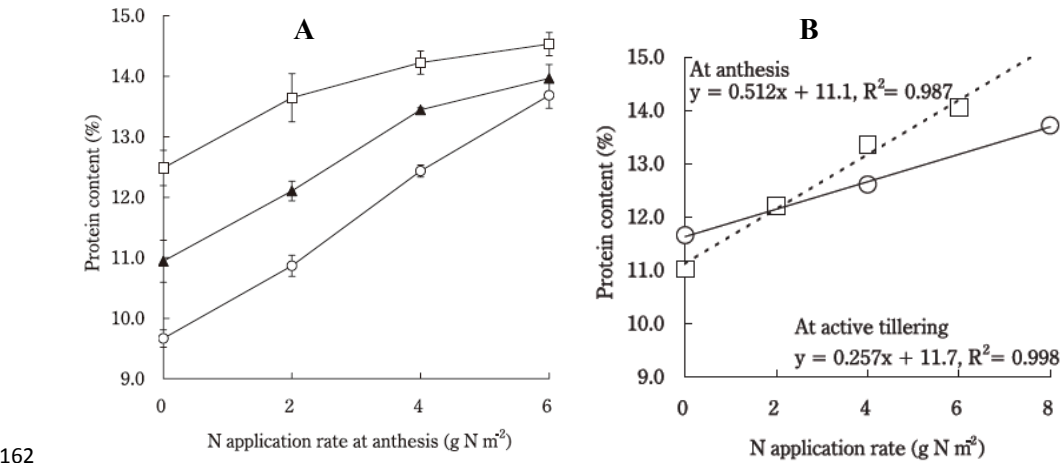
#### 4.5. Temperature and Rainfall

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

150 **5. How to ameliorate grain protein content?**

151 **5.1. Managing Nitrogen fertilization**

152 In agricultural crop production, nitrogen might be applied in different forms like compost,  
153 manures and urea. Optimally supply in multiple doses and timed to supply of nitrogen fertilizer at  
154 different developmental stages of a crop is important. Late season nitrogen application made  
155 between booting and early milky stage has proven effective to increase grain protein content  
156 (Clain and Kathrin, 2012). In dryland condition, protein content was increased by about two folds  
157 higher when nitrogen was applied before or during flowering than after flowering (Woodard,  
158 2003; Clain and Kathrin, 2012). This could be partially explained through the fact late season  
159 nitrogen application mainly benefits protein buildup than grain starch deposition (Sowers et al.,  
160 1994). The benefit of late season nitrogen application have not limited by only improving the  
161 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  $\text{m}^2$ , **▲** 4 gram N application  $\text{m}^2$ , **□** 8 gram N application  $\text{m}^2$  (Figure 2A). Figure 2B illustrates, **O**: N applied at active tillering, **□**: N applied at anthesis (Hiroshi et al., 2008).

With the application of 4 g N  $\text{m}^{-2}$  at active tillering, grain protein content increased linearly at a rate of about 0.5% per 1 g N  $\text{m}^{-2}$  [from 10.9% to 14.0%] with increasing N application rate [from 0 to 6 g N  $\text{m}^{-2}$ ] 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 2B. 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 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).

### 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 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. 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.

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219 Foliar application of zinc sulphate and iron sulfate can increase grain protein concentration in the  
220 harvested grain. Hence, application of micronutrient containing fertilizers could be the best  
221 approach to ameliorate grain protein content if used in combination with crop varieties with  
222 known genetic response to the applied micro fertilizers. On the basis of the available information  
223 it can further be conclude that the success of grain protein improvement can be influenced by seed  
224 rate under practice as optimal seeding rate is needed to efficiently utilize the applied fertilizers  
225 without competition or underutilization.

#### 226 **Conflict of Interest**

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

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## 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. and 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. <http://dx.doi.org/10.1094/CCHEM-87-1-0010>
- 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 Per la Ricerca e 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 and tillage research*, pp.69-78
- 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

260 Areas. Durum Wheat, Semolina and Pasta Quality, Montpellier (France), Ed. INRA, Paris  
 261 (Les Coloques, no: 99). Paris, France. pp. 67–82.

262 Hiroshi N., Satoshi M., and Osamu K., (2008) Effect of nitrogen application rate and timing on  
 263 grain yield and protein content of the bread wheat cultivars ‘Minaminokaori’ in  
 264 southwestern Japan. *Plant Prod.Sci.* 11 (1): 151-157.

265 Lopez-Bellido, L., Fuentes, M., Castillo, J.E., Lopez-Garrido, F.J., (1998) Effects of tillage, crop  
 266 rotation and nitrogen fertilization on wheat-grain quality grown under rainfed  
 267 Mediterranean conditions. *Field Crops Res.* 57:265–276.

268 Lopez-Bellido, L., Lopez-Bellido, R.J., Castillo, J.E., Lopez-Bellido, F.J., (2001) Effects of long-  
 269 term tillage, crop rotation and nitrogen fertilization on bread-making quality of hard red  
 270 spring wheat. *Field Crops Res.* 72:197–210.

271 Mitra, B., Payam, M., and Behzad, S., (2015) The Effect of iron nano-particles spraying time and  
 272 concentration on wheat. *Biological Forum an International Journal*, 7(1): 679-683.

273 Mukhtar Ahmed and Fayyaz-ul-Hassan (2015) Response of Spring Wheat (*Triticum aestivum* L.)  
 274 Quality Traits and Yield to Sowing Date. *PLoS ONE* 10(4)  
 275 <https://doi.org/10.1371/journal.pone.0126097>

276 Nesan, S., Ali, K., Hadis, N., and Shirani-Rad, A., (2012) The Effect of Iron Sulfate Spraying on  
 277 Yield and Revitalizing Health and Agriculture Nuffield Australia Project No. 0911.

278 Ozturk, L., Torun, B., Ozkan, H., Kaya, Z. and Cakmak, I. (2001) Tolerance of 65 Durum Wheat  
 279 Genotypes to Zinc Deficiency in a Calcareous Soil. *Journal of Plant Nutrition*, 24, 1831-  
 280 1847. <http://dx.doi.org/10.1081/PLN-100107315>

281 Ozturk, L., Yazici, M.A., Yucel, C., Tourn, A., Cekic, C., Bagci, A., Ozkan, H., Braun, H.J.,  
 282 Sayers, Z., and Cakmak, I., (2006) Concentration and localization of zinc during seed  
 283 development and germination in wheat. *Physiology Plantarum*, 128 (1):144-152.

284 Pringas, C., Koch, H.J., (2004) Effects of long term minimum tillage on yield and quality of  
 285 winter wheat as affected by previous crop – results from 9 years of on-farm research.  
 286 *Pflanzenbauwissenschaften* 8:24–33.

287 Rosella M., Simonetta F., and Francesco G., (2007) Protein content and gluten quality of durum  
 288 wheat (*Triticum turgidum* subsp. durum) as affected by sowing date. *J Sci Food*  
 289 *Agric* 87:1480–1488

290 Ross M., Roger, A., and Murray (2008) High protein wheat production. Practical information for  
 291 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 Composition Rather Than Concentration. *Front. Plant Sci.* 7:738. doi: 10.3389/fpls.2016.00738

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