T.R.
ONDOKUZ MAYIS UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF FIELD CROPS

EFFECTS OF ZINC BIOFORTIFICATION STRATEGIES ON YIELD, YIELD COMPONENTS AND SEED ZINC CONTENT OF BREAD WHEAT UNDER DROUGHT AND ZINC DEFICIENT SOIL CONDITION

PhD Thesis

Mohaned Mohammed Ali MOHAMMED

Supervisor
Prof. Dr. Erkut PEKŞEN

SAMSUN
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THESIS ACCEPTANCE AND APPROVAL

This thesis entitled "**Effects of Zinc Biofortification Strategies on Yield, Yield Components and Seed Zinc Content of Bread Wheat under Drought and Zinc Deficient Soil Condition**" prepared by **Mohaned MOHAMMED ALİ MOHAMMED** under the supervision of **Prof. Dr. Erkut PEKŞEN** has been accepted with unanimously of votes by our jury as PhD thesis in the result of thesis defense exam conducted on January 08, 2021.

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Approval
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Director of Institute

DECLARATION OF COMPLIANCE WITH SCIENTIFIC ETHIC

I hereby declare and undertake that I complied with scientific ethics and academic rules in all stages of my PhD thesis, that I have referred to each quotation that I use directly or indirectly in the study and that the works I have used consist of those shown in the sources, that it was written in accordance with the institute writing guide and that the situations stated in the article 3, section 9 of the Regulation for TÜBİTAK Research and Publication Ethics Board were not violated.

08 /01 / 2021

Mohaned Mohammed Ali MOHAMMED

DECLERATION OF THESIS ORIGINALITY REPORT

Thesis title: Effects of Zinc Biofortification Strategies on Yield, Yield Components and Seed Zinc Content of Bread Wheat under Drought and Zinc Deficient Soil Conditions

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ABSTRACT

EFFECTS OF ZINC BIOFORTIFICATION STRATEGIES ON YIELD, YIELD COMPONENTS AND SEED ZINC CONTENT OF BREAD WHEAT UNDER DROUGHT AND ZINC DEFICIENT SOIL CONDITIONS

Mohaned Mohammed Ali MOHAMMED
Ondokuz Mayıs University
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Department of Field Crops
PhD Thesis, January/2021
Supervisor: Prof. Dr. Erkut PEKŞEN

Drought stress and Zinc (Zn) deficiency are serious abiotic stress factors restricting plant growth and agricultural production when they occur concurrently, especially in arid and semi-arid regions that wheat is the most commonly cultivated crop. The main aims of this study were to improve drought tolerance, grain yield, and increase seed Zn content through Zn biofortification in bread wheat. In experiment I, the effect of seeds priming (2.5 and 5 mM Zn) and seed coating (1.5, 2.5 and 5 g Zn/kg seeds) with Zn on seed germination and seedling growth parameters were determined under controlled-growth chamber conditions at day/night temperature of 24/20 °C and 65-75% relative humidity during early growth stages of two wheat varieties showing difference for their seed Zn content (Imam with average 29 mg/kg Zn and Altındane 25.5 mg/kg Zn). In each treatment, 25 seeds were placed into petri dishes to determine seed germination rate and sown into pots containing 700 g alluvial soil with low Zn content to monitor seedling growth for 21 days with three replications. Seed priming with Zn, particularly high dose (5 mM Zn) had relatively positive impact and good performance on seed germination rate, mean germination time and seedling growth parameters when compared with low Zn dose (2.5 mM Zn) and hydropriming in both two wheat varieties. Seed coating with Zn, particularly with low Zn concentration (1.5 g Zn/kg seeds) and in Altındane with less Zn content has shown good respond and improved seed germination parameters in comparison with untreated seeds for both wheat varieties.

Experiment II was conducted under controlled-growth chamber and greenhouse conditions to evaluate different of Zn application strategies including untreated seed (0 Zn), hydropriming (0 Zn), seed priming (5 mM Zn), seed coating (1.5 g Zn/kg seeds), soil application (10 kg Zn/ha), foliar spray 0.5% and two combinations of soil application with foliar (10 kg Zn/ha+0.5%) and seed coating with foliar (1.5 g Zn/kg seeds+0.5%) on grain yield, drought tolerance and grain biofortification with Zn for wheat varieties mentioned above. 30 seeds of each variety and treatment sown in pot with 8 kg Zn deficient soil (0.6 mg/kg), after 7 days of germination seedling were thinned to 20 per pot. Initially, pots were irrigated at 100% of field capacity (FC) by daily weighted and watering. After 60 days of sowing at booting stage, plants were subjected to drought stress by maintain the irrigation at 50% of FC, while pots in the well-watered treatment were maintained at 100% FC until harvest time. The results shown that, the losses of grains wheat yield because of drought stress reached up to 8% in Imam and 15% in Altındane comparison with well-watered yield for both varieties. However, Zn application through seed coating and combination of seed coating with foliar spray improved the yield under drought stress by 10.8 and 9.5% in Imam, and by 14 and 17% in

Altındane, respectively. Zn application mitigated negative effects of drought stress and Zn deficiency through ameliorates WUE and Ψ_w. Antioxidants enzymes like Superoxide dismutase (SOD) under drought stress displayed more activity in untreated seed treatment, but the activity was more evident when Zn applied in Altındane variety and was disappeared in Imam variety. In this study drought stress has shown the highest grain Zn content by 57.5 mg/kg recorded by seed coating+foliar spray and 42.3 mg/kg recorded by foliar spray treatment in Altındane and Imam variety, respectively. However, in comparison with well-watered condition, drought stress increased grain Zn content by 40 and 5.5% in Altındane and Imam varieties, respectively.

Experiment III was carried out in Research Field of Ondokuz Mayıs University, Agricultural Faculty, Samsun, Turkey during the 2018-2019 growing season under rainfed conditions to assess whether Zn foliar spray, seed coating with Zn alone or combine with foliar spray can improve grain yield, quality, and Zn content of wheat varieties. Foliar spray, seed coating and combine application with Zn improved grain yield and grain Zn content in Imam and Altındane varieties by 14.7, 10.8 and 5.3%, and 10, 0 and 19.2%, respectively when compared with control treatment. Moreover, there was decline of grain protein content of wheat with all Zn application treatments under rainfed conditions, and the correlation among them was highly negative significant ($r = -0.62$; $P < 0.001$).

Keywords: Wheat, Zn biofortification, Drought stress, Drought tolerance, Seed coating, Zn deficiency

ÖZET

ÇİNKO İLE ZENGİNLEŞTİRME STRATEJİLERİNİN, KURAKLIK VE ÇİNKO NOKSAN TOPRAK KOŞULLARI ALTINDA EKMEKLİK BUĞDAYDA VERİM, VERİM BİLEŞENLERİ VE TOHUMUN ÇİNKO İÇERİĞİ ÜZERİNE ETKİLERİ
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Kuraklık stresi ve çinko (Zn) eksikliği, özellikle eşzamanlı olarak gerçekleştikleri zaman kurak ve yarı kurak bölgelerde bitki büyümesini ve tarımsal üretimi kısıtlayan önemli abiyotik stres faktörleridir. Bu çalışmanın temel amacı, kuraklık toleransını geliştirmek, buğday tanelerinin Zn ile biyolojik olarak güçlendirilmesi yoluyla bu sorunları hafifletmek veya aşmaktır. Deneme I'de, çinko ile tohum ön uygulama (2.5 ve 5 mM Zn) ve tohum kaplamanın (1.5, 2.5 ve 5 g Zn/kg tohum) tohum çimlenmesi ve fide büyüme parametreleri üzerindeki etkileri, tohumlarındaki çinko miktarları bakımından farklılık gösteren iki buğday çeşidinde (Imam 29 mg/kg Zn ve Altındane 25.5 mg/kg Zn) erken gelişme dönemi sırasında gündüz/gece 24/20 °C sıcaklık ve %65-75 oransal nemde kontrollü büyüme odası koşullarında belirlenmiştir. Her uygulamada, 25 tohum çimlenme oranlarını belirlemek için Petri kaplarına yerleştirildi ve fide büyümesini 21 gün boyunca izlemek için düşük Zn içerikli 700 g alüvyal toprak içeren saksılara üç tekrarlamalı olarak ekilmiştir. Zn ile tohum ön uygulamalarının, özellikle yüksek doz (5 mM Zn) ile uygulamanın, düşük Zn dozu (2.5 mM Zn) ve her iki buğday çeşidinde de hidropriming ile karşılaştırıldığında, tohum çimlenme oranı, ortalama çimlenme süresi ve fide büyüme parametreleri üzerinde nispeten olumlu bir etkiye ve iyi performansa sahip olduğunu ortaya koymuştur. Özellikle düşük konsantrasyonda (1.5 g Zn/kg tohum) tohum kaplama ve daha düşük Zn içeriğine sahip Altındane çeşidinde yapılan kaplama, her iki çeşidin uygulama yapılmayan tohumları ile karşılaştırıldığında daha iyi yanıt vermiş ve tohum çimlenme parametrelerini artırmıştır.

Deneme II, muamele edilmemiş tohum (0 Zn), hidropriming (0 Zn), tohum ön uygulama (5 mM), tohum kaplama (1.5 g Zn/kg tohum), toprak uygulaması (10 kg Zn/ha), yaprağa püskürtme (%0.5 Zn) ve toprak uygulaması+yaprak püskürtme (10 kg Zn/ha+%0.5) ve tohum kaplama+yapraktan püskürtme (1.5 g Zn/kg tohum+%0.5) birlikte uygulamalarının da dahil olduğu çinko uygulama stratejilerinin yukarıda bahsedilen iki buğday çeşidinde tane verimi, kuraklık toleransı ve tanelerin çinko ile zenginleştirilmesi üzerine etkilerini değerlendirmek için kontrollü büyütme odasında ve sera koşullarında yapılmıştır. Her çeşit ve uygulamaya ait 30 tohum, Zn eksikliği gösteren 8 kg toprakla (0.6 mg/kg) doldurulmuş saksılara ekilmiş, çimlenmenin 7. gününden sonra fideler sayıları saksı başına 20 adet olacak şekilde seyreltilmiştir. Başlangıçta saksılar yapılan günlük tartım ve sulamalar ile %100 tarla kapasitesinde sulanmıştır. Ekimden 60 gün sonra, başaklanma öncesi döneminde, toprak suyu %50 tarla kapasitesi arasında tutularak kuraklık stresine maruz bırakılırken, iyi sulanan uygulamadaki saksılar hasat zamanına kadar %100 tarla kapasitesinde tutulmuşlardır. Sonuçlar, kuraklık stresi nedeniyle buğday verimindeki kayıpların her iki çeşidin iyi sulanan uygulamaları ile karşılaştırıldığında Imam'da %8'e, Altındane'de ise %15'e ulaştığını göstermiştir. Bununla birlikte, tohum kaplama ve tohum kaplamanın yaprağa püskürtme ile kombinasyonu kuraklık stresi altında tane verimini İmam'da sırasıyla %10.8 ve 9.5, Altındane'de ise %14 ve 17 artırmıştır. Zn uygulaması, WUE ve Ψw'yi iyileştirerek kuraklık stresi ve Zn eksikliğinin olumsuz etkilerini azaltmıştır. Öte yandan, mevcut çalışma, kuraklık stresi altındaki Süperoksit Dismutaz (SOD) gibi antioksidant enzimlerin, uygulama yapılmayan tohumlarda daha fazla aktivite sergilediğini, ancak Zn'nun Altındane çeşidinde uygulandığında daha belirgin olduğunu ve Imam çeşidinde görülmediğini göstermiştir. Bu çalışmada, kuraklık stresi, en yüksek tane Zn içeriği Altındane ve Imam çeşidinde sırasıyla tohum kaplama+yaprağa püskürtme (57.5 mg/kg) ve yaprağa püskürtme (42.3 mg/kg yaprak) uygulamasından elde edilmiştir. Bununla birlikte, iyi sulanan koşullara kıyasla kuraklık stresi, Altındane ve Imam çeşidinde tane Zn içeriğini sırasıyla %40 ve 5.5 artırmıştır.

Deneme III, Samsun Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi Araştırma sahasında yağmura dayalı koşullarda 2018-2019 yetiştirme sezonunda yapraktan Zn uygulaması ve Zn ile tohum kaplamanın tek başına veya birlikte uygulandığında buğdayda tane verimi, tane kalitesi ve Zn içeriği üzerine etkilerinin olup olmadığını belirlemek için yapılmıştır. Çinkonun yapraktan püskürtme, çinko ile tohum kaplama ve bunların birlikte uygulamalarının, kontrol uygulaması ile karşılaştırıldığında, Imam ve Altındane çeşitlerinde tane verimini ve tane Zn içeriğini sırasıyla %14.7, 10.8 ve 5.3 ve %10.0 ve 19.2 oranında artırdığını göstermiştir. Tüm Zn uygulama yöntemlerinde yağmura dayalı yetiştirme koşullarında ile buğdayın tane protein içeriğinde azalma olmuş, aralarındaki korelasyon negatif ve çok önemli bulunmuştur ($r = -0.62$; $P < 0.001$).

Anahtar Kelimeler: Buğday, Zn biyolojik zenginleştirme, Kuraklık stresi, Kuraklık toleransı, Tohum kaplama, Zn eksikliği

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LIST OF ABBREVIATIONS

AB	Abscisic acid
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
B	Boron
℃	Celsius
CAT	Catalase
CIMMYT	International Maize and Wheat Improvement Center
cm	Centimeter
DF	Degree of freedom
DI	Drought index
DM	Dry matter
DS	Drought stress
DTPA	Diethyl triamine penta-acetic acid
FC	Field capacity
Fe	Iron
g	Gram
GB	glycine betaine
GDP	Gross Domestic Product
GP	Germination percentage
h	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
HI	Harvest index
HMs	Heavy metals
HP	Hydropriming
kg	Kilogram(s)
L	Litter (s)
LA	Flag leaf area
LEA	Late embryogenesis abundant
mg	Milligram
MGT	Mean germination time
mM	Mill molar(s)
mm	Milliliter(s)
MPa	Mega pascal
MSI	Membrane stability index
RWC	Relative water content
P	Probability
RH	Relative humidity
%	Percent
SOD	Superoxide dismutase

ROS	Reactive oxygen species
SPAD	Value of chlorophyll content
t/ha	tons per hectare
w/v	Weight per volume
WUE	Water use efficiency
WUEGY	Water use efficiency for grain yield
WW	Well-watered
Mg	Microgram(s)
Ml:	Microliter(s)
Ψw	Leaf water potential
Mn	Manganese

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1. INTRODUCTON

Cereals are the world's most important food crops and provide over half of the daily human intake of calories. Additionally, they constitute the major source of Zn for world's population, especially for the poor people living in the rural areas (Boonchuay et al., 2013). In term of protein supply, wheat accounts for 21% of the daily protein consumption per capita in major cereal crops. Generally, wheat is most grown in arid and semi-arid areas as in Turkey and Sudan. However, under this condition, due to little and irregular precipitation, soil moisture is limited (Ekiz et al., 1998), and that making soil poor in plant-availability Zn concentration (Cakmak et al., 1999). Water deficit and Zn deficiency are the main limitation environmental factors to successful crops yield such as cereals (Karim and Rahman, 2015). However, lack of water and nutrients like Zn deficient could severely restricted the yield and nutrient quality of wheat and other cereals crops (Bell and Dell, 2008; Karim et al., 2012). Zn deficiency is the major constraint to cereals growth and productivity in the world (Hong and Jin, 2007). Zn plays crucial role in plant growth and several of biological, physiological and biochemical processes such as protein synthesis, photosynthesis, antioxidant function, growth regulation and cofactor of large number of enzymes (Römheld and Marschner, 1991; Brown et al., 1993; Hafeez et al., 2013). Also, Zn is required to gene expression which is essential for the tolerance of environmental stress in plant like drought stress. However, it has been reported that Zn acts as detoxifier through detoxification of Reactive Oxygen Species (ROS) in plant cells (Cakmak, 2000; Broadley et al., 2007). Zn deficiency has been noticed to be the widely prevalent micronutrients deficiency in the agricultural soils globally, particularly in cereals-growing lands (Gomez-Coronado et al., 2016) and the most widespread crop micronutrients deficiency constraint restricting yield of crops (yield loss can exceed 40%) (Noulas et al., 2018). Moreover, Zn deficiency in the crops mainly occur in the soil with high pH, low organic matter and soil with low Zn availability (Alloway, 2008a; 2008b). In this regards, Alloway (2009) mentioned that 30% of the soil worldwide is attributed to Zn deficiency, and 50% of cereal cultivated lands have low levels Zn available for plant (Marschner, 1993; Graham and Welch, 1996).

Counteract and overcame the yield loss and malnutrition resulting in Zn deficiency in developing countries, particularly those have combined of concurrently drought stress and zinc deficiency such as in Mediterranean and arid and semi-arid regions represent substantial challenge for researchers, genetic engineers and plant breeders around the world. To enhance production of grain and Zn content in stable food crops like wheat, several strategies and approaches have been suggested. These strategies including agronomic and genetic biofortification (White and Broadley, 2011). Agronomic biofortification of food crops can be achieved through enhancing soil Zn availability for plants including supplementation programs and fertilizers application. Since find out the importance of Zn for plants, Zn fertilization for crops have become more prevalence and applied in wide range in Zn-deficient agricultural soils (Cakmak and Kutman, 2018). HarvestPlus program have determined the target of Zn concentrations in cereal crops like wheat, maize and pearl millet by 38, 38 and 66 mg/kg DM, respectively (Bouis and Welch, 2010; White and Broadley, 2011). Generally, the leaf of many crop plants needs Zn concentrations between 15-30 mg/kg DM for better growth and yield, and their growth keep inhibition when Zn concentrations at leaf be more than 100-700 mg/kg DM (White and Brown, 2010; Fageria, 2016).

Among Zn fertilization strategies, foliar spray has been confirmed to be sustainable, effective and low cost-strategy to increment Zn level of stable food crops like wheat (Rengel et al., 1999). In green house experiment conducted in China to investigate the influence of foliar spray of Zn, Mn and B applied at late growth stage of wheat under drought stress and well-watered condition, it has found that Zn foliar spray increased yield by 13%, and also, grain Zn concentrations have been raised (Karim et al., 2012). Biofortification of foliar spray with Zn have reported to be effective in increment of grain with Zn concentration in either of Zn-sufficient or Zn-deficient soils (Hussain et al., 2012). Furthermore, Zn foliar spray improved number of grain per spike and water use efficiency (Karim et al., 2012). Yavas and Unay (2016) studied out Zn foliar spray increased wheat height, length of spike, 1000-grain weight, number of grains per spike, chlorophyll content and relative water content (RWC). Besides the activity of superoxide dismutase (SOD), peroxidase (POD and catalase (CAT) have been increased. Similarly, when seeds with high Zn concentration used under low Zn supply, SOD activity in seedlings is significantly improved (Candan et al., 2018). Soil application is the simplest agronomic biofortification to improve the Zn content in stable food crops (e.g. wheat, maize and sorghum) when these crops grown under Zn deficient soils as has been applied in Turkey and India (Cakmak, 2009). In study carried out by Hussain et al. (2012), it was found that soil application with Zn (9 mg Zn/kg) lead to increase grain production by 29% and whole-grain Zn concentration by 95% in wheat crop. Also, in other research soil application increased straw Zn concentration in wheat by 18.1% (Maqsood et al., 2009). Furthermore, in study carried out in pot experiment grain yield and grain Zn concentration of two wheat cultivars have increased by average 25 and 30%, respectively, when the soil treated and mixed with Zn (Qaswar et al., 2017). Ma et al. (2017) revealed that soil Zn application (14 mg Zn/kg soil) under well-watered, moderate drought and severe drought conditions increased grain yield and Zn concentration of wheat by 10.5 and 15.8%, 22.6 and 9.7%, and 28.2 and 32.8%, respectively. Also, this research shown that Zn soil application enhanced total phenolic compound in wheat flag leaf by 5.8, 7 and 13.3% under well-watered, moderate drought and severe drought conditions, respectively compared with corresponding control treatment. Moreover, several studies have proven that the combination of foliar spray and soil application with Zn could be more effective to increase yield Zn concentration for grains such as wheat (Yilmaz et al., 1997), pea (Poblaciones and Rengel, 2016) and rice (Ram et al., 2013). In the same regard, Gomez-Coronado et al. (2016) find out soil application alone have no any significant effect on increasing of grain Zn concentration, but improved yield by 10%. At the same research, when Zn soil application combined with foliar spray with Zn lead to increase more than 20 mg/kg and 7% in Zn concentration and grain yield of wheat crop, respectively. Other study displayed that soil + foliar application lead 80% enhance in grain Zn content (Bharti et al., 2013). Among Zn fertilization methods, seed treatments such as priming and coating seeds with Zn can be economical and alternative tool to foliar spray and soil application with Zn in term of delivered to plants with relatively small amount of materials is applied per hectare (Taylor and Harman, 1990; Farooq et al., 2012). Use seeds with adequate Zn concentration could ameliorate grain Zn content, germination rate and increase yield in wheat (Yilmaz et al., 1997; Reis et al., 2018), maize (Ajouri et al., 2004; Harris et al., 2007) and chickpea (Johnson et al., 2005; Hidoto et al., 2017). Arif et al. (2007) indicated that seed priming with $ZnSO_4$ (0.05%) solution resulted in remarkable improve in grain spike, 1000-grain weight, biological weight and grain yield in wheat crop. Moreover, seed priming with ZnO NPs (100 mg/L) improved leaf chlorophyll content as well as increased straw and Zn concentration of grain wheat by 65 and 64%, respectively (Munir et al., 2018).

On the other hand, seed coating is another cost-effective and efficient type of seed treatment strategy for supply mineral nutrients by adhering them to the seed surfaces using an adhesive material to improve seed effectiveness. It is direct application of material such as micronutrients to the seeds without change its shape and size (Taylor and Harman, 1990). Further, macro-and micronutrients have been coated with seeds and revealed positive effects to ameliorate early plant growth (Scott and Archie, 1978; Scott et al., 1987). Similarly, seed coating for cereal seeds have been used to achieve various purposes such as protect seedlings from biotic and abiotic stresses like diseases, insects and low temperature (Schneider and Renault, 1997). Likewise, different substance such as nutrients have been applied in seed coating (Silcock and Smith, 1982). Seed coating with Zn could be promising approach to ameliorate seed germination, grain yield and grain Zn contents (Farooq et al., 2012). Rehman and Farooq (2016) found that seed coating with 1.25 g Zn/kg improved chlorophyll and maximized grain yield and Zn grain contents from 33 to 55% and from 21 to 35%, respectively.

Drought is another one of the most important environmental stresses which restrict plant growth and development, reduce yield and quality particularly in arid and semi-arid regions across the world. Approximately, 40-60% of world agricultural lands are affected by drought; therefore low water amount could constitute the main obstacle facing the crop production in the near future. Moreover, mostly rain falls during autumn and winter in Mediterranean condition, but soil moisture content begin to reduce in spring season coinciding with the flowering and grain filling stages of wheat (Acevedo et al., 1999). However, 9-10% of national cereal production have been significantly reduced by the drought and extreme heat during 1964 to 2007 (Lesk et al., 2016). It has been reported that drought stress induced yield losses in wheat by 57% (Balla et al., 2011). Previous study showed that water deficit is able to induce reduction of wheat production by 20-30% (Daryanto et al., 2016; Zhang et al., 2018). Water deficit throughout grain filling stage of wheat decreased yield and yield component, while superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) increased. Furthermore, many of studies revealed that Zn sulphate heptahydrate ($ZnSO_4.7H_2O$) has ability to improve drought tolerance by promoting of efficient root system and finally improve crop yield as well as Zn widely have been used as fertilizer to increase grain Zn contents in several cereals crops to reduce malnutrition problem. There have been a few studies and attention related to this interaction of Zn deficiency and drought stresses. Consequently, this research was implemented with following objectives:

1. To determine the most proper Zn dose to use in seed priming and seed coating to be able to achieve high germination rate and improve seedling growth parameters of Imam and Altındane bread wheat varieties at early growth stage under controlled condition.

2. To reveal the influence of Zn deficiency and drought stress on grains yield and grain Zn content of Imam and Altındane bread wheat varieties under various Zn soil and watering regime conditions.

3. To improve drought tolerance of Imam and Altındane bread wheat varieties, and also to alleviate or minimize negative influence of drought or Zn deficiency through Zn biofortification strategies, ameliorate grain yield, increase grain Zn content under Zn-deficient soil and deficit water regimes.

4. To determine the best Zn application treatment/s or their combination among seed priming, seed coating, soil application, foliar spray, soil application + foliar spray to be recommended as Zn application strategy for zinc deficient soil and drought conditions.

5. To reveal the genotypic variation of Imam and Altındane bread wheat varieties in terms of drought stress tolerance in the presence of Zn- deficiency and drought stress condition.

6. To assess whether seed coating with Zn alone or combine with foliar spray would be improved grain yield, grain quality, and Zn content of Imam and Altındane bread wheat varieties under rainfed conditions.

2. GENERAL INFORMATION

2.1. Wheat

Wheat (*Triticum aestivum* L.) is one of the largest and most important and stable food crop in the world. It an annual plant crop that belongs to family *Poaceae* and tribe *Triticeae* (Kereša et al., 2001). The plurality of cultivated wheat varieties in the world belong to the three main species of genus *Triticum*. Which are; the diploid, *Triticum monococcum* L. (Einkorn wheat), (2n = 2x = 14, AB), tetraploid, *T. durum* (durum wheat), (2n = 4x = 28, AABB) has traditionally been used for pasta and couscous products and hexaploid, *T. aestivum* L. (bread wheat), (2n = 6x = 42, AABBDD) which has been consumed for bread (Kimber and Feldman, 1987) and account for about 95% of the world's wheat production. The rest 5% of the world's wheat production is durum.

Total world wheat production has risen up from 521 million tons in 1986/87 to 771.71 million tons in 2017/18. China (134.3 tons), India (98.5 tons), Russia (85.8 tons) and United States (47.3 tons) are the great wheat production countries of the world. The importance of wheat as a worldwide food crop is reflected by it's the large cultivated area which estimated about 218.55 million hectares and total production of 771.71 million tons with an average yield of 3.53 tons for one hectare in 2017 as reported by (FAOSTAT, 2017). In Sudan, wheat is considered as the second most crucial staple food crop after sorghum, cultivated in land area of 0.167 million hectares (0.56 million feddan) and 7.66 million hectares with total production of 0.46 and 21.5 tons and productivity of 2.76 and 2.8 tons for one hectare in Sudan and Turkey respectively (FAOSTAT, 2017). However, the growth habitat of wheat varieties is a significant agronomic characteristic, consequently wheat cultivars divided into two general types, winter wheat, which require for low temperatures between germination and stem elongation stages (vernalization) in order to occur heading. In contrast to winter wheat, spring wheat without requirement to cold temperature in order to change from vegetative growth to reproductive growth. Generally, winter wheat is sown in autumn, whereas, spring wheat is sown in spring or winter season (Brooking, 1996). Principally, spring wheat is cultivated and produced in countries and regions where winters are too warm or result in production is infeasible (Taylor and Koo, 2012). According to Zadok's scale Zadoks et al. (1974) wheat divided into ten major or primary development growth stages are;

germination, seedling, tillering, stem elongation, booting, heading, flowering, milk, dough and ripening stages. A sound understanding and knowledge of these stages is crucial and required for plant scientists and farmers. Where many of agricultural practices and proper application time for fertilizers, insecticides, herbicides, fungicides, irrigation and harvest are pretty much depend on crop growth stage rather than calendar date.

Wheat contributes and provides more than 30% of the world population with more than half of their calories and protein requirements (Dhanda et al., 2004). Grain of wheat has a good nutritional value with 70% total carbohydrate, 12.1% protein, 60% starch, 1.8% lipids, 1.8% ash, 2% reducing sugars and provides 314 kcal/100 g of food. It is also, good source of several minerals and vitamins viz., Zn (3 mg/100g), iron (4.1 mg/100g), calcium (37 mg/100g), thiamine (0.45 mg/100g), nicotinic acid (5.4 mg/100g) and riboflavin (0.13 mg/100g) (Lorenz and Kulp, 1991).

Soft wheat flour due to it contains high amount of gluten is well-convenient for bread, cakes, cookies and biscuits, whereas durum wheat is suite to making pasta products, couscous, macaroni and spaghetti. Despite of grains is primarily used for human consumption; likewise is considered outstanding feed for poultry and livestock. Also, besides grain, straw rich in fibrous materials. It is use for making textiles, sorbents, filters, packing materials as well as animal feed. Since it is gas emission are low, it might be use as comparatively clean energy source (Campbell et al., 1997). Generally, wheat production in one country various from that produced in other countries in term of quantity and quality due to differences in soil types and environmental condition (Taylor and Koo, 2012).

2.2. Drought

In the days of global warming, the impact of drought stress on crops productivity is expected to increase. Drought is one of the major restricting factors to crops productivity such as wheat, thus food security. It has been reported that approximately 47% of world agricultural lands affect by drought (Figure 2.1), (Karim and Rahman, 2015).

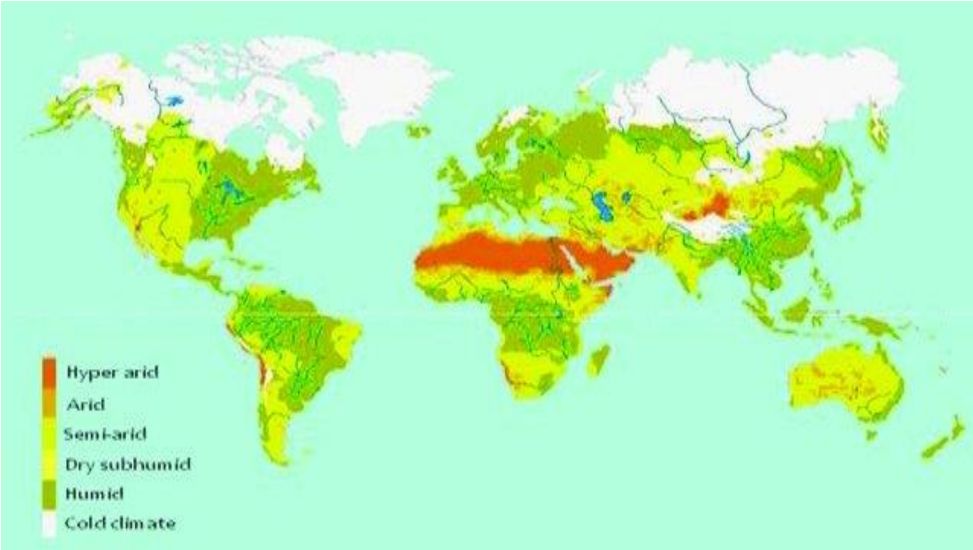


Figure 2.1. Global map of drought distribution (Karim and Rahman, 2015)

Wheat is most commonly cultivated in arid and semi-arid regions in Turkey and Sudan. However, under this condition soil moisture is limited due to low and irregular precipitation (Ekiz et al., 1998). Increasing water scarcity associated with climate change could negatively affected global wheat production, thus casing food insecurity and poverty (Tubiello et al., 2000). Further, the reduction of productivity in resulting of drought stress of the crop plants may differ from 50 to 73% (Berry et al., 2013). Severe drought cause significant losses and decline in crop yields via induce alteration at morphological, physiological and biochemical processes in all plant organs (Osmond and Grace, 1995). The impact of water stress varies based on the crop type, timing, duration and severity of drought (Pandey et al., 2001). Among cereal crops, sorghum and pearl millet are highly drought tolerant in comparison with wheat and maize, therefore they are predominantly cultivated in arid and semi-arid areas. Based on data collected in the duration from 1980 to 2015 examined to wheat and maize responses to drought stress in field experiments, the results observed that wheat had the lower yield reduction (20.6%) than maize (39.3%) (Daryanto et al., 2016). Also, they reported that wheat and maize contributed to more than 50% of cereal production in the world in 2013. Although, maize have been reported to be the most sensitive crop among cereals wheat is sensitive to Zn deficiency when grown on calcareous soils and rice in flooded soils also (Noulas et al., 2018). Drought stress have been reported to induce yield losses in wheat by 57% (Balla et al., 2011). Previous study showed that water deficit is able to induce reduction of wheat production by 20-30% (Daryanto et al., 2016; Zhang et al., 2018). Yield is fundamentally having complex interaction and correlation with wide range of processes such as chlorophyll content, water use efficiency etc. and most of these processes are adversely influenced by the water deficit (Fahad et al., 2017). Furthermore, drought negatively affected seed yield and its attributes such as plant height, number of grains/spike, spike length and 100-grain weight (Kilic and Yağbasanlar, 2010). Khan et al. (2015) found that leaf size and plant height were significantly reduced under the deficit-watered condition in maize. Drought stress was caused considerable loss in biomass production in wheat reached to 70% (Pant et al., 1998). Yavas and Unay (2016) reported that drought stress at grain filling stage significantly decreased height of plant, spike length, 1000-grain weight, number of grains/spike, chlorophyll content and relative water content. Furthermore, when water stress were imposed at flowering stages, it leads to affect and decrease seed yield, plant height and biological yield of safflower crop (Movahhedy-Dehnavy et al., 2009). It is known that drought stimulates and accumulates oxidative stress in plant tissues through increasing reactive oxygen species (ROS). These ROS constitutes critical issue to the cell functioning via damaging lipids and proteins (Fahad et al., 2017). The ROS are mostly produced in chloroplasts which are very susceptible to oxidative damage (Reddy et al., 2004).

Plants have evolved various mechanisms to deal with the harmful of drought stress which are throughout; 1) drought escape by completing the life cycle before the water in soil is depleted (Izanloo et al., 2008); 2) drought avoidance maintain relatively water balance despite a storage of soil moisture through stomata closure and reduce leaf area size (Yu et al., 2017); 3) drought tolerance withstand water deficit via osmatic adjustments and 4) drought resistance through altered metabolic changes such as increase antioxidants activities and phenolic compound metabolism (Reddy et al., 2004). In several parts of the world, drought stress often concurrently interfere with other of environmental stresses such as heat stress and microelement deficiency during growing season (Karim et al., 2012).

2.2.1. Effect of Drought Stress on Wheat

It is expected that the world population could attain about 9 billion at the end of this century. Consequently, it has been forecasted that demand for cereals, particularly wheat will rise by approximately 50% by 2030 (Borlaug and Dowswell, 2003). In order to fulfill this increasing of growing world population, the annual wheat production has also been increased. Wheat constitutes the paramount carbohydrate staples for people in several countries worldwide. However, the intermittent precipitation or irrigation at critical and sensitive stage of the crop in the season may cause considerable yield loss and crop failure (Ludlow and Muchow, 1990; Praba et al., 2009).

Drought is paramount environmental limited factor affect crop yield and poses a growing threat to sustainable agricultural and, altimetry, for food security in the world. Drought stress can induce changes and effects at any time and stage of wheat from sowing date to harvest time, but this influence become more critical and plants are sensitive during flowering and grain filling stages than early growth stages due to water deficit during these period could causes direct notable economical losses in yield and quality (Pavia et al., 2019). Approximately, one-third of wheat-growing regions in the developing countries are threat by drought throughout the growing season (Belaid and Morris, 1991; Van Ginkel et al., 1998). Under Mediterranean condition, wheat is mostly growing during the rainfall season (autumn), but at the end of the season, especially at grain-filling stage soil moisture begin to reduce and this ultimately, result in substantial lack of wheat production. For instance, about 30% of wheat cultivated-area is on drought-borne soil in United Kingdom (Foulkes et al., 2002). Based on meta-analysis, drought stress is able to induce reduction of wheat yield range between 20-30% (Daryanto et al., 2016; Zhang et al., 2018). In the fields particularly in the Mediterranean belt, wheat expose to various environmental stresses e.g. drought in arid and semi-arid countries. This deficit of water triggers a wide variety of changes at morphological, physiological and biochemical features in all parts of crop (Wang et al., 2016).

2.2.1.1. Impact of drought on morphological processes

Response of wheat to drought stress depends on the several categorical variables including; wheat type (winter or spring wheat), growth stage (vegetative or reproductive stage), drought intensity (mild, moderate or severe drought) and root environment (growing in pot or field condition) (Zhang et al., 2018). The initial and foremost impact of drought on wheat is poor germination and deteriorate seedling growth (Harris et al., 2002). Several studies have reported on the damaging impact of drought on germination and seedling growth of wheat (Farooq et al., 2009; Marcińska et al., 2013; Candan et al., 2018). It has been reported that decreases in available water induced damages to mitosis, expansion and elongation of cell which causes poor germination and production (Hussain et al., 2012). Also, a change in any agronomic traits including plant height, number of spikelet per spike, number of grains per spike and 1000-grain weight under low water condition lead to alters the final wheat yield. Similarly, drought stress during the early growth stages (vegetative phase) restricted plant height, leaf area and the number of tillers. Primarily, water stress causes reduction in wheat yield through diminish in the grain yield, either owing to a reduced amount of dry matter or direct impair to pollen during the reproductive phase (Prasad et al., 2008). Drought stress inhibits wheat performance at all growth stages from sowing till harvest period, but it is becoming more critical and damaging during the flowering and grain-filling phase (terminal drought) and causes significant reduction of yield (Farooq et al., 2014). At post-anthesis stage of wheat, water stress has strongly impact on grain filling and grain size (Jamieson et al., 1995; Yang et al., 2001; Ji et al., 2010). It has been demonstrated that the Mediterranean regions are more vulnerable drought stress in resulting decline of rainfall at the end of the season coincided with flowering and grain-filling stage, and this may lead to reduction of grain yield (Loss and Siddique, 1994). Severe water deficit in these stages have a number of serious impacts on wheat yield, reduced spikelet numbers, fertility of spikelet (Aspinall, 1984) and pollen sterility (Cattivelli et al., 2008). Previous result shown that moderate drought stress (40% reduction of water) caused low grain weight lead reduction of wheat yield, whereas under the severe drought stress (less than 40% water reduction) resulting in all yield components especially, number of grains per ear and number of fertile ears per unit (Giunta et al., 1993). Wheat yield and biomass decreased by 21, 25.8 and 32%, and by 11, 21 and 34.7% under mild, moderate and severe drought stress, respectively (Zhang et al., 2018). Yield ultimately has complex relation with several of physiological processes which negatively affected by drought.

2.2.1.2. Effect of drought stress on physiological processes

Many physiological processes in wheat are impaired by drought stress, induce a decrease in germination, growth, yield and seed quality. Among these important processes, photosynthesis is negatively limited by drought stress. These limitations cause reducing in leaf expansion, damage of photosynthetic machinery and premature leaf senescence (Wahid et al., 2005) and changes in structure of pigments and proteins (Menconi et al., 1995). Several studies have been reported that the decrease of net photosynthesis under drought stress attributed to stomatal and non-stomatal limitations (Ort et al., 1994; Ahmadi, 1998; Shangguan et al., 1999; Farooq et al., 2009). Drought primarily causes stomatal closure which in turn reduce the flow of CO_2 into mesophyll cells (Flexas et al., 2004; Kadam et al., 2014). Reduction of wheat yield by water stress may be due to reduce rate of photosynthesis (Flexas et al., 2004). Similarly, wheat genotypes which sustain flag leaf photosynthesis for a long period have ability to produce high yield (Larbi and Mekliche, 2004), because of flag leaf is considered main source of assimilates (30-50%) during the wheat grain yield development (Sylvester-Bradley et al., 1990). Chlorophyll is one of the major chloroplasts for photosynthesis, and any change or decrease in chlorophyll content in resulting environmental stress such as drought stress could have negative effect on photosynthesis and in turn yield (Anjum et al., 2011). The value of chlorophyll content of wheat varieties decreased by 6.5 and 37.3% under medium and harsh drought stress consisted for 20 days (Ma et al., 2017). Further, some research have revealed enhance in chlorophyll content in cereals under water stress (Estill et al., 1991). Leaf area index (LAI) is another parameter which affected by water stress, it also substantial factor to assess several plant processes like plant canopy, photosynthesis and evapotranspiration (Ahmad et al., 2015). Usually, genotypes with a large leaf area have ability to higher water uptake, would need to receive more water in each irrigation event (Puértolas et al., 2017). In a greenhouse experiment conducted by Karim et al. (2012), drought stress significantly reduced LAI, stomatal conductance, transpiration and photosynthesis in wheat. However, water relations are influenced by various significant characteristics which including; relative water content (RWC), leaf water potential (Ψ_{w}), transpiration rate and canopy temperature. Initially, relative water content during the early growth stages (the young leaves) is considered to be higher than the late stages of wheat growth (the mature leaves) (Siddique et al., 2000). A reduce of RWC in response to water deficit has observed in many of studies related to wheat (Praba et al., 2009; Roohi et al., 2013). Ψ_w define as ability of chemical potential of the water solution to do work. To absorb water, roots must generate internal water potentials low enough to overcome water potential in the soil (Torres-Ruiz et al., 2012). Ψ_w and transpiration rate significantly decreased under drought stress, which in turn enhanced the leaf canopy temperature (Fahad et al., 2017). Another significant feature for water status is water use efficiency (WUE) which is ratio of plant biomass produced to the total amount of water transpired. The temporary of stomatal closure should improve water use efficiency (Ludlow and Muchow, 1990). Abbate et al. (2004) mentioned that WUE of wheat under deficit-water supply was higher than in well-watered condition.

2.2.1.3. Impact of drought on biochemical processes

Initially, water deficit causes closure of stomata which ultimately, reduce uptake of carbon dioxide (CO_2) and eventually, occur inhibition of photosynthesis (Smirnoff, 1993). Reactive oxygen species (ROS) are usually generate by normal cellular metabolic processes such as photorespiration in mitochondria, chloroplast and peroxisomes (Apel and Hirt, 2004). Basically, there are four forms of cellular ROS, single oxygen (O_2), superoxide radical (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). These compounds continuously produce by plants under normal condition, but their overproduction increase and became harmful when plant subjected to drought stress (Xiong and Zhu, 2002). Reactive oxygen species are potentially dangerous under water stress and pose serious threat to plants by causing lipid peroxidation, enzyme inactivation, protein degradation, membrane injury, fragmentation and disruption of DNA and ultimately cell death (Davies, 1987; Imlay and Linn, 1988). Drought stress triggers the production and accumulation of ROS. Hence, they are detoxified by enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxidase (POX). And non-enzymatic antioxidants (ascorbic acid, glutathione (GSH), carotenoids, flavonoids, tocopherols, phenolics and alkaloids) are very important for protect plant against to drought stress (Apel and Hirt, 2004; Nayyar and Gupta, 2006; Farooq et al., 2009). Superoxide dismutase (SOD) acts as the first defense through converting O_2- into H_2O_2, thereafter H_2O_2 converts to H_2O and O_2 by catalase (CAT) (Wang et al., 2016). On the other hand, accumulation certain organic compounds of low molecular mass in various plant species in response to environmental stress such as drought refer to compatible solutes (Ashraf and Foolad, 2007). Among these compatible osmolytes produce by plants are proline, amino acid, polyols, tertiary and quaternary ammonium compounds (glycine betaine) (Rhodes and Hanson, 1993). Many studies have reported that accumulation of thus solutes contributed to enhance drought tolerance of wheat by elevate osmatic adjustment or detoxify ROS (Borojevic et al., 1980; Bahieldin et al., 2005; Wang et al., 2010;

Farooq et al., 2014). Plant growth regulators or phytohormones play vital role in regulation of plants physiological processes such as stomatal closure, photosynthesis, membrane permeability as well as act as early warning signal in response to water stress (Davies and Zhang, 1991; Khan et al., 2012). Under drought stress, endogenous contents of abscisic acid and ethylene increase, whereas those auxins, cytokinin and gibberellins decrease (Nilsen and Orcutt, 1996). Nevertheless, plant growth regulators play a significant role in drought tolerance of wheat (Farooq et al, 2014). Pervious researches have showed the influence of water stress on phytohormones of wheat (Morgan, 1983; 2000; Fischer et al., 2005).

2.2.2. Mechanisms of Drought Resistance

Plants have capability to adapt themselves to cope with drought by controlling several mechanisms, e.g., photosynthesis, transpiration and photorespiration. The ability plant to grow, survive and reveal economic yield at low environmental water availability refer to drought tolerance (Farooq et al., 2009). Improving drought tolerance in order to produce wheat varieties with high yield is a challenge and major goal of plant breeding programs (Blum, 1989). To cope up with drought stress, plants have adapted and evolved different strategies including morphological, physiological and biochemical mechanisms which remove or mitigate the harmful impacts of drought stress.

2.2.2.1. Morphological mechanisms

Plants have promoted different mechanisms to deal with the harmful of drought stress which are throughout 1) drought escape by completing the life cycle before depleting water in the soil. In this case, plant possess ability to store and assimilate water in some organs (stems and roots) and mobilize them for grains production as in cereals (Izanloo et al., 2008). 2) drought avoidance mainly occurs through minimizing water loss which via stomata closure, reduce leaf area size and shedding the old leaves (Yu et al., 2017), which contribute to water store. The second strategy of avoidance of water stress is maximizing water uptake through increase of roots density and length to use water more efficiently. These root systems help plant to extracting the water from considerable depth (Kavar et al., 2008). On the other hand, the ability of the plants to stay-green or delay senescence have been reported as drought-tolerance indicator in several studies (Thomas and Howarth, 2000). Stay-green have been extensive use in wheat breeding to ameliorate plant production in all environmental condition such as drought areas (Cattivelli et al., 2008; Lopes et al., 2011). Verma et al. (2004) has revealed the positive relationship between green flag-leaf area of wheat and yield under the terminal drought stress condition. Also, in the field experiment including two wheat genotypes (Seri M82 and Hartog), the study result demonstrated that flag leaf of Seri M82 have exhibited longer stay-green period during the grain filling stage and thus, gave more yield (6-28%) than that of Hartog genotype (Christopher et al., 2008). Usually susceptible wheat genotypes accumulate less biomass in leaves than tolerant-resistant ones (Kerepesi and Galiba, 2000).

2.2.2.2. Physiological and biochemical mechanisms

As an initial response to drought stress, plants prefer to closure their stomatal aperture as the first line defense in order to avoid extensive water loss (Deeba and Pandey, 2017). This response based on the species of genotype, the period and severity of water loss, the age and phase of genotype developmental and type of organ and cell (Barnabás et al., 2008). However, this response in resulting drought stress trigger by abscisic acid (ABA) which often synthesized in roots and translated to the xylem and leaves and acts as early warning signal (Davies and Zhang, 1991). Low soil moisture content causes ABA accumulation in roots or leaves exudes which ultimately reduce the shoot growth under deficit water condition (Davies et al., 2005). Several studies and research have shown that exogenous ABA increased drought tolerance of wheat through improved antioxidant defense (Du et al., 2013) and play significant role to ameliorate the wheat grain yield under low water regime (Travaglia et al., 2010). Other hormonal signals, such as ethylene, gibberellic acid and salicylic acid are also significant as regulators in signals transduction pathway under low water condition (Skirycz et al., 2010). The drought-tolerant species control stomatal function more efficiently to allow some carbon fixation under low water content thus, improving WUE (Yordanov et al., 2000). Efficient wheat varieties use water more efficiently than inefficient ones under drought stress. On the other hand, wide variety of plants synthesize and accumulate small molecular compounds in their cells as way of tolerating against stress define as osmolytes or osmoprotectans (Yokota et al., 2006). Many evidence referred to that accumulation of these compatible solutes have improved stresses e.g., drought in plants (Chen and Murata, 2002). Usually, compatible solutes are nontoxic at high concentrations. Mostly, they protect plants from stress via various routes, including detoxification of ROS, protection of membrane integrity and stabilization of enzymes and proteins (Bohnert and Jensen, 1996). Among the osmolytes most common and widely use and distribute are glycine betaine (GB) and proline. GB is synthesized in chloroplast and accumulate in response to dehydration stress (Yang et al., 2003). Furthermore, many studies have demonstrated that GB play significant role in increasing drought tolerance under water stress in wheat (Borojevic et al., 1980) and sorghum (Yang et al., 2003). Proline is also osmoprotectant accumulate in considerable amounts in leaves in response to environmental stresses. Under drought stress, accumulation of proline has been correlated with drought stress tolerance in many plant species. For instance, in rice crop exposed to drought stress the quantities of proline was risen up in leaves (Hsu et al., 2003). In other study carried out on a drought-tolerance, drought-sensitive wheat cultivars displayed that the rate of proline accumulation and utilization was notable higher in the drought-tolerant cultivar than drought-sensitive (Nayyar and Walia, 2003). Moreover, hazardous side-effect of drought stress is the overproduction of ROS, which resulting in an oxidative stress and negative injuries to different cellular compounds. Plants activate an antioxidant scavenging mechanisms to inactivate ROS (Gill and Tuteja, 2010; Dolferus et al., 2011). Plants initiate to increase activity of antioxidant defense systems in order to protect themselves against these toxic organs, These antioxidant systems comprising of enzymatic (SOD, CAT, GPX, APX and POX) and non-enzymatic (GSH, carotenoids, flavonoids and phenolic compounds) antioxidants (Gill and Tuteja, 2010). Several studies reported that the over-production of these antioxidants leads to increased tolerance in plants. For example, in experiment of wheat and maize exposed to low, moderate and harsh water stress showed that the moderate and severe drought stress have caused considerable damage to wheat than maize, also wheat revealed more malondialdehyde and H_2O_2 than maize under the moderate and severe water stress as well as, possessed more antioxidant defense (CAT) in the leave than maize (Nayyar and Gupta, 2006). However, in another study conducted with two wheat genotypes, drought tolerant genotype responded with significantly higher contents of antioxidants and lowest lipid peroxidation to water stress, whereas a susceptible genotype maintained lowest antioxidant and highest malondialdehyde (lipid peroxidation) (Sairam et al., 1998).

2.2.2.3. Molecular mechanisms

Plants have adapted themselves by various molecular mechanisms to endure different stresses. Drought stress induces change in expression of late embryogenesis abundant (LEA) genes. However, these LEA genes help plant to reduce damage and protectant protein from degradation (Ahmad et al., 2017) Accumulation of these genes play significant role in drought tolerance in many plant species (Gosal et al., 2009). It has been demonstrated that transformation of the LEA gene into various plant species can improve tolerance to water stress (Wang et al., 2016). Transferring LEA gene, *HVAI*, from barley crop into transgenic wheat enhanced growth and drought tolerance via elevated WUE and cell integrity (Sivamani et al., 2000). Protein synthesis also, is one of the major metabolic processes for plant to cope with drought stress. Usually, these proteins such as ribosomal protein accumulate greater in tolerant-genotype or plant species that the susceptible ones under deficit water condition (Wang et al., 2016). Several researches have revealed that drought-tolerant plants have adopted different signaling pathways that allow them to tolerate the harsh environmental condition.

2.3. Importance of Zinc

Zinc is one of the most crucial trace elements for the normal health growth, and plays numerous essential roles in reproduction of plants, animals and human. Zn reserved in seed must be in adequate rate to sustain crop growth. Furthermore, high Zn content in grain has also positive effects on seed germination and seedling vigor (Welch, 1999; Cakmak, 2008a). It is primarily present in all biological organisms as complexes with proteins and nucleic acids (Alloway, 2009) and playing catalytic, regulator, structural and co-factors roles in several enzymes and proteins (Alloway, 2008b). Zn is necessary and required for the activity of over 300 metalloenzymes (Gibson, 2012) such as SOD which is converting superoxide radicals to O_2 and H_2O and RNA for protein synthesis (Sharma and Dubey, 2005). World Health Organization (WHO) reported that an adult human body contains about 1.5 to 2.5 g of Zn with a daily intake requirement between 10-14 mg. It has been estimated that 17% of the world population is at risk of insufficient Zn intake according to food supply data, and this rate expect to increase (Wessells and Brown, 2012; Caldelas and Weiss, 2017). Approximately, 25% of this risk located in Africa in 2011 (Figure 2.2) (Kumssa et al., 2015). Moreover, it has been reported that 2-5% of the Gross

Domestic product of developing countries reduced by Zn and Fe deficiency (Kumssa et al., 2015). Zn deficiency could cause child mortality, mental impairment, stunting and poor health productivity. The reason of Zn deficiency of human predominantly returns to low amount of Zn in agricultural soil. Where, stable food crops such as wheat, maize and rice have grown. Therefore, agricultural tools (eg. Zn fertilization) will be root solution to solve this problem (Cakmak et al., 2017).

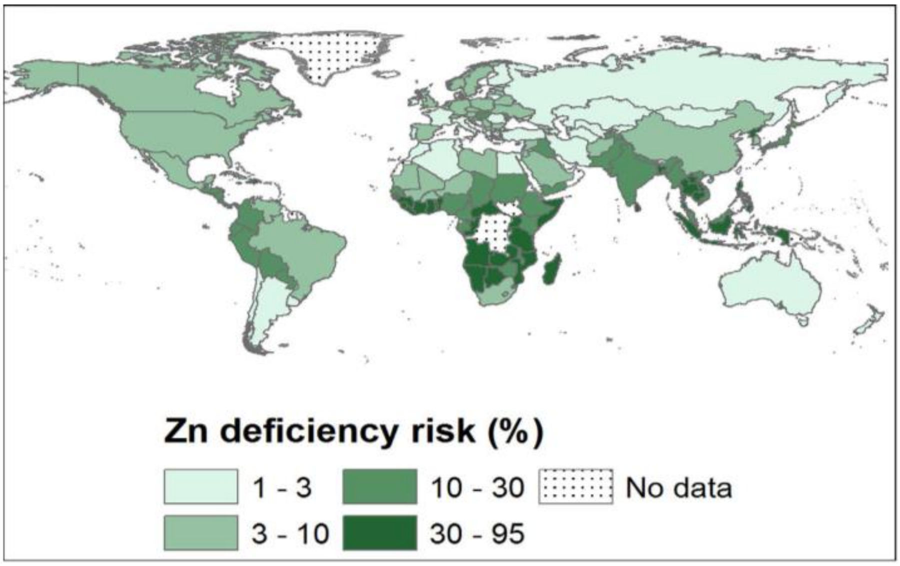


Figure 2.2. Global map of Zn deficiency risk (%) in 2011 (Kumssa et al, 2015)

2.4. Zinc Deficiency in Soil

Zn deficiency is common micronutrient deficiency occurred both in crops and human being. Zn reserved in seed must be in adequate rate to sustain crop growth. In addition to other trace elements which are not required for plants or animals, all soil types have Zn and other essential microelements (Alloway, 2008b). Globally, it is estimated that about one third of cultivated soils are low in plant-available Zn, where 50% of this area growing by cereals which traditionally reduce grain Zn concentration (Cakmak, 2008a). In many countries across the world such as Africa, south and south-East Asia, Zn deficiency in human primarily involving in low phytoavailable of Zn in soil (Figure 2.2) (Cakmak et al., 2017). In agricultural soil Zn content mostly range between 10-300 mg/kg with an average of 50 mg/kg (Kiekens, 1995). The lowest and highest Zn content were recorded in sandy and calcareous soils with the mean of 46 and 75 mg/kg, respectively (Noulas et al., 2018), with total average of soil 64 mg/kg (Kabata-Pendias and Pendias, 2001). Despite of high concentration of Zn in calcareous soil, it has low level of available

Zn and this is main reason for Zn deficiency of plants which cultivated in this soil particularly, in Turkey, Australia and India (Singh et al., 2005). Zn found in the soil solution and very low amount of Zn (< 1 mg/kg) can readily absorb and uptake by the plants (Kabata-Pendias, 2000). It has been reported that the critical deficient level of Zn extractable by diethyl triamine penta-acetic acid (DTPA) in the soil less than 1 mg/kg DW of soil (Lindsay and Norvell, 1978), and less than 0.6 for soil in which wheat cultivated (Bansal et al., 1990). The dominant factors which determining soil Zn distribution and Zn availability to crops are mainly: soil pH, organic matter content, calcium carbonate content, clay content, soil moisture status, presence of other elements as well as concentration of other macro-nutrients, especially phosphorus and climate factors (Alloway, 2008b). In some regions, low soils Zn content may be aggravated factor in resulting cereals may have lower Zn concentration when cultivated on Zn sufficient soil (Gibson, 2012). Zn deficiency distribution of soil in the world (Alloway, 2008b) is illustrated in Figure 2.3. To prevent or overcome soil Zn deficiency, for staple crops, many strategies like crops biofortification (Zn fertilizers) which is a common practice (Singh et al., 2005) and plant breeding through tolerant varieties to Zn deficiency might be help and alleviate the Zn deficiency in the soil. From this prospective, it is essential to rise our knowledge and understanding in term of mechanisms involved in Zn uptake, absorption and metabolism by plants (Caldelas and Weiss, 2017). Almost, half of the cereal-cultivating land in the world is influenced by low availability of Zn to plant roots resulting in a variety of negative effects of chemical and physiological conditions such as high pH level, calcium carbonate, low level of organic matter and soil moisture (Kutman et al., 2010), particularly in calcareous soil and arid and semi-arid region (Graham and Welch, 1996), suffering also from water deficit (Peleg et al., 2008). Up to 50% of wheat cultivated soil in the world is considered poor in plant-available Zn (Cakmak and Kutman, 2018).

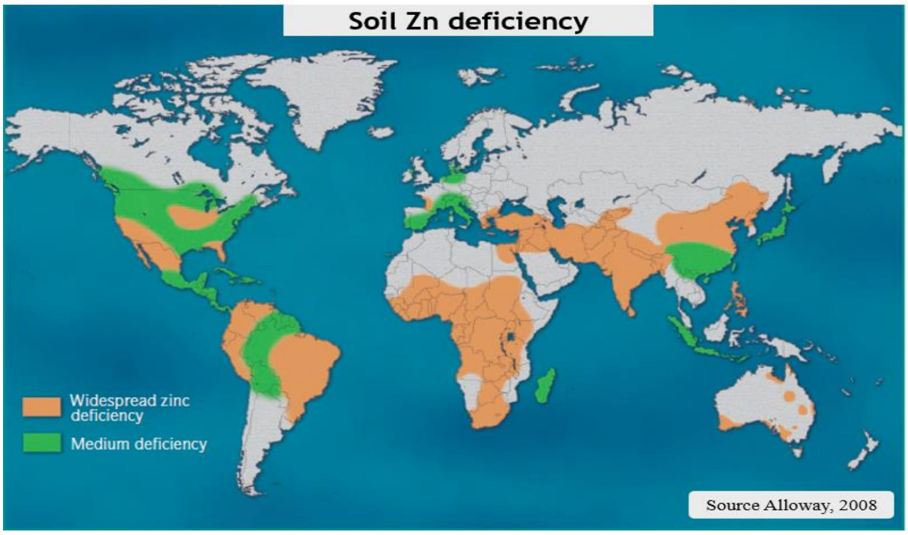


Figure 2.3. World maps illustrated distribution of soil Zn deficiency (Alloway, 2008b)

Moreover, about one-third people around the world are estimated to be at risk of Zn deficiency (Hotz and Brown, 2004). Distribution of human Zn deficiency in world (Wessells and Brown, 2012) is given Figure 2.4. This has considerable socio-economic impact (Stein, 2010). Human Zn deficiency is thought to be related to soil Zn availability and relative proportion of cereal grain in a diet (Alloway, 2009). This may be due to soil have limited Zn availability and applied Zn (Cakmak, 2008a). Thus, this limitation of soil Zn availability such as calcareous soil and grain Zn concentration can be overcoming through variety of interventions and strategies (Stein, 2010), these include both agronomic and genetic biofortification of cereal crops. Agronomic biofortification can be achieved by increasing soil phytoavailability or by application of Zn-fertilizers (White and Broadley, 2011), and this represent short-term solution to the problem (Cakmak, 2008a), while genetic biofortification is predicated on increasing Zn acquisition from soil and its accumulation in edible portions (White and Broadley, 2011).

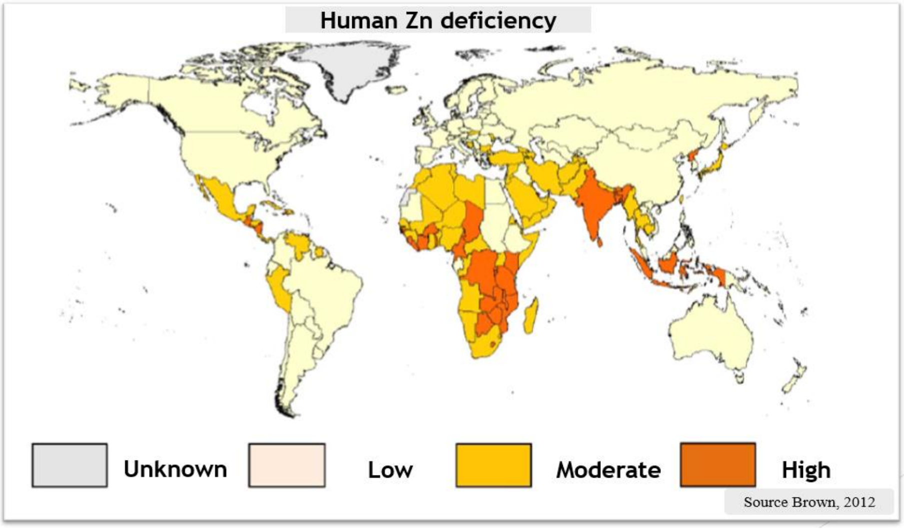


Figure 2.4. World maps illustrated distribution of human Zn deficiency (Wessells and Brown, 2012)

2.5. Zinc Deficiency in Wheat

In plant, Zn is required for normal growth, protein synthesis, gene regulation, protection of cells against to oxidative stress and high grain yield. Therefore, if Zn was in an inadequate in soil and insufficient amount in plant, this could expose plant to various physiological stresses (Sadeghzadeh, 2013). Zn deficiency is the most widespread micronutrients problem globally, affecting various staple food crops such as maize, rice and wheat (Alloway, 2008a). As the most widely cultivated crop on the planet, wheat (*Triticum aestivum* L.) is providing about 21% of the daily protein consumption per capita (Yilmaz et al., 2017), and bread wheat alone is stable food for 35% of the world's population (Cakmak and Kutman, 2018). Zn deficiency can be a main problem in wheat as found in Turkey, where the yield reduced by 50% (Alloway, 2008b). The main visual symptoms of Zn deficiency in wheat are converting of green leaf color to pale and yellow due to decrease of chlorophyll content and this cause chlorosis appearance (Bell and Dell, 2008) as well as short internode (Noulas et al., 2018) and reduction in shoot elongation. However, cereals species differ in their sensitivity to Zn deficiency, where maize and rice being highly sensitive than wheat. Bread wheat tend to be more tolerance to Zn deficiency rather than durum wheat (Marschner, 2011). It has been reported that the critical deficient concentration of Zn for shoot and whole plant of wheat were 15-20 and 20-25 mg Zn/kg dry matter, respectively (Singh et al., 2005; Marschner, 2011). Leaf Zn concentration less than 15 mg Zn/kg dry matter is considered as Zn-deficient (Singh et al., 2005). Usually, cultivated wheat has grain Zn concentration between 20-35 mg/kg, whereas this rate reduces less than 20 mg/kg when wheat grown on Zn-deficient soil (Rengel et al., 1999; Çakmak et al., 2004). For example, grain Zn concentration in Zn-deficient soil of Australia and Turkey was less than 10 mg/kg, while this concentration was rise to 20 mg/kg in Zn-sufficient or fertilized soil (Graham et al., 1992; Cakmak et al., 2010a). In general, the prevalence of Zn deficiency observed in the less developed countries, including sub-Saharan Africa (Gregory et al., 2017). Therefore, to meet the daily required Zn intake, these values has to increase, because wheat is a poor of Zn intake and also, it riches grain phytate which known to reduce bioavailability of Zn in human digestive tract (Lonnerdal, 2000; Cakmak et al., 2010b). This poor of Zn intake of wheat can be enhanced via variety of interventions including agronomic and genetic biofortification (Graham et al., 1999; White and Broadley, 2009; Bouis and Welch, 2010).

2.6. Role of Zinc as Drought-Tolerant of Wheat

Wheat is cultivated in arid and semi-arid environmental condition which requires use of available water efficiently. Within this context, there is many evidences that Zn deficiency stress in wheat become more apparent under limited water condition (Karim and Rahman, 2015). However, it known that soil moisture status plays significant role in Zn transfer through root and xylem from the soil to the upper part of plant, and this usually occur via diffusion (Marschner, 1995). Furthermore, several researches have displayed that drought stress increase content of reactive oxygen species (ROS) (Smirnoff, 1993; Alscher et al., 1997; Apel and Hirt, 2004). Plants response to water stress through accumulation of compatible solutes such proline and increase activity of anti-oxidative peroxidase (Marcińska et al., 2013). It has been reported that Zn effectively contribute to scavenging and detoxify ROS under drought stress through sustaining a high activity of Zn-containing superoxide dismutase (SOD), which is considered the main detoxifier of antioxidant enzymes against oxidative stress (Cakmak, 2000). Moreover, some morphological (number of grains per spike and grain yield) and physiological (chlorophyll content, photosynthesis and WUE) parameters of wheat have impaired by drought stress when imposed at booting stage, but all those parameters have improved in resulting when Zn foliar sprayed (Karim and Rahman, 2015). Zn fertilization have minimized lipid peroxidation of wheat flag leave, and ameliorated the antioxidant content (total phenolic, ascorbate and total flavonoids) and SPAD under deficit water condition (Ma et al., 2017). Zinc has critical roles to protect lipid membrane, protein, chlorophyll and DNA (Cakmak, 2000). Another research shown that conducted under water stress revealed that foliar Zn spray on wheat at booting until grain filling stage improved pollen viability, grain yield, Zn concentration and WUE, the result suggested that mitigated of WUE is major indicator to alleviate the harmful of drought stress (Karim et al., 2012). Recently, (Faran et al., 2019) reported that seed with high Zn concentration reduced malondialdehyde content and improved total antioxidant activity in wheat.

2.7. Biofortification of Zn

Biofortification of Zn is a strategy aims to enrichment Zn concentration in staple food crops such as wheat, in addition to enhanced bioavailability of Zn in human digestive tract either via plant breeding (genetic biofortification) or fertilization (agronomic biofortification) (White and Broadley, 2005). It has suggested that biofortification approach could be promising, cost-effective and sustainable technique for delivering of micronutrient (Zn) to human (Garg et al., 2018). Unfortunately, millions of people around the world have inadequate Zn in their diet (White and Broadley, 2009; Bouis and Welch, 2010). In food security report prepared by the Food and Agricultural Organization (FAO) and the World Food Program (WFP), have estimated that 795 million people across the world are malnourished, out of which 780 people living in the developing regions (Mc Guire, 2015). Also, it has reported that about 20% (1.2 billion) world's population suffer from Zn deficiency (Hotz and Brown, 2004), this resulting impaired development and reduction the Gross Domestic Product (GDP) by 2-5% in developing countries because of Zn and Fe deficiency (Kumssa et al., 2015). However, this has been attributed to the sourcing produce from soil with low bioavailable Zn, eating grains of crop with intrinsically least concentration of Zn (White and Broadley, 2011) and high phytate intakes that inhibit Zn assimilation human gut (Gibson, 2012; Joy et al., 2015). In pervious study carried out by Kumssa et al. (2015) phytate ratio to Zn (PA:Zn molar ratio) was higher than 15 in most of countries, the critical threshold level beyond which Zn absorption likely to be impeded. The majority of people living in rural regions of the world more exposure to Zn deficiency, and their consumption of animal-based foods is low because they rely on cereal-based foods as daily Zn intake (Wessells and Brown, 2012; Cakmak and Kutman, 2018). Several crops need certain amount of leaf Zn concentrations between 15-30 mg/kg DM for better yield, and their growth inhibited when Zn concentrations at leaf be more than 100-700 mg/kg DM (White and Brown, 2010; Fageria, 2016). Often, Zn concentration in wheat grain could be relatively less than 20 mg/kg (Alloway, 2009), and this value is too small to meet and achieve the target Zn concentration of 38 mg/kg which set by the HarvestPlus Program (Bouis and Welch, 2010; White and Broadley, 2011). Therefore, to achieve this target level of grain Zn concentration, variety of interventions including agronomic and genetic biofortification is required (Graham et al., 1999; White and Broadley, 2009; Bouis and Welch, 2010).

2.7.1. Agronomic Biofortification

Agronomic biofortification is defined as involve the application of Zn fertilizers to increase their bioavailable concentration in the edible parts of crops (White and Broadley, 2009; Gregory et al., 2017). Although, most cultivated soil, specially used for wheat have sufficient Zn content and absorb by crop, but its uptake via plant is often limited in resulting low phytoavilability or acquisition by root (White and Broadley, 2011; Cakmak and Kutman, 2018). Compared to genetic approach (plant breeding), agronomic biofortification (fertilization) is considered as short-term solution to Zn deficiency (Velu et al., 2014). Agronomic strategies look for improve Zn phytoavailability in soil, as well as enrich Zn concentration of grain through application of Zn-fertilizers to the soil, foliage or seeds (Fageria, 2016) and anther strategy such root-dipping of transplant seedlings have been noticed in rice (Alloway, 2008b). Furthermore, inorganic Zn-fertilizers like $ZnSO_4$, ZnO, and synthetic Zn-chelates are the most practices and used worldwide (White and Broadley, 2009). Selection of suitable source and rate of Zn, in addition to time and method of Zn application is considered imperative issue to achieve the target of Zn application. In several regions across the world, farmers have applied Zn-fertilizers in order to increase grain yield, but their understand and knowledge involved in the importance of rise Zn concentration also has to be in attention. Due to important influence on grain yield and Zn concentration in Turkey, the amount of Zn containing NPK fertilizers have considerable increased from 0 in 1994 to 400,00 tons per annum during following 10-15 years (Garg et al., 2018). Various Zn application strategies are mentioned below.

2.7.1.1. Soil application (Fertilization)

Soil application is the most widespread strategy among Zn application methods (Rehman et al., 2012). Zn can be applied to soil through different ways including broadcasting, banding placement, fertigation and sprayed onto the seedbed (Rehman, 2017). Usually, Zn apply to soil in the form of $ZnSO_4.7H_2O$ (21-22% Zn) which is widely used and one of the important source for ameliorate Zn deficiency due to its high water solubility, easy available and relatively low price (Singh, 2008), and was more effective in increasing grain Zn content, compared to other forms of Zn in many crops including wheat (Velu et al., 2014). Generally, soil application are typically in the range 4.5-34 kg Zn/ha with an average 10 kg Zn/ha (Alloway, 2008b). Moreover, the rate of Zn application is various considerable based on type of soil and cropping system (Liu et al., 2017). For instance, the recommended rate of Zn for wheat crop is about 23 kg Zn/ha in Turkey (Cakmak, 2008a), but this rate reduces to 2-5 mg Zn/ha in the Middle East (Rashid and Ryan, 2008). Mostly, Zn is applied as soil application in the field condition at the rate of 11-17 kg Zn/ha and this amount can alleviate the deficiency of Zn in calcareous soil of Australia (Martens and Westermann, 1991). Whereas, the recommended amount of Zn for wheat and rice is 11 kg Zn/ha in India (Singh, 2008). Several field and pot experiments for wheat have reported that grain Zn concentration as well as yield increases in response to soil Zn application. In Turkey, 45% of wheat production is obtained from Central Anatolia regions. Soil Zn application with 23 kg Zn/ha has provided increases in grain yield up to 260% compared to untreated soil (Yilmaz et al., 1997). In general, soil application of 5-17 Zn/ha as $ZnSO_4$ for one year is recommended. Ma et al. (2017) demonstrated that soil application of 14 mg Zn/kg as solution of $ZnSO_4.7H_2O$ before sowing under well water supply, moderate and severe drought enhanced grain yield by 10.5, 10.8 and 22.6% and Zn concentration by 9.7, 28.2 and 32.8%, respectively. In field experiment conducted in Mediterranean condition where low available water, soil fertilization did not significantly increase in Zn concentration, but it enhanced grain yield of wheat by about 10% (Gomez-Coronado et al., 2016). In green house experiments conducted using silt clay loam soil with low Zn content (0.7 mg/kg), soil application increased the grain Zn concentration from 30 to 39 mg/kg (Beebout et al., 2011). Soil with an adequate Zn nutrition may has protective role against oxidative stress like drought, because Zn possess Zn-containing superoxide dismutase (SOD), which is considered the major component of antioxidant defense system of plants, against drought stress (Candan et al., 2018). Within this context, it has been reported that Zn soil application improved the antioxidant enzymes in flag leaves of wheat, in contrary lipid peroxidation has been reduced under drought stress condition (Ma et al., 2017). Zhao et al. (2019) have showed that soil application of Zn would be effective method to enhance grain Zn concentration of wheat in the following years. Furthermore, repeated soil fertilization could potentially increase accumulation of heavy metals (HMs) in soil and grain of wheat thus, may resulting in threat for human food (Jiao et al., 2012). Many researches have revealed that, when soil application combined with foliar spray can be more effective in term of improving grain yield and Zn concentration for wheat (Hussain et al., 2012; Bharti et al., 2013; Gomez-Coronado et al., 2016). Compared to foliar spray, soil application is more effective to sustain productivity and ameliorate Zn concentration for wheat (Liu et al., 2019), but it is required in higher amounts (Fageria et al., 2009).

2.7.1.2. Foliar spray (Foliar fertilization)

Studies on foliar fertilizations was started in the late 1940s and early 1950s (Fritz, 1977), and in the 1980s examined for choice crops, including cereals (Girma et al., 2007). Moreover, high plants such as wheat, have ability to absorb Zn when applied as foliar spray in sufficient amount (Fageria et al., 2009). Use of foliar spray have increasing importance for field crops, particularly in developing regions (Bell and Dell, 2008). For successful foliar spray, mineral (Zn) should be in optimal concentration and stage of crop, in addition to source of Zn should be soluble in water to be more effective (Fageria et al., 2009). Further, there are several papers in the literature on agronomy studies that show the positive influence of Zn foliar spray on improving yield and enhancing grain Zn concentration for wheat. When Zn applied as foliar spray at late stages of wheat growth (e.g. grain-filling) rather than earlier developmental stages lead to ameliorating grain Zn concentration by 32-125% in various wheat varieties around ecological zones and countries (Cakmak, 2008a). For example, in China, foliar spray with 0.4% $ZnSO4.7H2O$ resulted in an increase on whole grain Zn content with 58% in wheat (Zhang et al., 2012). In addition to enrichment Zn concentration in whole grain, foliar spray, also enhanced the concentration of Zn in starchy endosperm (Velu et al., 2014). At another research, the average of experiments carried out in seven countries, foliar spray increased wheat grain yield by 5.2%, gave the higher grain Zn concentration with 41.2 mg/kg in compared with no Zn treatment (28 mg/kg). They are also reported that Zn foliar spray in combination with pesticides increased grain yield and grain Zn concentration for wheat with 7.7 and 35.7%, respectively (Ram et al., 2016). Based on meta-analysis data for 10 African countries, Joy et al. (2015) found that foliar Zn spray lead to an increase in Zn concentration in wheat, rice and maize grains of 63, 25 and 30% respectively, he also, reported that foliar spray is likely to be more cost-effective strategy to ameliorate Zn concentration in grains of cereal crops compared with soil application method. But, when foliar spray combine with soil application may result in about 3-fold increase in grain Zn concentration (Cakmak et al., 2010b). Therefore, the combined soil application with foliar spray together is recommended when high grain yield and Zn concentration are targeted at same time in addition to reduce cost of application, (Velu et al., 2014; Gregory et al., 2017).

2.7.1.3. Seed treatments

Recently, interest in an enrichment micronutrient, especially Zn in grain of staple food has been increased. Because grains with high Zn contents could have better germination, stress tolerance and thus, improve crops production particularly in Zn-deficient soils (Rengel and Graham, 1995). Additionally, higher Zn content in grains could have beneficial role to human health via overcome to malnutrition in population based on their diet on cereals (Cakmak, 2008a; Noulas et al., 2018). Therefore, commercial seed treaters are starting to look seed treatments as important method to increase value of seed as well as improve yield. However, seed treatments are economically not cost-effective strategy, required in very small amount, in addition to relatively nonpolluting ecosystem compared to others application strategies (Taylor and Harman, 1990), beside farmers can readily treat the seeds (Bell and Dell, 2008). The application of Zn through seed treatments have ameliorate grain yield and Zn content in wheat (Rehman et al., 2018). Several methods have been used to apply Zn to seeds which including seed priming and seed coating.

2.7.1.3.1. Seed priming

Seed priming define as soaking grains/seeds in water or nutrient solution aerated with a simple aquarium pump under determined time and conditions, thereafter drying back to their original weight. Priming seeds in Zn containing solution is a practical way to enhance grain Zn before sowing and ultimately, ameliorate crop establishment and plant yield (Noulas et al., 2018). It is a technique used to enhance seed Zn reserves for ameliorate seed quality, crops production, and increasing stress tolerance in crop plants (Imran et al., 2017). Moreover, many reports available in published literature have proven that Zn seed priming is effective for improving crops performance; priming of wheat grains with $ZnSO_4$ produced high grain yield than unprimed grains (Arif et al., 2007). Similarly, Rehman (2017) found that wheat seeds primed with Zn improved the stand establishment, and enhanced grain Zn concentration as well as grain yield. Zn priming increased endosperm Zn content 3-fold compared with hydropriming (water primed seed) in maize crop (Imran et al., 2017). Furthermore, grain yield and stress tolerance have been significantly improved via Zn primed seeds in various crops under different growth condition (Yilmaz et al., 1997; Slaton et al., 2001; Ajouri et al., 2004; Harris et al., 2007; Imran et al., 2013; Bradáčová et al., 2016). Zn seed priming have proven obviously their effectiveness for improving seed germination and seedling growth in wheat (Harris et al., 2008; Rehman et al., 2015; Reis et al., 2018), rice (Prom-u-thai et al., 2012), maize (Ajouri et al., 2004; Muhammad et al., 2015) and soybean (Goiba et al., 2018). In wheat, seed primed with 0.3% $ZnSO_4$ increased grain Zn concentration from 49 to 780 mg/kg, and significantly raised yield by 14% (Harris et al., 2008). Seed priming might be more efficient than soil application and has beneficial effect on yield (Slaton et al., 2001; Harris et al., 2008). Seed priming and coating are cost-effective as very small amount of Zn are sufficient to induce improvement in seed germination (Singh and Usha, 2003).

2.7.1.3.2. Seed coating

Seed coating is another cost-effective and efficient of seed treatment method for delivery mineral nutrients by adhering them to the seed surfaces using a sticky substance to increase seed performance (Freeborn et al., 2001). Seed coating is technology was developed for cereal seeds in the 1930s by British seed company, also it is increasing water availability to the seeds, thus might be improving its tolerant to drought stress (Gorim, 2014). Coating technologies have been investigated for application beneficial materials (Scott et al., 1987). Macro-and micronutrients have been applied in seed coating and revealed positive effects to improve early plant growth (Scott and Archie, 1978; Scott et al., 1987). Zn fertilizers should be dry powder and ground to <0.25 mm, to spray the seed surface in order to get promising results (Mortvedt and Gilkes, 1993). Seed coating with Zn has effectively ameliorated the yield of many staple crops such as rice (Rehman et al., 2012; Tavares et al., 2012) and barley (Zeḷonka et al., 2005). In Turkey, Zn-coating of wheat seeds significantly increased biological yield and grain yield, but had no notable effect on Zn concentration under Zn-deficient soil (Yilmaz et al., 1997; Cakmak, 2008b). Similarly, Rehman and Farooq (2016) displayed that seed coating with 1.25 g Zn/kg significantly improved chlorophyll and enhanced grain yield and Zn grain concentration from 33 to 55% and from 21 to 35%, respectively. Seed treatments including seed priming and seed coating might be easy and effective strategy of micronutrient application, as well as could be attractive option for poor farmers (Farooq et al., 2012).

2.7.2. Genetic Biofortification

Genetic biofortification is strategy using plant breeding to improve new genotypes of staple food crops with high density of micronutrient levels and low nutrient inhibitors with consideration to the amount of nutrient uptake and absorbed by the consumer (Bouis, 2003). Biofortification of wheat via conventional breeding is the most accepted method, cost-effective and sustainable solution alternative to transgenic and agronomic strategies (Garg et al., 2018). Hence, genotypic variation are necessary to be sufficiently enable plant breeders for screening and utilizing these variation in an increasing of major staple food crops such as wheat with concentration and bioavailability of Zn (Velu et al., 2014; Garg et al., 2018). The main aim of plant breeding program has been to enhance productivity and ameliorate new cultivars rich in micronutrients has frequently been breeding objective (Sadeghzadeh, 2013). However, conventional breeding can enhance Zn concentration without compromising yield and other desirable characteristics which preferred by farmer and consumer. Within this context, one of the three things that must occur for biofortification to be successful is high density of nutrient must combine with high yield and profitability (Bouis et al., 2011). Furthermore, there are many international organizations have initiated program to address micronutrient malnutrition of staple food crops through plant breeding program such as HarvestPlus program and International Maize and Wheat Improvement Center (CIMMYT) (Bouis and Welch, 2010). For example, (Monasterio and Graham, 2000) screened more than 300 germplasm accessions for Zn including hexaploid, tetraploid and diploid sources from gene bank of CIMMYT. Likewise, the most successful example of breeding wheat for enrichment Zn concentration is HarvestPlus program. They released about 11 genotypes or varieties of high-Zn wheat across the world, out of them in Asia continent and the rest one in Latin Americas (LAC) (http://www.harvestplus.org/). In India and Pakistan, Zn-biofortified wheat cultivars such as Shakti early maturity +14 ppm (40% increase) and Zincol +9 ppm (20% increase) have released, respectively (Singh et al., 2017). Previous studies carried out in Switzerland and Mexico have shown that absorption and assimilation of Zn from Zn-biofortified wheat is significantly greater than conventional or unfortified wheat (Signorell et al., 2019). Cakmak and Kutman (2018) suggested that genetic biofortification of wheat should be complemented with agronomic biofortification to avoid and overcome risk of accumulation grain Cd concentration. Genetic biofortification (identify and transfer desirable genes to targeted crop) is likely to be the most efficient approach and promising strategy in term of enrichment grain Zn content in wheat (Alloway, 2009; Noulas et al., 2018).

3. MATERIALS AND METHODS

3.1. Experiment I. Influence of Zn Seed Priming and Coating on Germination and Seedling Growth of Two Wheat Varieties under Controlled-Growth Conditions

3.1.1. Source of Wheat Varieties

Wheat varieties that were used in the experiments i.e. Imam variety which has average grain Zn content of 29 mg/kg and was obtained from Agricultural Research Corporation (ARC), Wad Madani, Sudan, where it is commonly cultivated in deficit-stressed water areas in the north part of Sudan. Turkish wheat variety Altındane which is commonly grown in Samsun province and has average grain Zn content of 25.5 mg/kg and was supplied from Black Sea Agricultural Research Institute.

3.1.2. Strategies of Zn Application

3.1.2.1. Seed priming

Seeds were soaked in distilled water for hydropriming and in 2.5 and 5 mM aerated solution of $Zn.SO_4.7H_2O$ for 12 h at 25 °C, in the dark for Zn priming. Aeration of the solutions was provided with a simple aquarium pump (Figure 3.1a). After priming duration ended, the primed seeds were washed thoroughly with distilled water, then surface dried and allowed to dry back to their original moisture content of 12% at room temperature.

3.1.2.2. Seed coating

Initially, seeds of wheat varieties were weighted before coated with Zn sulphate. Then, $ZnSO_4.7H_2O$ finely grinded (150 μm) and Zn solutions were prepared at the rates of 1.5, 2.5 and 5 g Zn/kg seeds. Finally, grounded of Zn sulphate mixed with Arabic Gum (AG) solution (5%, w/v) (Figure 3.1b) for 5 min to improve retention of Zn applied to seeds. Subsequently, seeds were soaked in slurry consist mix of Gum Arabic and Zn and incorporated sufficiently for 5 min and kept drying for the constant weight. The weight of applied coating material was determined by difference between the weight of dry coated seeds and initial weight of the raw seeds. Due to the Arabic Gum (AG) incorporated with the coating was generally less than 5% of the weight micronutrient (Zn), therefore, weight of AG was negligible and it was discharged (Figure 3.1c).

a) b) c)

Figure 3.1. a). Priming process with the solution of $ZnSO_4$ and aquarium pump providing aeration b) Arabic Gam (adhesive substance) c) coated seeds with $ZnSO_4$

3.1.3. Germination and Seedling Emergence Conditions

Seed germination and seedling emergence test were conducted in Petri dishes and pots, respectively. Seedling emergence experiment was established to study the effect of Zn seed coating and priming on seed germination parameters at early growth stage under growth controlled-condition at day/night temperature of 24/20 °C and 65/75% relative humidity. In each treatment, 25 seeds were sown into pots containing 700 g alluvial soil with low Zn content to monitor their germination and emergence for 21 days using three replications. Based on soil analysis, pre-sowing basal fertilizers were applied; phosphorus 75 mg/kg soil, nitrogen 100 mg/kg soil and potassium 25 mg/kg soil by using DAP, urea and K_2SO_4, respectively. After sowing completed, the pots were irrigated by 70% of water holding capacity until 7th day of seedling growth. The germination was monitored and the numbers of seedlings were determined by daily counting according to standard germination test (ISTA 1983). The seeds showing 2 mm radicle protrusion were considered as normal seedling. The treatments of this experiment are shown in Table 3.1.

Table 3.1. Treatments of germination and seedling emergence experiments conducted under controlled conditions

Treatments	Zn concentrations
Untreated seeds (Control)	0
Hydropriming (HP)	0
Seed priming	2.5 mM Zn
Seed priming	5 mM Zn
Seed coated with gum Arabic (5%)	0
Seed coating	1.5 g Zn/kg seed
Seed coating	2.5 g Zn/kg seed
Seed coating	5 g Zn/kg seed

3.1.4. Determination of Germination Parameters

The germinated seeds in each Petri dish were counted daily for 7 days. Then, the germination percentage was calculated at the 7th day. The germination percentage (GP) and mean germination time (MGT) were calculated according to the following equation (Zhang et al., 2007).

Germination percentage (G %) = No. of germinated seeds/ No. of total seeds

Mean germination time (MGT) = Σ (Gt × Tt)/Σ Gt

Where Gt is the number of germinated seeds on day t, Tt is time corresponding to Gt in days. Seedling vigor index after 7 days was calculated according to formula (Salah et al., 2015).

3.1.5. Measurement of Seedling Growth Parameters

At the end of the 21st days, five seedlings from each pot were selected, rooted and washed with distilled water. Then, their fresh roots and shoots were weighted and dry maters were determined after oven-drying for 48 hours at 70 °C.

3.1.6. Experimental Design and Data Analysis

The experimental design used in this study was a completely randomized design (CRD) with 48 treatments including 8 levels of Zn and two wheat varieties with three replications. Data analysis was performed with JMP software program and significant differences among mean were assessed using Fisher’s least significant differences (LSD) test at 0.01 probability level.

3.2. Experiment II. Impact of Different Zn Strategies on Drought Tolerance, Yield and Grain Biofortification in Wheat Grown under Zn- and Water- Deficient Conditions

3.2.1. Soil Properties and Analysis

Soil samples for the pot experiment were taken from Bafra, Samsun, through steel spade from depth of 0-30 cm, thereby brought to laboratory and kept at 4 °C. Samples were air-dried and sieved by passing through 2 mm sieve. Soil texture classified as silt clay loam with 62% silt, 27% clay and 11% sand. Furthermore, soil pH was 7.62 and 0.03% salinity. The DTPA-extractable Zn of the soil in experimental site was 0.6 mg/kg. Prior to the start of the experiment, the soil field capacity (FC) was determined as described by (Kammann et al., 2011). The whole pots were immersed in distilled water covered with plastic cap for 24 h and then let water to drain for another 24 h. Pre- and post-pot weight were recorded and determine then compared to calculate the FC in dry soil. However, the physical and chemical characteristics of the soils used in the greenhouse pot experiment are presented in Table 3.2.

Table 3.2. Physical and chemical characteristics of the soil used in the greenhouse pot experiments

Soil properties	Value	Degree
Soil texture		Silty clay loam
pH	7.62	Slightly alkaline
Salt %	0.038	Salt-free
$CaCO_3$ %	12.0	Limy
Phosphorus (P_2O_5), (kg/da)	2.43	Very low
Potassium (K_2O), (kg /da)	28.0	Middle
Organic matter (%)	1.0	Poor
Fe (mg/kg)	22.5	Middle
Cu (mg/kg)	2.7	High
Zn (mg/kg)	0.6	Very low
Mn (mg/kg)	7.4	Low

3.2.2. Strategies of Zn Application

Treatments and Zn concentration of the Experiment II are given in Table 3.3.

Table 3.3. The treatments and Zn concentrations in the Experiment II conducted under greenhouse condition

Wheat varieties	Watering regimes	Seed treatments	Zn Concentration
İmam	Well-watered (100% FC)	Untreated seeds (Control)	0
		Hydropriming	0
		Zn seed priming	5 mM
		Zn coating	1.5 g/kg seed
		Zn soil application	10 kg/ha
		Zn foliar application	0.5%
		Zn soil application + Zn foliar	10 kg/ha +0.5%
		Zn seed coating + Zn foliar	1.5 g/kg+ 0.5%
	Drought stress (50% FC)	Untreated seeds (Control)	0
		Hydropriming	0
		Zn seed priming	5 mM
		Zn seed coating	1.5 g/kg seed
		Zn soil application	10 kg/ha
		Zn foliar application	0.5%
		Zn soil application + Zn foliar	10 kg/ha +0.5%
		Zn seed coating + Zn foliar	1.5g/kg + 0.5 %
Altındane	Well-watered (100% FC)	Untreated seeds (Control)	0
		Hydropriming	0
		Zn seed priming	5 mM
		Zn seed coating	1.5 g/kg seed
		Zn soil application	10 kg/ha
		Zn foliar application	0.5%
		Zn soil application + Zn foliar	10 kg/ha +0.5%
		Zn seed coating + Zn foliar	1.5 g/kg seed + 0.5%
	Drought stress (50% FC)	Untreated seeds (Control)	0
		Hydropriming	0
		Zn seed priming	5 mM
		Zn seed coating	1.5 g/kg seed
		Zn soil application	10 kg/ha
		Zn foliar application	0.5%
		Zn soil application + Zn foliar	10 kg/ha +0.5%
		Zn seed coating + Zn foliar	1.5 g/kg seed + 0.5%

Based on the results obtained from Experiment I, the optimal concentration of Zn for seed coating and seed priming were 1.5 g Zn/kg seed and 5 mM Zn, respectively. Therefore, these doses have been chosen for the experiment II. The other Zn application strategies were including; Zn soil application (10 kg Zn/ha) applied by incorporated Zn with whole soil and mixed sufficiently before sowing (Figure 3.2a). In addition to Zn foliar application 0.5% (w/v) $ZnSO_4.7H_2O$ solution contained 0.01% Tween 20 as surfactant were sprayed on plants twice, the first one was at booting stage (Figure 3.2e), while the second was at the grain filling stage (Figure 3.2f). However, the control treatment was sprayed with distilled water. The volume of foliar solution used in this experiment was 500 L/ha. And two combinations of Zn were soil application+foliar spray and seed coating+Zn foliar spray (the method and time of application for these as the same as mentioned above).

Figure 3.2. Different developmental stages of wheat grown under greenhouse conditions a) Zn soil application, b) sowing process, c) tillering stage, d) stem elongation stage, e) booting stage, f) grain filling stage

3.2.3. Plant Material and Growth Condition

The same wheat varieties (Imam and Altındane) mentioned about in the Experiment I were used in the Experiment II. The effect of Zn application on drought tolerance, grain Zn content and grain yield of wheat varieties were studied in this experiment under controlled-growth room condition. Growth room was set to 450 (µmol/m^2/s) photo flux density, 16 h/8 h day/night period, 24/20 °C day/night temperature and 65±5/75±5% day/night RH. At tillering stage of plant growth, pots were moved to greenhouse from the controlled-growth room (Figure 3.2d).

According to soil analysis, basal fertilizers were added to all pots before sowing at the rate of 200 mg N/kg soil, 100 mg P_2O_5/kg soil and 25 mg K_2O/kg soil as urea, 20:20 compose and K_2SO_4, respectively, and mixed sufficiently. 30 seeds of each variety and treatment sown in an individual pot contained 8 kg Zn deficient soil (0.6 mg/kg) (Figure 3.2b).

After 7 days of germination, seedlings were thinned to 20 per pot. Initially, pots were irrigated at 80 to 100% of FC by daily weighted and watering. After 70 days of sowing at booting stage, drought stress imposed on plants by deficit irrigation between 40 to 50% of FC, while the control plants were maintained between 80 to 100% FC until harvest time. Booting stage was selected to impose drought stress because of winter wheat growth is often affected by water deficit due to sudden shortage of precipitation at this growth period in several wheat cultivation regions.

3.2.4. Measurement of Morphological Parameters

3.2.4.1. Plant height

At maturity stage of crop, five plants randomly were selected from each pot, thereby they measured from ground level to top of the plant with meter rod.

3.2.4.2. Spike length

The same plants selected above for determining of plant height have chosen to measure spike length. Spike length has taken as distance from the base to top of fertile spike excluding awns.

3.2.4.3. Number of spikelet per spike

Spikelets per spike were calculated from the spike of the main tiller of each selected plant at maturity stage.

3.2.4.4. Number of grains per spike

Grains per spike were determined by counting of total grains in the main tiller.

3.2.4.5. Weight of grains per spike

After grains per spike counted in the spike of main tiller, these grains were weighted on a digital electronic balance.

3.2.4.6. 1000 seeds weight

Weight of 1000 grains was determined according to ISTA (2015) rules by using an electronic balance in laboratory.

3.2.4.7. Biological yield

At maturity stage, aboveground biomass of plants in each pot were harvested, weighted and recorded separately to determine biological yield.

3.2.4.8. Harvest index

Harvest index was calculated by the ratio of seed or grain yield to above-ground biomass yield and expressed as percentage (Asif et al., 2017).

Harvest index = (Seed yield/biological yield) × 100

3.2.4.9. Seed yield

At seed maturity stage, plants in each pot were separately harvested and threshed manually, then seeds were cleaned and air dried to moisture content between 13-14% and the obtained seeds were weighted to determine the seed yield (g/pot) for each pot.

3.2.5. Measurement of Physiological Parameters

3.2.5.1. Chlorophyll content

Chlorophyll content as SPAD was measured with Apogee MC-100 meter on three full expanded flag leaves two times, the first one was measured five days after the first foliar application (late booting stage) and the second have been determined after one week of the last foliar application (grain filling stage).

3.2.5.2. Flag leaf area (LA)

Three days of the last foliar application, three flag leaves per pot were randomly selected and their leaf surface area were measured using leaf area meter and average was recorded as cm^2.

3.2.5.3. Membrane stability index (MSI)

Membrane stability index (MSI) was measured by using EC meter according to Sairam et al. (1997). Leaf sample of 100 mg from each treatment was thoroughly washed in double distilled water and placed in 100 ml tubes of distilled water. Then, tube was heated in water bath at 40 °C for 30 min. Then, the first electrical conductivity (EC1) value was determined. The same procedure was repeated through boiling leaf sample at 100 °C for 10 min and the electrical conductivity (EC2) was recorded. The MSI was measured with the following equation:

$$MSI = [1-(EC1/EC2)] \times 100$$

3.2.5.4. Relative water content (RWC)

Relative water content (RWC) was determined according to Barrs and Weatherley (1962). The fresh weight (FW) of flag leaf from each treatment was measured. Then, these leaves floated in 100 ml distilled water for one day at 4 °C darkness to determine turgid weight (TW). The same leaves were dehydrated in oven for 48 h at 80 °C to obtain dry weight (DW). Relative water content was determined as following formula:

$$RWC = [(FW-DW)/(TW-DW)] \times 100$$

3.2.5.5. Leaf water potential (Ψw)

Leaf water potential was determined according to Scholander (1964), with using a pressure chamber (PMS instrument CO, Corvallis, OR USA).

3.2.5.6. Water use efficiency (WUE)

Water use efficiency (WUE) for grain yield under greenhouse condition for each treatment calculated based on grain yield and total amount of water for each treatment with following equation (Karim et al., 2012) .

WUE (g/L) = Grain yield / Amount of total water used for irrigation.

3.2.5.7. Drought index (DI)

Drought index was estimated for grain yield of varieties according to method described by Abid et al. (2018):

$$DI = (YD/YW)$$

Where,
DI = drought index

YD = Seed yield of a variety/genotype under drought condition
YW = Seed yield of a variety/genotype under irrigation condition

3.2.6. Measurement of Biochemical Parameters

Antioxidants enzymes activities including Superoxide Dismutase (SOD), Catalase (CAT) and Ascorbate Peroxidase (APX) have been determined.

3.2.6.1. Homogenate preparation

In the preparation of homogenate, 1 g of the youngest leaf samples, which had completed their development, were freshly ground and thereby ground in liquid nitrogen and homogenized in 100 mM KH_2PO_4/0.5 mM EDTA pH (7.7) buffer containing 5 ml 1% (w/v) PVP. The supernatant was separated from the precipitate by centrifugation at 15.000 x g for 20 minutes at 4 °C in a cooled centrifuge. The resulting supernatant was maintained at -20 °C until use.

3.2.6.2. Measurement of superoxide dismutase (EC 1.15.1.1, SOD) enzyme activity

6-Hydroxidopamine (6-OHDA) is rapidly oxidized under physiological conditions and enzyme activity values were found by increasing absorbance inhibited by SOD as a result of measurements taken at 490 nm for 2 minutes (Heikkila and Cabbat, 1976).

3.2.6.3. Measurement of catalase (EC 1.11.1.6, CAT) enzyme activity

Determination of enzymatic activity, hydrogen peroxide (H_2O_2) measured at 240 nm for 2 minutes as a result of measurements taken by the interaction of the enzyme was carried out due to the decrease in time (Aebi, 1984).

3.2.6.4. Measurement of ascorbate peroxidase (EC 1.11.1.11, AP) enzyme activity

AP activity was determined by measuring ascorbate oxidation rate at 290 nm on Shimadzu UV-1800 spectrophotometer. The reaction mixture (1 ml) consists of 40 mM KH_2PO_4 buffer (pH 6.0), 1 mM EDTA, 20 mM H_2O_2, 2.5 mM L (+) ascorbic acid (ASA) and enzyme extract (Cakmak and Marschner, 1992).

3.2.7. Measurement of Quality Parameters

3.2.7.1. Determination of grain and shoot ash contents (%)

Firstly, samples of straws were washed with distilled water before dried at 70 °C for 48 h until constant weight was taken place. On the other hand, grains were milled and passed through 0.5 mm sieve. Thereafter, one gram from each sample of straws and grains were weighted (weight before ashing) and putted on oven at 550 °C for 12 to 15 h. Samples were weighted again after ashed and the ash contents of straws and grains were determined as in the following equation.

Ash content (%) = (Weight after ashing / Weight before ashing) x 100

3.2.7.2. Determination of Zn and Fe content in grain and shoot

Micronutrients (Zn and Fe) content in grains and shoots were determined after drying samples at 70 °C until constant weight was achieved. After that sample to pass through 0.5 mm sieve, 1 gram of powdered from each grains and straws was dry ash at oven at 550 °C for 12 to 15 h. The ashed samples were weighted, and acid digested with 4 ml of HCl. After digested 50 ml of distilled water was added. Zn, Fe, Cu and Mn were determined by Atomic Absorption Spectrophotometer (AAS).

3.2.7.3. Total Zn and Fe uptake

Total uptake of Zn and Fe (mg/pot) were determined by equation proposed by Graham et al. (1992) which as following

Total Zn Uptake = Seed yield (g/pot) × Zn content of seeds (mg/kg)

Total Fe Uptake = Seed yield (g/pot) × Fe content of seed (mg/kg)

3.2.8. Statistical Analysis

Data were analyzed and evaluated by JMP (13) Software SAS institute Inc., Cary. NC, USA. Experiment was subjected to a three-way ANOVA including; variety (two wheat varieties), Zn treatment (untreated seed, HP, priming, coating, soil, foliar, soil+foliar and coating+foliar), water regimes (well-watered and drought stress) and their interactions in the model. The figures have only conducted, if there were significant differences between interaction for the three factors (variety, Zn application methods and water regimes). Means showing significant differences were separated by using least-significant difference (LSD) test at $P<0.05$. The multivariate method was used to evaluate the correlation between the determined parameters.

3.3. Experiment III. Impact of Zinc Seed Coating and Foliar Spray on Yield and its Components, and Zinc Content of Wheat under Rainfed Conditions

3.3.1. Climatic and Soil Conditions of Research Area

This experiment was carried out in the Research Farm of Ondokuz Mayıs University, Samsun, Turkey during 2018-2019 growing period under rainfed condition. The total precipitation was 427 mm and monthly mean of temperature and relative humidity were 12.7 °C (Figure 3.3a and b) and 70% during the crop season, respectively.

At the 2018-2019 growing season, the precipitation and temperature during the flowering and grain filling stages (between April and May) started to increase till harvesting time (Figure 3.3a and b).

The chemical and physical properties of the soil are presented in (Table 3.4). Wheat varieties used in this experiment were the same to those in Experiments I and II.

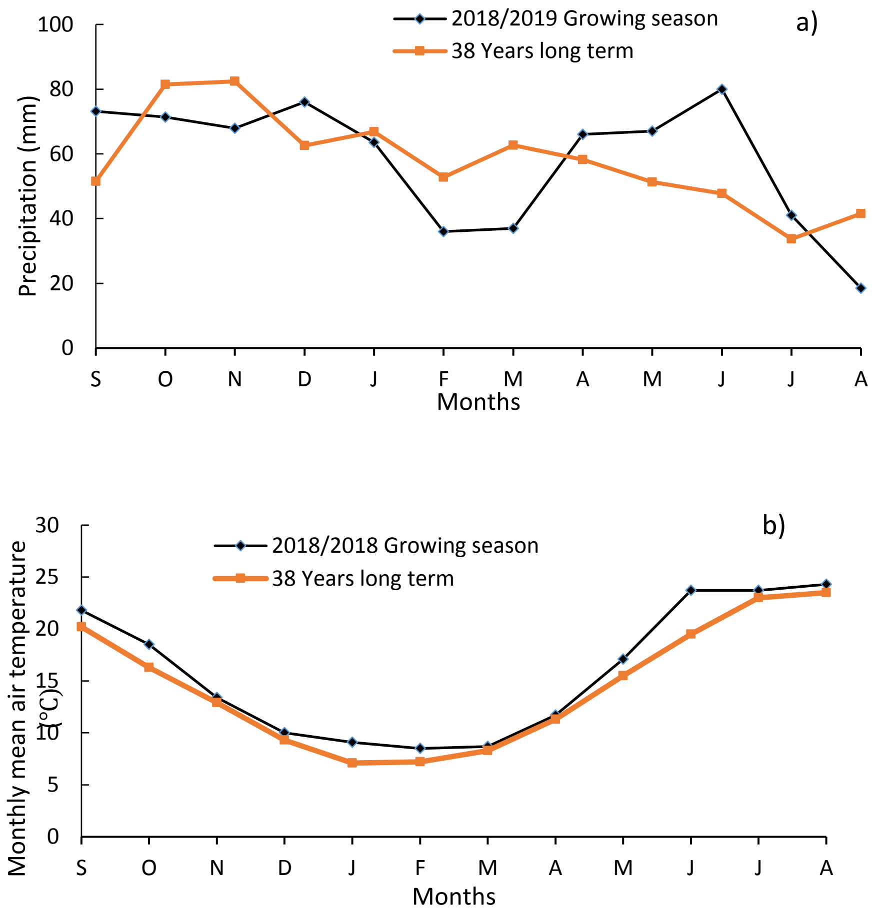


Figure 3.3. Total precipitation (a) and monthly mean air temperature (b) during growing season (2018/2019) and the average from 38 years period in Samsun province, Turkey

Table 3.4. Physical and chemical properties of the soil in the field Experiment III

Soil properties	Value	Degree
Soil texture		Silty clay loam
pH	7.24	Neutral
Salt (%)	0.03	Non-saline
$CaCo_3$ (%)	6.30	Moderate
Phosphorus (P_2O_5), (kg/da)	38.12	Very high
Potassium (K_2O), (kg/da)	138.00	Excessive
Organic matter (%)	1.99	Poor
Fe (mg/kg)	28.68	High
Cu (mg/kg)	2.27	High
Zn (mg/kg)	2.28	Low
Mn (mg/kg)	32.10	High

Sowing was done at the end of the October in plot size 3x4 m by hand (Figure 3.4a) and with seed rate 550 seeds per m^2 (Ekiz et al, 1998). Based on laboratory soil analysis, urea has been added to experiment plots as basal fertilizer with dose of 80 kg/ha pre-sowing while potassium and phosphorus had not added due to soil was sufficient and rich in these elements. Different developmental stages of wheat growth are presented in Figure 3.4.

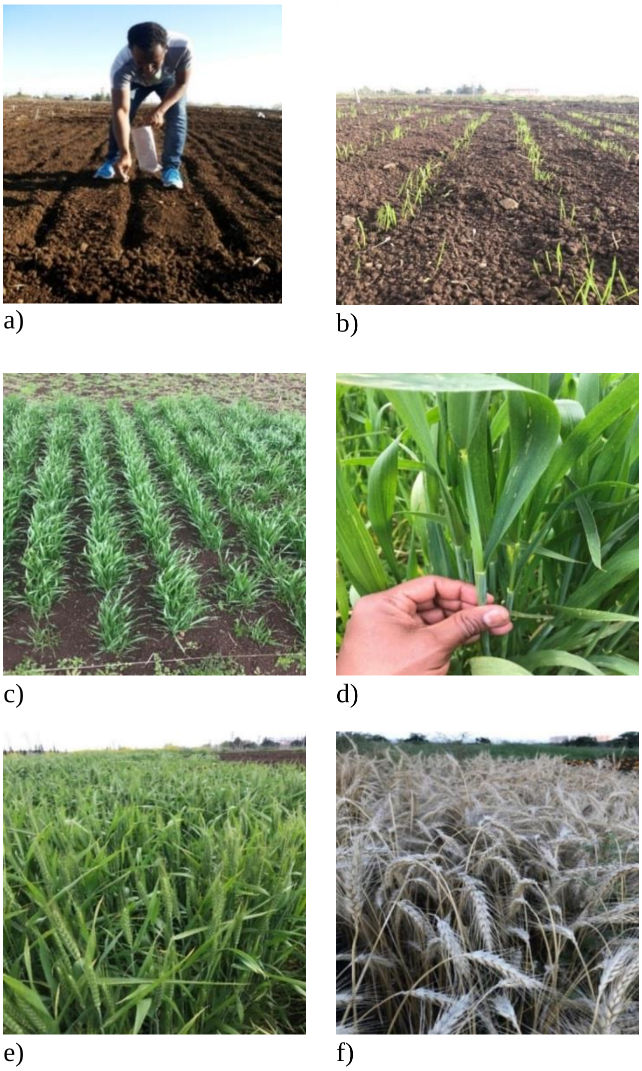


Figure 3.4. Different developmental stage of wheat growth showing a) sowing of seeds, b) starting of seed germination, c) tillering stage, d) booting stage, e) grain-filling stage and f) maturity stage

3.3.2. Treatments of the Experiment III

The impact of Zn application strategies on grain yield and Zn content of two wheat varieties were investigated in this experiment under the field condition. Zn application methods consisted of: 1) control (no Zn application); 2) foliar spray 0.5% of $ZnSO_4.7H_2O$ (w/v) sprayed twice on plants, first one at booting and the second at grain filling stage (Figure 5d and 5e, respectively) with 500 mm Zn solution until the solution started to run-off the leaves; 3) seed coating 1.5 g Zn/kg seeds; and 4) foliar+seed coating combined Zn application (0.5%+1.5 g Zn/kg seeds, respectively), foliar application were applied at the same growth stages which mentioned above in this experiment. The treatments have shown in Table 3.5. The experiment was designed in Completely Randomized Block Design with three replications as factorial including two wheat variety and four Zn application methods.

Table 3.5. Treatments and Zn concentration in the field experiment conducted under rainfed condition

Treatment	Zn concentration
Control	0
Zn seed coating	1.5 g Zn/kg seed
Zn foliar spray	0.5 %
Zn seed coating + Zn foliar spray	0.5%+ 1.5 g Zn/kg seed

3.3.3. Seed Yield and Yield Components

At full maturity stage of crop, ten plants were randomly selected from the middle rows of each plot for determining plant height, spike length, number of spikelet per spike, number of grain per spike and weight of grain per spike. Plants from the middle rows of each plot in a square meter area were harvested by hand to determine the biological yield (above ground biomass) and grain yield (ton/ha). Seeds were weighted after threshed manually and harvest index was determined and recorded. Harvest index was calculated according to the following equation.

Harvest index = (Seed yield/Biological yield) x 100

3.3.4. Measurement of Zn and Fe Content in Grain and Shoot

Zn and Fe content in grains and shoots were determined after drying of samples at 70 °C until constant weight was achieved. Samples were passed through 0.5 mm sieve. One gram of dry grains and shoots were ashed at oven at 550 °C for 12 to 15 h. The ashed samples were weighted, and acid digested with 4 ml of 3%

HCL. After digested 50 ml of distilled water was added. Zn and Fe contents were determined using by Atomic Absorption Spectrophotometer (AAS).

3.3.5. Determination of Seed Protein Content

In order to determine protein content, a standard graph was produced (Bradford, 1976). For this purpose, 1, 2, 4, 6, 10, 16 and 20 µl of bovine serum albumin (BSA) solution containing 1 mg protein in 1 ml were placed. The volume of all tubes was made up to 0.1 ml with distilled water and 0.9 ml of Coomassie Brillant Blue G-250 solution was added and mixed by vortexing. After incubation for 10 minutes, absorbance values were measured against 595 nm in 1 ml cuvettes. From the obtained results, the µg protein values corresponding to the absorbance values were transformed into a standard graph (Bradford, 1976). 96 µl of distilled water, 900 µl of Coomassie Brillant Blue G-250 solution and homogenate obtained as a result of 4 µl of the supernatant samples were placed in the cuvette absorbance values at 595 nm were read against the environment. According to the values obtained from each sample, protein contents were determined by using standard graph.

3.3.6. Statistical Analysis

Data were analyzed and evaluated by JMP (13) Software SAS institute Inc., Cary. NC, USA. Experiment was subjected to a two-way ANOVA including; variety (two wheat varieties), Zn treatment (control: untreated seed, Zn coating, Means showing significant differences were separated by using least-significant difference (LSD) test at $P<0.05$.

4. RESULTS AND DISCUSSION

4.1. Results of Experiment I

4.1.1. Seed Germination and Mean Germination Time

Zn is essential and remarkable element for seed germination and during the early establishment phase, particularly when seeds sown in Zn deficient soil. The germination percentage (GP) and mean germination time (MGT) were significantly influenced by Zn treatments of seed priming and coating (Table 4.1).

Table 4.1. Means of Zn treatments on germination percentage (GP) and mean germination time (MGT) in bread wheat varieties

Zn treatments	GP (%)		Mean	MGT (days)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	77.7 e	78.6 e	78.1 e	5.5 a	6.2 a	5.7 a
Hydropriming	78.0 e	92.0 a	85.0 d	4.4 c	4.3 e	4.4 d
Seed priming (2.5 mM Zn)	78.0 e	93.0 a	85.5 cd	4.4 c	4.8 cd	4.6 c
Seed priming (5 mM Zn)	85.4 cd	90.7 ab	88.0 bc	4.5 bc	4.3 e	4.5 cd
Seed coating with Arabic Gam	80.4 e	79.7 e	80.0 e	4.7 b	5.0 bc	4.9 d
Seed coating (1.5 g Zn/kg seed)	92.0 a	91.4 ab	91.5 a	3.6 e	4.6 de	4.1 e
Seed coating (2.5 g Zn/kg seed)	88.0 bc	89.7 ab	88.8 b	3.9 d	5.0 bc	4.5 cd
Seed coating (5 g Zn/kg seed)	89.0 ab	84.4 d	87.0 bcd	3.8 de	5.2 b	4.5 cd
Mean	83.5 b	87.4 a		4.4 b	4.9 a	
CV%	2.5			3.2		
LSD	3.6			0.25		
P value	Zn	**			**	
	V	**			**	
	ZnxV	**			**	

Means followed by the same letters are not statistically different. **: significant at $P<0.01$. V: varieties

Among Zn treatments, seed coating with 1.5 g Zn recorded the highest germination percentage and lowest MGT among other Zn treatments in Imam variety. Generally, seed coating and priming with Zn revealed remarkable increase in germination percentage and improved seedling growth in all Zn concentrations in compared to control (untreated seed) and hydropriming respectively, and for both varieties (Figure 4.1).

a) b) c) d)

Figure 4.1. Effect of priming and coating of wheat seeds with Zn on germination percentage and seedling growth. a) seedling from untreated seeds and b) seed coating with 1.5 g Zn, c) hydropriming and d) priming seed with 5 mM Zn

Seed coated with 1.5, 2.5 and 5 g Zn/kg seed in comparison with untreated seeds (control) enhanced seed germination with 18, 13 and 14% in Imam variety, and with 16, 14 and 7% in the Altındane variety, respectively. In comparison with hydropriming, seed priming particularly with 5 mM Zn had better germination for both varieties, especially for Imam variety which improved germination percentage with 10%. Moreover, Altındane variety didn’t show any significant difference between seed priming with Zn and hydropriming.

In seeds which treated with Zn priming and coating for both varieties, lower mean germination time (MGT) were determined in comparison with untreated seed and hydropriming. Furthermore, seed coating in all Zn concentration with 1.5, 2.5 and 5 g achieved important differences and less MGT by 3.6, 3.9 and 3.8 days were noted, respectively, when compared with untreated seeds 5.5 days in Imam variety. The lowest dose of seed coating (1.5 g) took less time (4.6 days) to complete its MGT and the higher doses than 1.5 g Zn/kg caused more MGT in comparison with seed coating with Gum Arabic (5 days) in Altındane variety. However, in case of seed priming, hydroprimed seeds (HP) have showed relatively decreasing in MGT and were less than those primed with Zn in both wheat varieties (Table 4.1).

Seedling from seed primed with water are known as hydropriming and to take less time to emergence and grow vigorously than those from non-primed seeds (Ajouri et al., 2004; Arif, 2005; Rashid et al., 2002). The data in Table 4.1 follow this pattern, but seed primed with 2.5 mM Zn in Imam variety and 5 mM Zn in Altındane variety have taken the same time to complete MGT in comparison with HP. In the present study, seed priming advanced all seedling growth parameters and has shown significant results particularly in Imam variety. On the other hand, seeds of Altındane variety have observed less responds Zn priming that might be return to that sufficient grain Zn content of this variety in comparison with low or medium Zn seed content or due to genetic variation.

The results of this study showed that seed priming with Zn high dose (5 mM) had relatively positive impact on seed germination and seedling growth parameters when compared with low Zn dose (2.5 mM) and hydropriming of two wheat varieties (Table 4.1). Similarly, seed primed with 5 mM or 10 mM $ZnSO_4$ generally mitigate the germination of barley seeds (Ajouri et al., 2004). On the other hand, seed priming with Zn up to 5 mM significantly enhanced seed growth, germination rate and dry weight of rice (Todeschini et al., 2011; Cambrollé et al., 2012; Prom-u-thai et al., 2012). Furthermore, several authors have described positive response to seed priming with Zn in seed germinating (Johnson et al., 2005; Mohsin et al., 2014; Reis et al., 2018). Seed priming with $ZnSO_4$ was very cost-effective in wheat and have widely applied and adopted by farmers for various crops like wheat. However, many results have shown that use seeds with adequate Zn concentration could increment grain Zn content, germination rate and increase yield in wheat (Yilmaz et al., 1997; Reis et al., 2018), maize (Ajouri et al., 2004; Harris et al., 2007) and chickpea (Johnson et al., 2005; Hidoto et al., 2017). The role of $ZnSO_4$ fertilizers as seed priming and seed coating in an improving seed germination and MGT of wheat has been also investigated by previous study carried out by Harris et al. (2007) and Rehman (2017) compared to HP and untreated seeds, respectively. Such published literatures as well as to our results in this study proven the importance of Zn in ameliorate the stand establishment of wheat germination. Another research indicated that ZnO (4 mg/kg of seeds) was significantly improved the seed germination, root, and shoot length of soybean (Montanha et al., 2020). The data from this study confirmed that the seed priming with Zn is an effective way to increase germination rate particularly with high Zn concentration (5 mM Zn) in variety with low Zn content (Table 4.1).

4.1.2. Shoot and Root Fresh Weight

Fresh roots and shoots weight were significantly ($P \geq 0.5$) affected by Zn application, variety and their interaction (Table 4.2). In Imam variety, seed coating with 1.5, 2.5 and 5 g Zn had positive effect on all growth seedling parameters and enhanced root fresh weight 44, 51 and 40%, shoot fresh weight 50, 43 and 43%, respectively. Rehman and Farooq (2016) pointed out seed coated with Zn improves the seedling weight due to better root and shoot growth.

Furthermore, in comparison with HP seed priming with 5 and 2.5 mM Zn had displayed significant influence through enhanced the weight of root fresh (29 and 23%) and shoot fresh (19 and 14%) in Imam variety. Across varieties, Altındane have shown higher weight for root and shoot fresh by 126.5 and 174.0 mg than that of Imam variety 107.3 and 154.0 mg, respectively (Table 4.2). That means that Altındane was the superior than Imam variety for fresh root and shoot weight.

Table 4.2. Means of Zn treatments on fresh root and shoot weight in bread wheat varieties

Zn treatments	Fresh root weight (mg)		Mean	Fresh shoot weight (mg)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	80.0 g	110.0 f	95.3 e	120.0 f	156.6 c	139.0 e
Hydropriming	90.0 g	121.0 cde	105.0 d	124.0 f	178.6 b	151.0 d
Seed priming (2.5 mM Zn)	113.6 ef	128.0 bc	120.0 bc	146.0 d	177.0 b	161.8 c
Seed priming (5 mM Zn)	116.0 c-f	133.0 b	125.0 ab	178.0 b	176.3 b	177.0 b
Seed coating with Arabic Gam	109.0 f	120.0 c-f	115.0 c	136.0 e	162.0 c	150.0 d
Seed coating (1.5 g Zn/kg seed)	114.6 def	146.0 a	130.6 a	180.0 ab	190.0 a	185.0 a
Seed coating (2.5 g Zn/kg seed)	121.0 cde	126.6 bc	124.0 ab	172.0 b	179.0 b	176.0 b
Seed coating (5 g Zn/kg seed)	112.4 ef	126.0 bcd	119.0 bc	172.0 b	172.0 b	172.6 b
Mean	107.3 b	126.5 a		154.0 b	174.0 a	
CV%	6			3.5		
LSD	11.8			9.7		
P value	Zn	**			**	
	V	**			**	
	ZnxV	**			**	

Means followed by the same letters are not statistically different. **: significant at P<0.01. V: varieties

The results obtained above apparently indicated that fresh weight of root and shoot increased in all of seed priming and seed coating levels compared to HP and untreated seeds respectively. The positive effect of Zn application in term of improving the fresh weight for root and shoot has been declared by Weisany et al. (2014) for soybean under salinity stress. Also, foliar spray of 2% $ZnSO_4$ was significantly enhanced the root and soot fresh weight of maize under pot experiment (Umar et al., 2020) which consistent with our results in this study.

4.1.3. Root and Shoot Length

When compared to HP and untreated seeds, seed priming and coating significantly improved seedling root and shoot length for both wheat varieties (Figure 4.2). Seed coating with 1.5, 2.5 and 5 g Zn enhanced roots length by 23, 22 and 20%, and shoots length by 26.5, 13 and 20% compare to untreated treatment. As indicated above, coated seed with 1.5 g Zn/kg seed had shown a slightly improvement in roots and shoots lengths when compared to other concentrations and untreated seeds (Table 4.3). Similarly, Zn coated seed make the nutrient available during the early establishment phase of seed germination and that leaded to faster the seedling growth (Taylor and Harman, 1990). Some studies reported that the value of Zn sufficient seeds for seed vigor based on seedling height (Cakmak, 2008a; Welch, 1999).

a) b) c) d) e) f) g) h) i)

Figure 4.2. Effect of priming and coating of wheat seeds with Zn on shoots and roots length (a, b and c seed coating with 5, 2.5 and 1.5 g Zn respectively, d and h hydropriming, e and i untreated seeds, f and g seed priming with 5 and 2.5 mM Zn, respectively)

In our present study, Zn deficiency in untreated seeds caused minimized of root and shoot length compared to all Zn treatments. It has been reported that one of the symptoms of Zn deficiency of wheat is short internode (Noulas et al., 2018) and this shorting might be leading to reduction in the shoot elongation. The significant of Zn application especially, in Zn deficient soil has previously carried out in rice by Malik et al. (2011).

Table 4.3. Means of Zn treatments on length of root and shoot in bread wheat varieties

Zn treatments	Root length (cm)		Mean	Shoot length (cm)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	8.6 e	8.6 e	8.6 b	15.0 de	15.0 de	15.0 d
Hydropriming	11.0 abc	11.0 ab	11.0 a	14.0 e	17.7 ab	16.0 cd
Seed priming (2.5 mM Zn)	11.0 abc	11.0 abc	11.0 a	18.0 ab	17.0 bc	17.5 b
Seed priming (5 mM Zn)	9.6 de	12.3 a	11.0 a	17.0 bc	17.3 bc	17.1 b
Seed coating with Arabic Gam	10.0 cde	10.3 bcd	10.3 a	17.3 bc	16.0 cd	16.6 bc
Seed coating (1.5 g Zn/kg seed)	10.6 bcd	11.3 abc	11.0 a	19.0 a	19.0 a	19.0 a
Seed coating (2.5 g Zn/kg seed)	11.6 ab	10.0 bc	10.8 a	17.0 bc	16.0 cd	16.5 bc
Seed coating (5 g Zn/kg seed)	11.0 abc	10.3 bcd	10.6 a	18.0 ab	15.3 de	16.6 bc
Mean	10.5	10.6		16.9	16.5	
CV%	8.6			5.5		
LSD	1.5			1.5		
P value	Zn	ns			**	
	V	**			ns	
	ZnxV	*			**	

Means followed by the same letters are not statistically different. *: significant at P<0.05, **: significant at P<0.01, ns: non-significant. V: varieties

4.1.4. Root and Shoot Dry Weight

Analysis of variance showed that the effect of Zn treatments, variety and their interaction on dry weight of roots and shoots were significant (P<0.05) (Table 4.4). Furthermore, in comparison with HP seed priming with 5 and 2.5 mM Zn had shown substantial effect via increasing the root dry weight by 21 and 28%, and shoot dry weight by 42 and 27% in Imam variety, respectively. On the other hand, Altındane variety had revealed significant results in Zn seed coated in all concentration, particularly low dose of 1.5 g Zn where improved and increased roots and shoots dry weight by 22 and 25% compare to untreated seeds, respectively. Altındane variety was the superior than Imam variety for dry roots and shoots weight (Table 4.4).

That could be return to that Altındane accumulated and assimilated Zn efficiently more than Imam. The result comply with Kaur et al. (2020) who shown that the high dry weight of leaf and tiller produced in efficient rice genotypes than insufficient one. Another research on chia plant displayed that soil Zn application (5 mg/kg Zn) achieved the highest dry matter among different rate of soil applications (Korkmaz et al., 2020).

Table 4.4. Means of Zn treatments on dry weight of root and shoot in bread wheat varieties

Zn treatments	Root dry weight (mg)		Mean	Shoot dry weight (mg)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	7.3 f	10.3 e	8.6 b	14.0 g	16.4 f	15.2 e
Hydropriming	10.3 e	11.0 cde	10.6 c	13.3 g	18.6 bcd	16.0 de
Seed priming (2.5 mM Zn)	11.0 cde	12.0 abc	11.5 b	17.0 ef	17.0 ef	17.0 cd
Seed priming (5 mM Zn)	13.2 a	11.6 bcd	12.3 a	19.0 bc	18.7 bcd	18.8 b
Seed coating with Arabic Gam	10.6 b	10.6 de	10.6 c	14.3 g	16.4 f	15.3 e
Seed coating (1.5 g Zn/kg seed)	10.4 e	12.6 ab	11.5 b	19.3 ab	20.6 a	20.0 a
Seed coating (2.5 g Zn/kg seed)	10.2 e	10.6 de	10.5 c	17.6 c-f	17.7 c-f	17.6 c
Seed coating (5 g Zn/kg seed)	11.3 cde	12.0 abc	11.6 b	17.3 def	18.0 b-e	17.6 c
Mean	16.5 b	18.0 a		16.9	16.5	
CV%	6.4			5.2		
LSD	1.1			1.5		
P value	Zn	**			**	
	V	**			**	
	ZnxV	**			**	

Means followed by the same letters are not statistically different. **: significant at P<0.01. V: varieties

The shoot and root dry weight of cotton seedling (30 day old) was significantly increased by Zn treatments (1 µM Zn) grown under hydroponic and drought stress experiment (Wu et al., 2015). Adding 0.05 µg Zn/kg soil remarkably enhanced shoot dry matter for chickpea by 21% compared to treatment with no Zn has added (Khan et al., 2003). In field experiment and Zn-deficient soil calcareous soil applied 5 mg Zn/kg soil was considerably improved shoot dry matter and grain Zn content of wheat (Esfandiari et al., 2018) this might be return to critical Zn deficient found in the soil where the effect of Zn could be more pronounced over sufficient Zn soil.

On bases on above study, it is concluded that seed priming in Zn containing solution and seed coating with Zn are simple and practical way to enhance seed Zn prior to sowing and contribute to better seedling growth. Moreover, seed with high Zn content can ameliorate seed germination, seedling vigor, sustain crop growth and stress tolerance particularly in Zn deficient soil. There was large difference between wheat varieties in term of the response to Zn priming and coating. The effectiveness was more pronounced in variety accumulated low Zn content than that have sufficient Zn content.

The results of this experiment also showed that seed coating with Zn gave good respond and revealed important results for all seed germination parameters in comparison with untreated seeds for both wheat varieties particularly variety with less Zn content of Altındane. This result clearly indicates to that seed coating with Zn could have more effective when applied to variety with low Zn content rather than those have sufficient Zn content. Moreover, seed coated with the highest dose of Zn 5 g/kg seed had a deleterious effect on seed emergence and seedling growth for both varieties, especially in Altındane (Table 4.1). Nevertheless, previous studies have noticed the same results reported by Dirginčiutė-Volodkienė and Pečiulytė (2011), Rehman and Farooq (2016), where that accumulation Zn at high concentration may induce Zn toxicity, which may affect plant growth.

Seed priming with Zn is a beneficial application to increase germination rate and improve seedling growth, particularly with high Zn concentration (5 mM) in comparison with low rate and hydropriming. Moreover, Zn coated seed with more than 1.5 g Zn/kg seed had no positive affect on seedling growth and seedling growth parameters. Therefore, the lowest dose of Zn coating at the rate of 1.5 g Zn/kg seed is considered as completely economic and environment friendly in agricultural production.

4.2. Results of Experiment II

4.2.1. Morphological Parameters

4.2.1.1. Plant height (cm)

As shown in Table 4.5, Zn treatments, water regimes and varieties significantly (P≥0.05) influenced the plant height. Variety × Zn treatments and variety × Zn treatment × water regimes interactions were found highly significant (Table 4.5).

Table 4.5. Analysis of variance for the effects of Zn treatments, varieties and water regimes and their interaction on plant height (cm)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.27	0.069	0.93
Varieties	1	5017.0	2661.0	<.0001
Zn treatments	7	101.3	7.7	<.0001
Variety×Zn treatments	7	88.6	6.7	<.0001
Water regimes	1	247.0	131.0	<.0001
Varieties×water regimes	1	1.5	0.8	0.3758
Zn treatments×water regimes	7	11.3	0.9	0.5462
Varieties×Zn treatments×water regimes	7	55.8	4.2	0.0007
Error	62	120.3		
Total	95	5643.4		

Plant height ranged from 77.4 to 70.0 cm and 62.0 to 54.0 cm for Imam and Altındane varieties under well-watered and deficit-watered condition, respectively. Among Zn application methods, seed coated with Zn had the highest plant height (74.4 cm) and the lowest was recorded in HP (56.0 cm) (Table 4.6). Similar results was also confirmed by Khan et al., (2008) who revealed that soil applied with Zn sulphate contributed in improve plant height of wheat compared to untreated soil with Zn under calcareous soil. This finding could be attribute to the role of Zn in an improve cell division of xylem which lead to ameliorated tillering, and ultimately increase wheat grain yield. Further, it has been reported that Zn deficiency causes stunting growth of crop (Hafeez et al., 2013). Plant height of Imam variety (73.4 cm) longer than Altındane variety (59.0 cm) (Table 4.6).

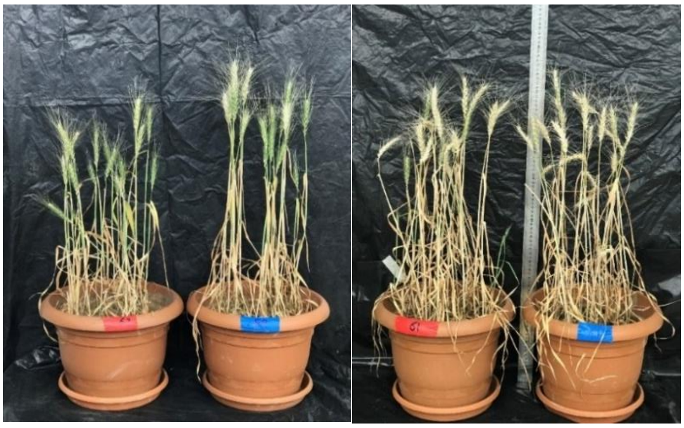
Table 4.6. Means of Zn treatments, varieties and water regimes for plant height (cm)

Varieties (V)	Zn treatments	Watering regimes (W)		Mean V×Zn
		WW	DS	
Imam	Untreated seeds	71.0 ef	70.3 ef	70.7 D
	Hydropriming	74.0 bcd	72.0 def	73.0 BC
	Seed priming	75.4 abc	73.7 cd	74.5 AB
	Seed coating	77.4 a	72.0 def	74.7 A
	Soil application	77.0 a	72.0 def	74.5 AB
	Foliar spray	75.7 abc	70.0 f	72.8 C
	Soil application + foliar spray	74.7 bc	72.4 de	73.5 ABC
	Seed coating + foliar spray	76.0 ab	71.0 ef	73.5 ABC
	Mean V×W	75.2 A	71.7 B	73.4 A
Altındane	Untreated seeds	60.4 ghij	56.0 lmn	58.2 GH
	Hydropriming	59.0 ijk	54.0 n	56.5 I
	Seed priming	58.4 jk	55.4 mn	56.8 HI
	Seed coating	61.4 gh	58.0 kl	59.7 EFG
	Soil application	59.4 hijk	59.4 hijk	59.4 FG
	Foliar spray	62.0 g	60.4 ghij	61.2 E
	Soil application + foliar spray	61.0 ghi	57.4 klm	59.2 FG
	Seed coating + foliar spray	62.0 g	59.4 hijk	60.7 EF
	Mean V×W	60.4 C	57.4 D	59.0 B
	Mean W	67.7 a	64.5 b	

Means followed by the same letters are not statistically different at $P<0.05$. WW: well-watered, DS: drought stress

It has been demonstrated that plant height of wheat was enhanced when soil treated with Zn, that could be attribute to that Zn involving in many of physiological processes such as activation of enzymes (Gibson, 2012) and regulator to stomatal (Oosterhuis and Weir, 2010) which eventually, reduce height of plant (Yaseen et al., 2011). In the combined study on the effect of drought stress and heat stress on the plant height of maize, the results revealed that drought stress had more adverse and severe damage on the plant height compared to those with heat stress and finally lead to decrease in the biomass and ultimately grain yield (Hussain et al., 2019).

Zn treatment x water regime interaction was found non-significant. Among interaction means where drought stress decreased plant height by 8%, compared with well-watered control treatment (Figure 4.3b), but seed coated with Zn has mitigate this reduction to only 5% (Figure 4.3c and Table 4.7).



a) b) c) d)

Figure 4.3. Effect of drought stress on plant height a) drought-untreated seeds, b) well-watered untreated seeds, c) drought seed-coating and d) well-watered untreated seed

Table 4.7. Means of Zn treatments × water regimes interaction for plant height (cm)

Zn treatments	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	65.7	63.2	64.4 d
Hydropriming	66.5	63.0	64.0 cd
Seed priming	66.8	64.5	65.0 bc
Seed coating	69.4	65.0	67.2 a
Soil application	68.2	65.7	66.9 a
Foliar spray	68.8	65.2	67.0 a
Soil application+foliar spray	67.8	64.8	66.0 ab
Seed coating+foliar spray	69.0	65.2	67.0 a
Mean	67.7 a	64.5 b	

Means followed by the same letters are not statistically different at $P<0.05$

4.2.1.2. Spike length (cm)

Spike length was not significantly affected by Zn treatments, while the interaction of Zn application with variety had highly significant difference (Table 4.8). Also, drought stress significantly affected spike length particularly for Imam variety.

Table 4.8. Analysis of variance for the effect of Zn treatments, varieties and water regimes and their interaction on spike length (cm)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.85	2.17	0.1200
Varieties	1	121.00	538.5	<.0001
Zn treatments	7	2.12	1.34	0.2451
Variety×Zn treatments	7	6.01	3.81	0.0016
Water regimes	1	6.00	26.59	<.0001
Varieties×water regimes	1	1.00	4.434	0.0392
Zn treatments×water regimes	7	1.69	1.075	0.3896
Varieties×Zn treatments×water regimes	7	1.87	1.185	0.3241
Error	62	12.10		
Total	95	160.00		

On the contrary of plant height, Altındane recorded higher spike length when compared with Imam variety. Zn application, the combination of treatments (soil+foliar and coating+foliar) resulted in significant increase in spike length under well-watered condition for Imam variety compared with untreated seed (control). But they have not capability to occur that for Altındane (Table 4.9).

Table 4.9. Means of Zn treatments, varieties and water regimes for spike length (cm)

Varieties (V)	Zn treatments	Watering regimes (W)		Mean V×Zn
		WW	DS	
Imam	Untreated seeds	11.0	10.3	10.7 D
	Hydropriming	11.5	10.3	11.0 CD
	Seed priming	11.5	10.5	11.0 CD
	Seed coating	11.4	11.0	11.2 CD
	Soil application	11.4	10.5	11.0 CD
	Foliar spray	11.6	11.2	11.4 C
	Soil application + foliar spray	11.7	10.9	11.3 C
	Seed coating + foliar spray	11.7	11.2	11.4 C
	Mean V×W	11.5 C	10.7 D	11.0 B
Altındane	Untreated seeds	13.8	13.7	13.8 A
	Hydropriming	13.7	13.7	13.7 A
	Seed priming	13.7	13.4	13.5 A
	Seed coating	13.5	13.1	13.4 AB
	Soil application	13.3	12.7	13.0 B
	Foliar spray	13.3	13.9	13.6 A
	Soil application + foliar spray	13.0	12.8	12.9 B
	Seed coating + foliar spray	13.5	12.6	12.9 B
	Mean V×W	13.5 A	13.2 B	13.3 A
	Mean W	12.5 a	11.9 b	

Means followed by the same letters are not statistically different at $P<0.05$. WW: well-watered, DS: drought stress

Spike length slightly decreased by 5% under deficit water condition as compared with well water treatment, respectively (Table 4.10). It has been demonstrated that drought stress at grain filling stage significantly reduced plant height and spike length of wheat (Yavas and Unay, 2016). Similarly in current study, water deficit caused reduction of plant height and spike length by 4.7 and 5% in compared with well-watered treatment, respectively.

The data regarding interaction effect of Zn treatments in combination with water regimes found non-significant. Also, the main effect of Zn treatments shown non-significant on spike length but, the main effect of water regimes revealed the notable impact on the spike length (Table 4.10). The results in consistent with previous study reported by Phuphong et al. (2018) who revealed that Zn foliar spray of $ZnSO_4.7H_2O$ (0.5%) had no any effect on panicle length of rice under field experiment.

Table 4.10. Means of Zn treatments×water regimes interaction for spike length (cm)

Zn treatments	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	12.4	12.0	12.2
Hydropriming	12.6	12.0	12.3
Seed priming	12.6	12.0	12.3
Seed coating	12.5	12.1	12.3
Soil application	12.3	11.6	12.0
Foliar spray	12.3	12.2	12.3
Soil application+foliar spray	12.2	11.7	12.0
Seed coating+foliar spray	12.6	11.8	12.2
Mean	12.5 a	11.9 b	

Means followed by the same letters are not statistically different at $P<0.05$

4.2.1.3. Number of spikelet per spike

The number of spikelet per spike were significantly influenced by Zn treatment, water regime and variety. Whilst, it has not observed any effect by interaction according to the data revealed on Table 4.11. The number of spikelet per spike statistically affected by water regime condition, where deficit-watered reduced the spikelet by 7.5% in comparison to well-watered. These results are line with (Daryanto et al., 2016) who reported that drought stress during anthesis stage caused important impact on number of spikelet per spike.

Table 4.11. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on number of spikelet per spike

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.66	1.07	0.34
Varieties	1	9.69	31.1	<.0001
Zn treatments	7	5.768	2.63	0.0185
Varieties×Zn treatments	7	3.205	1.46	0.1956
Water regimes	1	25.523	81.67	<.0001
Varieties×water regimes	1	0.023	0.07	0.7851
Zn treatments×water regimes	7	4.372	1.99	0.0689
Varieties×Zn treatments×water regimes	7	1.955	0.89	0.5166
Error	62	19.3		
Total	95	70.5		

Table 4.12. Means of Zn treatments, varieties and water regimes for number of spikelet per spike

Varieties (V)	Zn treatments	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	12.7	12.3	12.5
	Hydropriming	12.7	11.7	12.2
	Seed priming	13.0	11.7	12.3
	Seed coating	13.3	13.0	13.2
	Soil application	13.3	12.2	12.8
	Foliar spray	13.1	12.2	12.7
	Soil application + foliar spray	14.0	12.5	13.3
	Seed coating + foliar spray	13.7	12.3	13.0
	Mean V×W	13.2 B	12.2 D	13.4 A
Altındane	Untreated seeds	13.8	13.7	13.8
	Hydropriming	13.3	13.0	13.2
	Seed priming	13.3	12.7	13.0
	Seed coating	13.8	12.5	13.2
	Soil application	14.3	12.5	13.4
	Foliar spray	13.8	12.8	13.3
	Soil application + foliar spray	14.2	12.8	13.5
	Seed coating + foliar spray	14.5	12.7	13.6
	Mean V×W	13.9 A	12.8 C	12.7 B
	Mean W	13.5 a	12.5 b	

Means followed by the same letters are not statistically different at $P<0.05$. WW: well-watered, DS: drought stress

Furthermore, the combination of soil application+foliar spray, and seed coating+foliar spray recorded the highest number of spikelet per spike than control by 9.3 and 7.2%, respectively (Table 4.12). Whereas seed priming treatment had relatively more spikelet per spike than hydropriming under well-watered, but not for deficit-watered (Table 4.13).

Zn treatments alone or interaction with water regimes were found non-significant in the number of spikelet per spike (Table 4.11). The combination of seed coating+foliar spray, and soil application+foliar spray produced higher 14.1 and 14.1, and 12.5 and 12.7 number of spikelet per spike under well-watered and drought stress conditions, respectively (Table 4.13). Similar results were obtained in pervious study presented by Sangtarash (2010) who shown that drought stress imposed at different growth stages of wheat resulted in reduction in number of spikelet.

Table 4.13. Means of Zn treatments × water regimes interaction for the number of spikelet per spike

Zn treatments	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	13.2	13.0	13.2 a
Hydropriming	13.0	12.4	12.7 b
Seed priming	13.2	12.2	12.7 b
Seed coating	13.6	12.8	13.2 a
Soil application	13.8	12.4	13.0 ab
Foliar spray	13.5	12.5	13.0 ab
Soil application+foliar spray	14.1	12.7	13.4 a
Seed coating+foliar spray	14.1	12.5	13.3 a
Mean	13.5 a	12.5 b	

Means containing the different letters are significantly different at $P<0.05$

4.2.1.4. Number of grains per spike

The number of grain per spike has only influenced by main effects (Table 4.14).

Table 4.14. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on number grains per spike (grain/spike)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	1.51	0.83	0.43
Varieties	1	29.81	32.99	<.0001
Zn treatments	7	48.101	7.60	<.0001
Varieties×Zn treatments	7	7.289	1.15	0.3426
Water regimes	1	44.69	49.45	<.0001
Varieties×water regimes	1	1.62	1.80	0.1843
Zn treatments×water regimes	7	10.91	1.72	0.1188
Varieties×Zn treatments×water regimes	7	2.64	0.41	0.8877
Error	62	56.30		
Total	95	203.00		

Furthermore, the number of grain per spike in Imam variety ranged from 26.0 to 28.7 grain/spike under well-watered whereas in deficit-watered ranged from 25 to 28 grain/spike in control and coating treatment respectively. Interaction of Zn application and variety was found that Altındane variety observed more number of grains per spike (27.8 grain/spike) than Imam variety (26.7 grain/spike) (Table 4.15). Many studies shown effect drought stress on spikelet per spike and grains per spike reported by Khan et al. (2015) in maize, Kilic and Yağbasanlar (2010) in wheat, and Movahhedy-Dehnavy et al. (2009) in safflower.

Table 4.15. Means of Zn treatments, varieties and water regimes for number of grains per spike (grain/spike)

Varieties (V)	Zn treatments	Watering regimes (W)		Mean V×Zn
		WW	DS	
Imam	Untreated seeds	26.0	25.0	25.5
	Hydropriming	27.0	25.0	26.0
	Seed priming	27.7	24.7	26.1
	Seed coating	28.7	28.0	28.3
	Soil application	28.3	26.3	27.3
	Foliar spray	27.3	25.3	26.3
	Soil application + foliar spray	28.0	26.3	27.1
	Seed coating + foliar spray	27.7	27.0	27.3
	Mean V×W	27.6 B	25.9 C	26.7 B
Altındane	Untreated seeds	28.0	27.0	27.5
	Hydropriming	27.5	26.7	27.0
	Seed priming	27.7	26.0	26.8
	Seed coating	28.7	28.0	28.3
	Soil application	29.0	28.0	28.5
	Foliar spray	28.7	27.0	27.8
	Soil application + foliar spray	29.7	27.3	28.5
	Seed coating + foliar spray	28.3	28.7	28.5
	Mean V×W	28.4 A	27.3B	27.8 A
	Mean W	28.0 a	26.6 b	

Means followed by the same letters are not statistically different at $P<0.05$. WW: well-watered, DS: drought stress

This reduction of yield components (spikelet per spike and grains per spike) by drought might attribute to water deficit imposed during the flowering stages which is more critical and sensitive to water stress than one of the other stages. The effect of Zn treatments and water regimes on the number of grain per spike was non-significant (Table 4.16).

Table 4.16. Means of Zn treatments×water regimes interaction for number of grains per spike (grain/spike)

Zn treatments	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	27.0	26.0	26.5 c
Hydropriming	27.3	25.8	26.5 c
Seed priming	27.7	25.4	26.5 c
Seed coating	28.7	28.0	28.4 a
Soil application	28.7	27.3	28.0 a
Foliar spray	28.0	26.2	27.0 bc
Soil application+foliar spray	28.8	26.8	27.8 ab
Seed coating+foliar spray	28.8	27.8	28.0 a
Mean	28.0 a	26.6 b	

Means followed by the same letters are not statistically different at $P<0.05$

The yield attributes have notable effect on the final grain yield and any damage or injury on the one of these yield attributes such as number of grain per spike intend to induce adverse effect on the crop development and ultimately, grain yield (Gaju et al., 2009; Zulfiqar et al., 2020). However, Zn treatments × water regimes shown that maximum 28.8 number of grain per spike were found under well-watered conditions when seeds were treated with the combination of Zn treatments (seed coating+foliar spray, and soil application+foliar spray). Overall, Zn seed coating was recorded the highest 28.4 number of grains per spike rather than untreated seeds and the others Zn treatments, however, the result was non-notable (Table 4.16).

4.2.1.5. Weight of grains per spike

Analysis of variance pointed out a significant Zn application, water regime and variety for weight of grains per spike. Moreover, in interaction, weight of grains per spike was influenced by variety × Zn application and Zn application × water regime (Table 4.17).

Table 4.17. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on weight of grains per spike (g)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.0002	0.39	0.67
Varieties	1	0.0513	150.3	<.0001
Zn treatments	7	0.0533	22.3	<.0001
Varieties×Zn treatments	7	0.0062	2.6	0.0192
Water regimes	1	0.0782	228.9	<.0001
Varieties×water regimes	1	0.0012	3.5	0.065
Zn treatments×water regimes	7	0.0082	3.4	0.0036
Varieties×Zn treatments×water regimes	7	0.0041	1.7	0.1186
Error	62	0.0215		
Total	95	0.2244		

In Imam variety, seed coating recorded the highest percentage increased by 9 and 18.8% in weight of grains per spike under well-watered and drought stress respectively in compared to control (Table 4.18).

In an interaction of Zn treatments and water regime, the highest weight of grains was 0.84 g when seed treated with Zn coating and the lowest one was 0.77 g in control treatment (Table 4.19). Jalal et al. (2020) observed that grain size and grain per spike of wheat were increased when plant fertilized with 0.3% foliar spray Zn which is a similar finding in this study.

Table 4.18. Means of Zn treatments, varieties and water regimes for weight of grains per spike (g)

Varieties (V)	Zn treatments	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	0.77	0.73	0.75 i
	Hydropriming	0.80	0.76	0.78 h
	Seed priming	0.81	0.76	0.79 gh
	Seed coating	0.84	0.83	0.83 bc
	Soil application	0.83	0.79	0.81 ef
	Foliar spray	0.81	0.78	0.80 fgh
	Soil application + foliar spray	0.80	0.78	0.79 fg
	Seed coating + foliar spray	0.84	0.83	0.83 bcd
	Mean V×W	0.81 B	0.78 B	0.79 A
Altındane	Untreated seeds	0.84	0.80	0.82 de
	Hydropriming	0.83	0.81	0.82 de
	Seed priming	0.84	0.81	0.82 de
	Seed coating	0.87	0.86	0.86 a
	Soil application	0.86	0.84	0.85 b
	Foliar spray	0.85	0.83	0.84 b
	Soil application + foliar spray	0.87	0.83	0.85 b
	Seed coating + foliar spray	0.86	0.84	0.85 b
	Mean V×W	0.85 A	0.82 A	0.83 A
	Mean W	0.83 a	0.77 b	

Means followed by the same letters are not statistically different at P<0.05. WW: well-watered, DS: drought stress

Table 4.19. Means of Zn treatments × water regimes interaction for the number of grains per spike

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	0.80 d	0.74 e	0.77 d
Hydropriming	0.80 cd	0.75 e	0.78 cd
Seed priming	0.82 bcd	0.74 e	0.78 cd
Seed coating	0.85 a	0.83 abc	0.84 a
Soil application	0.84 ab	0.78 e	0.81 b
Foliar spray	0.83 abc	0.78 e	0.80 bc
Soil application+foliar spray	0.83 abc	0.78 e	0.80 bc
Seed coating+foliar spray	0.85 a	0.81 d	0.83 ab
Mean	0.83 a	0.77 b	

Means followed by the same letters are not statistically different at P<0.05

Zn treatments × water regimes interaction shown significant (P<0.05) differences in weight of grains per spike (Table 4.17). The interaction effect between Zn treatments and water regimes on the weight of grains per spike ranged from 0.80 to 0.85 g, and 0.74 to 0.81 g under well-watered and drought stress conditions respectively (Table 4.19). Among Zn treatments, the highest weight of grains per spike were 0.84, 0.83, and 0.81 g recorded by seed coating followed by seed coating+foliar spray and soil application, respectively (Table 4.19). The weight of grains per spike intends to ameliorate and enhance the grain yield, the same finding was obtained by Denčić et al. (2000) who described that the positive impact of weight of the grains per spike on the eventual wheat grain yield under optimal and drought conditions.

4.2.1.6. 1000 grains weight

1000 grains weight was significant except for varieties × water regimes (Table 4.20). In interaction effect of variety × water regime, 1000 grains weight was (31 and 25 g) and (33 and 27 g) under well-watered and deficit-watered for Imam and Altındane varieties respectively. In average deficit-watered treatment decreased the 1000 grains weight by 20.5% (Table 4.21).

Table 4.20. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on 1000 grains weight (g)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.020	0.99	0.3775
Varieties	1	1.192	113.32	<.0001
Zn treatments	7	1.762	23.92	<.0001
Varieties×Zn treatments	7	0.221	3.007	0.0087
Water regimes	1	8.942	849.71	<.0001
Varieties×water regimes	1	0.012	1.197	0.2780
Zn treatments×water regimes	7	0.244	3.32	0.0045
Varieties×Zn treatments×water regimes	7	0.394	5.36	0.0501
Error	62	0.65		
Total	95	13.44		

Zn application treatments significantly reduced 1000 grains weight when compared with control and hydropriming. The lowest 1000 grains weight (28 g) was recorded in the combination of coating+foliar whereas the control and hydropriming recorded the highest weight (31.5 g) (Table 4.22). Zn treatment (foliar spray) under field conditions did not show effect on 1000 grains weight of rice (Phuphong et al., 2018), this agree with our results.

Table 4.21. Means of Zn treatments, varieties and water regimes for 1000 grains weight (g)

Varieties (V)	Zn treatments	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	34.0	27.0	31.0
	Hydropriming	33.0	26.0	30.0
	Seed priming	31.0	24.0	27.0
	Seed coating	32.0	25.0	28.0
	Soil application	39.0	25.0	27.0
	Foliar spray	39.0	24.0	26.0
	Soil application + foliar spray	31.0	25.0	28.0
	Seed coating + foliar spray	27.0	24.0	25.0
	Mean V×W	31.0 C	25.0 D	28.0 B
Altındane	Untreated seeds	36.0	29.0	32.0
	Hydropriming	35.0	28.0	32.0
	Seed priming	33.0	27.0	30.0
	Seed coating	31.0	26.0	29.0
	Soil application	34.0	26.0	30.0
	Foliar spray	30.0	26.0	28.0
	Soil application + foliar spray	34.0	26.0	30.0
	Seed coating + foliar spray	33.0	26.0	29.0
	Mean V×W	33.0 A	27.0 B	30.0 A
	Mean W	32.0 a	26.0 b	

Means followed by the same letters are not statistically different at $P<0.05$. WW: well-watered, DS: drought stress

In present research, it was noted that Zn treatments under well-watered and drought stress conditions had no significant effect on 1000 grains weight, but drought stress had severe damage through reducing the 1000 grains weight by 23% compared to well-watered regime (Table 4.22). Previous research confirmed this results where that Zn fertilization via soil application (10 kg Zn/ha) did not show remarkable effect of 1000 grains weight of wheat under field condition experiment (Firdous et al., 2018). This results in accordance with who Arif et al. (2017) stated that Zn and potassium application improved the 1000 grains weight of wheat.

Table 4.22. Means of Zn treatments×water regimes interaction for 1000 grains weight (g)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	35.0 a	28.0 d	31.5 a
Hydropriming	35.0 a	28.0 d	31.5 a
Seed priming	32.0 b	26.0 e	29.0 b
Seed coating	32.0 b	26.0 e	29.0 b
Soil application	32.0 b	26.0 e	29.0 b
Foliar spray	29.0 c	25.0 e	27.0 c
Soil application+foliar spray	32.0 b	26.0 e	29.0 b
Seed coating+foliar spray	30.0 c	26.0 e	28.0 c
Mean	32.0 a	26.0 b	

Means followed by the same letters are not statistically different at $P<0.05$

4.2.1.7. Biological yield

Biological yield was statistically (P≥0.5) affected by main effect of Zn application, variety, water regime and only by three an interaction effect of variety × Zn application × water regime, but not for two interaction effects (Table 4.23).

However, Zn seed priming have shown slightly improving in biological yield (41.5 g) in comparison with hydropriming (41 g) but, there was no statistically difference (Table 4.24). A report of a meta-analysis revealed that wheat yield and biomass decreased by 21, 25.8 and 32%, and by 11, 21 and 34.7% under mild, moderate and severe drought stress (Zhang et al., 2018).

Table 4.23. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on biological yield (g/pot)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	3.77	1.21	0.28
Varieties	1	130.7	87.7	<.0001
Zn treatments	7	120.8	11.6	<.0001
Varieties×Zn treatments	7	11.80	1.1	0.3529
Water regimes	1	135.4	90.9	<.0001
Varieties×water regimes	1	54.00	36.3	<.0001
Zn treatments×water regimes	7	7.80	0.7	0.633
Varieties×Zn treatments×water regimes	7	45.20	4.3	0.0006
Error	62	91.56		
Total	95	600.90		

Table 4.24. Means of Zn treatments, varieties and water regimes for biological yield (g/pot)

Varieties (V)	Zn treatments	Watering regimes (W)		Mean V×Zn
		WW	DS	
Imam	Untreated seeds	42.7 efg	41.3 fghi	42.0
	Hydropriming	42.3 efgh	39.7 i	41.0
	Seed priming	43.3 de	39.7 i	42.0
	Seed coating	46.3 abc	41.3 fghi	43.8
	Soil application	4.07 ab	41.0 ghi	44.0
	Foliar spray	44.7 cd	40.7 hi	42.7
	Soil application + foliar spray	46.0 bc	41.3 fghi	43.7
	Seed coating + foliar spray	47.0 ab	4.03 def	44.8
	Mean V×W	44.8 B	41.0 C	42.9 B
Altındane	Untreated seeds	45.3 bc	42.3 e-h	43.8
	Hydropriming	44.7 cd	43.3 de	44.0
	Seed priming	44.7 cd	43.3 de	44.0
	Seed coating	45.7 bc	47.0 ab	46.2
	Soil application	44.7 cd	46.0 bc	45.3
	Foliar spray	48.0 a	44.7cd	46.3
	Soil application + foliar spray	46.3 abc	45.7 bc	46.0
	Seed coating + foliar spray	46.3 abc	46.7 ab	46.5
	Mean V×W	45.7 A	44.8 B	45.2 A
	Mean W	45.2 a	42.9 b	

Means followed by the same letters are not statistically different at P<0.05. WW: well-watered, DS: drought stress

The results of this report agree with our finding in this study where water deficit caused reduction of biological yield by 9.4% in compared with well-watered treatment. The reason could be due to indirect effect of drought through decrease biological yield or direct effect via impairing pollen grains during reproductive stage. Across treatments, biological yield increased under both of water regime condition with high increase of (10 and 4%) in combination of coating+foliar application in both well-watered and deficit-watered regimes over control, respectively (Table 4.25).

As revealed in Table 4.25 the main effect of Zn treatments showed notable effect on biological yield. Where, HP recorded the lowest biological yield 42.5 g among all treatments, whereas seed coating combined with foliar spray obtained the highest biological yield 45.7 g (Table 4.25). Similar results were reported that the combine application of sees application and foliar spray of Zn sulphate at stem elongation and early grain filling stages of wheat had positive effects and increased the biomass under Zn deficient calcareous soil (Abdoli et al., 2016). An anther research indicated that the combination Zinc with Boron produced the higher biological yield compared to using the nutrients separately (Wasaya et al., 2017).

Table 4.25. Means of Zn treatments × water regimes interaction for biological yield (g/pot)

Zn treatments	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	44.0	41.8	42.9 bc
Hydropriming	43.5	41.5	42.5 c
Seed priming	44.0	41.5	42.7 c
Seed coating	46.0	44.0	45.0 a
Soil application	45.8	43.5	44.7 a
Foliar spray	46.3	42.7	44.5 ab
Soil application+foliar spray	46.1	43.5	44.8 a
Seed coating+foliar spray	46.5	44.8	45.7 a
Mean	45.2 a	42.9 b	

Means followed by the same letters are not statistically different at $P<0.05$

4.2.1.8. Harvest index (HI)

Harvest index (HI) is crucial factor in determining yield production of wheat. HI was significantly affected by variety × water regime but, not by Zn application × water regime (Table 4.26). Where, Altındane variety had the higher HI (38.0%) under well-watered condition, while under deficit-watered regime Imam variety (37.6%) was higher harvest index (37.0%) than Altındane (35.6%) (Table 4.27).

Table 4.26. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on harvest index (%)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.26	0.1	0.901
Varieties	1	6.3	5.1	0.0271
Zn treatments	7	53.4	6.2	<.0001
Varieties×Zn treatments	7	16.7	1.9	0.0799
Water regimes	1	52.6	42.5	<.0001
Varieties×water regimes	1	19.8	16.0	0.0002
Zn treatments×water regimes	7	17.4	2.0	0.0677
Varieties×Zn treatments×water regimes	7	9.2	1.1	0.3988
Error	62	78.8		
Total	95	254.4		

Table 4.27. Means of Zn treatments, varieties and water regimes for harvest index (%)

Varieties (V)	Zn treatments	Watering regimes (W)		Mean V×Zn
		WW	DS	
Imam	Untreated seeds	37.4	35.5	36.4
	Hydropriming	37.5	35.3	36.4
	Seed priming	37.5	35.9	36.7
	Seed coating	38.2	39.5	38.9
	Soil application	37.5	37.4	37.4
	Foliar spray	37.6	36.9	37.3
	Soil application + foliar spray	36.9	37.9	37.4
	Seed coating + foliar spray	37.9	37.5	37.7
	Mean V×W	37.6 AB	37.0 B	37.2 A
Altındane	Untreated seeds	37.4	34.6	36.0
	Hydropriming	37.0	35.0	36.0
	Seed priming	37.0	33.9	35.5
	Seed coating	37.6	36.0	36.8
	Soil application	39.5	35.7	37.6
	Foliar spray	37.1	35.5	36.3
	Soil application + foliar spray	38.1	36.7	37.4
	Seed coating + foliar spray	40.1	36.8	38.4
	Mean V×W	38.0 A	35.6 C	36.7 B
	Mean W	37.7 a	36.2 b	

Means followed by the same letters are not statistically different at P<0.05. WW: well-watered, DS: drought stress

The interaction effect of Zn treatments and water regimes had non-significant effect on HI (Table 4.26). Under well-watered regimes seed coating+foliar spray recorded the highest HI by 39%, whereas the treatment of seed coating obtained the highest HI by 37.7% under drought stress. All Zn treatments were improved HI compared to untreated seeds, whilst Zn seed priming did not show such positive impact and recorded the lower HI compared to HP (Table 4.28).

Table 4.28. Means of Zn treatments × water regimes interaction for harvest index (%)

Zn treatments	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	37.3	35.0	36.2 c
Hydropriming	37.2	35.1	36.2 c
Seed priming	37.2	34.9	36.0 c
Seed coating	37.9	37.7	37.8 a
Soil application	38.4	36.5	37.4 ab
Foliar spray	37.3	36.2	36.7 bc
Soil application+foliar spray	37.5	37.3	37.4 ab
Seed coating+foliar spray	39.0	37.1	38.0 a
Mean	37.7 a	36.2 b	

Means followed by the same letters are not statistically different at $P<0.05$

Such results are in accordance with finding of Potarzycki and Grzebisz (2009) who demonstrated that foliar Zn spray on maize induced reduction of HI as compared to control treatment.

Overall, the highest and lowest HI were achieved by seed coating and control treatments, respectively (Table 4.28). Drought stress significantly affected and minimized HI by 6% rather than well-watered conditions. The important of HI into grains yield was identified in earlier study carried out in Mediterranean zone in Turkey and Syria by (Kobata et al., 2018). Also, it has been reported that seed primed with Zn improved harvest index of wheat grown under plough and Zero tillage systems (Zulfiqar et al., 2020). That might be to the crucial role of Zn in the early establishment of seedling growth through improving the radicle and coleoptile.

4.2.1.9. Grain yield (g/pot)

At the end of this century, the world population is expected to reach more than 9 billion. In order to fulfill this increasing of growing people globally, the annual wheat production has also to been increased. But, there are several factors that limit and obstacle this enhancing and extending of wheat production. Two of the major those factors are drought stress and Zn deficiency. They are the main restricting environmental factors to successful wheat production, especially in arid and semi-arid regions.

Analysis of variance indicated a significant difference among means of main effects (Zn application, variety and water regime) but not between means of combined effects (interaction effect) for grain yield (Table 4.29).

Table 4.29. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on grain yield (g/pot)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.36	0.41	0.6600
Varieties	1	9.69	22.45	<.0001
Zn treatments	7	50.8	16.84	<.0001
Varieties×Zn treatments	7	2.67	0.88	0.5236
Water regimes	1	57.19	132.5	<.0001
Varieties×water regimes	1	0.52	1.217	0.2741
Zn treatments×water regimes	7	3.03	1.004	0.4367
Varieties×Zn treatments×water regimes	7	2.31	0.767	0.6167
Error	62	27.24		
Total	95	153.9		

Compared with well-watered treatment, deficit-watered reduced grain yield by 10%. An interaction effect (variety × water regime) the grain yields due to water deficit treatment diminished by 10 and 8% in Imam and Altındane varieties respectively. Under well-watered condition grain yield varied substantially from 14 g/pot (hydropriming) to 16.3 g/pot (coating treatment) in Imam variety and from 14.7 g/pot (control) to 17.2 g/pot (coating+foliar treatment) in Altındane variety (Table 4.30).

Table 4.30. Means of Zn treatments, varieties and water regimes for grain yield (g/pot)

Varieties (V)	Zn treatments	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	15.9	14.7	15.3
	Hydropriming	15.9	14.0	15.0
	Seed priming	16.3	14.0	15.3
	Seed coating	17.7	16.3	17.0
	Soil application	17.6	15.3	16.5
	Foliar spray	16.8	15.0	15.9
	Soil application+foliar spray	17.0	15.7	16.3
	Seed coating+foliar spray	17.7	16.1	16.9
	Mean V×W	16.9	15.2	16.0 B
Altındane	Untreated seeds	16.9	14.7	15.8
	Hydropriming	16.5	15.2	15.9
	Seed priming	16.5	14.7	15.6
	Seed coating	17.2	16.8	17.0
	Soil application	17.6	16.4	17.0
	Foliar spray	17.8	15.9	16.8
	Soil application+foliar spray	17.6	16.8	17.2
	Seed coating+foliar spray	18.6	17.2	17.9
	Mean V×W	17.4	16.0	16.6 A
	Mean W	17.2 A	15.5 B	

Means followed by the same letters are not statistically different at $P<0.05$. WW: well-watered, DS: drought stress

Drought stress caused reduction of wheat yield by 20-30% (Daryanto et al., 2016; Zhang et al., 2018). Previous study was revealed by Balla et al. (2011) who found that water deficit is able to induce reduction of wheat production reach to 57%.

In this work, the loss of grains wheat yield because of drought stress reached up to 8% in Imam and 15% in Altındane comparison with well-watered yield for both test varieties (Table 4.31). But, Zn application through seed coating and combination of seed coating with foliar spray improved the yield under drought stress by 10.8 and 9.5% in Imam, and by 14 and 17% in Altındane, respectively. Similar finding was also confirmed by Gomez-Coronado et al. (2016) who reported that Zn soil application and combination of soil with foliar spray improved grain yield by 10 and 7% respectively under Mediterranean conditions. The major reasons of yield reduction in resulting effect of drought on yield components such as plant height, number of grain per spike, biological yield etc.

Table 4.31. Means of Zn treatments×water regimes interaction for grain yield (g/pot)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	16.4	14.6	15.5 d
Hydropriming	16.2	14.5	15.4 d
Seed priming	16.4	14.4	15.4 d
Seed coating	17.4	16.5	17.0 ab
Soil application	17.6	15.8	16.7 bc
Foliar spray	17.3	15.4	16.3 c
Soil application+foliar spray	17.3	16.2	16.7 bc
Seed coating+foliar spray	18.1	16.6	17.3 a
Mean	17.2 a	15.5 b	

Means followed by the same letters are not statistically different at P<0.05

The data regarding effect of Zn treatments on grain yield has shown highly significant (Table 4.29). Where, the least grain yield was observed when no Zn treatments were applied (untreated seeds and HP), whereas the highest grain yield was observed by the combination of seed coating with foliar spray (Table 4.31). The role of Zn as required and necessary particularly, under soil Zn deficient was recorded in different crops such as wheat (Tao et al., 2018), maize (Liu et al., 2016), rice and common bean (Rashid et al., 2019) under various and Zn treatments conditions. On the other hand, seed priming in this study did not display significant differences as compared to HP (Table 4.31). Seed with high Zn content enhanced wheat grain yield by 12 to 21% under drought stress compared to seed with medium and less Zn content (Faran et al., 2019). This reflects the crucial of Zn and its effect on the crops production, especially these corps cultivated under low water content with Zn deficient soil.

4.2.2.10. Correlation among grain yield and its attributes

Under well-watered condition grain yield have shown strongly positive linear relationship with biological yield, harvest index, No. of spikelet/spike, and weigh of grains/spike (r= 0.81, 0.72. 0.69 and 0.60, respectively) However, there was no significant correlation between other parameters and grain yield (Figure 4.4).

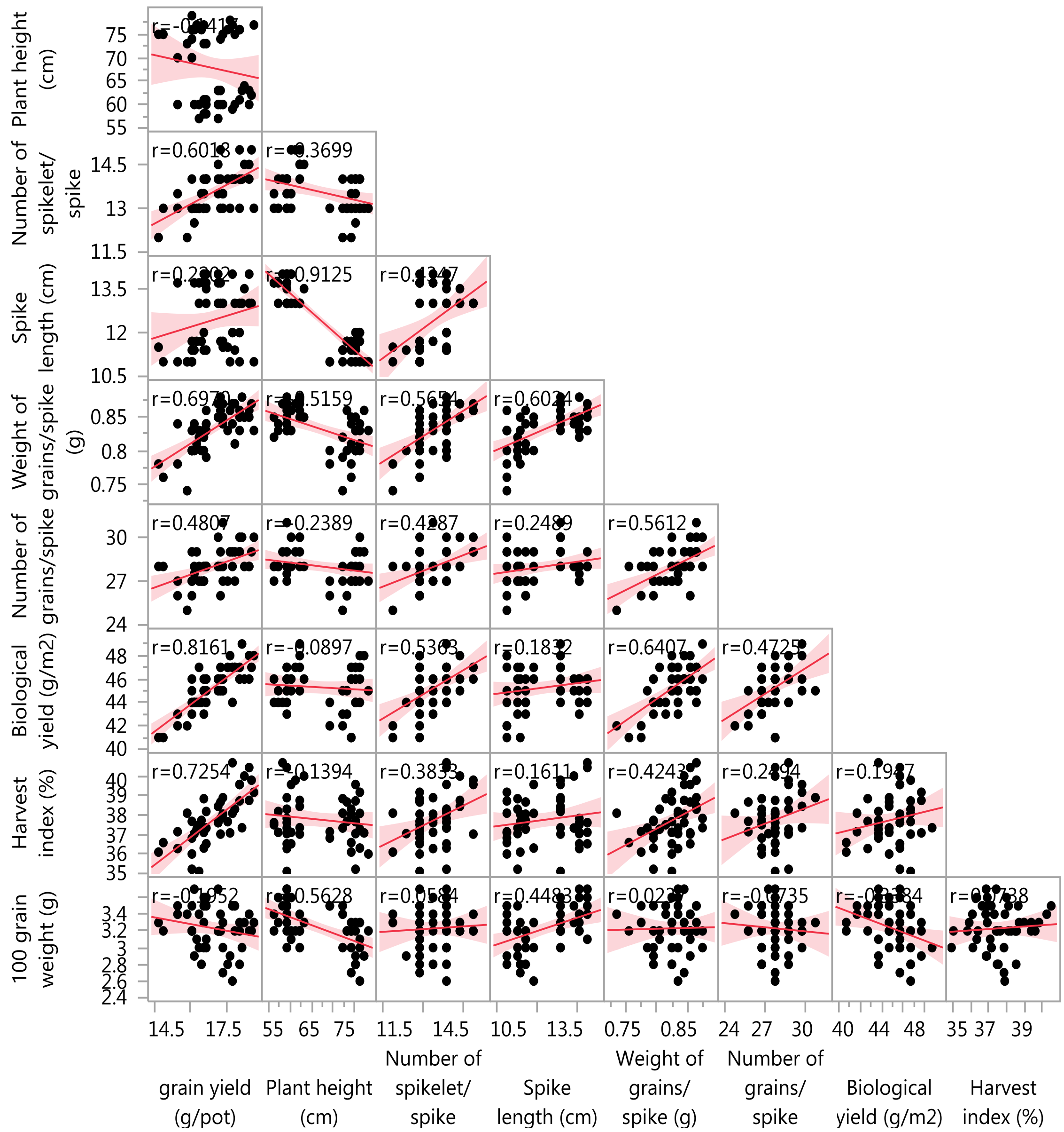


Figure 4.4. Correlations between grain yield and its components under well-watered conditions

Several authors also revealed such positive correlation between grain yield of wheat and aboveground biomass and harvest index (Bogale and Tesfaye, 2016) and weight of grain per spike (Leilah and Al-Khateeb, 2005). This positive relationship among grain yield and biological yield due to availability of assimilates to the grain via weight dry re-allocation (Shakhatreh et al., 2001).

Whereas, in deficit-watered treatment, negative correlation was noticed in plant height with spike length and weight of grain/spike. However, biological yield, No. of grains/spike and weight of grains/spike had positive relationship with grain yield (Figure 4.5).

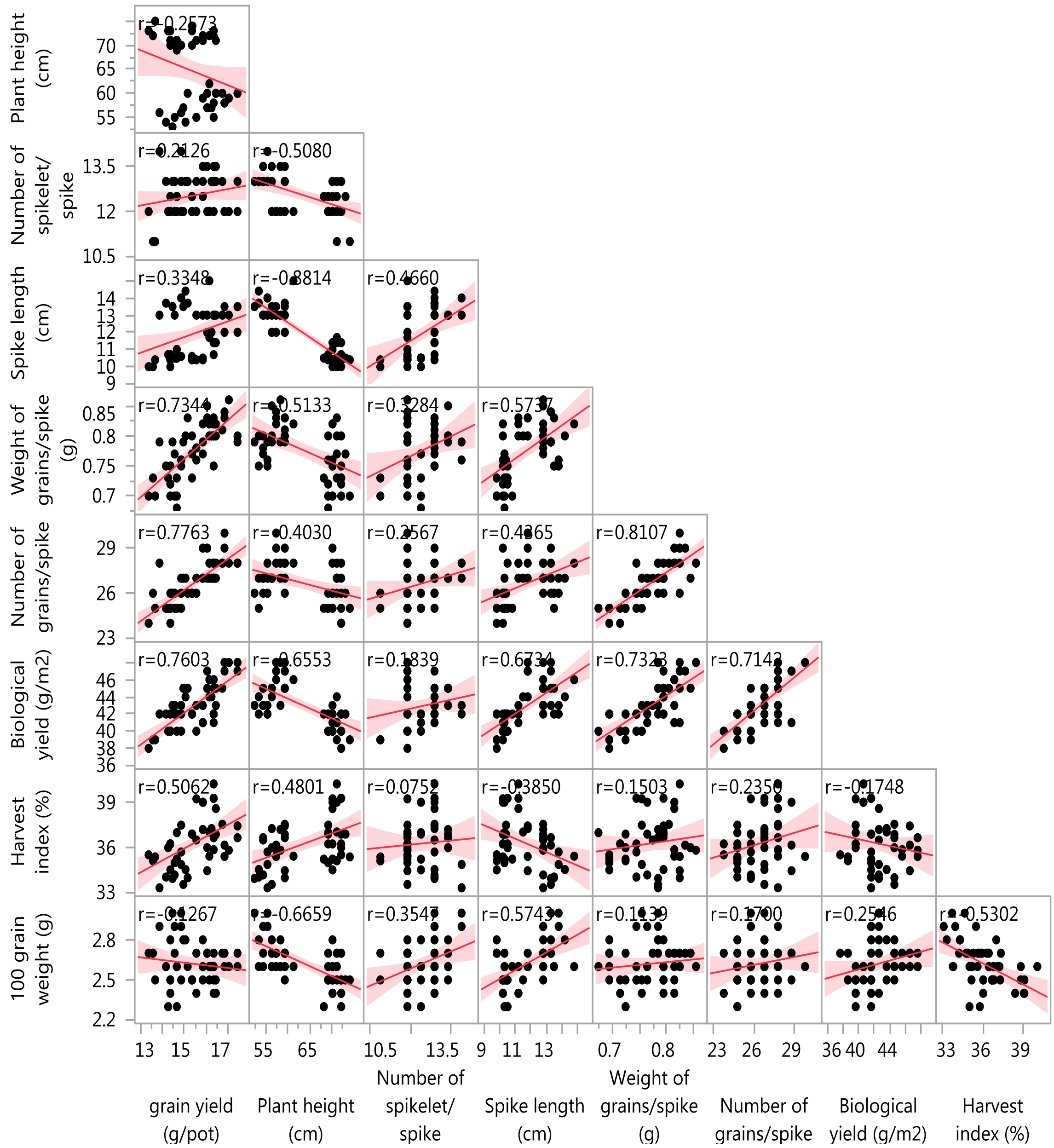


Figure 4.5. Correlations between grain yield and its components under drought stress conditions

4.2.2. Physiological Parameters

Yield is primarily having complex interaction and correlation with wide range of physiological processes such as photosynthesis, chlorophyll content, water use efficiency etc. And most of these processes are adversely influence by the water deficit. Among the important of these processes is photosynthesis which is negatively limited by drought stress. Chlorophyll is considered as main chloroplasts for synthesis photosynthesis. Therefore any change of chlorophyll content due to water stress could have negative impact on photosynthesis and ultimately, production.

4.2.2.1. Flag leaf area (LA)

Flag leaf area (LA) significantly affected by all main effects (Zn treatments, varieties and water regimes) and combined effects (varieties × Zn treatments, Zn application×water regimes and varieties × Zn treatments × water regimes) except of variety × water regimes interaction (Table 4.32 and 4.33).

Table 4.32. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on flag leaf area (cm^2)

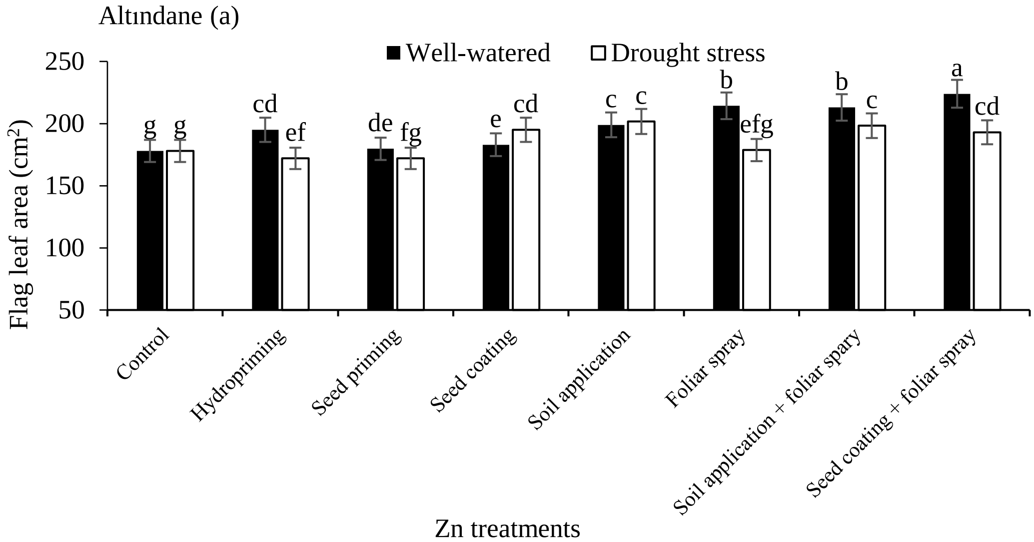
Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	118.27	2.61	0.081
Varieties	1	237407.04	9982.9	<.0001
Zn treatments	7	8921.67	53.59	<.0001
Varieties×Zn treatments	7	2800.62	16.82	<.0001
Water regimes	1	1204.17	50.63	<.0001
Varieties×water regimes	1	9.37	0.39	0.5323
Zn treatments×water regimes	7	3896.83	23.40	<.0001
Varieties×Zn treatments×water regimes	7	1777.63	10.67	<.0001
Error	62	1402.40		
Total	95	257670		

In Altındane variety, the biggest LA was resulted in by seed coating+foliar spray (224 cm^2) under well-watered condition, while the lowest was obtained by control treatment in well and deficit-watered regime (178 cm^2). As shown in Figure 4.6a, seed coating and soil application had not affected by water deficit condition because they had relatively the same value of LA that recorded under well-watered condition. Furthermore, in Imam variety, LA was ranged from 77 cm^2 (control) to 120 cm^2 (foliar spray) in well-watered condition and from 63 cm^2 (control) to 120 cm^2 (soil application+foliar spray) under deficit-watered condition (Figure 4.6b).

Table 4.33. Means of Zn treatments × water regimes interaction for flag leaf area (cm^2)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	123.5 g	121.8 g	122.7 e
Hydropriming	140.8 d	133.8 ef	137.3 d
Seed priming	138.3 de	132.8 f	153.5 d
Seed coating	146.5 c	147.5 c	147.0 c
Soil application	149.5 bc	147.1 c	148.3 bc
Foliar spray	167.4 a	137.8 def	152.5 a
Soil application+foliar spray	154.2 b	149.1 bc	151.7 ab
Seed coating+foliar spray	162.5 a	137.3 def	150.0 abc
Water regimes mean	147.8 a	138.4 b	
	Imam	Altındane	
Varieties mean	93.6 b	192.6 a	

Means followed by the same letters are not statistically different at P<0.05



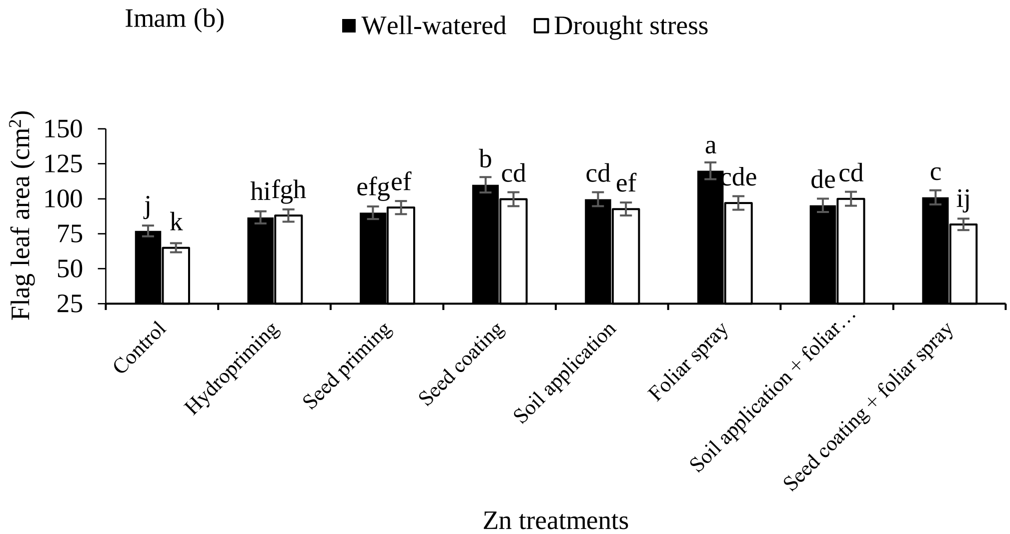


Figure 4.6. The effects of Zn treatments × water regimes interaction on flag leaf area (LA) a) Altındane and b) Imam varieties. The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

Across varieties Altındane have shown wide LA over Imam. However, leaf area index also reduced and affected by drought stress in this study, Altındane shown bigger leaf are index than Imam, which could have impact on the yield. Previously study was reported that varieties with a large LA have ability to higher water uptake as well as absorb more light which important in photosynthesis process (Ahmad et al., 2015). Wasaya et al. (2017) demonstrated that the combine effect of Zn and B was significant on LA of maize when applied as foliar spray under rainfed conditions. Further, the effect of drought stress on LA for Altındane did not indicate to significant differences as compared to well-watered regime, where in case of Imam, it shown notable effect and reduced the LA by 18% in comparison with well-watered conditions (Figure 4.6). Such results well agreement with these observation reported by Karademir et al. (2012) and Mohammadian et al. (2005) who shown that LA was decreased by 30% in cotton and 14.1% in sugar beet crops, respectively.

4.2.2.2. Chlorophyll content

Zn treatments, water regimes and variety × Zn treatments interaction on chlorophyll content (SPAD) of flag leaves at booting stage (15 days after drought imposed) was significant (Table 4.34).

Table 4.34. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on chlorophyll content at booting stage

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	1.27	0.69	0.503
Varieties	1	2.7	2.94	0.0911
Zn treatments	7	38.0	5.99	<.0001
Variety×Zn treatments	7	14.5	2.29	0.0383
Water regimes	1	80.7	89.01	<.0001
Varieties×water regimes	1	2.7	2.94	0.0911
Zn treatments×water regimes	7	5.2	0.81	0.5787
Varieties×Zn treatments×water regimes	7	12.2	1.92	0.0811
Error	62	56.72		
Total	95	213.83		

Across of treatments, seed coating and seed coating+foliar spray with Zn had improved chlorophyll content when compared with control at booting stage. Also, seed priming with Zn have demonstrated improving in chlorophyll content than hydropriming treatment (Figure 4.7). However, in case of chlorophyll contents which have taken at grain filling stage (21 days after drought imposed), the interaction effect (Zn applications × variety × water regimes) shown significant impact on chlorophyll content of flag leaves at grain filling stage (Table 4.35), where seed coating with Zn in Imam variety have recorded the highest chlorophyll content on the flag leaves and improved chlorophyll content by 8.5% than control (Figure 4.8). A study by Ma et al. (2017) has been observed that moderate and severe drought stress consisted for 20 days reduced the value of chlorophyll content of wheat varieties by 6.5 and 37.3%, respectively. Also in this research, the chlorophyll content of our wheat varieties at booting stage (15 days after drought imposed) and grain filling stage (21 days after drought imposed) decreased by 5 and 10% due to drought stress respectively. Variety has no any effect on chlorophyll content at both of growth stages.

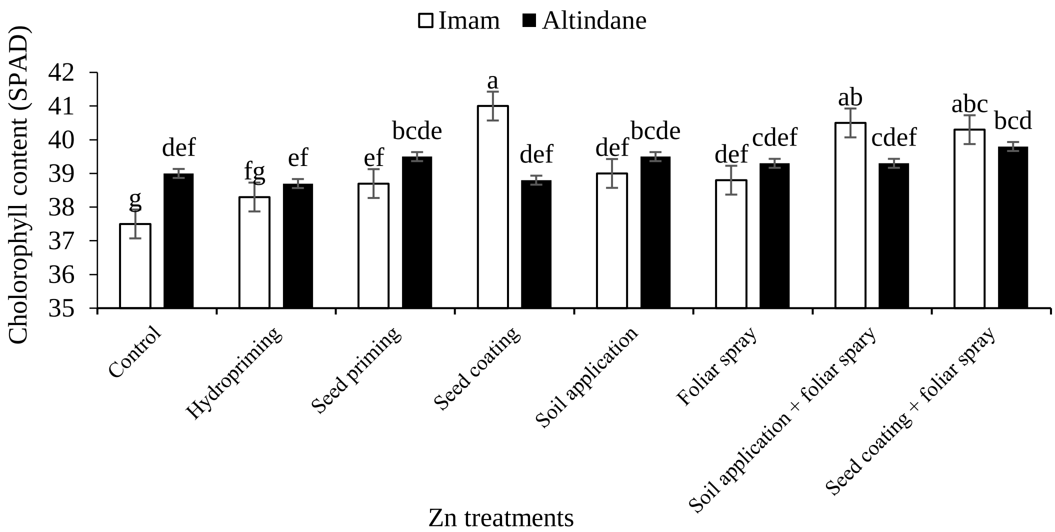


Figure 4.7. The effects of Zn treatments × varieties interaction on chlorophyll content (SPAD) at booting stage. The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

Table 4.35. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on chlorophyll content (SPAD) at grain filling stage

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	2.77	1.59	0.2114
Varieties	1	0.01	0.012	0.9132
Zn treatments	7	38.40	6.31	<.0001
Variety×Zn treatments	7	29.57	4.86	0.0002
Water regimes	1	184.26	211.96	<.0001
Varieties×water regimes	1	31.51	36.24	<.0001
Zn treatments×water regimes	7	29.65	4.87	0.0002
Varieties×Zn treatments×water regimes	7	16.40	2.69	0.0166
Error	62	53.90		
Total	95	386.48		

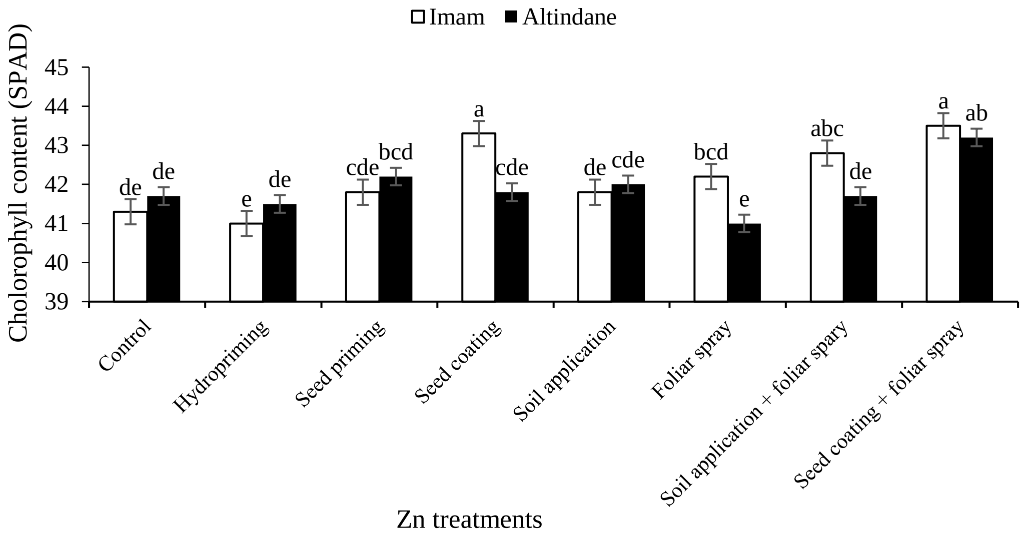


Figure 4.8. The effects of Zn treatments×varieties interaction on chlorophyll content (SPAD) at grain filling stage. The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

Yield has crucial relationship with many physiological processes such as photosynthesis and chlorophyll pigments, and any damage and deleterious effects on such processes would to induce adverse alteration in the plant growth and ultimately yield (Mohammed and Pekşen, 2020). In regard of chlorophyll content which obtained in the grain filling stage, the main effect of Zn treatments and water regimes revealed notable influence on chlorophyll content, however, varieties did not show difference (Table 4.36).

Table 4.36. Means of Zn treatments×water regimes interaction for chlorophyll content (SPAD) at grain filling stage

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	42.5	40.5	41.5 cd
Hydropriming	41.6	40.8	41.2 d
Seed priming	42.8	41.0	42.0 bcd
Seed coating	43.5	41.6	42.5 ab
Soil application	42.8	41.0	42.0 bcd
Foliar spray	42.5	40.6	41.5 cd
Soil application+foliar spray	43.2	41.3	42.2 bc
Seed coating+foliar spray	44.7	42.0	43.3 a
Water regimes mean	43.0 a	41.0 b	
	Imam	Altındane	
Varieties mean	42.2	41.8	

Means followed by the same letters are not statistically different at P<0.05

Overall, Zn treatments, the combination of seed coating+foliar spray compared to control and other Zn treatments was achieved the highest chlorophyll content for Imam and Altındane varieties (Figure 4.8). Likewise, the positive effect of Zn on the chlorophyll content was indicated in several results described by Liu et al. (2016) and Wang et al. (2009) in maize, Samreen et al. (2017) in mung beans, and Kandoliya et al. (2018) in wheat. Zn applied through soil application was played significant role in an enhanced photosynthesis rate through improving stomatal conductance and chlorophyll rate in maize (Liu et al., 2016). Stomatal conductance and transpiration rate parameters were reduced in cowpea crop under drought stress, however, the application of Zn mitigated this damage throughout enrichment these parameters (Dehnavi and Sheshbahre, 2017) which ultimately improve the chlorophyll content.

4.2.2.3. Membrane stability index (MSI)

It is known abiotic stresses such as drought stress injury and damage MSI, and this last widely uses as indicator for screening and evaluating genotypes and varieties for their drought resistance. In present study, MSI statistically affected only by all main effects and variety×water regime an interaction effect (Table 4.37). Water stress negatively influenced MSI of both two varieties in comparison with well-watered. The reduction of MSI was obtained by Rakhra et al. (2015) who determined that tolerant cultivar of wheat had more MSI compared to sensitive cultivar during post anthesis stage.

Table 4.37. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on membrane stability index

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	1.93	0.40	0.066
Varieties	1	610.04	259.13	<.0001
Zn treatments	7	66.46	4.03	0.001
Variety×Zn treatments	7	11.46	0.70	0.6757
Water regimes	1	1066.67	453.10	<.0001
Varieties×water regimes	1	130.67	55.50	<.0001
Zn treatments×water regimes	7	22.50	1.37	0.2354
Varieties×Zn treatments×water regimes	7	11.17	0.68	0.6903
Error	62	148.7		
Total	95	2070		

According to these results, Imam variety had higher MSI (72.5 and 63.5%) than Altındane variety (65 and 60%) under well-watered and deficit-watered condition, respectively (Figure 4.9). There was substantial decrease in MSI value due to drought stress conditions, particularly for Altındane variety. The reduction of MSI may be attribute to closure of stomatal conductance during the abiotic stresses like severe drought stress.

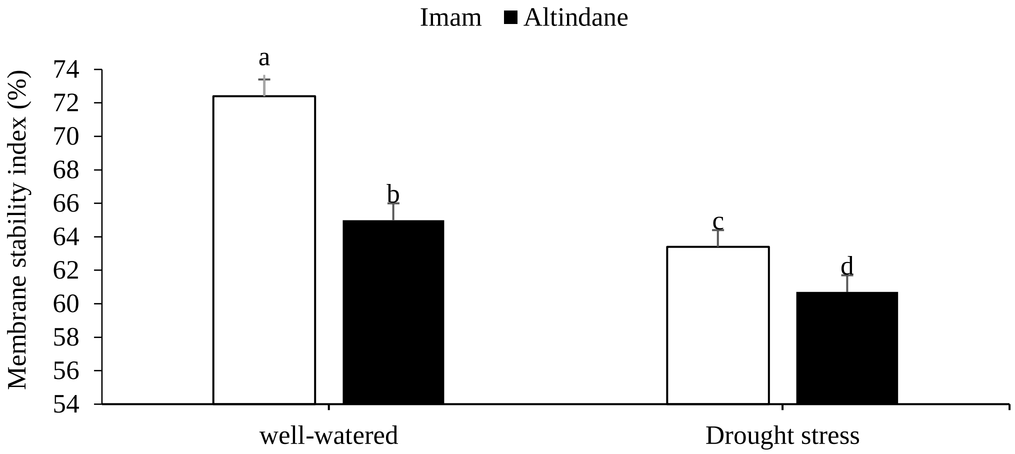


Figure 4.9. The effect of varieties×water regimes interaction on membrane stability index. The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

The effect on leaf cell membrane stability index resulted in drought stress was more pronounced, where it reduced MSI by 14 and 8% for Imam and Altındane, respectively. According to data revealed in Table 4.38, Zn treatments was showed relatively remarkable effect on MSI. This ameliorating and enhancing due to applying micronutrient such as Zn could be return to the role of these micronutrients to accelerate the chlorophyll synthesis of plant (Tufail et al., 2018).

Table 4.38. Means of Zn treatments×water regimes interaction for membrane stability index (%)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	68.7	60.4	64.5 b
Hydropriming	68.0	61.0	64.5 b
Seed priming	68.5	61.7	65.0 b
Seed coating	70.2	63.4	66.7 a
Soil application	67.8	61.8	64.8 b
Foliar spray	69.0	64.4	66.7 a
Soil application+foliar spray	68.8	62.2	65.7 ab
Seed coating+foliar spray	69.2	62.2	65.5 ab
Water regimes mean	68.7 a	62.1 b	
	Imam	Altındane	
Varieties mean	68.0 a	63.0 b	

Means followed by the same letters are not statistically different at P<0.05

4.2.2.4. Relative water content (RWC)

There was no significant difference effect of Zn treatments × variety × water regime an interaction on RWC (Table 4.39). Moreover, the interaction of Zn treatments × water regime revealed that deficit-watered treatment reduced RWC in comparison with well-watered although of Zn application, but this reduction due to water deficit not effected when seed primed and seed coated with Zn have been applied (Figure 4.10).

Table 4.39. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on relative water content (%)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	4.93	0.89	0.412
Varieties	1	2.04	0.75	0.3912
Zn treatments	7	141.63	7.39	<.0001
Variety×Zn treatments	7	54.63	2.85	0.0119
Water regimes	1	35.04	12.79	0.0007
Varieties×water regimes	1	26.04	9.51	0.003
Zn treatments×water regimes	7	72.63	3.79	0.0017
Varieties×Zn treatments×water regimes	7	28.29	1.48	0.1921
Error	62	170.30		
Total	95	535.60		

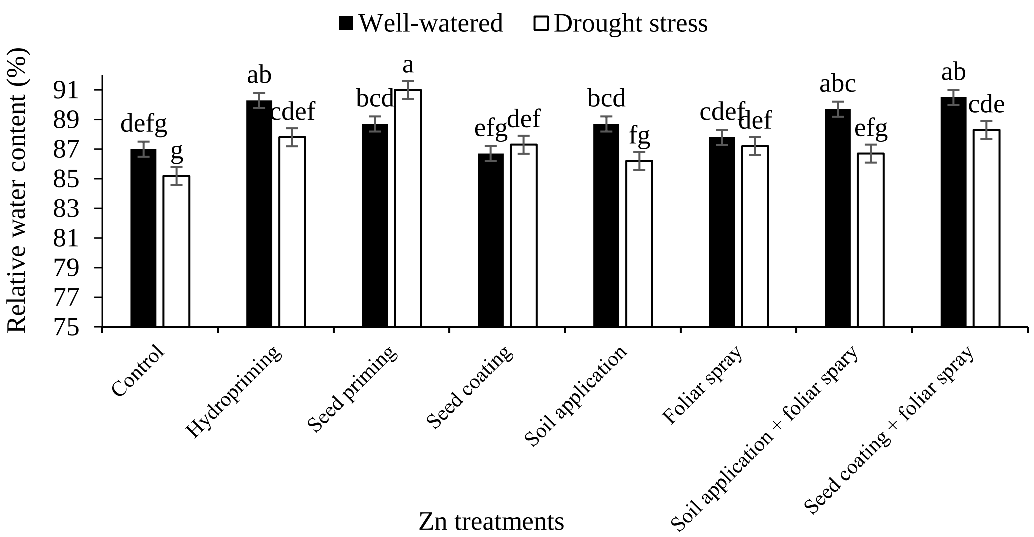


Figure 4.10. The effects of Zn treatments×water regimes interaction on relative water content (RWC). The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

The reason of drought stress impact on RWC might return to loss turgor during exposure to drought stress and this could lead to appearance of wilting symptoms on the crops. Screening of RWC as tool and indictor for drought tolerance in wheat has observed previously in researches reported by (Larbi and Mekliche, 2004; Sofy, 2015).

As shown in Figure 4.10 drought stress was slightly reduced relative water content in control treatment as compared to control of well-watered regime. Farooq et al. (2009) declared that the reduction of RWC is the primary signs and indicator to the drought stress. However, application of Zn particularly, seed priming treatment alleviated the effect of drought stress through enhanced RWC as compared with HP, control and others Zn treatments (Figure 4.10). The application of Zn under drought stress ameliorated the antioxidant activities which in return enhances the stability of membrane and ultimately induces increasing in RWC (Umair Hassan et al., 2020). In regarded of well-watered regime, the combination of seed coting+foliar spray treatment recorded the highest RWC as compared with others Zn treatments strategies. In general, drought stress minimized RWC for HP, control, and all Zn treatments except the seed priming treatment (Figure 4.19).

4.2.2.5. Leaf water potential (Ψw)

Leaf water potential ($\Psi_{w)}$ had shown significant ($P \geq 0.05$) effects by Zn application × water regime and variety × Zn application interaction effects (Table 4.40).

Table 4.40. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on leaf water potential (MPa)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.0056	0.31	0.72
Varieties	1	1.450	166.75	<.0001
Zn treatments	7	0.570	9.36	<.0001
Variety×Zn treatments	7	0.203	3.34	0.0043
Water regimes	1	0.602	69.17	<.0001
Varieties×water regimes	1	0.000	0.000	1
Zn treatments×water regimes	7	0.167	2.74	0.0148
Varieties×Zn treatments×water regimes	7	0.125	2.060	0.0609
Error	62	0.55		
Total	95	3.67		

Drought stress increased Ψw in an untreated seeds and HP treatments by 12 and 10% compared to well-watered condition respectively. All Zn treatments have enhanced Ψw in drought stress than well-watered condition. Among Zn treatments seed coating+foliar recorded the lowest Ψw by 1.32 followed by soil application+foliar spray by 1.47 MPa (Figure 4.11).

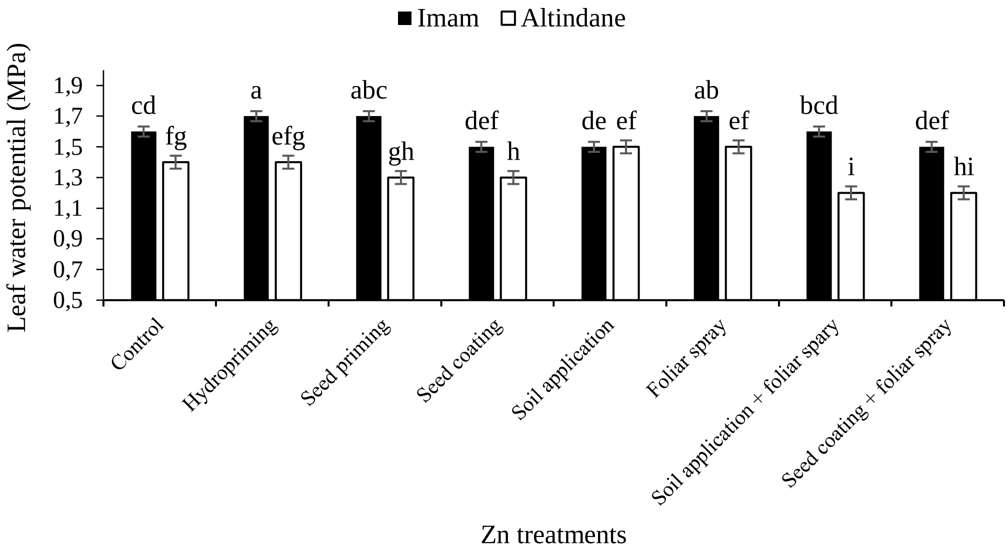


Figure 4.11. The effect of Zn treatments × varieties interaction on Ψw. The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

This reflect the role of Zn to decreasing Ψw under drought stress and such could be sign to improve drought tolerance through Zn treatments. Among varieties, Imam has recorded the lowest Ψw (1.48 MPa) by seed coating and foliar spray application and the highest was in hydropriming treatment (1.70 MPa), whereas in Altındane variety the lowest Ψw was obtained by seed coating with foliar spray(1.5 MPa), and the highest observed in foliar spray treatment (1.2 MPa). In general, Imam variety had higher Ψw than Altındane (Figure 4.12; Table 4.41).

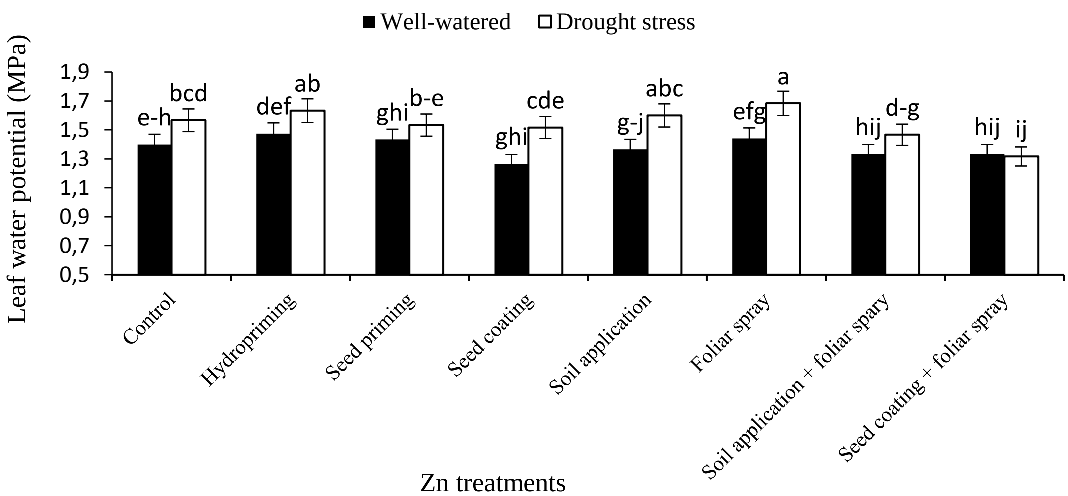


Figure 4.12. The effects of Zn treatments × water regimes interaction on Ψw. The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

Table 4.41. Means of Zn treatments × water regimes interaction for leaf water potential (MPa)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	1.40 f-i	1.56 bcd	1.48 b
Hydropriming	1.47 def	1.63 ab	1.55 ab
Seed priming	1.43 e-h	1.53 b-e	1.48 b
Seed coating	1.26 j	1.51 cde	1.39 c
Soil application	1.36 g-j	1.60 abc	1.48 b
Foliar spray	1.44 efg	1.68 a	1.56 a
Soil application+foliar spray	1.33 hij	1.46 d-g	1.40 c
Seed coating+foliar spray	1.33 hij	1.31 ij	1.32 c
Water regimes mean	1.3 b	1.5 a	
	Imam	Altındane	
Varieties mean	1.6 a	1.3 b	

Means followed by the same letters are not statistically different at $P<0.05$

In the present study, water relation such as RWC and leaf water potential (Ψ_w) reduced in response to drought stress. Similar trend was observed by Guóth et al. (2009) and Praba et al. (2009) for effect of water stress on RWC and leaf water potential (Ψ_w), respectively. Leaf water potential is one of the crucial water relation traits that using as main indicator in identifies the drought stress in the crop leaves. As shown in Figure 4.11, Altındane had the low negative (higher) value of Ψw that revealed its low dehydrated leaf rather than Imam. Thus, the high negative value shows lower Ψw in the leaves (Parkash and Singh, 2020). In present study, the combination of seed coating+foliar and soil application+foliar spray treatments enhanced the value of Ψw by 19 and 7%, respectively, under drought stress (Figure 2.12). This reflects that Zn must be in adequate rate in the soil with low water content. Similar study as accordance with our study reported that Ψw of maize was significantly decreased with low level of Zn rate, however, when Zn was added to moderate level as $ZnSO_4.7H_2O$ that lead to increased Ψw by 30% compared to non-Zn treatment under water stress conditions (Zhang et al., 2020b). The decreasing of Ψw under drought stress was indicated in the previous researches for various crops (Ashraf and O'Leary, 1996; Chowdhury et al., 2017).

4.2.2.6. Water use efficiency (WUE)

Analysis of variance in (Table 4.42) revealed that WUE statistically affected by main effects of variety and Zn application methods, but not by the interaction effects. Across varieties, Altındane was used water more sufficiently (1.15 g/L) than Imam variety (0.99 g/L) (Table 4.43).

Table 4.42. Analysis of variance for the effects of Zn treatments, varieties, water regimes and their interaction on water use efficiency (g/L)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.048	6.76	0.002
Varieties	1	0.590	138.55	<.0001
Zn treatments	7	0.280	9.47	<.0001
Variety×Zn treatments	7	0.027	0.91	0.501
Water regimes	1	0.001	0.41	0.5256
Varieties×water regimes	1	0.001	0.27	0.6065
Zn treatments×water regimes	7	0.045	1.51	0.1788
Varieties×Zn treatments×water regimes	7	0.020	0.681	0.6876
Error	62	0.222		
Total	95	1.233		

On the other hand, all of Zn application methods were slightly improved WUE compared to control. In this regard, the highest WUE value was recorded in seed coating+foliar spray (1.14 g/L), followed by soil application (1.12 g/L) and seed coating treatment (1.11 g/L), while the lowest WUE value was in control. However, seed priming had observed relatively increase in WUE in compared with hydropriming treatment, but this increasing has not displayed statistical difference (Table 4.43).

Table 4.43. Means of Zn treatments × varieties interaction for water use efficiency (g/L)

Zn treatments	Variety (V)		Mean
	Altındane	Imam	
Untreated seeds	1.00	0.93	0.99 C
Hydropriming	1.10	0.89	1.00 C
Seed priming	1.10	0.96	1.03 C
Seed coating	1.18	1.05	1.11 AB
Soil application	1.17	1.10	1.12 AB
Foliar spray	1.15	1.00	1.08 B
Soil application+foliar spray	1.21	1.00	1.11 AB
Seed coating+foliar spray	1.23	1.10	1.14 A
Mean	1.15 a	0.99 b	

Means followed by the same letters are not statistically different at $P<0.05$

Water use efficiency did not effect by water regimes in the present study. Zn application method have revealed increasing of WUE for both Imam and Altındane varieties, this finding confirmed with previous study carried out by Karim et al. (2012) who found out that Zn foliar spray at late stage on wheat lead to elevated the harmful effect of drought stress. This reflecting the important of Zn application in drought environmental conditions such arid and semi-arid regions which are more vulnerable drought stress in resulting decline of rainfall at the end of the season coincided with flowering and grain-filling stage, this may lead to reduction of grain yield.

Water use efficiency (WUE) defined as the amount of water that using and utilizing to produce the biomass and grain yield for specific crop (Lipiec et al., 2013) or total amount of water transpired to produce shoot biomass (Ludlow and Muchow, 1990). The interaction of varieties and water regimes did not show notable effects on WUE (Table 4.43). The resistance-drought wheat genotype shown higher WUE than susceptible-drought genotype (Marcińska et al., 2013). As appeared in Table 4.43 Zn deficiency (control) resulted in reduction of WUE, however, Zn treatments especially, seed coting+foliar spray enhanced WUE by 15% compared to control treatment. Khan et al. (2004) indicated that Zn deficiency caused minimizing of WUE for chickpea which consistent with our results in this study. Further, Zn as main effect shown significantly effect on WUE, but such significant effect in presence of drought stress has been disappeared. It has been reported that Zn application on maize remarkably effected on grain yield and WUE, but the effects was varied based on the water conditions (Zhang et al., 2020a).

4.2.2.7. Drought index (DI)

There was no outstanding effect in term of drought index among Zn application methods and variety (Table 4.44). Higher value of DI was recorded in seed coated with Zn (0.95) than control (0.88). Over all, Imam variety was revealed low DI (0.90) than Altındane variety (0.92). However, there was no significant difference between both varieties (Table 4.45).

Table 4.44. Analysis of variance for the effects of Zn treatments, varieties and their interaction on drought index

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2			
Variety	1	0.00624	2.355	0.1347
Zn treatments	7	0.03018	1.627	0.1637
Variety×Zn treatments	7	0.02108	1.136	0.3661
Error	32	0.084		
Total	49	0.142		

Table 4.45. Means of Zn treatments × varieties interaction for drought index

Zn treatments	Variety (V)		Mean
	Altındane	Imam	
Untreated seeds	0.85	0.92	0.88
Hydropriming	0.92	0.84	0.88
Seed priming	0.89	0.88	0.88
Seed coating	0.98	0.92	0.95
Soil application	0.93	0.88	0.90
Foliar spray	0.89	0.89	0.89
Soil application+foliar spray	0.96	0.92	0.94
Seed coating+foliar spray	0.92	0.91	0.92
Mean	0.92	0.90	

Among several abiotic stresses, drought is one of the common and widely distributed factors. Hence, seeking resistance wheat varieties consider and remain the crucial goal to avoid losses of yield production. There are wide selection criteria to evaluate and investigate crop drought tolerance such DI. Among various maize genotypes (Hao et al., 2011) and wheat cultivars (Abid et al., 2018) tested under deficit water conditions, it has been reported that genotypes and cultivars with more DI are highly resistance than ones with low DI.

Many selection criteria have been using to investigate and evaluate the drought resistance varieties or genotypes for crops. Drought resistance index was used to identify and screening several wheat inbred lines for their drought tolerance and grain yield as well as others selection criteria (Nouraein et al., 2013).

4.2.3. Biochemical Parameters

4.2.3.1. Antioxidant enzymes activities

It is known that drought stimulates and accumulates oxidative stress in plant tissues through increasing reactive oxygen species (ROS). Plants initiate to increase activity of antioxidant defense systems such as CAT, SOD and APX in order to protect themselves against these toxic organs. The antioxidant enzymes activities of CAT and APX were highly significantly influenced by wheat varieties and Zn treatments, while SOD affected by just Zn treatments (Table 4.46, 4.47, 4.48, 4.49, 4.50 and 4.51).

Table 4.46. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on CAT activity

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	91.083	5.09	0.0089
Variety	1	51.042	5.70	0.0199
Zn treatments	7	34772.6	555.68	<.0001
Variety×Zn treatments	7	25854.2	413.16	<.0001
Water regime	1	24.000	2.68	0.1064
Variety×water regime	1	11484.3	1284.67	<.0001
Zn treatments ×water regime	7	34916.0	557.97	<.0001
Variety×Zn treatments×water regime	7	17237.62	275.46	<.0001
Error	62	554		
Total	95	12498		

Table 4.47. Means of Zn treatments × water regimes interaction for CAT (U/g protein)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	37.8 k	55.8 i	46.8 e
Hydropriming	70.0 ef	38.8 k	54.4 d
Seed priming	75.4 d	71.2 e	73.2 c
Seed coating	62.5 h	130.6 a	96.5 a
Soil application	86.8 c	107.0 b	97.0 a
Foliar spray	85.2 c	28.5 l	56.8 d
Soil application+ foliar spray	45.8 j	64.8 gh	55.4 d
Seed coating+foliar spray	107.8 b	66.5 fg	87.2 b
Water regimes mean	71.4	70.4	
	Imam	Altındane	
Varieties mean	71.7 a	70.1 b	

Means followed by the same letters are not statistically different at P<0.05

Table 4.48. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on SOD activity (U/g protein)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.33	0.36	0.96
Variety	1	1.6	3.6	0.0638
Zn treatments	7	1152.3	365.6	<.0001
Variety×Zn treatments	7	807.1	256.1	<.0001
Water regime	1	0.1	0.1	0.739
Variety×water regime	1	717.2	1592.7	<.0001
Zn treatments ×water regime	7	607.0	192.6	<.0001
Variety×Zn treatments×water regime	7	212.8	67.5	<.0001
Error	62	28.4		
Total	95	3526		

Table 4.49. Means of Zn treatments × water regimes interaction for SOD (U/g protein)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	3.9 l	9.3 g	6.6 g
Hydropriming	11.0 f	6.8 j	9.0 e
Seed priming	8.4 hi	8.3 i	8.3 f
Seed coating	9.1 gh	15.3 b	12.2 c
Soil application	12.7 d	15.4 b	14.0 b
Foliar spray	12.0 e	5.5 k	8.7 ef
Soil application+ foliar spray	9.0 ghi	12.6 de	10.8 d
Seed coating+foliar spray	21.7 a	14.3 c	18.0 a
Water regimes mean	11.0	10.9	
	Imam	Altındane	
Varieties mean	10.8	11.1	

Means followed by the same letters are not statistically different at P<0.05

Higher CAT and APX enzyme activities were found in Imam variety than that of Altındane variety (Table 4.47 and Table 4.51).

Table 4.50. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on APX activity (U/g protein)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.0366	1.1755	0.3154
Variety	1	4.5	287.5	<.0001
Zn treatments	7	19.9	181.5	<.0001
Variety×Zn treatments	7	22.1	201.2	<.0001
Water regime	1	0.2	11.5	0.0012
Variety×water regime	1	5.7	363.3	<.0001
Zn treatments ×water regime	7	7.6	69.4	<.0001
Variety×Zn treatments×water regime	7	2.9	26.5	<.0001
Error	62	0.96		
Total	95	63.83		

Table 4.51. Means of Zn treatments×water regimes interaction for APX (U/g protein)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	0.23 k	1.31 e	0.77 f
Hydropriming	1.64 c	1.0 gh	1.36 b
Seed priming	0.57 j	0.65 ij	0.61 g
Seed coating	0.75 i	1.15 fg	0.95 e
Soil application	1.0 gh	1.28 ef	1.18 cd
Foliar spray	1.29 ef	0.95 h	1.12 d
Soil application+foliar spray	0.95 h	1.50 d	1.22 c
Seed coating+foliar spray	2.50 a	1.80 b	2.20 a
Water regimes mean	1.13 b	1.22 a	
	Imam	Altındane	
Varieties mean	1.4 a	0.96 b	

Means followed by the same letters are not statistically different at P<0.05

Under adequate water, CAT activity enhanced by Zn application methods in Imam and Altındane as comparison with control except for soil application+foliar spray and foliar treatments. Similarity, Zn application under drought stress increased the activity of CAT particularly, seed coating and soil application strategies in both of varieties (Figure 4.13).

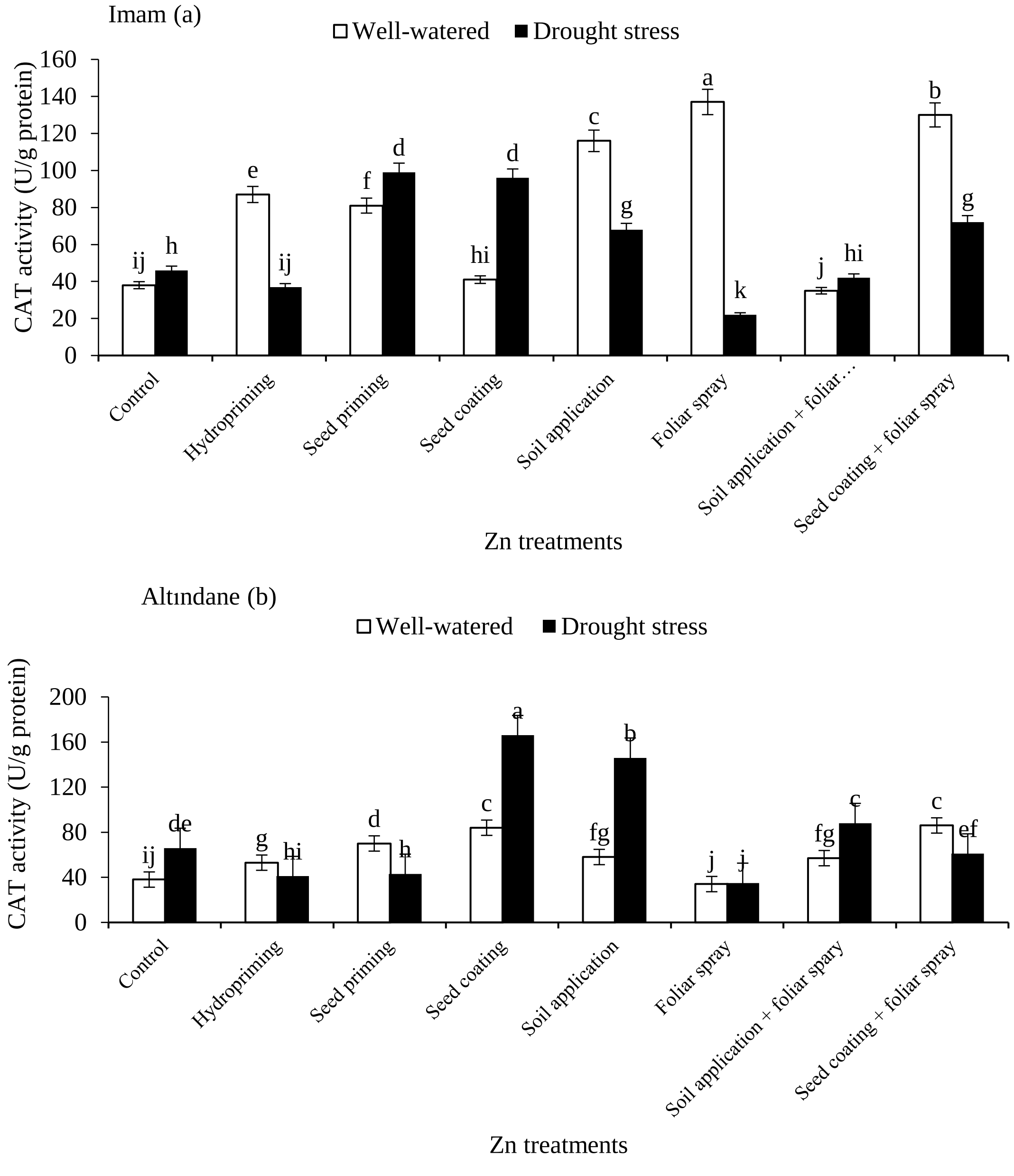
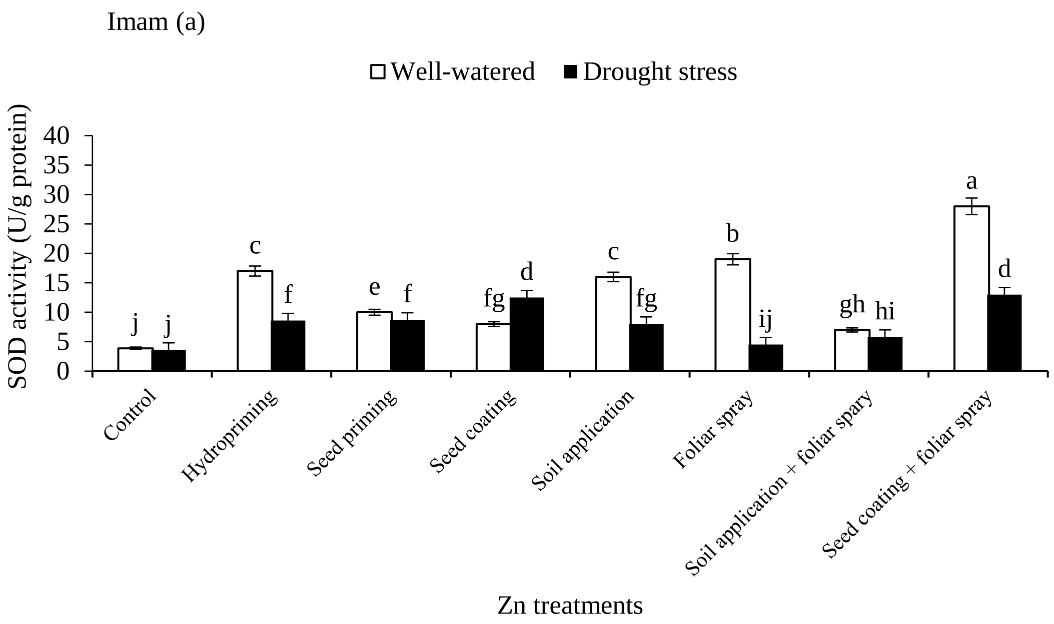


Figure 4.13. The effects of Zn treatments × water regimes interaction on CAT activity of (a) Altındane and (b) Imam. The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

SOD activity shown increasing by Zn application under well water conditions in Imam variety, but this enhancing of SOD activity suppressed and reduced by drought stress in comparison with well-watered condition (Figure 4.14a). Conversely, in Altındane variety drought stress displayed higher SOD activity than well-watered conditions in control and Zn application treatments except in seed priming treatment (Figure 4.14b).



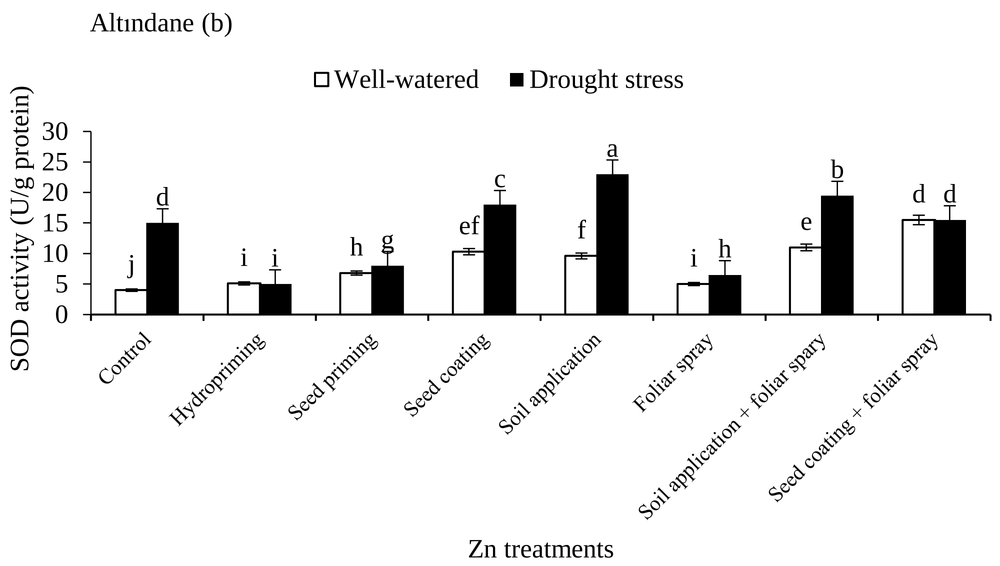


Figure 4.14. The effects of Zn treatments × water regimes interaction on SOD activity of (a) Imam and (b) Altındane. The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

On the other hand, APX enzyme activity minimized by seed priming treatment compared with hydropriming under well-watered and drought stress conditions for both of varieties. In Imam variety, Zn application methods shown higher APX activity compared with control under adequate water conditions (Figure 4.15a). The highest activity was recorded by seed coating+foliar spray under well-watered.

Under various water regimes, APX activity was higher in normal water conditions than drought stress in Imam variety. On the contrary, Altındane showed more activity of APX under deficit water than well-watered conditions (Figure 4.15b).

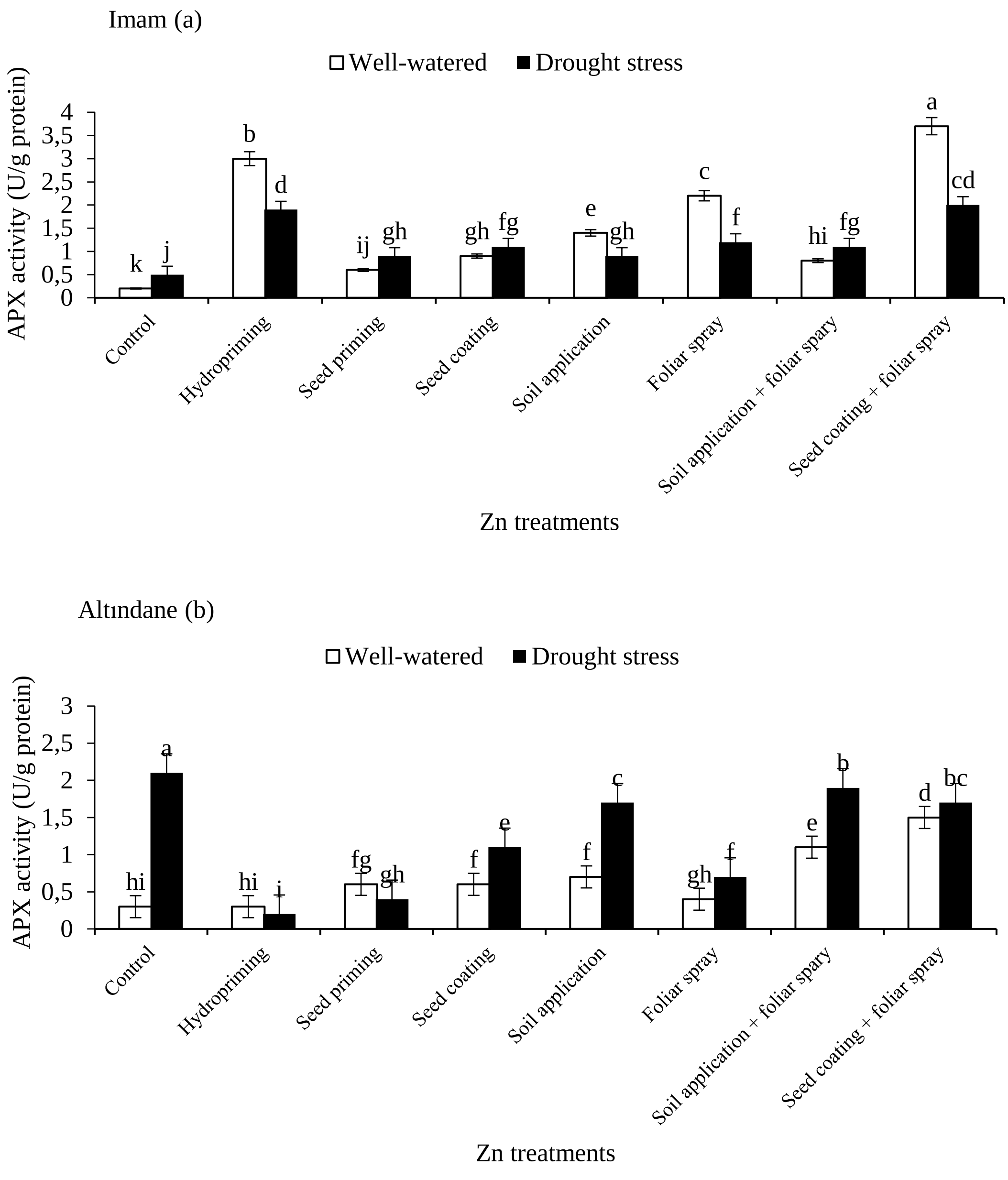


Figure 4.15. The effects of Zn treatments × water regimes interaction on APX activity (a) Imam and (b) Altındane. The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

Many studies reported that the over-production of these antioxidants leads to improve tolerance in wheat crop. Accumulation of these antioxidants under water stress has been reported by Zn application in several prior studies. For instance, pervious study demonstrated by Ma et al. (2017) indicated that Zn fertilization have minimized lipid peroxidation of wheat flag leave, and ameliorated the antioxidant content under deficit water condition. Recently, Faran et al. (2019) reported that seed with high Zn concentration reduced malondialdehyde content and improved total antioxidant activity of wheat. The current study showed that flag leave of wheat varieties has different results in regard of antioxidants activities.

The increasing of antioxidant enzymes activities especially under abiotic stress like drought is a good indicator to ability of plant to tolerant against this stress. Where the activity of CAT was significantly increased in drought-tolerant wheat cultivar compared to control (Huseynova, 2012). SOD represents the initial line defense against toxic substance of raised levels of ROS (Alscher et al., 1997; Gill and Tuteja, 2010). Several antioxidant enzymes activities in response to outer water stress largely increased in wheat crop which in return lead to reduction of ROS (Caverzan et al., 2016). In current research, Altındane demonstrated higher activities for CAT, SOD and APX enzymes under drought stress rather than Imam. The same finding was reported by Csiszár et al. (2007) who stated that resistance variety of *Allium cepa* had higher activities of SOD and CAT than susceptible variety under oxidative stress. SOD activity in the leaf of *Trifolium repens* was significantly enhanced under water stress conditions (Chang-Quan and Rui-Chang, 2008). Moreover, when the seedling of rice subjected to *in vitro* drought stress for 24 h lead to increase activity of SOD in the seedling, which attributed to enhancing of ROS production (Sharma and Dubey, 2005).

4.2.4. Quality Parameters

In several part of the world drought stress often concurrently interaction with other of environmental stresses such as Zn deficiency during the growing season. Zn deficiency in the soil result in low amount of Zn content of stable food crops such as wheat which ultimately causes Zn deficiency in human. Therefore, correcting the main cause of problem by agricultural tools will be preferable choice. Among these agricultural methods to overcome Zn deficiency in the soil is agronomic biofortification (Zn fertilizers) which is a common practice (Singh et al., 2005).

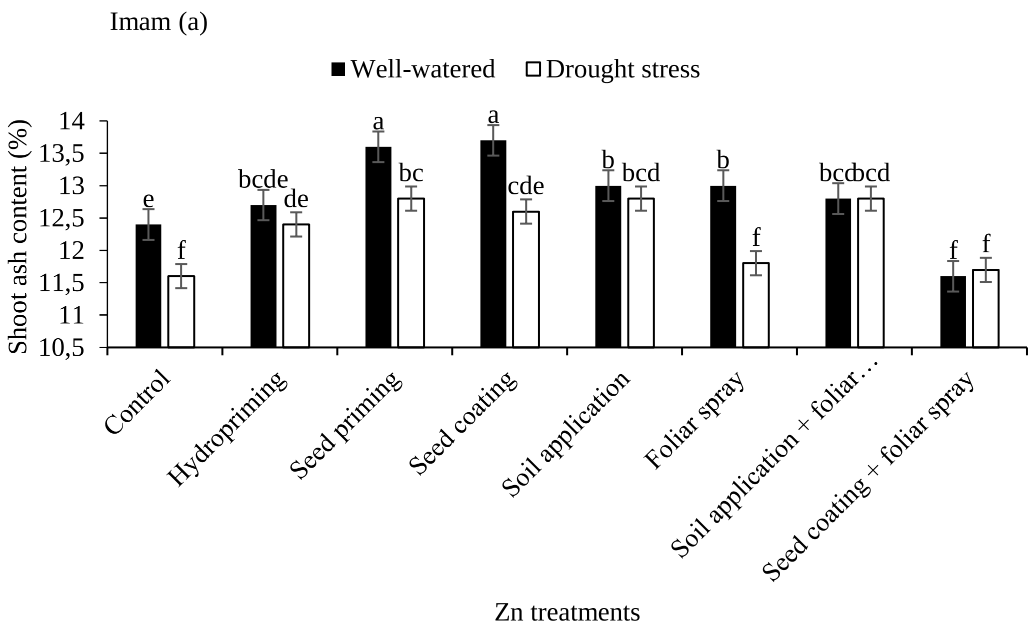
4.2.4.1. Shoot and grain ash contents (%)

It was found that wheat varieties, Zn treatments and water regimes had significant effects on shoot Zn content. Except for the varieties × water regimes interaction, all interactions were highly significant for shoot ash content (Table 4.52).

Table 4.52. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on shoots ash content (%)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.140	1.65	0.2002
Variety	1	25.73	593.77	<.0001
Zn treatments	7	12.34	40.67	<.0001
Variety×Zn treatments	7	4.57	15.06	<.0001
Water regime	1	7.99	184.44	<.0001
Variety×water regime	1	0.08	1.75	0.1903
Zn treatments ×water regime	7	2.82	9.31	<.0001
Variety×Zn treatments×water regime	7	1.80	5.92	<.0001
Error	62	2.63		
Total	95	58.09		

Drought stress has reduced shoots ash content by 6 and 9% in control treatment in Imam and Altındane varieties respectively. In general, Imam variety was observed more ash content than Altındane under deficit-watered condition. Zn application treatments have improved the ash content under well-watered and deficit-watered for both varieties particularly Imam variety (Figure 4.16a). However, the highest shoot ash content was recorded by seed priming in Imam (13.5%) and Altındane variety (12.9%) under well-watered condition. Under deficit-watering condition, the highest ash content was obtained from seed priming (12.8%) and foliar spray (11.6%) treatments in Imam and Altındane varieties, respectively (Figure 4.16a and b).



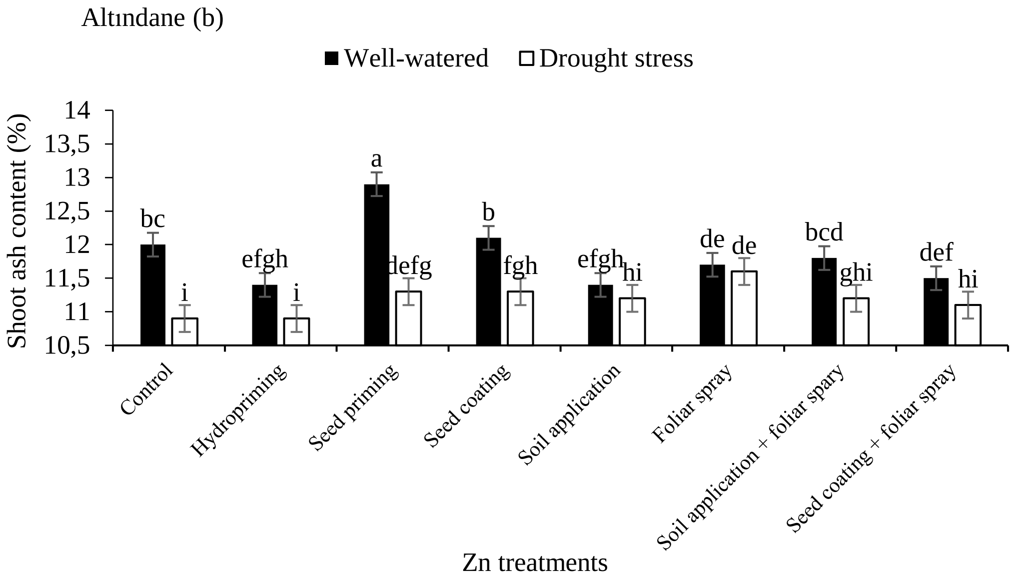
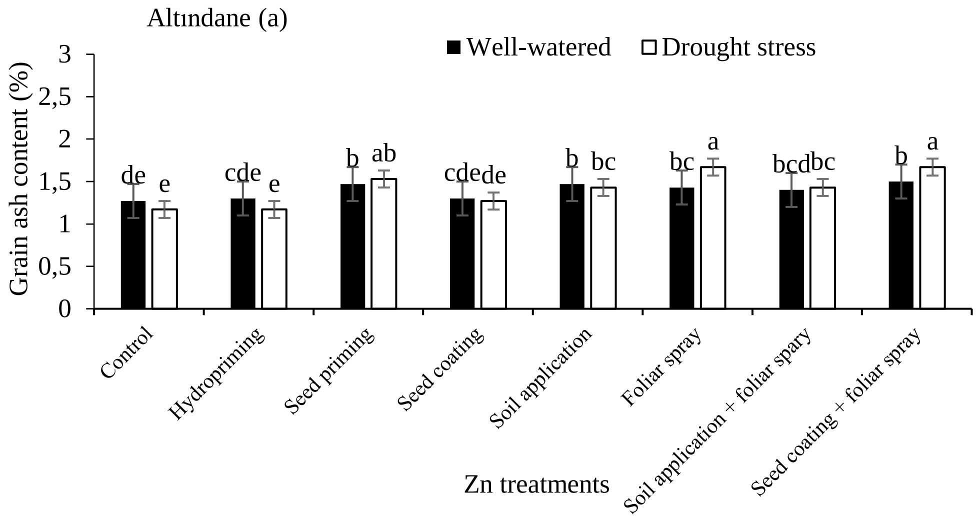


Figure 4.16. The effects of Zn treatments × water regimes interaction on shoots ash content of Imam (a) and Altındane (b). The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

On the other hand, drought stress reduced grain ash content by 9 and 24% in Altındane and Imam variety in comparison with control treatment, respectively (Table 4.53). But this reduction has decreased by Zn application methods. Furthermore, all of Zn applications strategies have revealed more grain ash content under drought stress than well-watered condition in both of varieties (Figure 4.17a). Soil application and foliar spray treatments had recorded maximum and minimum level of grain ash content in Imam variety respectively, in return foliar spray had biggest level, while both of control and hydropriming recorded the least levels of grain ash content in Altındane variety under drought stress condition (Figure 4.17b).

Table 4.53. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on grains ash content (%)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.005	0.34	0.70
Variety	1	0.088	11.52	0.0012
Zn treatments	7	1.391	26.12	<.0001
Variety×Zn treatments	7	0.813	15.27	<.0001
Water regime	1	0.113	14.91	0.0003
Variety×water regime	1	0.055	7.24	0.0091
Zn treatments ×water regime	7	0.697	13.10	<.0001
Variety×Zn treatments×water regime	7	0.406	7.62	<.0001
Error	62	0.46		
Total	95	4.07		



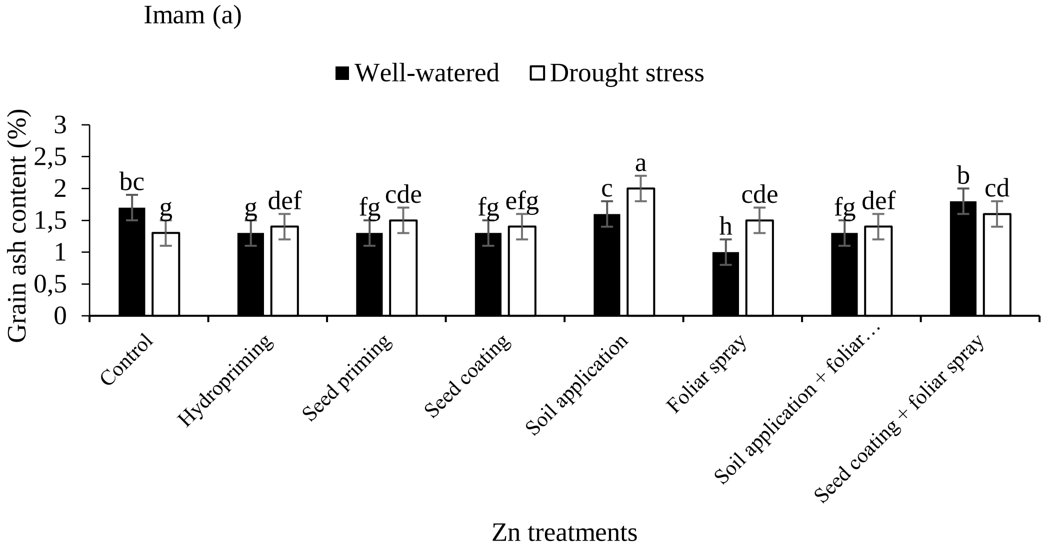


Figure 4.17. The effects of Zn treatments × water regimes interaction on grains ash content of Imam (a) and Altındane (b). The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

Similar trend was observed by (Gomez-Coronado et al., 2016) who find out that Zn soil application+foliar spray increased ash content in season that received low precipitation rate (drought) compared to the highest rainfall season.

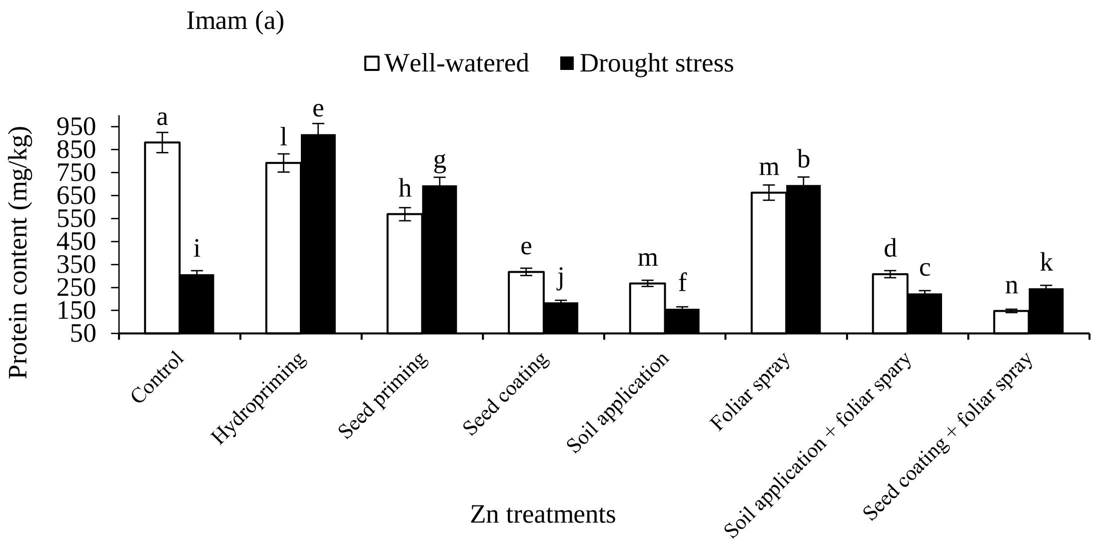
Statistically, shoot and root ash contents were significantly reduced by drought stress as compared to well-watered regime (Table 52 and 53). Ash content in shoot or root indicator to accumulate nutrients contents in these parts of plant and any increase of ash content will be positively increasing with mineral content. Allahdadi and Bahreininejad (2020) reported that ash content of globe artichoke decreased by 16 and 21% under moderate and severe drought stress conditions.

4.2.4.2. Protein content of the flag leaf

As shown in Table 4.54, all of main factor and their combine effects revealed highly remarkable influence on the protein in the flag leaf. Furthermore, the interaction of Zn×water regime × variety shown that drought stress enhanced the protein content rather than adequate water regime. This increase was relatively clear in Imam variety when compared with Altındane variety which demonstrated higher protein under well-watered condition. Under low water condition, Zn application methods decreased protein in comparison with control, unless this reduction disappeared under well water conditions (Figure 4.18). The reduction of protein content in the wheat flag leaf due to biotic stresses such as drought, heat and their combined stress shown in previous study reported by Sattar et al. (2020).

Table 4.54. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on protein content of wheat flag leaf (mg/kg)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	45.6	2.23	0.115
Variety	1	13860	1289	<.0001
Zn treatments	7	2057164.2	27330.9	<.0001
Variety×Zn treatments	7	1748784.4	23233.9	<.0001
Water regime	1	4681.2	435.4	<.0001
Variety×water regime	1	139120.5	12938.2	<.0001
Zn treatments ×water regime	7	1644664.7	21850.5	<.0001
Variety×Zn treatments×water regime	7	311943.8	4144.4	<.0001
Error	62	633		
Total	95	6226770		



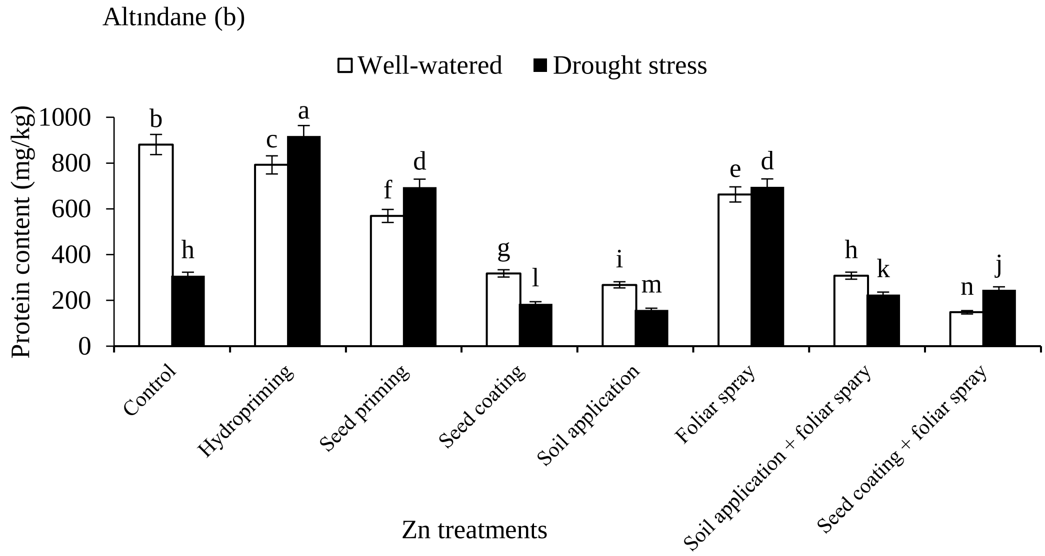


Figure 4.18. The effects of Zn treatments × water regimes interaction on grains protein content of Imam (a) and Altındane (b). The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

The enhancing of protein in the flag leaf in response to Zn application was clearly observed on the HP, foliar spray, and seed priming which increased the protein in the flag leaf by 200, 126, and 125% under drought stress conditions for both varieties. In this regard, our result comply with previous research conducted by Noreen et al. (2020) who revealed that protein content was increased by 102% in the barley crop under salinity stress, but the Zn foliar spray combined with ABA increased protein by 20%. The accumulation of protein in response as initial defense against oxidative stress such as drought stress (Hameed et al., 2015). Furthermore, Zn treatments under well-watered regimes did show any remarkable effect on the protein in the flag leaf (Figure 4.18).

4.2.4.3. Zn content in shoots and grains

The importance of Zn content of wheat shoots or whole plant as it is major food source for animals in rural regions have to put in consideration. Hence, to obtain good growth and high grain yield from wheat, Zn content has not to be less than 15 mg Zn/kg dry matter. Otherwise, the value less than this amount considers as Zn deficient and may be affect plant growth, thus grain yield. Wheat varieties, water regimes and Zn application methods and their interactions had highly significant differences in terms of shoot (Table 4.55 and Table 4.56) and grain Zn content (Table 4.57 and Table 4.58).

Table 4.55. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on shoot Zn content (mg/kg)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	3.70	0.47	0.62
Variety	1	694.45	181.52	<.0001
Zn treatments	7	67656.13	2526.3	<.0001
Variety×Zn treatments	7	2225.075	83.087	<.0001
Water regime	1	59.85	15.64	0.0002
Variety×water regime	1	515.22	134.67	<.0001
Zn treatments ×water regime	7	819.84	30.61	<.0001
Variety×Zn treatments×water regime	7	3749.41	140.01	<.0001
Error	62	241.10	75964	
Total	95			

Table 4.56. Means of Zn treatments × water regimes interaction for shoot Zn content (mg/kg)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	14.1 f-i	13.5 g-j	13.8 cde
Hydropriming	12.0 ij	13.1 g-j	12.5 e
Seed priming	12.1 hij	14.3 fgh	13.2 de
Seed coating	11.8 j	17.8 e	14.8 cd
Soil application	16.1 ef	14.7 fg	15.4 c
Foliar spray	64.5 c	59.1 d	61.8 b
Soil application+ foliar spray	72.3 b	72.6 b	72.5 a
Seed coating+foliar spray	78.5 a	63.6 c	71.0 a
Water regimes mean	35.2 a	33.6 b	
Varieties mean	Imam	Altındane	
	37.1 a	31.7 b	

Means followed by the same letters are not statistically different at P<0.05

Altındane variety had higher shoot and grain Zn content (31.7 and 38.6 mg/kg) than that in Imam variety (37.1 and 32.8 mg/kg). Shoot Zn content was found higher well-watered condition (35.2 mg/kg), while grain Zn content was high in drought stress condition (39.5 mg/kg) (Table 4.56 and Table 4.58).

Table 4.57. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on grain Zn content (mg/kg)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	13.90	3.08	0.052
Variety	1	810.84	337.0	<.0001
Zn treatments	7	2514.99	149.3	<.0001
Variety×Zn treatments	7	450.07	26.7	<.0001
Water regime	1	1342.51	557.9	<.0001
Variety×water regime	1	810.84	337.0	<.0001
Zn treatments ×water regime	7	107.74	6.4	<.0001
Variety×Zn treatments×water regime	7	167.41	9.9	<.0001
Error	62	140.0		
Total	95	6358.4		

Table 4.58. Means of Zn treatments × water regimes interaction for grain Zn content (mg/kg)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	25.3 h	29.8 f	27.5 f
Hydropriming	27.3 g	33.8 de	30.5 e
Seed priming	30.8 f	37.7 c	34.2 c
Seed coating	29.3 f	53.0 d	32.1 d
Soil application	37.6 c	45.8 a	41.7 a
Foliar spray	33.8 de	43.0 b	38.4 b
Soil application+ foliar spray	32.8 e	44.6 ab	38.7 b
Seed coating+foliar spray	39.1 c	46.4 a	42.7 a
Water regimes mean	32.0 b	39.5 a	
	Imam	Altındane	
Varieties mean	32.8 b	38.6 a	

Means followed by the same letters are not statistically different at P<0.05

Under well-watered condition, Altındane and Imam variety have revealed the highest Zn content in shoots by 81 mg/kg which recorded by foliar spray and 100 mg/kg recorded by seed coating+foliar spray respectively (Figure 4.19a and b). Likewise, under deficit-watered regime, foliar spray caused significant increasing in grain Zn content by 63% in Imam variety, whereas in Altındane variety seed coating+foliar spray had the best increasing in grain Zn content by 70% when compared to the control treatments for both varieties and under the same water regime.

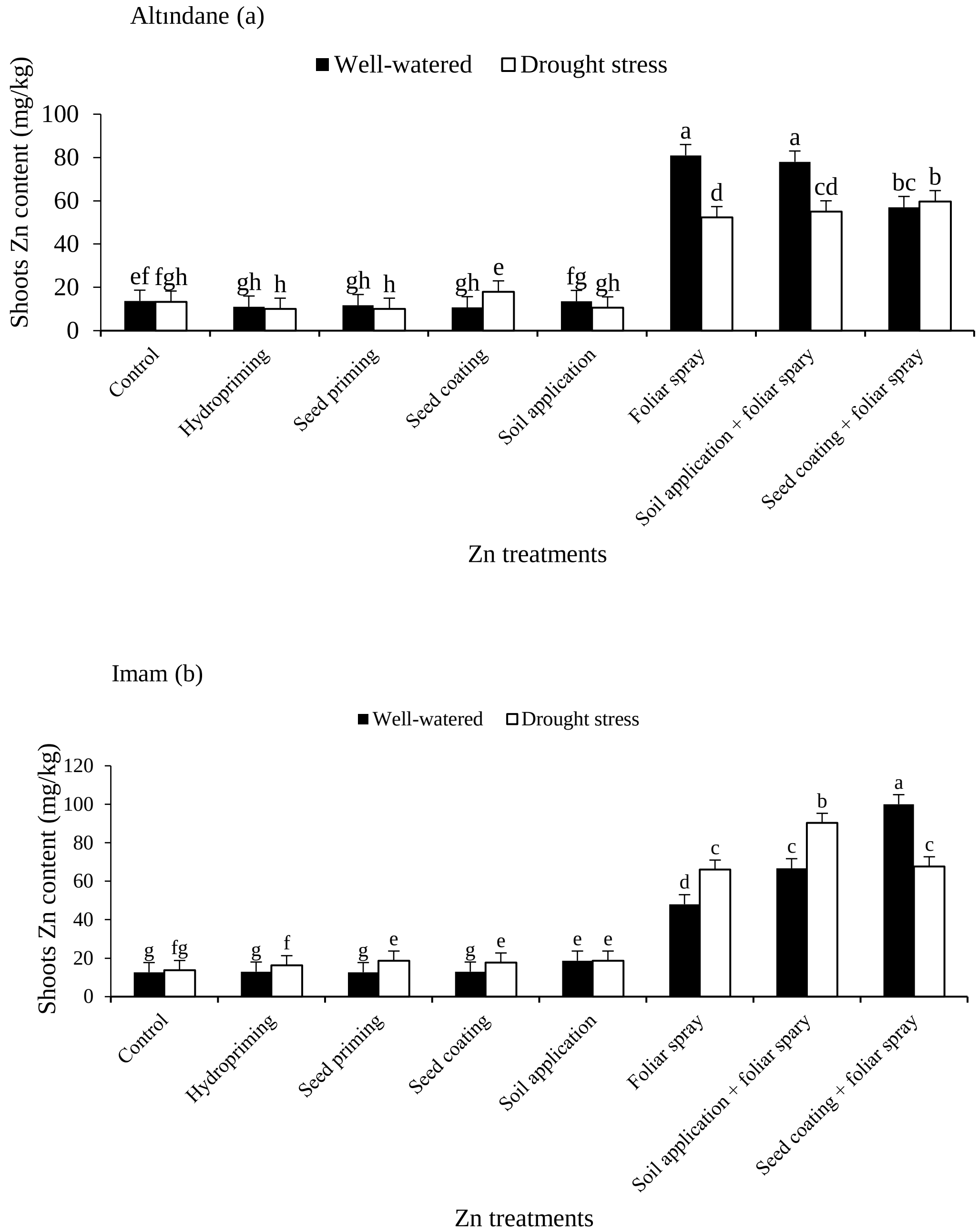
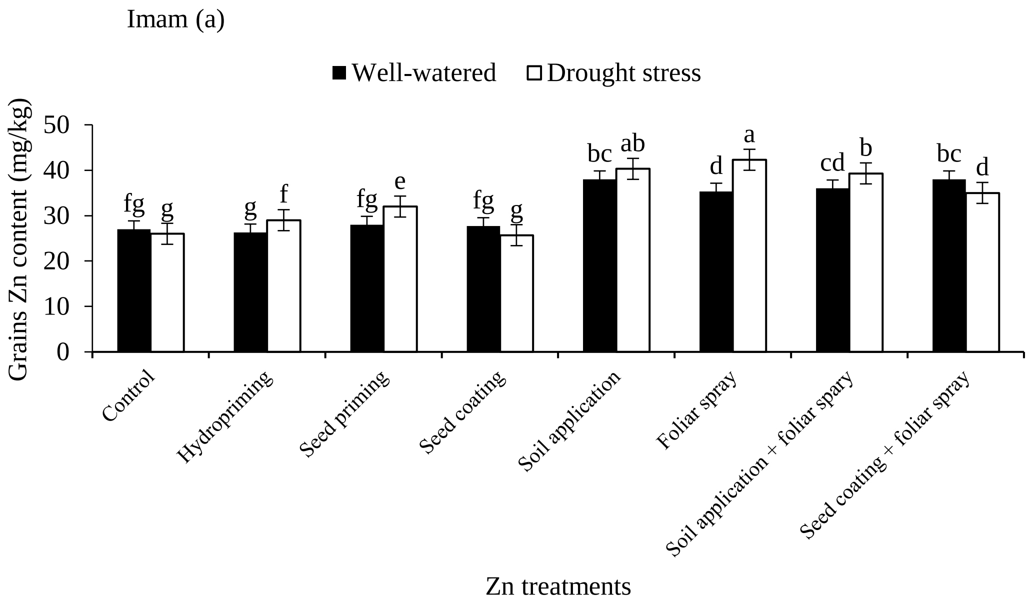


Figure 4.19. The effects of Zn treatments × water regimes interaction on shoots Zn content of Altındane (a), and Imam (b). The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

In this study, drought stress has shown the highest grain Zn content by 42.3 mg/kg recorded by foliar spray and 57.5 mg/kg recorded by seed coating+foliar treatment in Imam and Altındane variety, respectively (Figure 4.20a and b). However, in comparison with well-watered condition, drought stress increased grain Zn content in Altındane (40%) and Imam variety (5.5%).



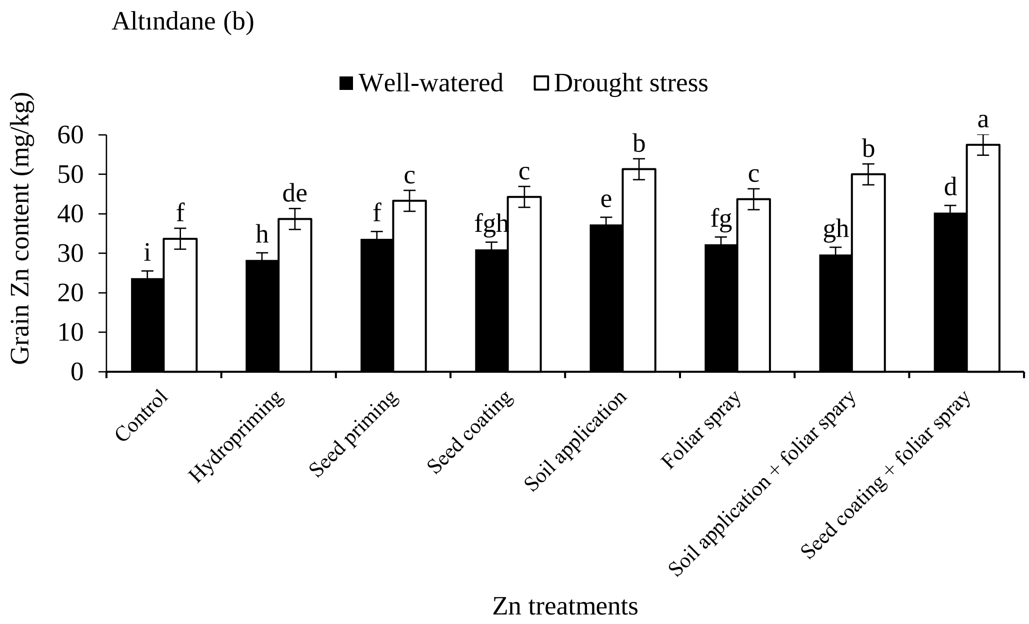


Figure 4.20. The effects of Zn treatments × water regimes interaction on shoots Zn content of Imam (a) and Altındane (b). The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

Similar results were shown by (Gomez-Coronado et al., 2016) who find out that grain Zn concentration of wheat varieties increased by 46% under low rainfall condition. At other study reported by Ma et al. (2017) indicated that grain Zn concentration of wheat increased from 38 mg/kg in adequate water to 42 mg/kg in severe drought stress (Alloway, 2009). Often, Zn concentration in grain of wheat could be relatively between 20-35 mg/kg, but this rate reduces less than 20 mg/kg when wheat cultivated on Zn-deficient soil (Çakmak et al., 2004; Rengel et al., 1999). The results of the present study revealed that the highest grain Zn content in Imam was 42.3 mg/kg achieved by foliar spray under drought stress which is lower than target level for grain Zn biofortification (45 mg/kg) (Liu et al., 2017), but higher than final target content (37 mg/kg) set by harvest program (Bouis and Saltzman, 2017). Correspondingly, the combination of seed coating with foliar spray in Altındane variety observed the highest Zn content in grain (57.5 mg/kg) under low water regime which is highest of recommended value of grain Zn biofortification set by HarvestPlus and (Liu et al., 2017).

In the present study, the treatments of foliar spray, seed coating+foliar spray, and soil application+foliar spray were recorded highly significant effect on the shoot Zn content for both varieties and under the well-watered and drought stress conditions (Figure 4.19). Shoot Zn content of maize was significantly increased through Zn seed priming with 10 mM with or without subjected to drought stress (Nawaz et al.). Likewise, shoot Zn content of wheat increased by 18.1% when Zn applied to soil with Zn rate 6 mg/kg (Maqsood et al., 2009). Cowpea shoot Zn content increased 3-fold via 0.3% foliar spray as compared to control (Kumar andDhaliwal, 2020). Zn application as foliar spray at late of developmental stages of crops such as wheat was more efficient in an enhancing shoot and grain with Zn as compared to early developmental stages (Olsen and Palmgren, 2014). Zn foliar spray (0.4% $ZnSO_4.7H_2O$) was more effective than soil application in an enrichment grain with Zn content, where increased the whole grain Zn content by 58% (Zhang et al., 2012). That could be associated with relatively the high rate of Sodium carbonate as well as PH which restrict the Zn absorption and uptake through roots. In this study, seed coating+foliar spray increased grain Zn content by 46 and 66% for Imam and Altındane under well-watered conditions respectively. Thus, this strategy (Zn seed coating+foliar spray) could be the effective method whether under adequate water or drought stress conditions to elevate the levels of Zn in the grains especially in the soil with critical Zn deficient such as calcareous soil. Zn Seed coating offers essential Zn element which required for early germination and improve seedling germination. It has been indicated that seed with high or adequate Zn ameliorated root and coleoptile dry weight in rice (Boonchuay et al., 2013), and such in turn facilitate and contribute to Zn uptake from the soil. And in return, foliar spray accumulates Zn rapidly after two weeks from the flowering stage of the crops (Jaksomsak et al., 2018).

4.2.4.4. Shoot and grain Fe content

Analysis of variance shown that all of main factors and their interactions had highly significant (P≥0.05) effects on shoot (Table 4.59 and 4.60) and grain Fe content (Table 4.61 and 4.62).

Table 4.59. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on shoot Fe content (mg/kg)

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	70.58	1.41	0.251
Variety	1	95508.16	3820.12	<.0001
Zn treatments	7	12367.79	70.66	<.0001
Variety×Zn treatments	7	9901.66	56.57	<.0001
Water regime	1	1584.37	63.37	<.0001
Variety×water regime	1	6936.00	277.42	<.0001
Zn treatments ×water regime	7	13085.7	74.77	<.0001
Variety×Zn treatments×water regime	7	8495.5	48.54	<.0001
Error	62	1550		
Total	95	149500		

Table 4.60. Means of Zn treatments×water regimes interaction for shoot Fe content (mg/kg)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	130.5 d	137.7 c	134.0 cd
Hydropriming	150.6 b	136.5 c	143.5 b
Seed priming	167.7 a	153.4 b	160.5 a
Seed coating	170.0 a	155.0 b	162.3 a
Soil application	118.5 e	150.0 b	134.3 cd
Foliar spray	152.4 b	123.7 e	138.0 c
Soil application+ foliar spray	170.8 a	124.1 e	147.5 b
Seed coating+foliar spray	123.1 e	137.8 c	130.5 d
Water regimes mean	148.0 a	139.7 b	
	Imam	Altındane	
Varieties mean	175.3 a	122.3 b	

Means followed by the same letters are not statistically different at P<0.05

Shoot Fe content was higher in Imam variety (173.3 mg/kg) that that in Altındane variety (122.3 mg/kg). Both Fe content in wheat shoot and grain were found higher in well-watered (148 and 37.5 mg/kg) than that of drought stress condition (139.7 and 32.5 mg/kg) (Table 4.60 and Table 4.62)

Table 4.61. Analysis of variance for the effects of Zn treatments, varieties, water and their interaction on grain Fe content (mg/kg)

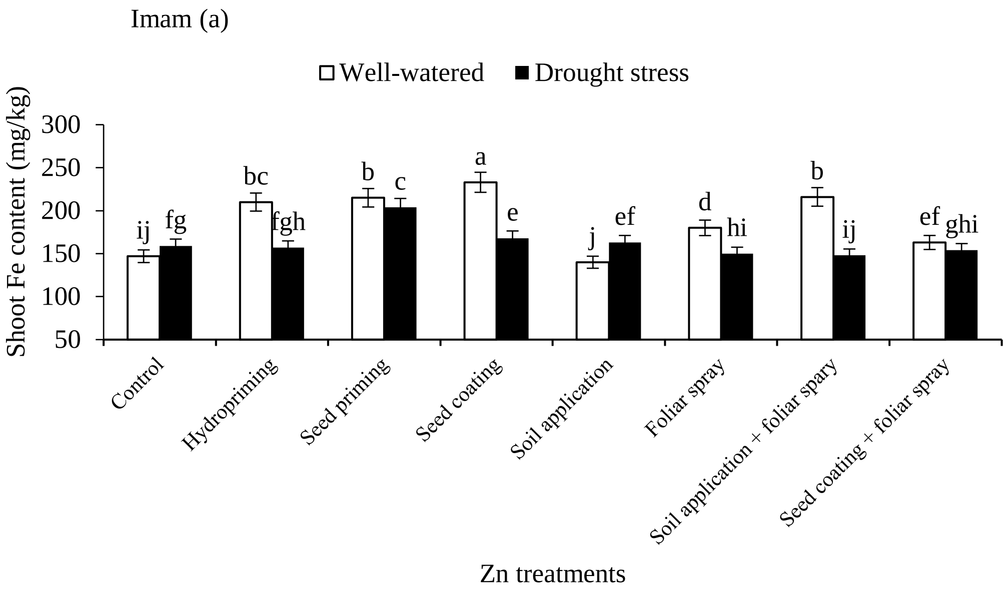
Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	5.14	0.50	0.61
Variety	1	1971.1	383.0	<.0001
Zn treatments	7	480.8	13.3	<.0001
Variety×Zn treatments	7	776.3	21.6	<.0001
Water regime	1	605.0	117.6	<.0001
Variety×water regime	1	2025.8	393.7	<.0001
Zn treatments ×water regime	7	484.1	13.4	<.0001
Variety×Zn treatments×water regime	7	1264.2	35.1	<.0001
Error	62	324		
Total	95	7936		

Table 4.62. Means of Zn treatments×water regimes interaction for grain Fe content (mg/kg)

Zn treatments	Water regimes (W)		
	Well-watered	Drought stress	Mean
Untreated seeds	32.0 fgh	35.5 d	33.7 cd
Hydropriming	39.5 c	32.8 efg	36.1 ab
Seed priming	40.3 bc	34.5 def	37.4 a
Seed coating	34.3 def	29.5 h	31.9 d
Soil application	34.8 de	34.1 def	34.5 bc
Foliar spray	42.1 ab	31.3 gh	36.7 a
Soil application+foliar spray	43.4 a	32.6 efg	38.0 a
Seed coating+foliar spray	34.0 def	29.8 h	31.9 d
Water regimes mean	37.5 a	32.5 b	
	Imam	Altındane	
Varieties mean	30.5 b	39.5 a	

Means followed by the same letters are not statistically different at P<0.05

In regards of Fe shoots content, the highest rate were recorded by seed coating in well-watered and drought stress conditions for Imam and Altındane varieties (Figure 4.21a and b). However, drought stress had negative impact and reduced shoots Fe content in Imam variety, but this reduction have relatively decreased in Altındane variety.



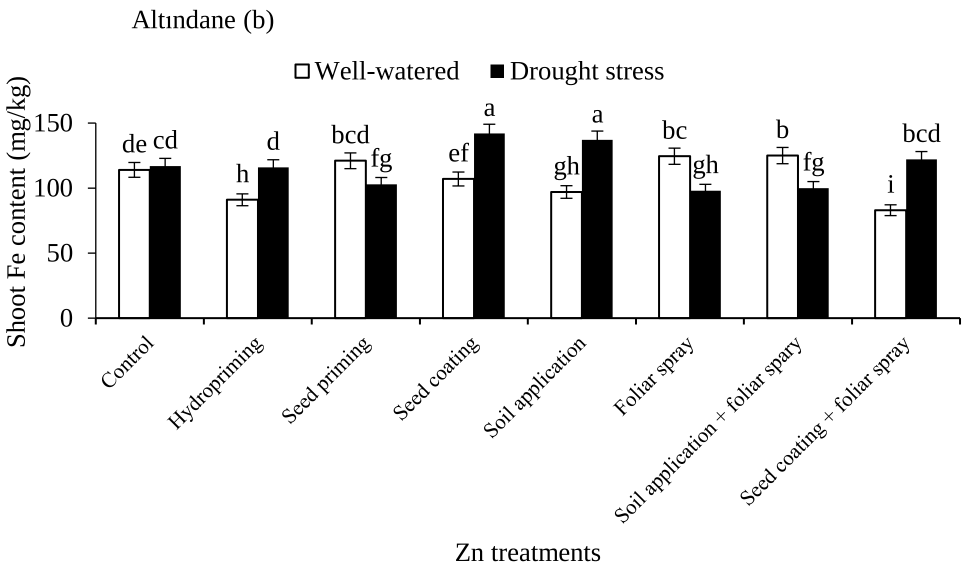
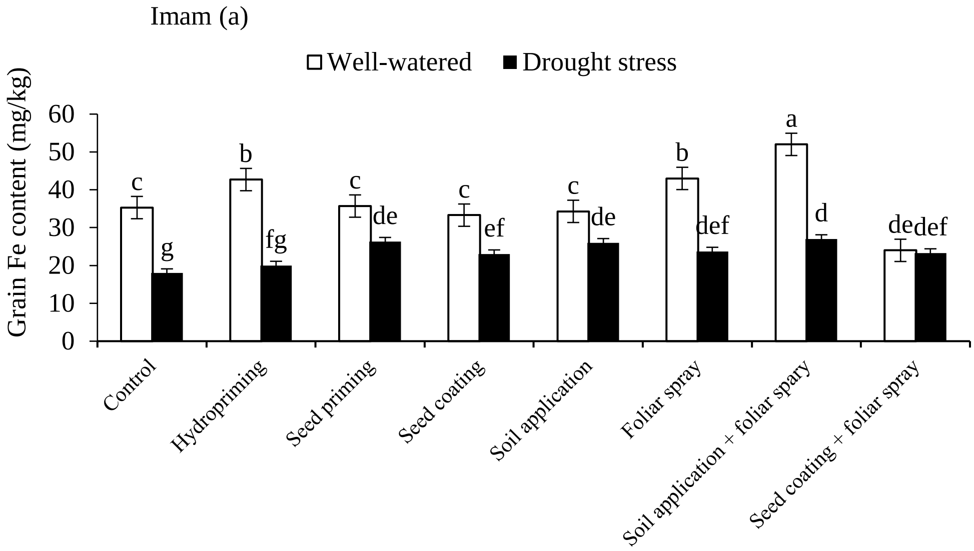


Figure 4.21. Influence of different Zn treatments on shoot Zn content of Imam (a) and Altındane (b). The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

On the other hand, Fe content grains ranged from 24 to 52 mg/kg with an average 37.5 mg/kg and from 18 to 26.3 mg/kg with an average 23.5 mg/kg in well-watered and drought stress conditions of Imam variety, respectively (Figure 4.22a), whereas in Altındane were ranged from 29 to 45 mg/kg with an average 37.5 mg/kg, and from 36 to 53 mg/kg with an average 41.5 mg/kg in well-watered and drought stress conditions respectively (Figure 4.22b). Under drought stress, Imam variety have shown highly response to Zn application treatments that throughout enhanced shoots Fe content when compared with the same treatments under well-watered condition. On average, low water regime minimized grains Fe content by 59 and 10.6% in comparison with adequate water regime in Imam and Altındane variety, respectively.



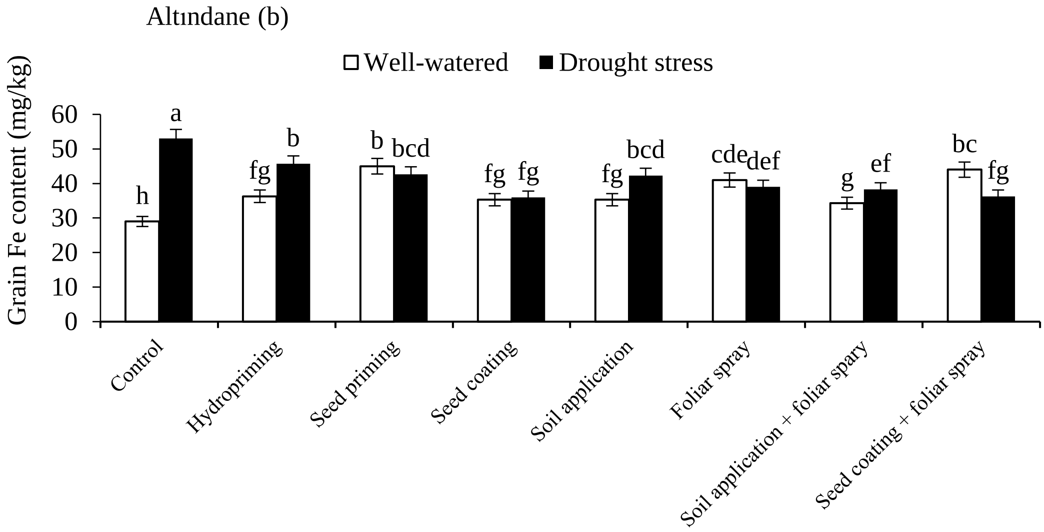


Figure 4.22. Influence of different Zn treatments o grain Fe content of Imam (a) and Altındane (b). The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

In general, Zn application methods did not show inverse effect with grains Fe content in well-watered condition for both varieties, but in case of drought conditions, Zn application strategies have revealed the negative effect with grains Fe content in Altındane, but not for Imam variety. Our results in this study agree with previous research conducted by Jalal et al, (2020) who reported that higher Fe content of wheat grain was obtained by lower rate of foliar spray with Zn. This inhibition of Fe uptake due to the antagonism relationship between Zn and Fe at the high Zn levels.

Increasing Fe and Zn content in the crops (grain and shoot) is considered as major and crucial goal to overcome malnutrition health problem for human, particularly who are based in their food on the crops such as wheat as main sources for such micronutrients (Fe and Zn). Under well-watered regime Zn treatments of soil application+ foliar spray and foliar spray were significantly enhanced grain Fe content as compared to control for Imam variety (Figure 4.22a). Our results in agreement with Gomez-Becerra et al. (2010) who displayed the strong associations between grain Zn concentration with grain Fe content. Also, similar study was reported by Zhang et al. (2010) who show the significant and positive relationship among Zn and Fe grain contents. Likewise, there was highly significant and linear positive correlation between grain concentration for Zn and Fe under dry and wet treatments have been found by Peleg et al. (2008). On the other hand, the antagonism relationship observed in Altındane variety under drought stress, where grain Fe content remarkably reduced with all Zn treatments compared to control treatment (non Zn) (Figure 4.22b). similarly, this opposite correlation among Zn and Fe was observed by Saha et al. (2017) in wheat grains. This reduction of Fe content in grain resulted in the competition between two Fe and Zn in uptake and absorption by roots in soil was earlier described in study reported by Dutta et al. (1989).

4.2.4.5. Zn and Fe uptake

Zn application showed significant effects on Zn and Fe uptake by shoots and grains. Among varieties, in Imam shoot Zn uptake ranged from 0.54 (untreated seeds) to 4.67 mg/pot (seed coating+foliar spray) under well-watered condition, and from 0.57 (untreated seeds) to 3.73 mg/pot (soil application+foliar spray) under drought stress condition (Table 4.63). While shoot Zn uptake was ranged from 0.50 (seed priming) to 3.65 mg/pot (soil application+foliar spray) in Altındane in well-watered condition, it was varied from 0.43 (seed priming) to 2.66 mg/pot (seed coating+foliar spray) in drought stress condition (Table 4.63).

Table 4.63. Means of Zn treatments, varieties and water regimes for shoot Zn uptake (mg/pot)

Varieties (V)	Zn treatments (Zn)	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	0.54 k	0.57 k	0.55 e
	Hydropriming	0.55 k	0.64 ef	0.60 e
	Seed priming	0.55 k	0.74 k	0.64 e
	Seed coating	0.60 k	0.73 k	0.66 e
	Soil application	0.87 jk	0.77 k	0.82 e
	Foliar spray	2.14 fgh	2.68 d-g	2.41 c
	Soil application + foliar spray	3.06 b-e	3.73 b	3.40 ab
	Seed coating + foliar spray	4.67 a	2.90 c-f	3.78 a
	Mean V×W	1.62 A	1.60 A	1.61
Altındane	Untreated seeds	2.03 gh	1.76 hi	1.89 cd
	Hydropriming	0.61 k	0.52 k	0.56 e
	Seed priming	0.50 k	0.43 k	0.47 e
	Seed coating	0.51 k	0.61 k	0.56 e
	Soil application	0.53 k	0.70 k	0.62 e
	Foliar spray	1.65 bc	1.81 ijk	1.41 d
	Soil application + foliar spray	3.65 bc	2.35 e-h	3.00 b
	Seed coating + foliar spray	3.40 bcd	2.66 d-g	3.03 b
	Mean V×W	1.61 A	1.27 B	1.44
	Mean W	1.61	1.43	

Means followed by the same letters are not statistically different at P<0.05. WW: well-watered, DS: drought stress

Table 4.64. Means of Zn treatments and water regimes interaction for shoot Zn uptake (mg/pot)

Zn treatments (Zn)	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	1.28 e	1.17 ef	1.22 c
Hydropriming	0.58 g	0.58 g	0.58 d
Seed priming	0.52 g	0.58 g	0.55 d
Seed coating	0.56 g	0.67 fg	0.61 d
Soil application	0.70 fg	0.73 efg	0.72 d
Foliar spray	1.90 d	1.93 d	1.91 b
Soil application+ foliar spray	3.36 b	3.0 bc	3.20 a
Seed coating+foliar spray	4.03 a	2.78 c	3.40 a
Mean	1.61	1.43	

Means followed by the same letters are not statistically different at P<0.05

Altındane absorbed Zn in grains sufficiently under drought stress than well-watered. In contrast, Imam uptaked more Zn under well-watered compared to drought stress (Table 4.65). Zn uptake by shoot and grain was evaluated as shoot dry weight multiply shoot Zn content, in return grain Zn uptake was calculated via grain dry weight multiply grain Zn content. Generally, shoot and grain Zn uptake through the combination treatments was more pronounced than control and others Zn treatments (Table 4.63 and 4.65).

Zn accumulation in grains of Altındane variety (0.64 mg/kg) was significantly higher than Imam varity (0.62 mg/kg). Grains of wheat varieties have accumulated more Zn under drought stress (0.60 mg/kg) than that in well-watered condition (0.56 mg/kg) (Table 4.65).

Table 4.65. Means of Zn treatments, varieties and water regimes for grain Zn uptake (mg/pot)

Varieties (V)	Zn treatments (Zn)	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	0.43 mno	0.38 o	0.40 f
	Hydropriming	0.42 no	0.40 no	0.41 f
	Seed priming	0.46 l-o	0.45 l-o	0.45 f
	Seed coating	0.49 k-o	0.42 no	0.45 f
	Soil application	0.66 c-h	0.61 f-j	0.64 bc
	Foliar spray	0.59 g-k	0.63 e-i	0.61 cd
	Soil application + foliar spray	0.60 g-j	0.61 f-j	0.61 cd
	Seed coating + foliar spray	0.67 c-g	0.57 g-l	0.61 cd
	Mean V×W	0.54 C	0.51 D	0.62 B
Altındane	Untreated seeds	0.78 bc	0.55 g-l	0.66 bc
	Hydropriming	0.40 no	0.54 i-m	0.47 ef
	Seed priming	0.50 j-n	0.58 g-k	0.54 de
	Seed coating	0.56 g-k	0.73 b-f	0.64 bc
	Soil application	0.57 g-k	0.76 bcd	0.66 bc
	Foliar spray	0.64 d-i	0.80 ab	0.72 ab
	Soil application + foliar spray	0.55 h-l	0.75 b-e	0.64 bc
	Seed coating + foliar spray	0.62e-i	0.90 a	0.76 a
	Mean V×W	0.58 B	0.70 A	0.64 A
	Mean W	0.56 B	0.60 A	

Means followed by the same letters are not statistically different at P<0.05. WW: well-watered, DS: drought stress

Table 4.66. Means of Zn treatments and water regimes interaction for grain Zn uptake (mg/pot)

Zn treatments (Zn)	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	0.60 cde	0.46 fg	0.53 c
Hydropriming	0.41 g	0.47 fg	0.44 d
Seed priming	0.47 fg	0.52 ef	0.50 cd
Seed coating	0.52 ef	0.57 de	0.55 c
Soil application	0.62 cd	0.69 abc	0.65 ab
Foliar spray	0.61 cd	0.71 ab	0.66 ab
Soil application+ foliar spray	0.58 de	0.68 abc	0.63 b
Seed coating+foliar spray	0.64 bcd	0.73 a	0.70 a
Mean	0.56 B	0.60 A	

Means followed by the same letters are not statistically different at P<0.05

There was significant difference among Zn treatments for total Zn uptake, while no significant differences found between watering regimes and wheat varieties (Table 4.67) The highest total Zn uptake values were obtained from seed coating+foliar spray 4.10 mg/kg and 5.33 and soil application + foliar spray treatment by 3.83 mg/kg (Table 4.68)

Table 4.67. Means of Zn treatments, varieties and water regimes for total Zn uptake (mg/pot)

Varieties (V)	Zn treatments (Zn)	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	0.97 l	0.95 l	0.96 e
	Hydropriming	0.97 l	1.05 l	1.01 e
	Seed priming	1.00 l	1.20 kl	1.09 e
	Seed coating	1.09 l	1.15 l	1.12 e
	Soil application	1.54 jkl	1.38 kl	1.46 e
	Foliar spray	2.73 ghi	3.31 d-g	3.02 c
	Soil application + foliar spray	3.67 b-e	4.34 b	4.01 ab
	Seed coating + foliar spray	5.33 a	3.47 c-g	4.40 a
	Mean V×W	2.16	2.10	2.13
Altındane	Untreated seeds	2.81 fgh	2.32 hij	2.56 cd
	Hydropriming	1.00 l	1.06 l	1.04 e
	Seed priming	1.02 l	1.02 l	1.01 e
	Seed coating	1.07 l	1.34 kl	1.21 e
	Soil application	1.11 l	1.46 kl	1.28 e
	Foliar spray	2.30 hij	1.98 ijk	2.14 d
	Soil application + foliar spray	4.21 bc	3.10 e-h	3.65 b
	Seed coating + foliar spray	4.02 bcd	3.56 b-f	3.79 b
	Mean V×W	2.19	1.98	2.09
	Mean W	2.18	2.04	

Means followed by the same letters are not statistically different at P<0.05. WW: well-watered, DS: drought stress

Table 4.68. Means of Zn treatments and water regimes interaction for total Zn uptake (mg/pot)

Zn treatments (Zn)	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	1.89 d	1.63 de	1.76 c
Hydropriming	0.99 f	1.05 ef	1.02 d
Seed priming	1.00 f	1.10 ef	1.05 d
Seed coating	1.08 ef	1.24 ef	1.16 d
Soil application	1.32 def	1.42 def	1.37 cd
Foliar spray	2.51 c	2.65 c	2.58 b
Soil application+ foliar spray	3.94 b	3.72 b	3.83 a
Seed coating+foliar spray	4.68 a	3.51 b	4.10 a
Mean	2.18	2.04	

Means followed by the same letters are not statistically different at P<0.05

In general, the combination of Zn application were the high efficient to absorb Zn from the soil by crop compared to other Zn and untreated seeds treatments. Furthermore, shoots were more Zn uptake than grains under well-watered and drought conditions for both varieties that could be return to Zn dissolved in grains as it required for protein synthesis and metabolism. Same finding of results in earlier research presented by (Graham et al., 1992) who shown that genotypes absorb more Zn from soil are commonly the most efficient to uptake Zn in their tissue and grain.

In general, the combination of Zn application were the high efficient to absorb Zn from the soil by crop compared to other Zn and untreated seeds treatments. Furthermore, shoots were more Zn uptake than grains under well-watered and drought conditions for both varieties that could be return to Zn dissolved in grains as it required for protein synthesis and metabolism. Same finding of results in earlier research presented by (Graham et al., 1992) who shown that genotypes absorb more Zn from soil are commonly the most efficient to uptake Zn in their tissue and grain.

However, soil under Zn deficiency and low water content such as in arid and semi-arid, in addition to Mediterranean regions, agronomic biofortification (fertilizations) could be the effective, applicable, promising, cost-effectiveness and beneficial approach and strategy in biofortification wheat grain and subsequently overcome to human health problem resulting in Zn deficiency under thus conditions.

Water regimes did not show notable effect on the shoot Zn uptake, it demonstrated significant effect on grain Zn uptake, where drought stress reduced the grain Zn uptake by 6% compared to well-watered conditions for Imam variety (Table 4.65). However, this reduction was more pronounced in Altındane variety where as compared to well-watered regime, drought was decreased grain Zn uptake by 20% (Table 4.65 and Table 4.66). In this study, increasing Zn uptake through roots from the soil to the shoot via phloem and ultimately to the grain could attribute to the very low levels of phosphorus element in the soil. the similar results was observed by Korkmaz et al. (2020) who reported that the high levels of phosphorus intend to reduce and inhibit Zn uptake due to the antagonism between Zn and phosphorus and eventually decreased grain Zn content. Also, exceed phosphorous fertilizers, especially in the soil with low extractable Zn in tend to minimize Zn uptake and content in the grain (Marschner, 1993). It has been suggested that the increasing Zn uptake from roots to shoots has not ensure enrichment of grain content that based on the translocation of Zn (Kaur et al., 2020). Zn through foliar spray readily uptake by plant compared to soil application that due to losses of Zn during the roots uptake (Phattarakul et al., 2012). The second reason, in the foliar spray Zn absorb from the leaf as Zn^{2+} and enter inside the plant tissue through stomata pore and translocate via phloem into the others sink tissues (fruit and grain) (Gupta et al., 2016).

There were highly significant differences for shoot Fe uptake among wheat varieties, watering regimes (Table 4.69) and Zn treatments (Table 4.70).

Table 4.69. Means of Zn treatments, varieties and water regimes for shoot Fe uptake (mg/pot)

Varieties (V)	Zn treatments (Zn)	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	6.25 ghi	6.56 gh	6.40 e
	Hydropriming	8.90 cd	6.23 ghi	7.56 bc
	Seed priming	9.30 bc	8.10 de	8.70 a
	Seed coating	10.78 a	6.95 fg	8.87 a
	Soil application	6.58 gh	6.70 g	6.64 de
	Foliar spray	8.05 de	6.10 g-j	7.06 cd
	Soil application + foliar spray	9.95 ab	6.10 g-j	8.02 b
	Seed coating + foliar spray	7.62 ef	6.62 gh	7.12 cd
	Mean V×W	8.42 A	6.66 B	7.55 A
Altındane	Untreated seeds	5.28 j-n	3.88 o	4.58 h
	Hydropriming	4.68 l-o	5.00 k-n	4.88 gh
	Seed priming	4.62 mno	4.80 lmn	4.71 h
	Seed coating	5.26 j-n	5.42 i-m	5.34 fg
	Soil application	4.70 l-o	6.748 gh	5.59 f
	Foliar spray	5.17 k-n	5.53 i-l	5.35 fg
	Soil application + foliar spray	5.75 h-k	4.46 no	5.10 fgh
	Seed coating + foliar spray	5.14 k-n	5.14 k-n	5.14 fgh
	Mean V×W	5.10 C	5.07 C	5.10 B
	Mean W	6.75 A	5.88 B	

Table 4.70. Means of Zn treatments and water regimes interaction for shoot Fe uptake (mg/pot)

Zn treatments (Zn)	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	5.77 e-h	5.22 h	5.49 e
Hydropriming	6.78 bc	5.65 fgh	6.22 cd
Seed priming	6.96 b	6.44 bcd	6.70 ab
Seed coating	8.02 a	6.19 c-f	7.10 a
Soil application	5.64 fgh	6.59 bc	6.11 d
Foliar spray	6.60 bc	5.81 d-h	6.21 cd
Soil application+foliar spray	7.85 a	5.28 gh	6.56 bc
Seed coating+foliar spray	6.38 b-e	5.88 d-g	6.13 cd
Mean	6.75 A	5. 88 B	

Means followed by the same letters are not statistically different at P<0.05

Highly significant differences were determined for grain Fe uptake among wheat varieties, watering regimes (Table 4.71) and Zn treatments (Table 4.72). For grain Fe uptake, Altındane variety (0.64 mg/kg) was superior than Imam variety (0.49 mg/kg). More grain Fe uptake was realized in well-watered condition (0.63 mg/kg) than that of under drought stress (0.51 mg/kg) (Table 4.71).

Table 4.71. Means of Zn treatments, varieties and water regimes for grain Fe uptake (mg/pot)

Varieties (V)	Zn treatments (Zn)	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	0.56 ghi	0.26 l	0.41 de
	Hydropriming	0.67 b-e	0.28 kl	0.47 cd
	Seed priming	0.58 f-i	0.37 jk	0.47 cd
	Seed coating	0.59 e-i	0.37 jk	0.48 c
	Soil application	0.60 d-i	0.40 j	0.50 c
	Foliar spray	0.72 bc	0.35 jkl	0.54 c
	Soil application + foliar spray	0.89 a	0.42 j	0.65 ab
	Seed coating + foliar spray	0.42 j	0.38 j	0.40 e
	Mean V×W	0.63 B	0.35 C	0.49 B
Altındane	Untreated seeds	0.54 hi	0.70 bc	0.62 b
	Hydropriming	0.52 i	0.75 b	0.64 ab
	Seed priming	0.65 c-g	0.65 c-g	0.65 ab
	Seed coating	0.70 bc	0.68 b-e	0.69 a
	Soil application	0.64 c-g	0.60 d-i	0.63 ab
	Foliar spray	0.64 c-g	0.68 b-e	0.66 ab
	Soil application + foliar spray	0.67 b-e	0.64 c-g	0.65 ab
	Seed coating + foliar spray	0.70 bc	0.65	0.67 ab
	Mean V×W	0.63 B	0.67 A	0.65 A
	Mean W	0.63 A	0.51 B	

Means followed by the same letters are not statistically different at P<0.05. WW: well-watered, DS: drought stress

Table 4.72. Means of Zn treatments and water regimes interaction for grain Fe uptake (mg/pot)

Zn treatments (Zn)	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	0.55 ef	0.48 g	0.51 c
Hydropriming	0.60 cde	0.51 fg	0.56 bc
Seed priming	0.61 cde	0.51 fg	0.56 bc
Seed coating	0.65 bc	0.52 fg	0.59 b
Soil application	0.62 bcd	0.50 fg	0.56 bc
Foliar spray	0.68 b	0.51 fg	0.60 b
Soil application+foliar spray	0.78 a	0.53 fg	0.65 a
Seed coating+foliar spray	0.56 def	0.51 fg	0.53 c
Mean	0.63 A	0.51 B	

Means followed by the same letters are not statistically different at P<0.05

The best treatment for the grain Fe uptake was Soil application+foliar spray by 0.65 mg/kg (Table 4.72).

Wheat varieties, watering regimes (Table 4.73) and Zn treatments showed highly significant differences for total Fe uptake (Table 4.74). Imam variety has accumulated more Fe (8.04 mg/kg) in total than Altındane variety (5.74 mg/kg). Total Fe uptake was higher under well-watered condition (7.39 mg/kg) than drought stress condition (6.40 mg/kg).

Table 4.73. Means of Zn treatments, varieties and water regimes for total Fe uptake (mg/pot)

Varieties (V)	Zn treatments (Zn)	Watering regimes (W)		Mean
		WW	DS	V×Zn
Imam	Untreated seeds	6.82 f-i	6.82 f-i	6.82 fg
	Hydropriming	9.57 bc	6.50 f-j	8.04 cd
	Seed priming	9.88 b	8.47 d	9.17 ab
	Seed coating	11.37 a	7.33 ef	9.35 a
	Soil application	7.18 ef	7.10 fg	7.14 ef
	Foliar spray	8.77 cd	6.44 f-j	7.60 de
	Soil application + foliar spray	10.84 a	6.50 f-j	7.52 de
	Seed coating + foliar spray	8.05 de	7.00 fgh	3.78 a
	Mean V×W	9.06 A	7.02 B	8.04 A
Altındane	Untreated seeds	5.83 j-m	4.58 n	5.21 j
	Hydropriming	5.21 lmn	5.85 j-m	5.52 ij
	Seed priming	5.27 lmn	5.45 k-n	5.36 j
	Seed coating	5.96 i-m	6.10 h-l	6.04 hi
	Soil application	5.34 k-n	7.09 a	6.22 gh
	Foliar spray	5.81 j-m	6.21 j-k	6.01 hi
	Soil application + foliar spray	6.41 f-j	5.10 mn	5.76 hij
	Seed coating + foliar spray	5.85 j-m	5.80 j-m	5.82 hij
	Mean V×W	5.71 C	5.77 C	5.74 B
	Mean W	7.39 A	6.40 B	

Means followed by the same letters are not statistically different at P<0.05. WW: well-watered, DS: drought stress

The highest total Fe uptake was obtained in soil application+foliar spray treatment by 7.70 mg/kg (Table 4.74).

The interaction of total Zn uptake with total Fe uptake shown highly variation for both varieties under different water regime. In Imam variety, total Zn uptake shown linear negative correlation with total Fe uptake under both water conditions, but the correlation was not significant (Figure 4.23a and b). On the other hand, in Altındane, the interaction between them was negative, however was not significant (Figure 4.23c) under drought stress, whereas it was significant (r= 0.5870) under well-watered condition (Figure 4.23d).

Table 4.74. Means of Zn treatments and water regimes interaction for total Fe uptake (mg/pot)

Zn treatments (Zn)	Water regimes (W)		Mean
	Well-watered	Drought stress	
Untreated seeds	6.32d-g	5.70 g	6.01 e
Hydropriming	7.39 b	6.17 efg	6.78 cd
Seed priming	7.58 b	6.96 bcd	7.27 ab
Seed coating	8.67 a	6.72 cde	7.70 a
Soil application	6.26 efg	7.09 bc	6.68 d
Foliar spray	7.29 bc	6.33 d-g	6.81 cd
Soil application+foliar spray	8.63 a	5.81 fg	7.22 bc
Seed coating+foliar spray	6.94 bcd	6.40 def	6.67 d
Mean	7.39 A	6.40 B	

Means followed by the same letters are not statistically different at P<0.05

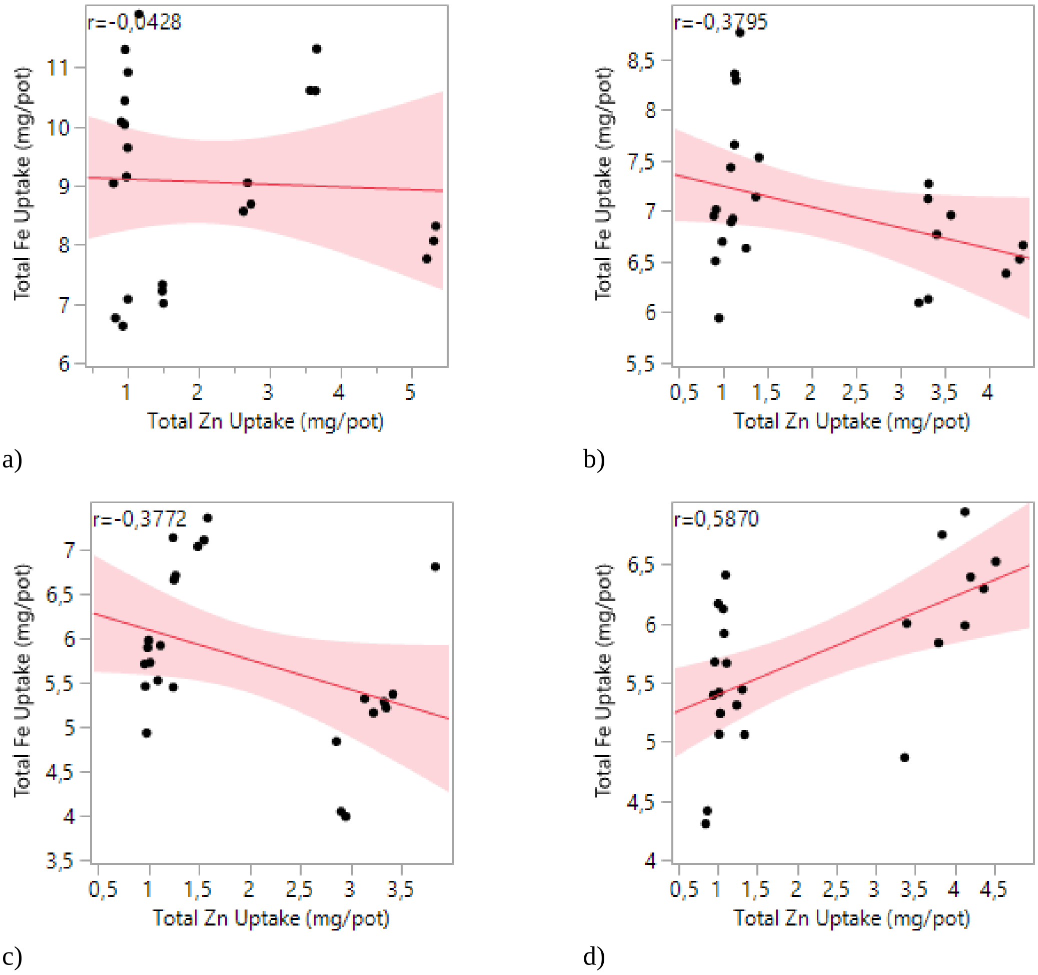


Figure 4.23. Correlations between total Zn uptake with total Fe uptake for Imam variety under well-watered (a) and drought (b), and Altındane variety variety under well-watered (c) and drought (d)

In conclusion, the results of this experiment have revealed the considerable losses of wheat yield and grains Zn content that caused throughout drought stress and soil Zn deficiency, especially when they take place concurrently, but Zn fertilization with different strategies have shown ameliorate in term of grain yield, as well as mitigate the negative and damage impacts of drought stress via improvement various of physiological processes. Additionally, under these environmental stresses conditions (drought and soil Zn deficiency) different Zn application methods have observed good performance and results related in an increment of Zn biofortification in wheat grain, particularly the combinations of seed coating with foliar spray and soil application with foliar spray which achieved values highest than determined target set by HarvestPlus.

4.3. Results of Experiment III

The prevalence of Zn deficiency became globally, especially in the less developed countries such as Sub-Saharan Africa, the major reason is that people rely in their consumption on cereal-based foods as major source of Zn intake. The best and effective approach to deal with or overcome this problem is agricultural tools like agronomic and breeding biofortification which became more common and worldwide use strategy.

4.3.1. Morphological Traits and Yields Components

All Zn treatments relatively contributed to improve and enhance grain yield and its components. Table 4.75 shown that Zn treatments and interactions (Zn application × variety) had played significant role in affecting plant height. Zn application improved plant height when compared with control. In case of interaction effects were significant and maximum plant height was recorded in Altındane variety with Zn coating. While in main affect (Zn application) all Zn treatments significantly shown increase in plant height in comparison with control (Table 4.75). In this context, foliar, coating and combine application with Zn increased plant height with 3.8, 5.1 and 5.3%, respectively. From this results, seed coating, foliar spray and their combination (seed coating+foliar spray) have increased grain yield of wheat varieties through ameliorated the yield components such as plant height biomass and 1000 grains weight. Similar results have been reported by Esfandiari et al. (2016) who found that foliar spray result in considerable increased in grain yield, No. of grain/spike and plant high in different growth stage of wheat crop. However, the main effect of variety has shown highly statistically significant for spike length (Table 4.75) where, Altındane variety (16.2 cm) had longer spike than Imam variety (13.2 cm). Also, the combined effect has observed significant effect on spike length, where the highest spike length (16.8 cm) was recorded in Altındane variety when treated with Zn coating and the least (12.8 cm) was recorded in control of Imam variety (Table 4.75). In research conducted on maize, foliar spray of 2% $ZnSO_4$ solution remarkable achieved enhance in plant height by 7% as compared to control in the field experiment (Umar et al., 2020). Similarly, in our results the foliar spray was significantly increased plant height by 7% when it compared to control. In regard of spike length, Zn treatments did not show notable effect on spike length, however, there was significant effect on spike length characteristic of wheat when Zn treatments × varieties interaction was considered (Table 4.75). There is evidence that Zn application improve yield attributes such as plant height and spike length as indicated in the earlier research observed by Das et al. (2020).

Table 4.75. Means of Zn application through seed coating and foliar spray for plant height (cm) and spike length (cm) of bread wheat varieties grown under rainfed condition

Zn application methods	Plant height (cm)			Spike length (cm)		
	Variety (V)		Mean	Variety (V)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	93.7 c	93.0 c	93.3 B	12.8 e	15.8 bc	14.3
Foliar spray	99.4 ab	94.7 c	97.0 A	14.0 d	15.5 c	17.7
Seed coating	96.4 bc	100.4 a	98.4 A	13.0 de	16.8 a	14.9
Seed coating+foliar spray	98.4 ab	98.7 ab	98.5 A	13.2 de	16.5 ab	14.9
Mean	97.0	96.7		13.2 B	16.2 A	
LSD value	Zn	*			ns	
	V	ns			*	
	Zn xV	*			*	

Means followed by the same letters are not statistically different at P<0.05. ns: non-significant

Moreover, number of spikelet per spike and number of grains per spike were highly significant affected by variety, but the interaction effects have no displayed this significant on these parameters. Among varieties, Altındane had more number of spikelet per spike than Imam. The main effect of Zn application methods had highly significant effect on number of grains per spike, the more of grains per spike was recorded in Zn foliar spray (45.5 grains per spike), whereas the minimum was recorded in control (37.6 grains per spike) (Table 4.76).

Table 4.76. Means of Zn application through seed coating and foliar spray for the number of spikelet/spike and number of grains/spike of bread wheat varieties grown under rainfed condition

Zn application methods	Number of spikelet per spike			Number of grain/spike		
	Variety (V)		Mean	Variety (V)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	16.2	20.5	18.3	34.0	41.3	37.6 B
Foliar spray	17.5	21.1	19.3	39.4	51.6	45.5 A
Seed coating	16.4	21.2	18.8	35.4	47.0	41.2 AB
Seed coating+foliar spray	17.1	21.5	19.3	40.0	49.0	44.5 AB
Mean	16.8 B	21.1 A		37.2 B	47.2 A	
LSD value	Zn	ns			*	
	V	*			*	
	Zn xV	ns			ns	

Means followed by the same letters are not statistically different at P<0.05. ns: non-significant

Recently, in the five field experiment Zn seed coating (0.3 kg Zn/ha used as ZnO ethanediol flowable suspension) significantly increased the 1000 seeds weight and number of grain per m^2 for maize crop in some locations (Martínez-Cuesta et al., 2021). In our study, the number of grain per spike was not significantly affected by the interaction effect (Zn treatments × varieties), it was significantly increased by Zn treatments as only main effect (Table 4.76).

The effect of variety and Zn treatments contributed significantly to variation in weight of grain per spike and 1000 seeds weight (Table 4.77). Furthermore, among Zn treatments foliar spray and combine application appeared improved in weight of grain/spike and 1000 grains weight with 49.0 and 52.5 g compared with other treatments, respectively. 1000 grains weight was significantly affected by Zn treatments and varieties, but not by the interaction effect. The highest 1000 grains weight (52.5 g) was determined in combination of seed coating+foliar spray (Table 4.77). Seed treatment with 1% $ZnSO_4$ solution and 1% $FeSO_4$ solution enhanced the 1000 seeds weight of wheat (40 g) as compared to control (30 g) (Barman et al., 2020). However, Zn treatments through soil application, foliar spray and their combination were significantly improved and raised the 1000 grains weight of maize in experiment carried out under field conditions (Khalid et al., 2019).

Table 4.77. Means of Zn application through seed coating and foliar spray on weight of grains/spike and 1000 grains weight (g) of bread wheat varieties grown under rainfed condition

Zn application methods	Weight of grains/spike			1000 grains weight (g)		
	Variety (V)		Mean	Variety (V)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	15.6	17.5	16.5 C	50.0	48.0	49.0 C
Foliar spray	20.3	23.6	22.0 A	52.0	49.0	50.0 BC
Seed coating	17.3	20.3	18.8 BC	52.0	51.0	52.0 AB
Seed coating+foliar spray	19.1	22.6	20.9 AB	53.0	52.0	52.5 A
Mean	18.0 B	21.0 A		52.0 A	50.0 B	
LSD value	Zn	*			*	
	V	*			*	
	Zn xV	ns			ns	

Means followed by the same letters are not statistically different at $P<0.05$. ns: non-significant

As shown in Table 4.78 both Zn application and variety had positive role to mitigate harvest index. Generally, in comparison with control all of Zn treatments seed coating, foliar and combination had enhanced harvest index by 10.6, 16 and 11%, respectively. However, Imam variety had the higher harvest index than

Altındane variety. On the other hand, the number of tillers/plant was significantly influenced by Zn treatment. Where, the highest number of tillers/plant was recorded in Zn combine treatment, while the least number was in control treatment. Harvest index and number of tillers per plant were not remarkable effect by the interaction of Zn treatments and varieties (Table 4.78). Such results comply with previous study reported by Firdous et al. (2018) who found that Zn treatments through foliar spray of $ZnSO_4.7H_2O$ (0.5%) did not achieved notable effect on HI.

Table 4.78. Means of Zn application through seed coating and foliar spray harvest index (%) and number of tillers/plant of wheat varieties grown under rainfed condition

Zn application methods	Harvest index (%)			Number of tillers/plant		
	Variety (V)		Mean	Variety (V)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	33.2	31.0	32.1 B	6.3	6.0	6.2 B
Foliar spray	36.3	32.3	34.3 AB	7.4	6.7	7.0 B
Seed coating	37.7	34.2	36.0 A	7.0	7.0	7.0 B
Seed coating+foliar spray	36.7	32.2	34.4 AB	8.0	8.7	8.3 A
Mean	36.0 A	32.4 B		7.2	7.1	
LSD value	Zn	*			*	
	V	*			ns	
	Zn xV	ns			ns	

Means followed by the same letters are not statistically different at P<0.05. ns: non-significant

Grain yield and biological yield were only significantly influenced with variety and Zn application respectively. Compared with control treatment foliar spray, seed coating and combine application with Zn improved grain yield by 14.7, 10.8 and 5.3% respectively, but this increased in yield was not significant. Similarly, in previous study, it has been reported that foliar spray 2% improved plant high, 1000-grains weight, biological yield, harvest index and grain production in maize crop (Mohsin et al., 2014).

Variety have shown notable effect on grain yield where, Imam variety displayed more respond to Zn application through achieving high yield (6.7 t/ha) than Altındane (5.7 t/ha). However, in an interaction effects the highest and lowest biological yield was obtained from foliar spray and seed coating with Zn, respectively (Table 4.79). In the present study, as shown in Table (4.79) Zn application through all treatments slightly increased grain yield, however, this increasing was not significant. This none increasing in grain yield might be due to the high amount of water content throughout precipitation season of growth (Zhang et al., 2012). The same finding was show by Phuphong et al. (2018) who revealed that foliar spray of $ZnSO_4.7H_2O$ (0.5%) on booting stage of rice under field condition did not significantly affect grain yield. In the other research, when foliar spray with 0.5% of $ZnSO_4.7H_2O$ applied on various developmental stage growths of rice had no demonstrated notable effect on the yield (Boonchuay et al., 2013) which is in close accordance with results revealed in this study.

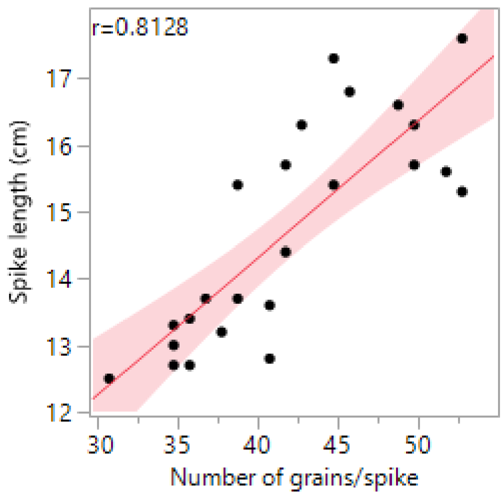
Table 4.79. Means of Zn application through seed coating and foliar spray for biological yield (g/m^2) and grain yield (t/ha) of wheat varieties grown under rainfed condition

Zn application methods	Biological yield (g/m^2)			Grain yield (t/ha)		
	Variety (V)		Mean	Variety (V)		Mean
	Imam	Altındane		Imam	Altındane	
Untreated seeds (Control)	1826 abc	1736 bcd	1781	6.1	5.4	5.7
Foliar spray	1833 abc	2016 a	1925	6.6	6.5	6.6
Seed coating	1953 ab	1536 d	1745	7.3	5.3	6.3
Seed coating+foliar spray	1806 abc	1696 cd	1751	6.6	5.5	6.0
Mean	1855	1746		6.7A	5.7 B	
LSD value	Zn	ns			ns	
	V	ns			*	
	Zn xV	*			ns	

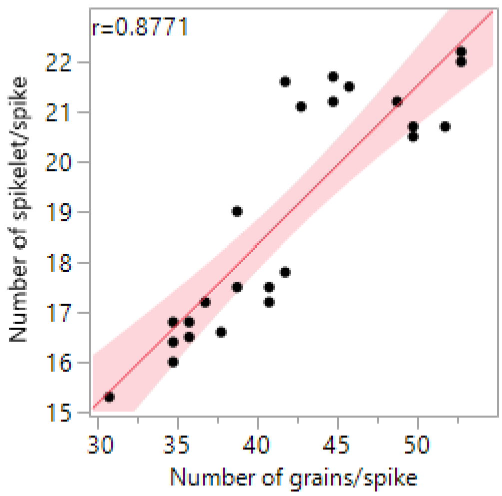
Means followed by the same letters are not statistically different at P<0.05. ns: non-significant

4.3.2. Correlation Among Grain Yield and Its Attributes

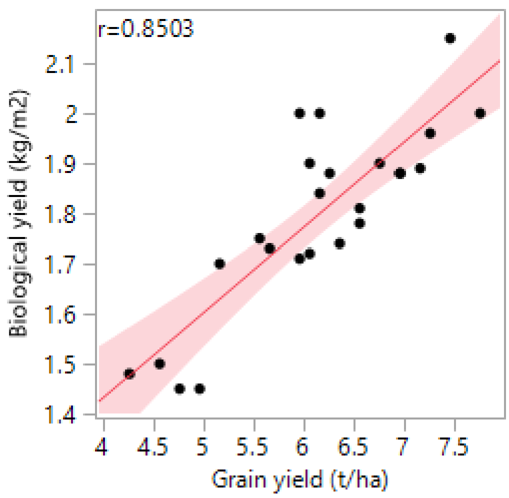
The correlation among grain yield and its attributes is given in (Figure 4.24). There was highly significant and linear positive relationship between grain yield with biological yield (r= 0.85; P<0.0001) and harvest index (r= 0.78; P<0.0001). Whereas, both of spike length (r= - 64; P<0.001) and number of spikelet/spike have shown significant and linear negative correlation with grain yield (Figure 4.24). Moreover, grain yield in association with and number of grain per spike and 1000-grains weight showed non-significant correlation. On the other hand, grain yield did not show significant relationship with grain Zn content and the correlation among them was negative (r= -0.022), but was not significant. In contrast, shoot Zn content revealed highly positive significant correlation (r= 0.58) with grain yield (Figure 4.25). It is concluded from above results that grain yield with its components have revealed a significant positive relationship between grain yield with biological yield and harvest index. Whereas, both of spike length and number of spikelet per spike have shown significant negative correlation with grain yield. On the other hand, grains yield has shown negative correlation with grain Zn content, but this relationship was not significant. Our current results agree with studies previously revealed by Gomez-Coronado et al. (2016) who found that grain yield have observed slightly negative relationship with grain Zn content. In contrast, shoot Zn content shown highly positive significant correlation (r= 0.58) with grain yield. This might be due to the importance role of Zn as regulator and developing root systems of plants, therefore the poor roots could have limit Zn uptake from soil, which adversely effect on the transported of this element to upper surface and reaches to grain of the crop, thus reducing the yield.



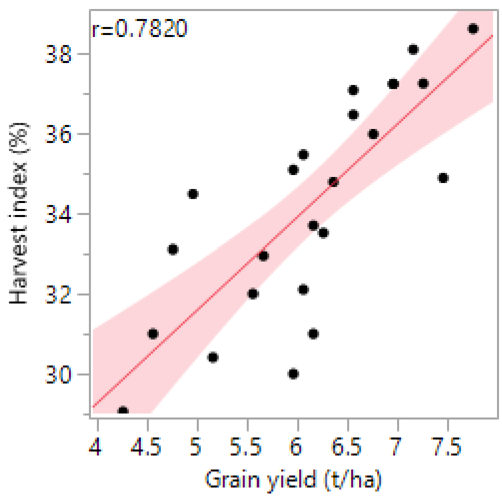
a)



b)

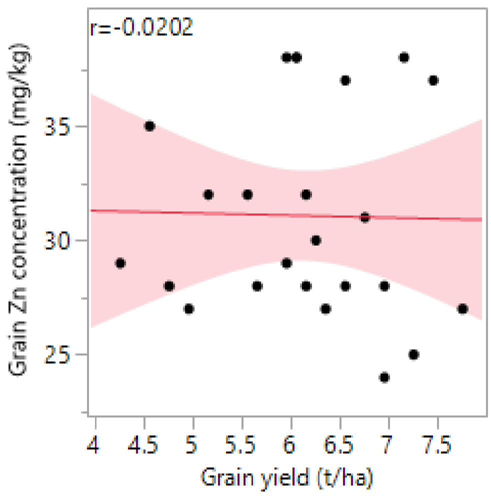


c)

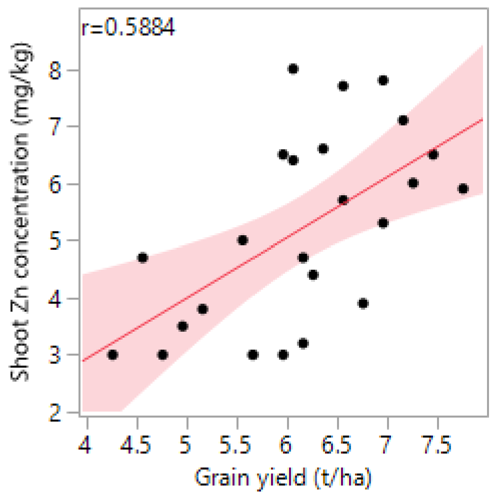


d)

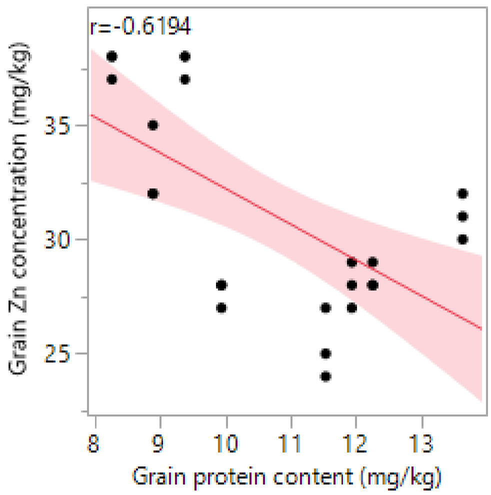
Figure 4.24. Correlations between grain yield with spike length (a), number of spikelet per spike (b), biological yield (c) and harvest index (d) under rainfed conditions



a)



b)



c)

Figure 4.25. Correlations between grain yield with grain (a) and shoot Zn contents (b), and grain protein content with grain Zn contents (c) under rainfed conditions

The positive correlation among wheat grain yield and harvest index and spikelet length was previously observed by (Barman et al., 2020; Leilah and Al-Khateeb, 2005). In this study the relationship between grain yield and grain Zn content was negative, but this antagonism relationship was disappeared and positive between grain yield and shoot Zn content (Figure 4.25). Likewise, it has been demonstrated that a significant positive correlation was between grain yield with its stover Zn content of rice crop (Singh et al., 1983). Also, in previous research wild emmer wheat genotype observed higher grain yield, whereas their Zn content was much reduced (Yilmaz et al., 2017).

4.3.3. Shoot and Grain Zn Content

Apparently in Table 4.80, all of Zn application relatively improved shoot Zn content. In this work, Zn content in shoots was very low ranged from 4 to 7.4 in Imam and from 3.1 to 7 mg/kg in Altındane. Across Zn application treatments, foliar spray recorded the highest content of Zn in shoots by 7 and 7.4 mg/kg in Altındane and Imam, respectively. Likewise, foliar spray with Zn increased both of shoots Zn content by 85% in Imam variety and 80% in Altındane variety in comparison with controls for each variety (Figure 4.26).

Table 4.80. Analysis of variance for the effect of main factors and their interaction on shoot Zn content (mg/kg) of two wheat varieties grown under rainfed condition

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.015	0.027	0.9734
Variety	1	11.34	38.75	<.0001
Zn application methods	3	44.27	50.42	<.0001
Variety×Zn application methods	3	3.98	4.53	0.0202
Error	14	4		
Total	23	63.7		

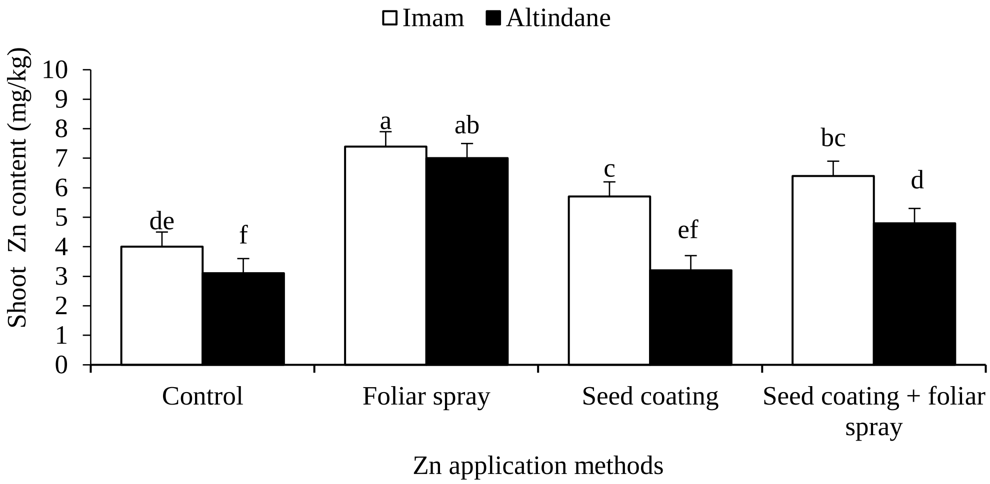


Figure 4.26. Influence of interaction effect of Zn treatments × varieties on shoot Zn content under rainfed condition. The data represent means of three replicate, and different letters indicate to substantial different among treatments at P<0.05

On the other hand, grains Zn content had significantly affected by Zn application strategies (Table 4.81).

Table 4.81. Analysis of variance for the effect of main factors and their interaction on grain Zn concentration (mg/kg) of two wheat varieties grown under rainfed condition

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	5.33	3.111	0.076
Variety	1	10.66	12.44	0.0033
Zn application methods	3	252.5	98.19	<.0001
Variety×Zn application methods	3	193.3	75.18	<.0001
Error	14	12		
Total	23	473.8		

In the present study, the range of Zn content of untreated Zn-grains for Imam and Altındane varieties range between (27 to 31 mg/kg) with average 29 mg/kg and (24 to 28 mg/kg) with average 26 mg/kg, respectively. Application of Zn seed coating alone had no a significant effect and reduced grain Zn content of both varieties. Similar of previous study carried out in Turkey under soil Zn deficient have reviewed by Cakmak (2008b) who demonstrated that coating grains of wheat with Zn had no any significant influence on grain Zn content. Nevertheless, when the seed coating combined with foliar spray have reflected notable results and enhanced grain with Zn content, especially Imam variety which shown highly respond to this combination that through its reached the final target content of Zn (37.7 mg/kg). In case of Altındane variety foliar spray alone without combined with seed coating have achieved the determined value (37.7 mg/kg) which as set by HarvestPlus program (Bouis and Saltzman, 2017).

However, grains Zn content in the field experiment ranged between 25 and 37.7 mg/kg in Imam and 27.7 and 37.7 mg/kg Altındane. The combination of seed coating with foliar spray and foliar spray showed increasing in the amount of Zn content by 51 and 36% in Imam and Altındane variety, respectively (Figure 4.27). Our results in this work confirmed the several studies discussed previously in term of importance of foliar spray as the best and effective strategy in an enrichment wheat grain with Zn. For instance, Joy et al. (2015) found that foliar spray increased Zn concentration in grains of wheat, rice and maize by 63, 25 and 30%, respectively. Ram et al. (2016) demonstrated that the average of experiments carried out in seven countries, foliar spray increased wheat grain yield by 5.2%, and recorded the higher grain Zn concentration with 41.2 mg/kg in compared with no Zn treatment (28 mg/kg). Zn fertilizers such as $ZnSO_4.7H_2O$ applied through foliar spray on the surface of leave crops transport and move via epidermis of leaves and reach to grain through phloem, and this type of transport called symplast. The reason of respond and an enrichment of wheat grains with Zn through foliar spray could be resulting in wheat has highly efficient in term of remobilized Zn from leaves to grains. Zn content centration in Altındane (31.8 mg/kg) slightly higher than that in Imam variety (30.4 mg/kg). Similarly, foliar spray with seed coating and foliar spray with Zn displayed the highest value of grain Zn content by 37.7 and 37.7 mg/kg in Imam and Altındane variety, respectively. Seed coating alone have shown unfavorable effect on grain Zn content whereas when coupled with foliar spray with Zn had revealed and achieved curtail results in term of Zn content for both varieties (Figure 4.27). In general, farmers have applied Zn-fertilizers in order to increase their grain yield of crops in many parts of the world, thus their understand and awareness involved in the importance of rise Zn content also has to be in attention. Furthermore, the concentration of Zn in wheat generally less than 20 mg/kg (Alloway, 2009). And this value is too small to achieve and reach to targeted Zn concentration (37 mg/kg) which determined by HarvestPlus program.

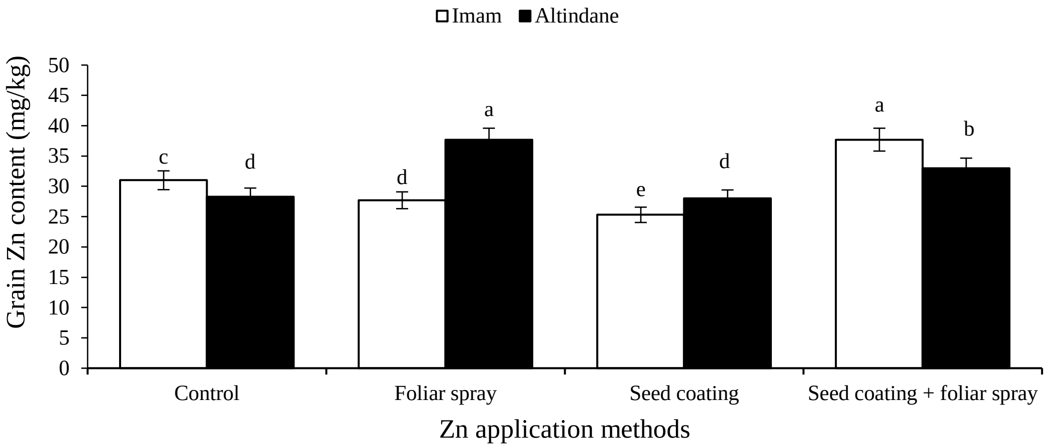


Figure 4.27. Influence of interaction effect of Zn treatments × varieties on grain Zn content (mg/kg) under rainfed condition. The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

Statistically, shoot and grain were influenced by Zn treatments and varieties (Table 4.80 and 4.81). across Zn treatments, foliar spray had the most shoot and grain Zn content compared to control and others Zn treatments for both varieties (Figure 4.26 and 4.27). The foliar spray with nitrogen at late growth stage of wheat cultivars was significantly ameliorated and enrichment shoot and grain content (Wang et al., 2015). Wide variation among varieties in terms of their shoot and grain of Zn content, where Imam variety was relatively pronounced highest Zn shoot and grain content as compared to Altındane variety (Figure 4.26 and 4.27). In soil Zn deficient experiment conducted under field condition 0.5% of $ZnSO_4.7H_2O$ increased grain Zn content of chickpea by 21% (Hidoto et al., 2017).

4.3.4. Shoot and Grain Fe Content

Analysis of variance indicated a significant difference among means of interaction effects for shoots Fe content (Table 4.82), but not for grains Fe content (Table 4.83). However, the highest shoot Fe content was obtained by seed coating (58 mg/kg) in Altındane variety, whilst the combination of seed coating with foliar spray followed by control and foliar spray have shown the lowest Fe content by 56, 54 and 53 mg/kg, respectively. In Imam variety, Zn application methods of seed coating and the combination treatment have displayed increasing in shoot Fe content in comparison with control (Figure 4.28).

Table 4.82. Analysis of variance for the effect of main factors and their interaction on shoot Fe content (mg/kg) of two wheat varieties grown under rainfed condition

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	38.58	1.79	0.20
Variety	1	1751.0	148.4	<.0001
Zn application methods	3	267.1	7.55	0.0023
Variety×Zn application methods	3	458.1	1.30	0.0002
Error	14	150		
Total	23	2664		

Table 4.83. Analysis of variance for the effect of main factors and their interaction on grain Fe content (mg/kg) of two wheat varieties grown under rainfed condition

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	5.1	1.32	0.29
Variety	1	3.92	1.94	0.1824
Zn application methods	3	23.35	3.85	0.0298
Variety×Zn application methods	3	12.94	2.13	0.1355
Error	14	27.1		
Total	23	72.5		

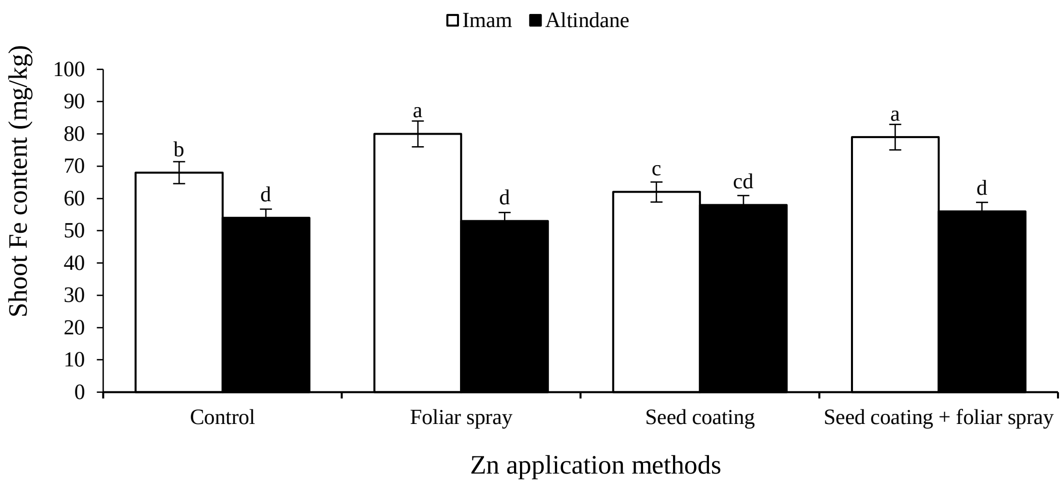


Figure 4.28. Influence of interaction effect of Zn treatments × varieties on shoot Fe content (mg/kg) under rainfed condition. The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

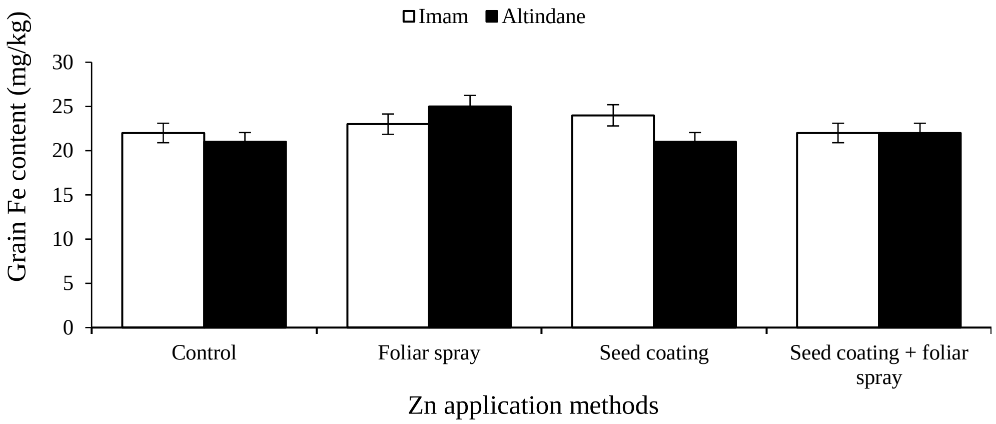


Figure 4.29. Influence of interaction effect of Zn treatments × varieties on grain Fe content (mg/kg). The data represent means of three replicate

Several studies reported by (Morgounov et al., 2007; Gomez-Becerra et al., 2010; Zhang et al., 2010; Wang et al., 2015) indicated that Zn foliar spray had remarkable effect in an improving grain Fe content, also they mentioned the positive correlation among micronutrients (Zn and Fe). Our results observed partially similar trend where the Zn application methods slightly increased grains Fe content for both varieties in comparison with control (no Zn application). The accumulation and enhancing micronutrients such as Zn and Fe in grains of staple food crops like wheat through biofortification are essential and remain the considerable challenge toward plant breeders and scientists to encounter and overcome the malnutrition problems facing people especially those living in rural areas across the world. Hence, to enrichment these grains with Zn and Fe concurrently are important, but to combine these increasing of micronutrients with quality traits such as protein content is considered as crucial challenge and become great achievements if it has been achieved.

Across variety, Imam had revealed the more Fe content in shoot than Altındane (Figure 4.28). In case of grain Fe content, interaction of Zn application with varieties significantly did not display any effect on grain Zn content (Figure 4.29).

Zn treatments significantly affected shoot Fe content, but this effect of Zn treatments absented on grain Zn content. Positive relation between grain Zn content and grain Fe content in wheat cultivars was reported by Gomez-Becerra et al. (2010) and Zhang et al. (2010). In different Zn application rate of $ZnSO_4$ applied as seed pelleting and foliar spray accumulated the highest leaves and seeds Fe content of common bean as compared to treatment without Zn application (Poshtmasari et al., 2008). In the field experiment, Zn foliar spray applied on different wheat developmental stages (booting, anthesis and early grain filling stages) of wheat was increased Fe content in the first year of season, however, this increasing was greater and produced in the second year (Niyigaba et al., 2019). Similarly, the positive association between Zn and Fe was indicated in durum wheat where Zn application no inhibit the Fe uptake (Cakmak et al., 2010) which confirmed the results in this study. The application of Zn-sulfate through priming seed was greater than soil Zn-sulfate in an enrichment grain wheat with Fe content and this effectiveness due to the type of amino acid (Seddigh et al., 2016).

4.3.5. Grain Protein Content

Another important part in term of grain quality is protein content. Based on data of analysis of variance illustrated in Table 4.84 both of main effects and interaction effect have observed highly statistical remarkable influence on protein content. However, the highest protein content was obtained by untreated seed (control) 13.7 and 12.3 mg/kg for Imam and Altındane, respectively (Figure 4.30). Whereas, Zn application e.g. foliar spray, seed coating and combination of seed coating with foliar spray have shown the lowest rate of protein content by 10, 11.6 and 9.44 mg/kg for Imam and 8.3, 12 and 8.9 mg/kg for Altındane. Within this context, Zn application methods have revealed negative effect on protein content for both of varieties. For instance, foliar spray and seed coating with foliar spray in Imam variety reduced protein by 37 and 40% in comparison with untreated seed, but this reduction was relatively less and mitigated by seed coating (18%). Across variety, Imam had revealed the more grain protein content than Altındane. It seems that seed coating relatively has the higher protein content in comparison with foliar spray and combination of seed coating with foliar, but slightly less than control treatment (Figure 4.30).

Table 4.84. Analysis of variance for the effect of main factors and their interaction on protein content of grain (mg/kg) under rainfed condition

Source of variance	DF	Sum of Squares	F Ratio	Prob > F
Replication	2	0.258433	1.0012	0.3923
Variety	1	4.550104	35.2546	<.0001
Zn application methods	3	66.576113	171.9456	<.0001
Variety×Zn application methods	3	3.255446	8.4078	0.0019
Error	14	1.80		
Total	23	76.4		

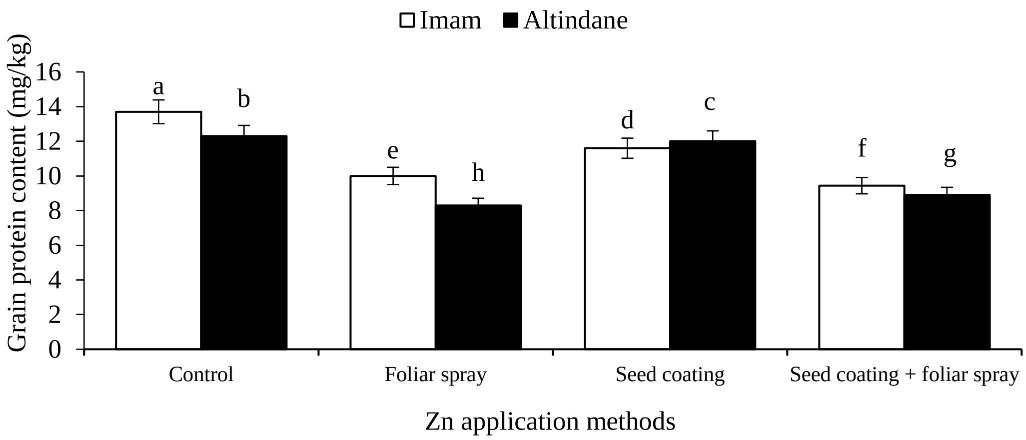


Figure 4.30. Influence of interaction effect of Zn treatments × varieties on grain Fe content (mg/kg) under rainfed condition. The data represent means of three replicate, and different letters indicate to substantial different among treatments at $P<0.05$

In present study, there was decline of grain protein content of wheat with all Zn application methods under rainfall condition. This reduction of protein content was also reported by Peleg et al. (2008) who find out that grain protein content have shown positive correlation with Zn and Fe concentration only under low water condition, but not in an adequate water supply. Additionally, pervious study carried out by Melash et al. (2019) shown that foliar application with $ZnSO_4.7H_2O$ decreased grain protein content of wheat and but this affect was not statistically significant. Consequently, it could be saying that our current results in this work agreement and accordance with those studies. The reason might be due to Zn application improve growth parameters and thus grain yield, it has been reported that high grain yield has inverse association with grain protein content (Rharrabti et al., 2001). Similar results was obtained by Ramzan et al. (2020) who reported that protein content was affected by Zn application, where the maximum grain protein content was observed in control (treatment with Zn), whereas the application of $ZnSO_4$ and $FeSO_4$ was suppressed grain Zn content in wheat.

In conclusion of field experiment, the results showed that the treatments of Zn, especially, improved grain yield and its components in Altındane and Imam wheat varieties foliar spray. In respect to biofortification of grains with Zn neither seed coating no foliar spray alone greatly increased grain Zn content. However, these treatments gave relatively demonstrated good and promising results through enhancing grain with Zn content when combined together. Whereas, Fe content did not show any difference by Zn application. Further, there was genotypic variation among varieties in response to Zn application where Imam variety given high grain yield and protein content than Altındane variety. On the other hand, there was antagonistic relation among Zn application methods and grain protein content where the content of protein reduced in the treatments which had high content of Zn. In this regard, the relationship between grain micronutrients contents with grain protein content are debatable, largely inconspicuous and required more researches in the future.

5. CONCLUSIONS AND FUTURE RECOMMENDATIONS

On the basis of this study, seed priming with 5 mM Zn was effective way to increase germination rate and improve seedling growth in comparison with hydropriming treatment. Whereas, Zn coated seed with Zn more than 1.5 g Zn/kg seed had no positive effect on growth parameters. Nevertheless, germination and seedling growth had depressed relatively. Therefore, coating of seeds with Zn applied at the smallest rate (1.5 g Zn/kg seed) may be considered as completely economic and safe for ecosystem. Furthermore, the wheat yield losses due to drought stress reached up to 8% in Imam and 15% in Altındane, when compared with well-watered treatment yield. But, Zn application through seed coating and combination of seed coating with foliar spray improved the seed yield under drought stress by 10.8 and 9.5% in Imam, and by 14 and 17% in Altındane, respectively.

There was a wide variation between Altındane and Imam bread wheat varieties in term of biofortification of grains with Zn and seed yield under greenhouse conditions. Altındane showed slightly increase in seed yield and had higher Zn content in seed in comparison with Imam variety. Altındane used water more sufficiently and had less Ψ_w than Imam variety. In contrary, Imam variety gave high seed yield and protein content than Altındane under rainfed conditions. There was antagonistic relationship between Zn application methods and seed protein content, where the content of protein reduced when the seed Zn content increased. Based on our results, following suggestions can be recommended;

1- Seed priming (5 mM Zn) and seed coating (1.5 g/kg seed) with Zn are recommended to improve seed germination rate and seedling growth parameters.

2- Zn biofortification through foliar spray is suggested to increase grain Zn grains Zn content. However, combined application of seed coating and foliar spray is also recommended in order to obtain high grain yield and grain Zn content drought stress and Zn deficient soil conditions.

3- To ensure and success of grain biofortification with Zn, it should be considered that the varieties with low Zn content in its grains gives response to Zn application than those with sufficient Zn content under soil with Zn-deficient and low water content.

4- The relationships between Zn and Fe contents with grains protein content are debatable, largely inconspicuous and required more research in the future.

5- As it known that endosperm of seeds represent the major nutrition for several people globally, hence the content of Zn in this part of seeds under these stresses conditions are recommended to investigate.

6- The effect of soil Zn deficiency and drought stress on wheat seed yield and nutrient content should be carried in the field conditions to obtain more accurate and reliable results.

7- In this study, role of Zn as a tool to improve drought tolerance has investigated. Nevertheless, the role of Zn to alleviate deleterious effect of biotic stress especially soil borne pests on seed and seedling also should be investigated and evaluated in the future.

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