

T.R.
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**IMPACT OF CADMIUM STRESS ON AGRO-
MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS
IN WHEAT LANDRACES OF TÜRKİYE**

DOCTOR OF PHILOSOPHY

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IMPACT OF CADMIUM STRESS ON AGRO-MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS IN WHEAT LANDRACES OF TÜRKİYE submitted by Awatef Ali Ahmed SHLIBAK and defended before the Examining Committee Members listed below in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Department of Biology, Institute of Graduate Studies of Bolu Abant Izzet Baysal University** in **6.06.2024** by

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Awatef SHLIBAK

ABSTRACT

IMPACT OF CADMIUM STRESS ON AGRO-MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS IN WHEAT LANDRACES OF

TÜRKİYE

PHD THESIS

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Wheat is vital for global food security, but its growth and development can be significantly affected by environmental stress factors such as heavy metals. This study was conducted to evaluate the effects of cadmium exposure on seedling growth and the accumulation of antioxidant and non-antioxidant enzymes in different wheat varieties. The study found that the effects of Cd on seed germination decreased as Cd concentrations increased, but there was no significant difference in the response of all wheat types to Cd stress. In *T. aestivum*, *T. monococcum*, *T. dicoccum*, and *T. turgidum*, 500 µM Cd application was found to reduce seed viability. However, there was no significant effect on the dry weight of plants grown in petri dishes. In soil experiments, different Cd concentrations were found to have no significant effect on root and shoot growth, but in *T. dicoccum*, different Cd concentrations caused a decrease in fresh and dry shoot weight. Additionally, it was determined that the total protein content increased in wheat varieties exposed to Cd stress, while CAT activity either increased or decreased depending on the wheat species.

Furthermore, as part of this research, sequence analyses were conducted to effectively distinguish the phylogenetic relationships among local wheat varieties in Turkey by sequencing the ITS2, matK, and rbcL regions. The study reveals that local wheat varieties have a close relationship with Triticum and that their growth and development can be significantly affected by environmental stress factors such as cadmium. It has been found that ITS2 sequences, particularly, are highly effective in resolving phylogenetic relationships due to their high interspecies variation. Similarly, rbcL sequences have also been proven effective, as evidenced by high bootstrap support values and clear clade differentiation. Although matK sequences were not obtained for *T. aestivum*, the matK tree still provided valuable insights into the relationships between *T. monococcum* and *T. dicoccum*.

KEYWORDS: Wheat Varieties, Seedling Growth, Antioxidant Enzymes, Proline, Phenolics, Catalase, Hydrogen Peroxide, Superoxide Dismutase, Heavy Metal Tolerance

ÖZET

**TÜRKİYE’NİN BUĞDAY ÇEŞİTLERİNDE KADMIYUM STRESİNİN
AGRO-MORFOLOJİK VE FİZYOLOJİK PARAMETRELER
ÜZERİNDEKİ ETKİSİ
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**BOLU, HAZİRAN - 2024
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Buğday, küresel gıda güvenliğinde hayati öneme sahiptir, ancak büyüme ve gelişimi ağır metaller gibi çevresel stres faktörleri tarafından önemli ölçüde etkilenebilir. Bu çalışma, kadmiyumun farklı buğday çeşitlerinde, fide büyümesi, antioksidan ve antioksidan olmayan enzimlerin birikimi üzerindeki etkilerini değerlendirmek için yapılmıştır.

Çalışma, Cd'nin tohum çimlenmesi üzerindeki etkilerinin Cd konsantrasyonları arttıkça azaldığını ancak tüm buğday tiplerinin Cd stresine verdiği tepkide anlamlı bir farklılık görülmediğini bulmuştur. *T. aestivum*, *T. monococcum*, *T. dicoccum* ve *T. turgidum*'da, 500 µM Cd uygulamasının tohum canlılığında azalmaya neden olduğu belirlenmiştir. Ancak, petride yetiştirilen bitkilerinin kuru ağırlığı üzerinde anlamlı bir etki görülmemiştir. Toprak deneylerinde, farklı Cd konsantrasyonlarının, kök ve sürgün uzaması üzerinde anlamlı bir etkisinin olmadığı ancak *T. dicoccum*'da farklı Cd konsantrasyonlarının taze ve kuru sürgün ağırlığında azalmaya neden olduğu bulunmuştur. Ayrıca, Cd stresine maruz bırakılan buğday çeşitlerinde toplam protein içeriğinin arttığı, CAT aktivitesinin ise buğday türüne bağlı olarak artış veya azalış gösterdiği belirlenmiştir.

Ayrıca, bu araştırmanın bir parçası olarak, Türkiye'deki buğday yerel çeşitleri arasındaki filogenetik ilişkilerin etkin bir şekilde ayrımını sağlamak için ITS2, mat K ve Rbc L üç bölgesini dizileyen dizi analizleri yapılmıştır. Çalışma, buğday yerel çeşitlerinin *Triticum* ile yakın bir ilişkiye sahip olduğunu ve büyüme ve gelişimlerinin kadmiyum gibi çevresel stres faktörleri tarafından önemli ölçüde etkilenebileceğini ortaya koymaktadır. ITS2 dizilerinin, özellikle, yüksek türler arası varyasyonları nedeniyle filogenetik ilişkileri çözmede son derece etkili olduğu bulunmuştur. Benzer şekilde, rbcL dizilerinin de yüksek bootstrap destek değerleri ve net klad farklılaşması ile etkili olduğu kanıtlanmıştır. *T. aestivum* için matK dizileri elde edilememiş olsa da matK ağacı yine de *T. monococcum* ve *T. dicoccum* arasındaki ilişkilere dair değerli bilgiler sağlamıştır.

ANAHTAR KELİMELELER: Buğday Çeşitleri, Fide Büyümesi, Antioksidan Enzimler, Prolin, Fenoller, Katalaz, Hidrojen Peroksit, Süperoksit Dismutaz, Ağır Metal Toleransı

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LIST OF ABBREVIATIONS AND SYMBOLS

mm	: Millimeter
μM	: Micro Mole
CAT	: Catalase
SOD	: Superoxide Dismutase
H₂O₂	: Hydrogen Peroxide
ROS	: Reactive Oxygen Species
DNA	: Deoxyribose Nucleic Acid
ITS	: Internal Transcribed Spacers
MATK	: Megakaryocyte-associated Tyrosine Kinase
RBCL	: Ribulose-1,5-bisphosphate Carboxylase
NR DNA	: Nuclear Deoxyribose Nucleic Acid Region
MAD	: Malondialdehyde
APX	: Ascorbate Peroxidase
GPX	: Glutathione Peroxidase
BSA	: Bovin Serum Albumin
PVP	: Polyvinylpyrrolidone
TCA	: Trichloroacetic Acid

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1. INTRODUCTION

Wheat (*Triticum* spp.) is one of the most important cereal crops in the world, and in Türkiye, it serves as a staple food for a significant portion of the world's population. In Türkiye, wheat cultivation has led to the emergence of diverse landraces adapted to various environmental conditions. Due to modern agricultural practices and high-yielding varieties, many of these traditional landraces are at risk of disappearing, along with their unique genetic traits that offer resistance to various biotic and abiotic stresses, making them essential resources for the development based on future wheat crop types (1,2). Biotic or abiotic factors during germination are very critical because they worsen crop establishment and reduce yield. Salt, drought, and cold are the most devastating abiotic factors, during germination and early seedling stages. Soil-driven stresses, such as heavy metal accumulation - cadmium, are also the main agronomic challenges threatening wheat germination, growth, and quality. Abiotic stress factors such as extreme temperature, cold or high salinity, heavy metals, and drought worsen plant growth and productivity (1,2). Growth and yield are limited up to 50 % due to abiotic stresses. Under stress conditions, morphological and physiological characteristics deteriorate. Industrialization leads to heavy metal increase in soil as industrial waste consists of various organic, inorganic, radioactive, and heavy metal mixtures. When their amount exceeds the limits in the soil, they generate toxins in plants. Cadmium (Cd) is one of the most important contaminants in the food chain because it is readily taken up by different species of plant cells (3). Wheat landraces are traditionally crop varieties that farmers cultivate in Türkiye. Furthermore, the decline of these landraces poses a threat to food security and the ability to adapt to changing environmental conditions and emerging pests as well as diseases (4,5). According to Zencirci and Özberk (2021) (6), the genetic diversity found in wheat landraces of Türkiye highlights the significance of landraces in maintaining a wide array of traits, including drought tolerance, disease resistance, and adaptability to diverse climatic conditions. Furthermore, the conservation of these landraces is crucial for the sustainability and resilience of wheat production in Türkiye. Conservation strategies are important for establishing community-based seed banks to preserve and propagate landraces at the local level. Therefore, involving farmers in conservation efforts, as they possess valuable knowledge about the landraces, can

play a critical role in their preservation. Similarly, conservation strategies are divided into two concepts which are In-situ and Ex-situ conservation. In-situ conservation on-farm allows sustainable development and determination. On the other hand, ex-situ conservation in gene banks and research institutes of seeds are important, ensuring the availability for future breeding and researchers (6,7).

1.1 Wheat Landrace

Landrace collections contain a higher amount based on genetic variety than elite types, which is an accepted requirement for breeding procedures. Additionally, this genetic diversity has been utilized by adding landrace-derived cultivars to these programs (8). Furthermore, the revolution of historical wheat landraces was the domestication process, through which the brittle rachis of wild emmer (*Triticum turgidum* spp. *dicoccoides* Körnick.) and einkorn (*Triticum monococcum* spp. *aegilopoides*) evolved to the non-brittle branches of emmer wheat (*Triticum turgidum* spp. *dicoccon* Schrank Thell.) $2n = 4x = 28$, AABB and wheat (*Triticum monococcum* spp. *monococcum*) $2n = 2x = 14$, AmAm, respectively. Wheat species and the whole Triticeae tribe have been known to have $n = 1x = 7$ chromosomes since the 1900 (9). Cultivated wheat includes Einkorn, Emmer, Durum, Rivet, Polish, Persian, Spelt, Bread, Club, and Indian Shot. Einkorn (*Triticum monococcum* spp. *monococcum*) has diploid $2n = 2x = 14$, AA chromosomes, emmer (*Triticum dicoccon*) and durum (*Triticum durum*) have $2n = 4x = 28$, BBAA, and bread wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta*) have hexaploid $2n = 6x = 42$, BBAADD chromosomes. The Triticeae genome contains three A in wheat landraces that are part of current wheat evolution; for example, *T. urartu* is the donor to the A genome, and the donor B genome is thought to be significantly derived from *Ae. speltoides* Tausch. There is also a consensus about the donor of D which comes from *Ae. tauschii* Coss. Those with AA, BB, and DD chromosomes have $2n = 14$, those with AABB chromosomes have $2n = 28$, as well as those with AABBDD chromosomes have $2n = 42$ chromosomes. Modern wheat cultivars are classified into two types: hexaploid bread wheat (*Triticum aestivum* L.), and tetraploid or durum wheat (*T. turgidum* L. Thell.) (9). Furthermore, emmer came from the hybridization of *T. urartu* and *Ae. speltoides* ($2n = 2x = 14$). Based on the evolution process, first, the emmer *T. dicoccoides* Körn. ex Asch. as well as *Graebn. Schweinf.* transformed to emmer *T. dicoccon* and then to tetraploid wheats

such as *T. polonicum* L., *T. turgidum* L., *T. carthlicum* Nevski, as well as *T. durum* Desf. Therefore, bread wheat emerged from the hybrid of *T. dicoccoides* and *Ae. Tauschii* (10,11,12). After the domestication process, traditional farmers started to be cultivators of wheat varieties. Even though traditional farmers did not participate in formal breeding programs, selection was still in progress due to natural selection in the environment and farmers' interest in the wheat varieties they grew. In addition, farmers utilized these primitive relatives of wheat for their domestic purposes, such as feeding their livestock and home utilization for making bulgur as well as macaroni (6).

A previously conducted study demonstrated new cultivar variants that explain the relationship between *Aegilops* and *Triticum* species. As a result, 12 samples from four *Triticum* species and 24 samples from eight *Aegilops* species were studied by using nuclear *ITS* rDNA region to sequence and partial sequence of the chloroplast *matK* gene. Therefore, the tree produced using nuclear component (*ITS*) sequences demonstrates a tight link among polyploid types based on wheat and *Aegilops speltoides* species, providing further support for the origin of the enigmatic B genome donor *Ae. speltoides*. The grouping of *Ae. cylindrica* and *Ae. tauschii*, as well as their close proximity to polyploid wheat, suggested that one of these species was the source of the D genome (13). As previously study reported, diploid *Triticum* species, such as *T. urartu*, have been identified as the A genome donor, and their location on the constructed tree is useful. The constructed tree based on chloroplastic *matK* sequences revealed the similar relationship between polyploid wheat and *Ae. speltoides* species, indicating that the latter species provided chloroplasts for polyploid wheat (13). As a result, Raveendar et al. (2019) (13) found that the concept of coinheritance of nuclear and chloroplast genomes has been largely validated, with *Ae. speltoides* serving as the maternal donor. Furthermore, for both trees, the remainder of *Aegilops* species formed a separate cluster, but diploid *Triticum* species, with the exception of *T. urartu*, had a monophyletic structure (14).

The phylogenetic relationships among plant species have been identified by utilizing various genes from nuclear and chloroplast genomes (15). Additionally, Nucleolar Organizing Regions (NORs) are located at the chromosomal sites.

Similarly, ribosomal RNA genes (rDNA) are found as parts of repeat units that are arranged in tandem arrays. These repeat unit contain transcribed regions which are 18S, 5.8S, as well as non-transcribed spacer (NTS) regions 26S rRNAs (16) (14). Thus, the reconstruction of the phylogeny of *Triticum* is based on the variation in many repeated nucleotide sequence families, which is explained by the donor of the B genome towards hexaploid wheat using sequences of three low-copy DNA fragments (17). The evolution of the ITS region is more complicated in hybrid and polyploid species. Transcribed region internal transcribed spacers (*ITS*) are located on either side of 5.8S rRNA gene and are termed as *ITS1* and *ITS2*(18). Additionally, the *matK* gene is approximately 1500 base pairs (bp) long, located within the intron of the chloroplast gene *trnK*, and involved in the splicing of group II introns. Moreover, the Chloroplastic *MaturaseK* (*matK*) coding sequence is an important region as it is the most rapidly evolving plastid gene, used to identify the phylogenetic relationships at the intragenic level (16). In the same way, (17) DNA markers have provided new information to discover genetic relationships on various *Triticum* and *Aegilops* species.

1.2 Some Species of Wheat Landraces

1.2.1 Bread Wheat Landraces

Triticum aestivum L., also known as bread wheat (hexaploid), is one of the "big three" cereal crops, with 25,000 varieties. Additionally, bread wheat has evolved via environmental adaptation to a variety of conditions (19). Bread wheat landraces account for 53% of the total average, while durum wheat landraces have slightly decreased to 38.0%. Moreover, the organization of wheat average has nearly disappeared by 2.2%. In the same direction, the loss of genetic diversity can be estimated through the frequency of rare species. The number of morphotypes listed by Gökgöl for the provinces and districts under a research study was 213 in the (1920s) (20). In those same provinces, about 63 morphotypes have been found recently, resulting in a 70% reduction in genetic diversity. Furthermore, depending on the area, up to 80 percent of farmers utilize current cultivar styles, which is associated with the majority of cultivars continuing to be grown alongside landraces. In the middle Black Sea area 45 to 55% of the percentage was produced by farmers in the field whereas in the southern coastline zone, it was 98%. Generally, farmers

express satisfaction with wheat landraces production, although 25% of producers grow bread wheat and 30% of grow durum wheat. Similarly, *Triticum aestivum* L., 83% of respondents and for durum *Triticum turgidum*, 93%, expressed satisfaction with the grain's quality and appropriateness for making handmade food production (20). Furthermore, the germplasm of landraces is expanded and their genetic diversity is increased through the seed exchange system utilized by traditional farmers. In conclusion, morphological and agronomical trait characterization is an important factor in determining genetic diversity in wild and local plant populations related to cultivation for breeding purposes and agricultural need (1,11).

1.2.2 Einkorn Wheat Landraces

The cultivation of hulled wheats including einkorn (*T. monococcum* var. *monococcum*) which is associated with emmer wheat (*T. dicoccum* L.), decreased globally as well as in Türkiye because of their low yield levels until the 1960s. However, after the 1990s, interest in natural as well as organic products, and the health benefits of hulled wheats were recognized due to their high starch-resistant content (21).

The diploid wheat *Triticum monococcum* L. (einkorn) was overshadowed by the high-yielding tetraploid and hexaploid wheat varieties and largely forgotten by modern breeders (22). Furthermore, according to the molecular research based on the nuclear and chloroplast microsatellites, 50 einkorn wheat (*Triticum monococcum* L.) accessions from various regions of the world, such as Europe, North Africa, and the Near East were identified to have two primary gene pools. One originated in Morocco and the Iberian Peninsula, while the other came from Europe and the Near East (7,13) in Türkiye, particularly around Sinop, the region surrounding Kastamonu, the Western Black Sea, Samsun, and Bolu, *Triticum monococcum* spp. *monococcum*, commonly known as IZA (einkorn) wheat, is a single-grain wheat variety that originated in Germany and is mostly used for bulgur. Furthermore, einkorn wheat contains single-grain, husky spikes, after it has been boiled and split in stone mills by utilizing an entirely conventional method (23). Einkorn wheat (*T. monococcum*) showed less intraspecific DNA variations of its nuclear genomes compared to Aegilops species which are *Ae. speltoides* and *Ae. Squarrosa* which presented the largest nuclear genome variation, while its

chloroplast genome variation was the least, according to research via Kilian et al. (2007) (18, 7). Moreover, compared to current wheat cultivars, landraces contain greater amounts of nutrients including copper, iron, magnesium, manganese, phosphorus, selenium, and zinc (25). Thus, these landrace cultivars have high concentrations of lutein, carotenoids, and tocopherols, which are known to be beneficial against chronic diseases. Furthermore, they are resilient to saline, cold, and drought stress (1). In addition, einkorn, emmer, and spelt should be utilized as modern wheat breeding projects as they are potential sources of drought, salt, cold, and biotic stress factors (26). Furthermore, the 'A' genome based bread and durum wheat can benefit from increased genetic diversity through the utilization of einkorn (27).

1.2.3 Emmer Wheat Landraces

Emmer wheat started in Türkiye and extended to several other nations, including North Africa, the Balkans, Italy, and Spain (26). According to earlier research, emmer wheat was domesticated in the Karacadağ Mountains located southeast of Türkiye and to the west of the Diyarbakır province (27). The dispersal of wheat was accompanied by adaptation to different environments, leading to the evolutionary success of this species (28). Nowadays, the most economically significant wheats, such as hexaploid bread wheat and tetraploid durum wheat (*T. turgidum* subsp. *Durum*), are descended from domestic emmer wheat (*Triticum turgidum* subsp. *dicoccon*, *Triticum turgidum* subsp. *aestivum*). Accordingly, these species differ from their ancestors in a trait highly significant to agriculture, such as the free-threshing phenotype. Similarly, emmer is a hulled wheat that does not undergo free-threshing, and significant mechanical processing is needed to remove the seeds from the husks (28). A major obstacle impeding emmer's resurrection and maintenance is a lack of market prospects, despite the plant's minimal input requirements and capacity to adapt to poor soils. Nonetheless, emmer's nutritional and health benefits have opened new commercial prospects, and as niche customers' appreciation of these qualities grows, so do the opportunities to preserve this important species (29). Emmer wheat germplasm is a key to modern wheat variety improvement. Also known as *Triticum dicoccon*, it is critical to wheat domestication and current variety development. Its tremendous genetic diversity and different gene combinations enhance its potential. The evaluation of polymorphism for gliadin proteins in Turkish-cultivated emmer wheat (*Triticum*

dicoccum spp. *dicoccum* Schrank Thell.) populations revealed high genetic diversity ($He = 0.92$) as well as displayed 27 distinct patterns made up of various gliadin protein combinations. Furthermore, the majority of Turkish-cultivated emmer wheat populations have the α -45 and w-35 gliadins, which are strongly linked to dough quality; as a result, emmer wheat germplasm bears desirable traits related to quality (7,29).

However, the Gene Bank, in order to assess genetic diversity using DNA markers (SSRs), investigated samples of emmer wheat germplasm preserved at the Aegean Agricultural Research Institute in Izmir, Türkiye. This material yielded 100% polymorphism in total (497) alleles and recorded significant genetic variation ($He = 0.9$). Furthermore, landraces displayed higher genetic diversity estimates for the A genome than the B genome, in contrast, the SSR loci at telomeric and sub-telomeric regions displayed lower genetic diversity than other parts of the chromosomes. The microsatellite marker x-gwm-312, documented as having a linkage with the salinity tolerance in wheat, displayed the highest polymorphism ($He = 0.97$) among the SSR markers utilized for analysis. Therefore, Turkish emmer wheat germplasm conserved in the Gene Bank might have potential salinity tolerance exploitable in formal wheat breeding programs (30).

1.2.4 Durum Wheat Landraces

Triticum durum Desf. (Durum wheat) related to the tetraploid group and designed from a genome combination of *Triticum durum* cells (AABB), consist of 28-chromosomes within the durum wheat species. It is announced as the second most important wheat after bread wheat (31) especially, in food manufacturing for macaroni-making quality. In fact, durum wheat is grown on about 22 million hectares worldwide including in the Mediterranean Sea, in the U.S.S.R., Iraq, Iran, Pakistan, India, China, Canada, U.S.A., Mexico, Argentina, Uruguay, Chile, as well as North Africa (31). *Triticum turgidum* L. convar. *durum* Desf. M.K. is an important crop in Türkiye and Mediterranean countries and its significance has increased recently in the world. *Triticum durum* Desf. (durum wheat) in Türkiye has covered 65–70 % of the total wheat area during the 20th century (during the Ottoman Empire) (18). Due to the scarcity of high-quality raw materials for the pasta industry, its growing importance in global trade, and the food shortages that

many developing nations face, landraces of durum wheat in Türkiye have a higher level of genetic diversity, as shown by RAPD markers for observed heterozygosity and gene diversity (7,27). Furthermore, to comprehend the genetic processes underlying wheat domestication and geographic dissemination, and to develop fundamental approaches for future usage of the special qualities of adaptability, performance, stress tolerance, and end-utilization quality that landrace cultivars display (28,1). Due to their sensitivity to zinc deficiency, which occurs in many soils in Türkiye, durum wheat cultivars are less tolerant to cold (18). Additionally, cultivation is suited considering some key cultivation factors, such as improved grain yield, yield stability, a better understanding of genotype-environment interactions (GxE) and adaptation mechanisms, responsiveness to inputs and efficient use of resources in various cultivation systems, resistance to biotic stresses (pests and diseases), and tolerance to abiotic stresses (drought, salinity, etc) (34). Furthermore, these techniques may be used with minor modifications to boost productivity and shorten the breeding program's duration (18,34).

1.3 Strees in Wheat

A wheat landrace can be defined as a conventional variety which includes stress tolerance and high yield stability with moderate crop yield under minimal input conditions (35,3) These landraces have emerged as a diverse and heterogeneous population as they provide a resisting capacity against biotic and abiotic stresses (5).

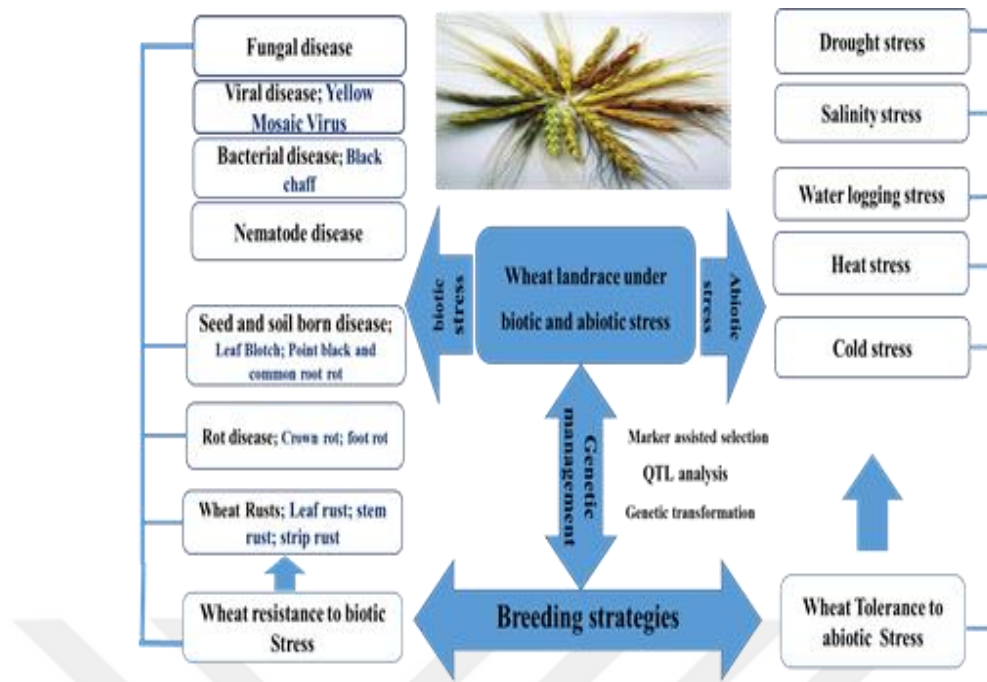


Figure 1.1. Stress factors of wheat landraces (extended from (36,37))

Several abiotic factors affect wheat and decrease its production via stress responses: 31.56% by heat, 26.61% by drought, and 23.38% by salinity. In addition to salinity, drought, and cold adversely affect the quality of water and water absorption reduce soil osmotic potential, induce water deficiency, and cause morphological, physiological, and biochemical deterioration, eventually reducing yield (36,37). Industrialization leads to an increase heavy metal in soil as industrial waste consists of various organic, inorganic, radioactive, and heavy metal mixtures (38). Such heavy metals behave as vital micronutrients and play an essential role in plants, e.g. as cofactors of metabolic enzymes. When their amount exceeds the limits in the soil, they generate toxins in plants (39).

Cadmium (Cd) is one of the most significant pollutants in the food chain because it is rapidly absorbed by many types of plant cells (3). Cadmium promotes oxidative damage in plants by decreasing or increasing levels of antioxidative enzymes (40). As a result, it reduces seedling growth, which is plumule and radical in plant rice (42), and has a greater impact on root than shoot growth of plant wheat (40). Therefore, cadmium is a highly mobile element that is readily absorbed by plants, it has the ability to contaminate soil and create taken up in various plant parts when it comes to heavy metal issues. It is recycled by anthropogenic activities (38).

Cheng et al. (2006) (42) discovered that plants are the primary transporter of harmful substances from the soil to humans. High Cd solubility and toxicity harm plant development(42). According to Gouia et al. (2003) (43), Cd toxicity in plants is produced by an interaction with the SH group that inhibits enzyme activity. Furthermore, it inhibits plant nutrient uptake and reduces the availability of minerals in the soil (38). Many assays are used to measure the phototoxicity of Cd and Pb, including root elongation, seedling growth, and seed germination (44). Plants lack the ability to perceive the physical characteristics of metals, such as their particular weight (45). Furthermore, heavy metal contamination is a serious environmental problem that limits plant productivity and threatens human health. Likewise, Cd enters the environment mostly via industrial processes, wastewater irrigation, the use of metal-containing pesticides in municipal composts with phosphatic fertilizers, and evaporation into the atmosphere, resulting in phytotoxicity and threats to animal and human health throughout the food chain (46). Cadmium is rapidly absorbed and transported to other plant parts by the roots. Changes in physiological and biochemical processes, as well as responses from organs and whole plants, such as reduced growth and development, reduced productivity, chlorosis, and changes in the structure, shape, and many metabolic activities in different cell compartments, like chloroplasts, are indicative of the toxic symptoms of metals in plants (47).

One of these detrimental impacts is the suppression of photosynthesis, which includes the production of chlorophyll as well as how photochemical processes are carried out. Because Photosystem II (PSII) is so susceptible to Cd, it was significantly more inhibited than Photosystem I (PSI) in terms of function. Additionally, recent research has shown that Cd has a variety of impacts on PSII's donor and acceptor sites. Cd stops electrons from moving from Q_A^- to Q_B^- on the acceptor site. It also stops the oxygen-evolving cycle, which leads to oxygen evolution on the donor site (48). Based on the chemical and physical characteristics of heavy metals, there is evidence that plants react to their toxicity in three distinct ways at the molecular level: **(a)** autoxidation-induced generation of reactive oxygen species (ROS), **(b)** biomolecules' critical functional groups being blocked, and **(c)** vital metal ions being removed from biomolecules. Since Cd is a non-redox metal, its toxicity may be linked to oxidative damage brought on by ROS, including

hydroxyl radicals (OH), singlet oxygen ($^1\text{O}_2$), superoxide radicals (O_2^-), and hydrogen peroxide (H_2O_2). Depending on its concentration, Cd can either inhibit or stimulate the activity of several antioxidative enzymes before any visible symptoms of toxicity appear (44,49). These very reactive ROS harm membrane lipids, proteins, pigments, and nucleic acids, which drastically lowers plant growth and production and ultimately results in plant death. These kinds, due to leakage of the electron transport chain and subsequent reaction cascades, are unavoidable by products of all aerobic life in normal physiological control (49). However, they usually remain at an appropriate level and do not cause oxidative damage. Plants may produce more ROS in stressful conditions such as heat, drought, and exposure to heavy metals. Oxidative stress would result from an imbalance between the production and elimination of ROS (50). In different plants, cadmium stress may result in modifications to the antioxidant system and oxidative damage, such as lipid peroxidation. Changes in the antioxidant system are typically used to infer ROS generation generated by Cd (46). Moreover, the responsiveness of antioxidant enzymes to Cd and metals might vary among different species and tissues. Plants become more stress-tolerant as their anti-oxidative enzyme activity increases. Superoxide dismutase (SOD; EC 1.12.1.1), is the primary step of cellular defense. It dismutates O_2^- to H_2O_2 and O_2 . Furthermore, the ascorbate-glutathione cycle, (APX; EC 1.11.1.11) ascorbate peroxidase, which decreases H_2O_2 , or the activity of catalase (CAT; EC 1.11.1.6), or glutathione peroxidase (GPX; EC 1.11.1.9) in peroxisomes limit H_2O_2 accumulation (52, 50).

1.3.1 The Mechanisms of Toxicity and Tolerance in Wheat Plants

The decrease of wheat plant growth caused by exposure towards Cd-stress might be due to various toxicity mechanisms in wheat at physiological and molecular levels, including excessive production of ROS and overproduction of malondialdehyde (MDA) contents, associated with increased thiobarbituric acid reactive substances (TBARS), as documented by Chen et al. (2010) (52). Cadmium toxicity may cause membrane damage in wheat plants, resulting in decreased nutrient and mineral uptake. Milone et al. (2003) (53) found that the durum wheat cultivars Adamello and Ofanto had more (K) leakage ions, indicating free circulation of Cd-stress ions. The Ofanto cultivar's (K) leakage ions remained lower, possibly due to vacuolar compartmentalization under Cd stress.

Furthermore, damage to the composition and functioning of photosynthetic pigments may result in a reduction in chloroplasts' photosynthetic pigment in leaves due to leaf Cd content. According to Groppa et al. (2008) (54), the reduced rate of wheat root development under Cd stress may be related to the production of nitric oxide (NO) in the roots of the plant. The authors proposed that the distribution of Cd inside subcellular structures is a major determinant of Cd toxicity in wheat roots. The excessive amount of cadmium (Cd) resulted in changes to the structure of proteins and hindered the functioning of certain genes (54,55).

Similar to this, Li et al. (2011) (56) found that while Cd-stress concentrations were reduced in detoxified fractions and heat-stable protein Cd-stress-rich granules, they increased in heat-denatured protein organelles and metal-sensitive fractions, increasing the Cd-stress levels in the growth medium. Consequently, one of the main contributing factors to the toxicity of Cd-stress to wheat roots is the distribution of subcellular Cd-stress. Protein structural abnormalities were also generated by excessive Cd stress based on certain genes, as demonstrated by Cebeci et al. (2008) (57). In addition, structural alterations, genotoxicity, and excessive ROS production are the main processes that cause Cd to be hazardous to wheat plants.

Researches conducted that the concentration of cadmium in wheat roots remains significantly higher than in the shoot (55). According to Ci et al. (2011) (58), there was a decrease in Cd-tolerant wheat varieties compared to Cd-sensitive varieties, and the accumulation of Cd changed across different times of exposure to Cd. Several types of wheat plants have a tolerance mechanism that involves decreased absorption and accumulation of Cd. Therefore, enzymatic and non-enzymatic antioxidant enzyme activity are the main mechanisms of Cd tolerance in wheat cultivars, when wheat plant cultivars are under Cd stress, diminishing ROS (56). Furthermore, the activities of peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) increased in wheat shoots and roots with increasing Cd concentrations, as declared by Chen et al. (2014) (59). On the other hand, at low concentrations of Cd (50 μ M Cd stress/24 days), leaf CAT activity decreased in the Cd-sensitive types of wheat while increasing in tolerant types (58). Also, at lower Cd levels, there was no effect on the activities of GPX, GR, APX, CAT, or SOD.

As found by Milone et al. (2003) (53), the roots exhibited higher levels of APX, GPX, and syringaldazine peroxidase (SPOD) activity in response to Cd stress compared to leaves.

According to Yannarelli et al. (2007) (60), Glutathione reductase (GR) activity increased in wheat roots under Cd stress. Ahmad et al. (2009) (61) state that the overproduction of phytochelatins (PC) has a major impact on the resistance to cadmium. Additionally, wheat plants subjected to elevated Cd levels generated higher amounts of the low-molecular-mass component glutathione (GSH). Previous studies showed that Cd stress raised GST activity, GSH levels, and the production of non-protein thiol (NPT) when Cd concentrations in the solution reached 2.0 mM, of the concentration, as well as the increased production of phytochelatins (PC-SH) in the roots and shoots of wheat plants (55). Also, wheat seedlings accumulate more proline under Cd stress, although excess Cd-induced proteins in leaves might lead to tolerance and enhance Cd-detoxifying protein levels (55).

Moreover, by changing plant growth regulators (PGR), the plant may tolerate high levels of Cd in plants in various ways. According to Asgher et al. (2015)(62), the hormones gibberellic acid (GA), salicylic acid (SA), nitric oxide (NO), ethylene, brassinosteroids BRs, and polyamines Pas constitute some of the primary plant growth regulators (PGRs). Other significant plant growth regulators include abscisic acid ABA, cytokinins CKs, jasmonic acid JA, indole-3-acetic acid IAA, and ascorbic acid (AsA). Research suggests that wheat can enhance PGR production in response to Cd stress, potentially enhancing its tolerance to high Cd levels (55).

2. AIM AND SCOPE OF THE STUDY

This research study aimed to investigate the effect of Cadmium toxicity with different concentrations on the early-stage germination and development of seedling characteristics of wheat landraces from Türkiye. Additionally, it sought to explore and evaluate the effect of Cd stress on physiological parameters of seedling growth of wheat landraces.

Furthermore, the study aimed to identify and evaluate the origin and phylogenetic relationships of the wheat landrace species in Türkiye to determine strategies for preservation. Such germplasm is valuable for its local adaptations acquired through selection in specific geographic region over many generations.

3. MATERIALS AND METHODS

3.1 The Sample of Wheat Landraces Selected to Study the Physiological Experiments

The experiment was carried out at the Plant Genetic and Pathology Lab in the Department of Biology at Bolu Abant İzzet Baysal University at Bolu, Türkiye. Four wheat landrace cultivars -Einkorn wheat, Emmer wheat, Bread wheat (Ekiz), and Durum wheat- were collected from the Türkiye region in Bolu field as presented in Table 3.1 below. Furthermore, the wheat cultivars were selected to evaluate cadmium nitrate ($\text{Cd}(\text{NO}_3)_2$) at different concentrations (50 μM , 100 μM , 250 μM , and 500 μM) on seed germination and seedling growth in Petri dishes and treatments in the soil. The following parameters were measured: germination percentage, seed vigour index, shoot and root toxicity, tolerance index, root and shoot elongation, fresh and dry weight of the wheat landraces, and chlorophyll (a, b) and carotenoid contents, phenolic content, proline contents, protein content, CAT, SOD and H_2O_2 determined. In addition, the study included the detection and determination of some elements (K, Mg, P, Fe, and trace Cd toxic) in the leaves of the wheat landraces.

Table 3.1. The seed samples of wheat landraces were collected from Türkiye.

Common name of Species	Scientific name of species	Region
Ekiz	<i>Triticum aestivum</i> L.	Uluslararası Tar. Araş. Enst.
Einkorn	<i>Triticum monococcum</i> spp. <i>monococcum</i>	Seban Güneyce köyü
Emmer	<i>Triticum dicoccum</i> spp. <i>dicoccum</i> Schrank	Sinop Durağan Gölgerişi
Durum	<i>Triticum turgidum</i> L. var. <i>durum</i>	Uluslararası Tar. Araş. Enst. Müd.

3.1.1 Sterilized and Treatment by Cadmium Nitrate

Prior to germination, seeds were surface sterilized by adding 5 drops of Tween20 (Merck, Darmstadt, Germany) to 100 mL of water for 1 minute and 15 minutes with 30% sodium hypochlorite and then rinsed three times with distilled water (63). The seed samples of wheat landraces were sterilized and treated with four levels of Cadmium stress concentration: control (0 μM and 50 μM , 100 μM , 250 μM , and 500 μM) Cadmium Nitrate ($\text{Cd}(\text{NO}_3)_2$) (98% Sigma-Aldrich).

3.1.2 Physiological Experimental Analysis: Studying the Effect of Cadmium ($\text{Cd}(\text{NO}_3)_2$) on Morphological Traits of wheat landraces on Petri Plate.

The experimental on the Petri plates, was conducted using four replicates and 200 seeds for each population, presented in Tabel 1.3. Each sample was treated with different concentrations of cadmium: control (0 μM , 50 μM , 100 μM , 250 μM , and 500 μM). Additionally, they were incubated under controlled conditions in a growth chamber ($20\pm 2^\circ\text{C}$, 16 hours of light and 8 hours of dark) (64). During the germination, 2 ml of dH_2O was added to the control, while 2 ml from different concentrations of Cd were added every fourth-eight hours for seven days on a Petri plates (65). After 8 days, the germination percentage was evaluated, and coleoptile (shoot) elongation, root elongation, root fresh weight, root dry weight, and seedling vigour under cadmium stress were measured according to Kim et al. (1994) (66). Germination percentage was calculated by multiplying normal emergence by seedling shoot length and dividing by 100. Similarly, seedling vigour index (S.V.I.) = Seedling length (mm) $\times$ germination percentage / (100%). The fresh root of wheat landrace cultivars was oven-dried at 65°C for 48 hours to measure dry weight according to Weatherley, (1950) (67).



Picture 3.1. The seed germination and seedling growth of wheat landraces with $\text{Cd}(\text{NO}_3)_2$ treatment on Petri plate.

3.1.3 Physiological Experimental Analysis, designed to Study the Effect of Cadmium ($\text{Cd}(\text{NO}_3)_2$) on Morphological and Physiological Traits of wheat landraces in Soil

To investigate the impact of cadmium nitrate on wheat seedling germination characteristics, shoot length, root length, root fresh weight, and root dry weight, 20 surface-sterilized seeds from each wheat landrace population mentioned in Table 1.3 were utilized. The wheat seeds were grown in sterilized plastic pots (diameter 40 cm, 2.5 kg soil) containing heat-sterilized soil. Different experimental setups were designed, either combined or separate, under various concentrations of cadmium stress inducers. The cultivars were subjected to four levels of cadmium treatments (0 as control (0 μM , and 50 μM , 100 μM , 250 μM , and 500 μM) for treated plants in plastic pots). Additionally, they were incubated under controlled conditions in a growth chamber (20 $\pm$ 2 $^\circ\text{C}$, 16 hours of light conditions and 8 hours of dark conditions).

Similarly, during the germination seedling, 150 ml of dH_2O was added to the control group while 150 ml of the different concentrations of Cd solution was applied to each plastic pot containing seedling cultivars for 7 days. After seven days of plant growth and the emergence of two leaves, the Cd treatment solution was initiated by adding 150 ml from different concentrations to each seedling cultivar. Then, after 28 days' wheat cultivars were harvested. Subsequently, the leaves and roots were oven-dried at 70 $^\circ\text{C}$ for eighty-four hours to obtain their dry weight (65). The germination percentage shoot and root toxicity, and tolerance index were calculated below:

$$\text{Germination (\%)} = \frac{\text{total seed germination}}{\text{total seed of plant}} \times 100$$

$$\text{Shoot toxicity (\%)} = \frac{\text{Shoot length of cotrol} - \text{Shoot length of treatment}}{\text{Shoot leght of control}} \times 100$$

$$\text{Root toxicity (\%)} = \frac{\text{Root length of cotrol} - \text{Root length of treatment}}{\text{Root leght of control}} \times 100$$

$$\text{Tolerance toxicity\%} = \frac{\text{Mean root length in stress}}{\text{Mean Root leght in control}} \times 100$$



Picture 3.2. The germination of seedling growth of wheat landraces with $\text{Cd}(\text{NO}_3)_2$ treatment in soil.

3.1.4 Determination of Chlorophyll (a, b) and Carotenoids

After 28 days of harvesting of wheat, chlorophyll contents were determined after extracting the pigment from 0.5 g plant leaves macerated in a pestle–mortar with 10 ml of methanol then centrifugation at 15,000g for 10 minutes. The chlorophyll in the supernatant quantified by using Helios α UV/VIS spectrophotometer at 470, 653 and 666 nm. The concentrations of carotenoids and chlorophylls α and β were determined according to the equations used by Lichtenthaler and Wellburn (1983) (67) as follows:

$$\text{Total carotenoids (mg/L)} = 1000 \text{ Abs}_{470} - 2.860 \text{ Ca} - 129.2 \text{ Cb}/245$$

$$\text{Chlorophyll a (mg/L)} = 15.65 \text{ Abs}_{666} - 7.340 \text{ Abs}_{653}$$

$$\text{Chlorophyll b (mg/L)} = 27.05 \text{ Abs}_{653} - 11.21 \text{ Abs}_{666}$$

3.1.5 Determination of Total Phenolic Content

To extract of phenolics from wheat landraces, 0.5 g leaf tissue was macerated in a pestle and mortar with 10 ml of methanol/water (1:1, v/v). The suspended macerate was then ultrasonicated for 15 min at room temperature followed by centrifugation at 1500×g for 15 min. The clear greenish supernatant was collected, and cell debris was subjected to another extraction for 4 hours. The

extraction procedure followed the approach outlined by Waterhouse (2002) (69) approach as mentioned in the first step below.

For each sample, 20 μL of the solution or blank was mixed with 1.58 ml water and 100 μl of 2 N Folin-Ciocalteu, which was then mixed for five minutes. Subsequently, 300 μl of 20% (w/v) Na_2CO_3 was added for twenty minutes at 40° C.

The absorbance was measured at 765 nm in spectrophotometer and the values were plotted against the concentration using methanol as the blank.

The content of phenolic plant extraction was expressed in gallic acid equivalents (GAE) / (dry extract (dE)) and was determined using the following equation:

$C = cV/m$ C=total phenolic compounds, (mg GAE/g) (dry extract (dE)); c = the concentration of gallic acid obtained from the calibration curve, (mg/ml); V=the volume of extract, ml=the weight of extract of phenolic from wheat landraces.

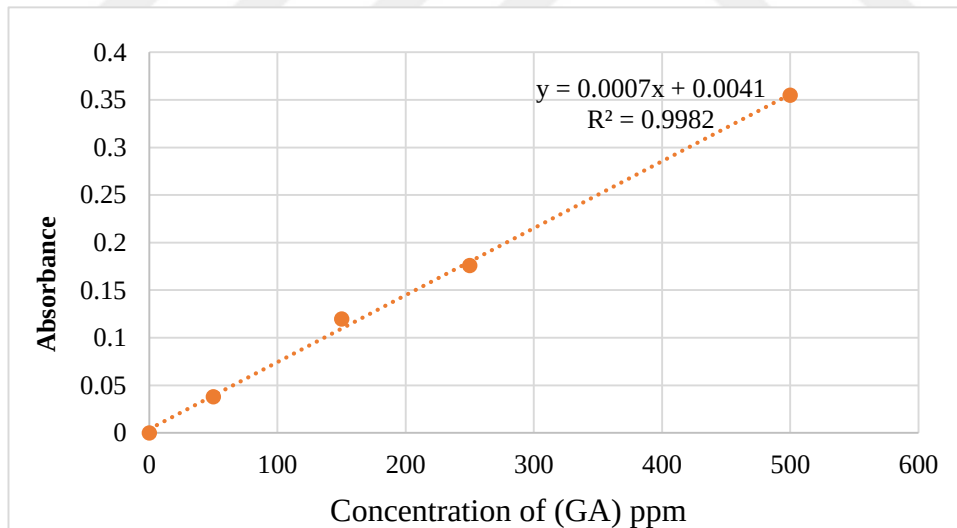


Figure 3.1. Standard curve of gallic acid (ppm).

3.1.6 Determination of Proline Content

The Proline method consisted of extracting 0.2 g of dry wheat leaves with 10 mL of 3% (sulfosalicylic acid) and centrifuging the mixture at (3000 $\times$ g) for 20 minutes, as performed by Bates et al. (1973) (70). Then, 2 mL of the extracted

solution was added with 2 mL of (ninhydrin) and 2 mL of (glacial acetic acid). The reaction was spotted after 1 hour by using an ice bath after exposure at a temperature of 100 °C. In the next step, the reaction mixture was extracted with 4 mL of toluene, mixed for 15-20 seconds, and determined spectrophotometrically at 520 nm against standard proline.

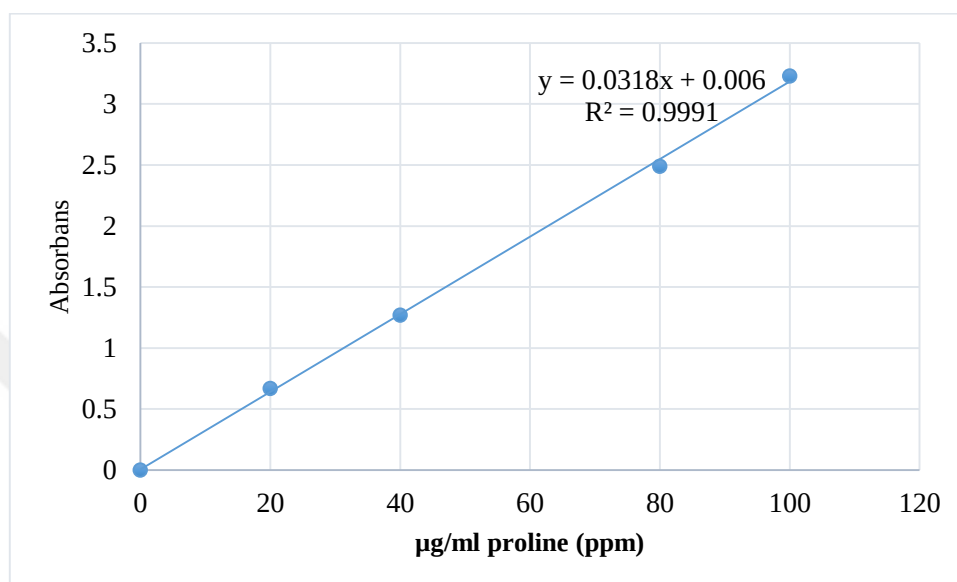


Figure 3.2. Standard curve of proline (ppm).

3.1.7 Extraction of wheat Leaves for Enzyme Assays

The extraction of wheat leaves to analyze their Catalase (CAT) and Superoxide Dismutase (SOD) activities has been performed as described by Şahin (2019) (71). In the first step of preparing the enzyme analysis, 0.5g of weight fresh leaves was mixed with 2 mL of 50 (mM K-phosphate) buffer, 2 (mM Na-EDTA) and 1% (w/v) polyvinylpyrrolidone (PVP) The mixture or extraction was then centrifuged at 4 °C at 12000 rpm (min^{-1}) for 10 min, and the resulting extraction was kept at -20 °C for further determination of CAT and SOD.

3.1.8 Determination of Soluble Protein Content

As documented by Lowry et al. (1951) (72) for the determination of the soluble protein content, it was announced that from each sample, 0.2 ml was put into a test tube. Furthermore, 2 ml of an alkaline copper sulphate reagent was added to the extract. Therefore, after 10 minutes of incubation at room temperature, the absorbance was measured at 660nm by a spectrophotometer against a blank. A

standard curve of protein was prepared using concentrations of 0, 50, 100, 200, 600, and 800 mg/l (ppm) of Bovin Serum Albumin (BSA).

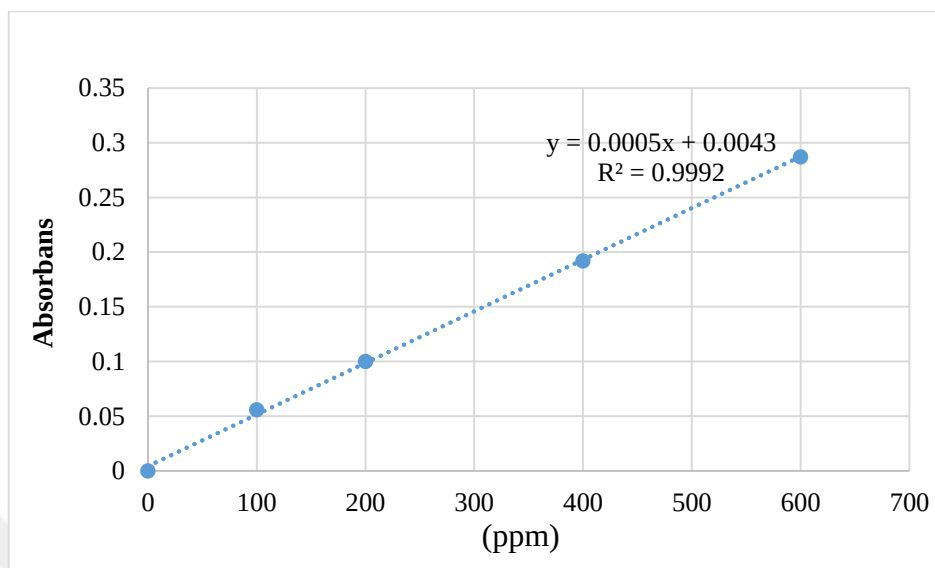


Figure 3.3. Standard curve of bovine serum albumin (BSA).

3.1.9 The Measurement of Catalase Activities

According to Lartillot et al. (1988) (73), the activity of Catalase (CAT) has been determined utilizing spectrophotometry to calculate the amount of (H_2O_2) absorbed at 240 nm. The reaction was examined by adding 0.5 ml of a 1M hydrochloric acid (HCl) solution (Ar). The initial absorbance of H_2O_2 was detected by measuring the absorption rate of the reaction mixture at zero time (A_s). The reaction mixture consisted of 2.5 ml of the substrate and 0.5 ml of 1 M hydrochloric acid (HCL). In the same way, 2.5 ml of buffer and 0.5 ml of hydrochloric acid (HCL) were added to each tube. Spectrophotometry was used to measure the protein absorbance (A_t) of the solution that had 20 μ l of enzyme extracted. The absorbance variation caused by the enzymatic activity was calculated:

$$A = (A_s + A_t) - A_r$$

The Enzyme activity solution in Uml^{-1}/mg protein was calculated.

$$Activity = (A \times Vt) / (\epsilon \times t \times Ve) \times (1 / mg \text{ proten})$$

Vt = The total volume of reaction(ml)

Ve = volumof enzme extraction(ml)

ε = extinction coefficient of

t = Reaction time

3.1.10 The Measurement of Superoxide Dismutase (SOD) Activities

The research technique as reported by Sun et al. (1988) (74) has been utilized to evaluate the activity of Superoxide Dismutase (SOD, EC 1.15.1.1), also known as superoxide oxidoreductase. This evaluation method is based on the enzyme's capacity to prevent the photochemical reduction of nitro blue tetrazolium (NBT). Additionally, the SOD measure involves the following reagents: In a 200-mL beaker, mix 40 mL of xanthine, 20 mL of EDTA, 20 mL of 150 $\mu\text{mol/L}$ nitro blue tetrazolium, 12 mL of 400 mmol/L (Na_2CO_3), as well as 6 mL of 1 g/L bovine serum albumin. The enzyme extract was combined with 1.425 mL of SOD assessment reagent, and 0.05 mL of an enzymatic extract from each tube was put into a water bath set at 25°C. In each tube, was added 0.025 μL of xanthine oxidase solution and incubated for 20 minutes. Afterward, the enzymatic reaction was ended by adding 0.05 mL of 0.8 mmol/L CuCl_2 solution to each of the tubes, which were then detected at 560 nm spectrophotometrically. Further, the following equation was used to get the inhibition values:

$$\% \text{Inhibition} = \frac{(\text{Absorbance blank} - \text{Absorbance sample}) \times 100}{\text{Absorbance blank}}$$

$$\text{Activity (U/ml)} = (\% \text{Inhibition}) / (50 \times 0.1)$$

$$\text{Specific activity} = (\text{Activity (U/ml)}) / (\text{mg/ml protein})$$

3.1.11 The Measurement of H_2O_2 Activities

The approach used by Velikova et al. (2000) (75) to evaluate H_2O_2 content involved the following steps: 0.5 g of fresh leaf wheat was homogenized with 5 mL 0.1% (TCA) trichloroacetic acid and centrifuged at 10,000 g for 15 minutes at 4°C. Next, 1 mL from the supernatant and mix it with 10 mL of phosphate buffer and 2 mL of potassium iodide (KI). The absorbance at 390 nm was then obtained. The H_2O_2 level was calculated using a standard curve.

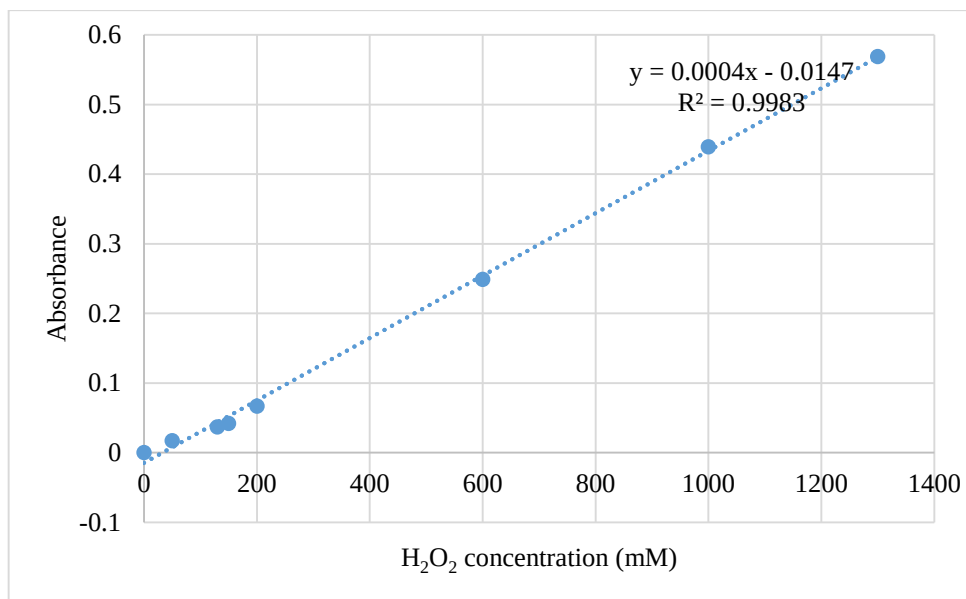


Figure 3.4. The H₂O₂ standard curve.

3.1.12 Determination of Elemental Composition and Cadmium Toxicity in Wheat Landraces

A 0.5 g sample of dry wheat was treated with (Cd (NO₃)₂) at concentration of 100 µM, 500 µM, with a control group for comparison. The samples were digested using Milestone microwave equipment under the pressure of 100 bar and 300°C for 54 minutes. The digestion reaction was prepared by mixing 8 mol/L nitric acid and H₂O₂ (33% (w/v)) with the sample, following the procedures outlined in the certified standard reference material (NIST SRM 1567b) for wheat obtained from the National Institute of Standards and Technology (NIST, USA) (75). Calibration solutions were prepared daily by serial dilution of a stock solution, followed by ICP multi elements standard solution to determine the concentration of Mg, K, P, Fe, and Cd (76) in wheat landraces collected from different region in Türkiye.

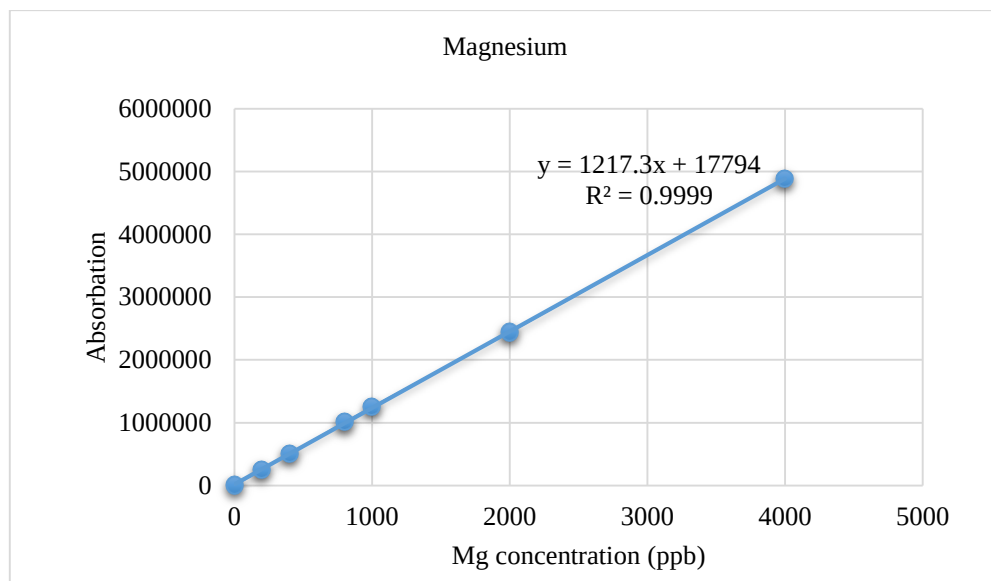


Figure 3.5. The standard curve of magnesium in wheat leaves.

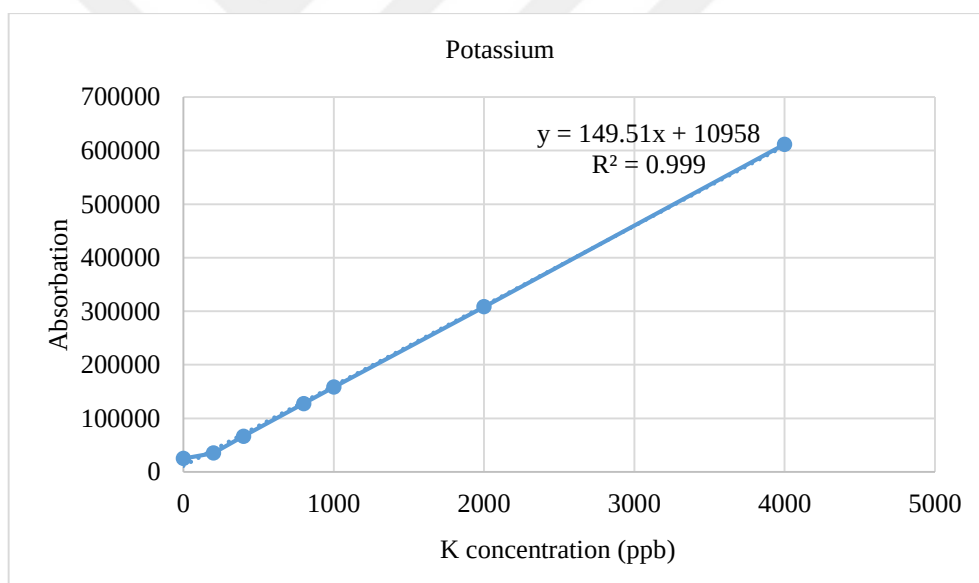


Figure 3.6. The standard curve of potassium in wheat leaves.

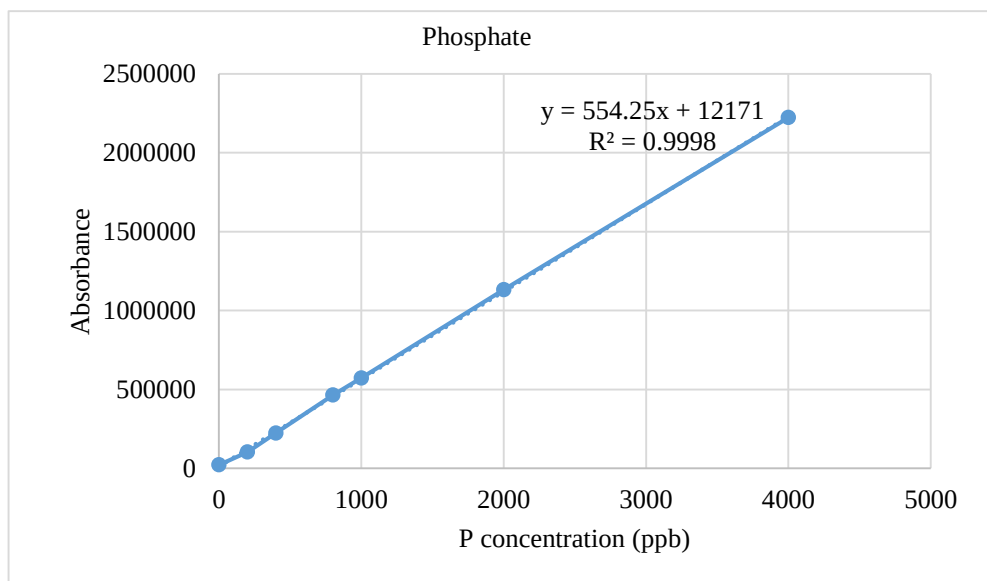


Figure 3.7. The standard curve of phosphate in wheat leaves.

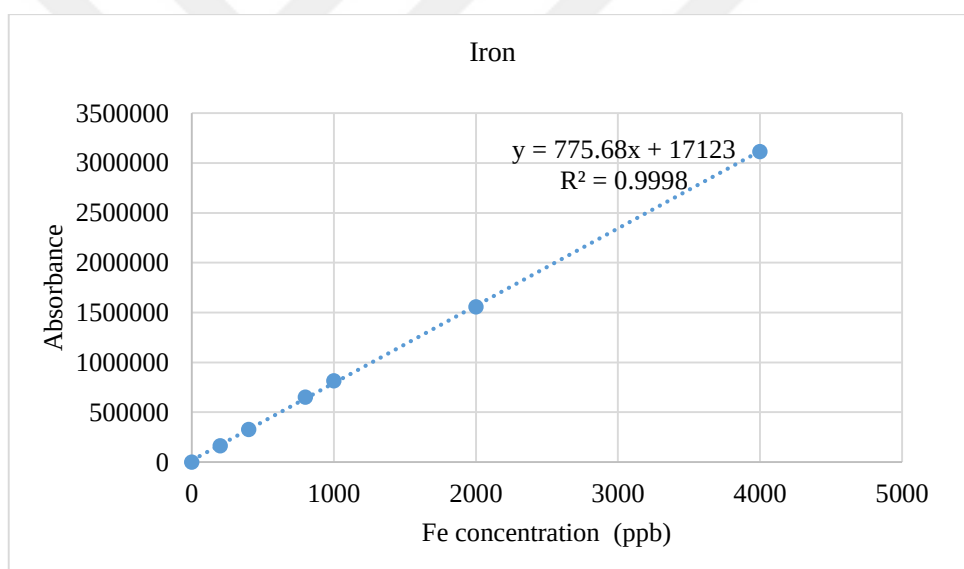


Figure 3.8. The standard curve of iron in wheat leaves.

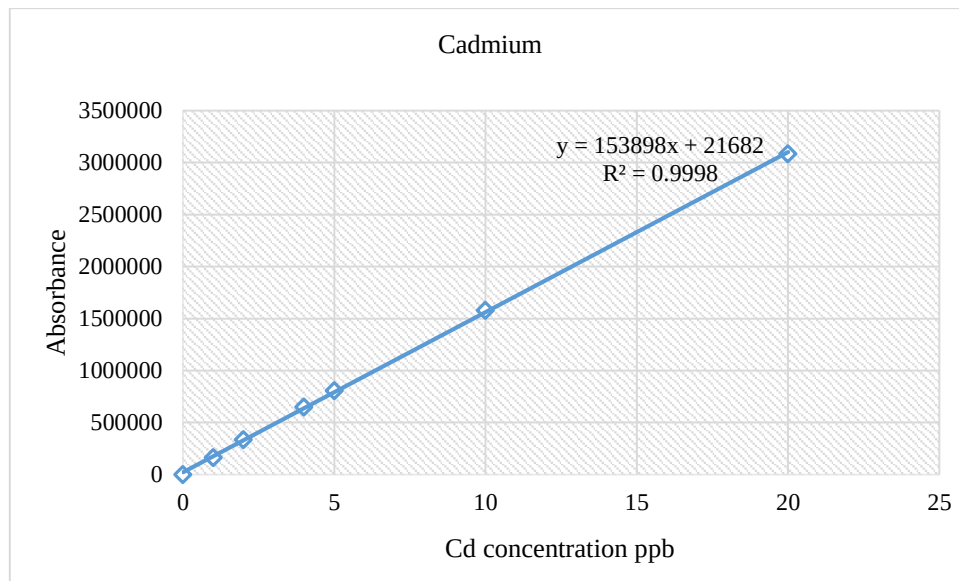


Figure 3.9. The standard curve of cadmium in wheat leaves.

3.2 Statistical Analysis

The data were evaluated by using an ANOVA test, and mean values were compared using Duncan's test with IBM SPSS (Version: 22.0). Furthermore, based among the treatments (different concentration of Cd) and the control was indicated by $P \leq 0.05$. The experiments involving Petri dishes for the analysis of growth parameters were performed in four replicates. Additionally, the analyses were performed to determine the contents of oxidative stress indicators, and antioxidant enzyme activities were carried out in three replicates in the soil.

3.3 The Sample of Wheat Landraces Selected to Study the Molecular Experiments

This study analyzed seventeen accessions of wheat landraces representing *Triticum* spp., collected from Bolu field and the Plant Genetic and Pathology Lab at Bolu Abant İzzet Baysal University in Türkiye as presented in Table 3.2.

Table 3.2. The seed samples of wheat landraces were collected from Türkiye.

N	Co de	Scientific name of wheat landrace	Common name	Region	Location by GPS
1	16	<i>Triticum monococcum</i> spp.	Siyez	Kastamonu /İhsangazi koçcugazkoyu	41°12'48" N 33°36'18" E
2	30	<i>Triticum dicoccum</i>	Gernik	Kastamonu Akkaya Köyü (Değirmenbaşı Mahallesi)	41°13'11"N 33°28'20"E
3	31	<i>Triticum dicoccum</i>	Gernik	Sinop/ Durağan / Gölgerişi Köyü/Galuk Mevkii	41°26'5 N 35°22'48"E
4	32	<i>Triticum dicoccum</i>	Gernik	Sinop/ / Durağan /Gölgerişi Köyü/ Tenler Mevkii	41°26'15"N 35°22'54"E
5	33	<i>Triticum dicoccum</i>	Gernik	Sinop/ / Durağan /Gölgerişi Köyü/ Caulk Mevkii	41°25'58"N 35°22'48"E
6	34	<i>Triticum dicoccum</i>	Gernik	Sinop/ / Durağan / Köseli Köyü/ Kurugöl / Mevkii	41°27'55"N 35°16'50"E
7	35	<i>Triticum monococcum</i> spp.	Siyez	Bolu/Seben IZA 17 A (Mö)	40°23'17"N 31°32'20"E
8	36	<i>Triticum monococcum</i> spp.	Siyez	Bolu/Seben/Musasofular Köyü IZA (MO)	40°21'19"N 31°31'7"E
9	37	<i>Triticum monococcum</i> spp.	Siyez	Bolu/Seben/Musasofular Köyü IZA (MO)	40°23'17"N 31°32'20"E
10	39	<i>Triticum monococcum</i> spp.	IZA	Bolu/ Seben/ Güneyce köyü / Kas Mevkii	40°20'45.0" N 31°28'03.0" E
11	43	<i>Triticum monococcum</i> spp.	IZA	Bolu Yakuplar Köyü Büyük Tarla (köy içi)	40°46'32.0" N 31°39'30.0" E
12	44	<i>Triticum monococcum</i> spp.	IZA	Bolu/ Yakuplar Köyü Mevkii	40°46'32.0" N 31°39'34.0" E
13	45	<i>Triticum monococcum</i> spp.	IZA	Bolu/ Yakuplar Köyü Mevkii	40°46'30.3" N 31°39'30.7" E
14	47	<i>Triticum monococcum</i> spp.	IZA	Bolu/ Seben/ Musasofular/ köyü	40°21'19.0" N

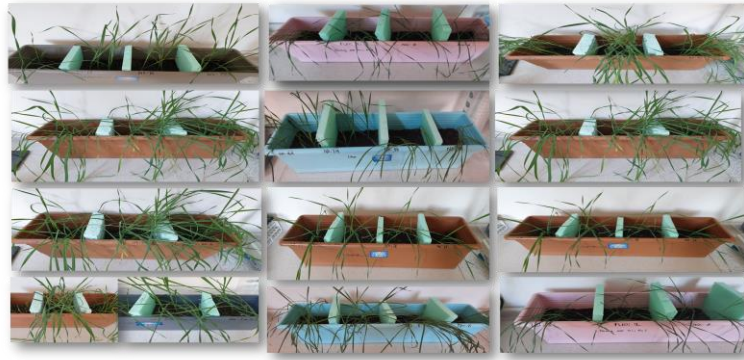
					31°31'10.0" E
1 5	48	<i>Triticum monococcum</i> spp.	IZA	Bolu/ Seben Haccağız köyü Beylik mevkii	40°21'23.8" N 31°30'01.0" E
1 6	I	<i>Triticum aestivum</i>	Gallbasan	Karabük Safranbolu Ouacuma bağçgez koyü	41°25'15"N 32°48'4"E
1 7	II	<i>Triticum aestivum</i>	SARI Serez	Samsun Ladik Çamlıköy	40°52'39"N 35°46'11"E

3.3.1 Molecular Analysis

Each sample of wheat landraces seeds has been grown in plastic pots containing sterilized soil, autoclaved for 15 minutes at 121 °C. After that 5 seed samples from each population of wheat landraces were germinated and placed under controlled conditions (growth chamber at 22 °C $\pm$ 2, 16 hours in light and 8 hours in dark) for 1 month. Distilled water was added to the plants day by day after 1 month -old plants, which were grown in the plastic pots. 1 g of leaf tissue from each variety was weighed in triplicate and stored at -20 °C for DNA extraction from dry leaves.



Picture 3.3. Five seeds samples for each population (wheat landraces).



Picture 3.4. The planted of wheat landraces after 1 month.

3.3.2 The DNA Isolation and Primer Selection

- Fresh leaf tissue was harvested from 4-week-old germinated seedlings, grounded well Dellaporta et al. (1983) (77) and total DNA was extracted using the Gene MATRIX Plant & Fungi DNA Purification according to the manufacturer's instructions.

- 1- Weighing 0.5 g of fresh wheat leaf, it was finely ground using a cooled mortar and pestle. Additionally, 100 mg wet weight tissue was taken in 2 ml Eppendorf tube and centrifuged. Subsequently, 500 μ l of the buffer Lyse CT was added to thoroughly suspended the precipitate.
- 2- RNase and proteinase K were added, followed by vortexing, addition of chloroform, and centrifugation, and transfer of the lysate to a new tube.
- 3- Then, 600 μ l of the lysate is transferred to a DNA binding spin-column, centrifuged at 11,000 $\times$ g for 1 minute, and then 500 μ l of Wash PX buffer is added. The spin-column is then removed and resuspended in Wash PX buffer. Place spin-column into new receiver tube 1.5-2 ml and Added 50-150 μ l of Elution buffer to elute bound DNA. Incubate the spin-column/collection tube assembly for 2 min at room temperature. Then, centrifuge at 11,000 $\times$ g for 1 min. Remove spin column, cap the receiver tube.
- 4- Finally, the extracted DNA was resuspended in 100 μ L water, and dilutions were made to 10 ng/ μ L followed by storage at -20°C . Genomic DNA was quantified via utilizing a Thermo Scientific NanoDrop 2000c Spectrophotometers instrument, and the A260/A280 nm ratio was determined (13). DNA quality was confirmed on a 0.8% agarose gel.

3.3.3 DNA Amplification and Sequencing

DNA quality was confirmed on a 0.8% agarose gel, Sequences of the universal primers for three sequence regions which are nuclear (nrDNA) region ITS2, with the chloroplast (cpDNA) region (*mat K* and *rbcL*) for efficient discrimination of *Triticum* species (13); the primer sequences are presented in Table 3. 3.

DNA sequence for three regions (*ITS2*, *matK* and *rbcL*) as represented in Table 2. PCR amplifications were carried out in 20 µl reaction volumes which PCR the reaction mixture consisted of 0.2 mM MgCl₂, 1× PCR buffer, 0.5 mM primers, 0.2 mM each dNTP, 2 U Taq DNA polymerase and 100 ng of template DNA. The amplification results obtained by PCR (kyratec thermocycler) were run in 1.0% agarose gel prepared with 1x TAE buffer at 100 volt current for 60 minutes, the bright bands in agarose gel, are purified by using the protocol (MAGBIO HighPrep™ PCR Clean-up) The PCR products were purified by using a PCR (MAGBIO HighPrep™ PCR Clean-up) and purified samples were sent to a company (BMLabosis, Ankara, Türkiye) for sequencing analysis.

Table 3.3. Primer sequences for (ITS2, matK and rbcL) PCR conditions.

Regions	Primer sequences 5'-3'	PCR conditions	Amplified region
<i>ITS2</i>	ATGCGATACTTGGTGTGAAT GACGCTTCTCCAGACTACAAT	95°C 5 min; 95°C 40s, 56°C 40s, 72°C 30s, 35 cycles; 72°C 5 min	~480 bp
<i>matK</i>	CGTACAGTACTTTTGTGTTTACGAG ACCCAGTCCATCTGGAAATCTTGGTTC	95°C 5 min, 95°C 40s, 56°C 40s, 72°C 45s, 35 cycles; 72°C 5 min	~900bp
<i>rbcL</i>	ATGTCACCACAAACAGAGACTAAAGC GAAACGGTCTCTCCAACGCAT	95°C 5 min, 95°C 40s, 56°C 40s, 72°C 45s, 35 cycles; 72°C 5 min	~650 bp

3.3.4 Alignment of Sequences and Haplotype Determination

“In this study, Sequences obtained from the company (BMLabosis, Ankara, Türkiye) in the form of chromatograms were visually inspected for quality. Subsequently, the corresponding DNA sequences were aligned and trimmed using Geneious Prime 2024.0 (<https://www.geneious.com/>). DnaSP 6 Rozas et al. (2017) (78) was employed to collapse similar sequences into unique haplotypes.

3.3.5 Phylogenetic and Phylogeographic Analysis

Phylogenetic relationships among haplotypes were investigated using Maximum Parsimony (MP) analysis implemented in PAUP 4.0b10 (Swofford, 2002). The MP analysis employed a heuristic search strategy with TBR branch-swapping and 1000 random addition replicates. To assess branch support, the analysis was conducted with 1000 bootstrap replicates (Swofford, 2002). The resulting tree was visualized with FigTree v1.3.1 (Rambout, 2009).

4. RESULTS

The effect of cadmium toxicity on seed germination and seedling growth parameters in Petri plate.

4.1 Effects of Cadmium Stress on Coleoptile and Root Elongation of Wheat Landraces Grown in Petri Plates

The results observed for coleoptile length of different wheat species under varying concentrations of $\text{Cd}(\text{NO}_3)_2$ (0 μM , 50, 100, 250 and 500 μM) indicated that the maximum inhibition of coleoptile length in *T. aestivum* ($13.34^g \pm 4.9$) and *T. turgidum* ($13.5^g \pm 3.3$) and in *T. monococcum* ($14.5^{gh} \pm 3.6$) at a concentration of 500 μM $\text{Cd}(\text{NO}_3)_2$ compared with the control ($16.14^{efg} \pm 4.4$, $16.7^{defg} \pm 4.6$ and $17.5^{def} \pm 2.7$, respectively. However, similar results were recorded at a concentration of 250 μM $\text{Cd}(\text{NO}_3)_2$ in *T. aestivum*, *T. monococcum* and *T. turgidum* ($15.03^{fgh} \pm 3.9$, $16.3^{defg} \pm 4.1$ and $16^{efg} \pm 2.5$) as presented in Figure 4.1 and Table 4.1. On the other hand, *T. dicoccum* showed greater tolerance and minimal inhibition compared with the control under different concentrations of $\text{Cd}(\text{NO}_3)_2$ at 50, 100, 250 μM ($30.0^{bc} \pm 4.9$, $31.9^{ab} \pm 5.6$, $29.6^{bc} \pm 5.4$) and at 500 μM ($34.0^c \pm 2.8$), which had no significant effect compared with the control ($33.9^a \pm 5.22$), as presented on Figure 4.1 and Table 4.1. In contrast, at the 100 μM concentration of $\text{Cd}(\text{NO}_3)_2$, *T. aestivum* and *T. monococcum* showed minimal effect, with a slight increase in coleoptile length compared with the control and other wheat landrace species.

Table 4.1. The Effects introduced of heavy metal cadmium stress ($\text{Cd}(\text{NO}_3)_2$) under different concentrations (0 μM , 50 μM , 100 μM , 250 μM , and 500 μM on seedling growth length, germination rate (%) and seedling vigour (SVI) of wheat landraces in Petri plates are presented as the mean $\pm$ SD based four replicates.

Species of wheat landraces	$\text{Cd}(\text{NO}_3)_2$ treatment	Coleoptile length (mm)	Root elongation (mm)	Germination %	Seedling vigour index (SVI)
<i>Triticum aestivum</i> L. (Ekiz)	0 μM	16.14 ^{efg} $\pm$ 4.4	29.1 ^{def} $\pm$ 14.4	87.5 ^{abcd} $\pm$ 15	125.8 ^{cde} $\pm$ 39.4
	50 μM	15.07 ^{fgh} $\pm$ 5.3	23.3 ^{efgh} $\pm$ 12.5	75.0 ^{bcd} $\pm$ 21	106.1 ^{cde} $\pm$ 30.7
	100 μM	17.6 ^{def} $\pm$ 4.7	26.3 ^{efg} $\pm$ 9.8	72.50 ^{cd} $\pm$ 9.6	86.3 ^{de} $\pm$ 18.5
	250 μM	15.03 ^{fgh} $\pm$ 3.9	17.5 ^{hij} $\pm$ 6.3	72.50 ^{cd} $\pm$ 9.6	79.8 ^{de} $\pm$ 13.7
	500 μM	13.34 ^g $\pm$ 4.9	14.3 ^{ij} $\pm$ 7.8	70.0 ^d $\pm$ 0.0	67.8 ^d $\pm$ 2.2
<i>Triticum monococcum</i> spp. (Einkorn)	0 μM	17.5 ^{def} $\pm$ 2.7	25.5 ^{efg} $\pm$ 12.4	95.0 ^{ab} $\pm$ 5.8	158.6 ^c $\pm$ 23.3
	50 μM	17.1 ^{def} $\pm$ 2.7	27.9 ^{def} $\pm$ 12.4	90.0 ^{abcd} $\pm$ 8.2	139.3 ^{cd} $\pm$ 24.9
	100 μM	17.8 ^{de} $\pm$ 3.7	24.8 ^{efgh} $\pm$ 7.9	85.0 ^{abcd} $\pm$ 5.8	129.2 ^{cd} $\pm$ 24.8
	250 μM	16.3 ^{defg} $\pm$ 4.1	17.9 ^{hij} $\pm$ 6.9	75.0 ^{bcd} $\pm$ 7.9	138.1 ^{cd} $\pm$ 8.9
	500 μM	14.5 ^{gh} $\pm$ 3.6	11.4 ⁱ $\pm$ 6.7	80.0 ^{abcd} $\pm$ 8.2	93.7 ^{de} $\pm$ 23.8
<i>Triticum dicoccum</i> spp. (Emmer)	0 μM	34.0 ^a $\pm$ 5.22	63.5 ^a $\pm$ 17.6	100.00 ^a $\pm$ 0.0	340.0 ^a $\pm$ 14.2
	50 μM	30.0 ^{bc} $\pm$ 4.9	48.1 ^b $\pm$ 17.9	90.0 ^{abcd} $\pm$ 8.2	254.0 ^b $\pm$ 72.4
	100 μM	31.9 ^{ab} $\pm$ 5.6	44.3 ^{bc} $\pm$ 21.5	90.00 ^{abcd} $\pm$ 8	288.4 ^b $\pm$ 63.5
	250 μM	29.6 ^{bc} $\pm$ 5.4	34.9 ^{de} $\pm$ 16.8	90.0 ^{abcd} $\pm$ 8.2	240.0 ^b $\pm$ 68.0
	500 μM	27.9 ^d $\pm$ 2.8	21.7 ^{fghi} $\pm$ 9	92.5 ^{abc} $\pm$ 9.6	234.9 ^b $\pm$ 54.0
<i>Triticum turgidum</i> L. var. <i>durum</i> (Durum)	0 μM	16.7 ^{defg} $\pm$ 4.6	30.4 ^{def} $\pm$ 16.1	85.0 ^{abcd} $\pm$ 10	121.5 ^{cde} $\pm$ 26.9
	50 μM	18.8 ^d $\pm$ 2.8	40 ^{cd} $\pm$ 19.8	72.5 ^{cd} $\pm$ 5.0	91.6 ^{de} $\pm$ 12.1
	100 μM	16.3 ^{defg} $\pm$ 4.3	27.3 ^{ef} $\pm$ 13.6	72.5 ^{cd} $\pm$ 5.0	84.6 ^{de} $\pm$ 20.1
	250 μM	16 ^{efg} $\pm$ 2.5	18.8 ^{ghij} $\pm$ 14.1	75 ^{bcd} $\pm$ 12.9	87.1 ^{de} $\pm$ 29.4
	500 μM	13.5 ^g $\pm$ 3.3	14.5 ^{ij} $\pm$ 9	75.0 ^{bcd} $\pm$ 5.8	65.9 ^d $\pm$ 18.1

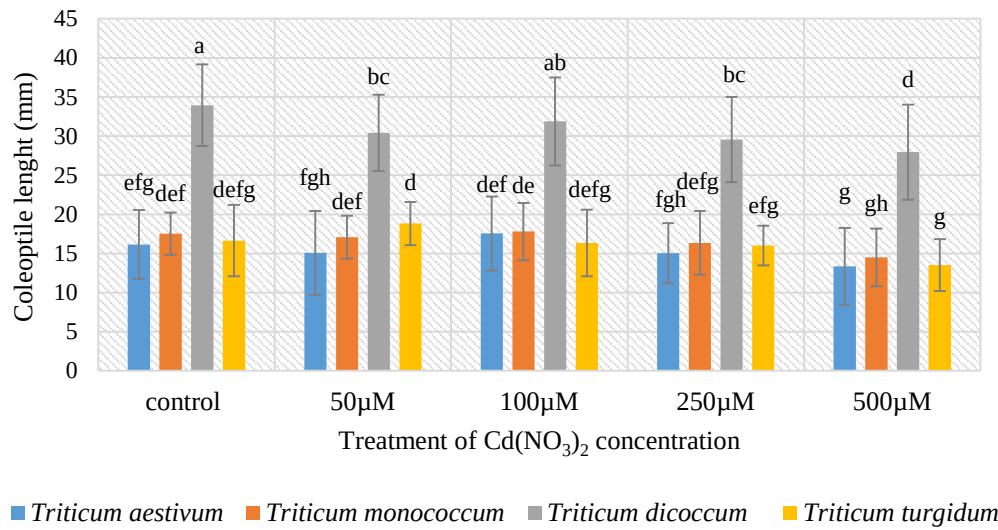


Figure 4.1. The effect of cadmium stress (Cd(NO₃)₂) concentrations (0 μM, 50 μM, 100 μM, 250 μM and 500 μM) on coleoptile length (in mm) of wheat landraces grown in Petri plates.

A gradual decrease in the plant root of wheat with different Cd (NO₃)₂ concentration was observed in all species of wheat *T. aestivum*, *T. monococcum*, *T. dicoccum* and *T. turgidum*. seedling growth (root elongation) exhibited more tolerance to Cd (NO₃)₂ in *T. dicoccum* (21.7^{fghi}±9 mm) than other wheat species *T. aestivum*, *T. monococcum*, *T. turgidum* (14.3^{ij}±7.8, 11.4ⁱ±6.7 and 14.5^{ij}±9 mm) at 500 μM Cd (NO₃)₂ respectively as presented in Figure 4.2 and Table 4.1. The results showed that Cd (NO₃)₂ at (250 μM) reduced the root elongation up to (17^{hij}±6.3, 17^{hij}±6.9, and 18.8^{ghij}±14.1) in *Triticum aestivum*, *T. monococcum*, and *T. turgidum* compared with control (29.1^{def}±14.4, 25.5^{efg}±12.4 and 30.4^{def}±16.1 mm) respectively as presented in Figure 4.2 below Table 4.1 above, whereas *T. dicoccum* showed a decrease (34.9^{de}±16.8 mm), at 250 μM in root elongation, as compared to the control (63.5^a±17.6 mm) as shown in Figure 4.2, Table 4.1. At 50 and 100 μM of Cd(NO₃)₂ shown a little decrease of root elongation of all *Triticum* species compared with other concentration and control. In contrast, *T. monococcum* and *T. turgidum* shown increase of root elongation (27.9^{def}±12.4 and 40^{cd}±19.8 mm) at 50 μM and compared with control.

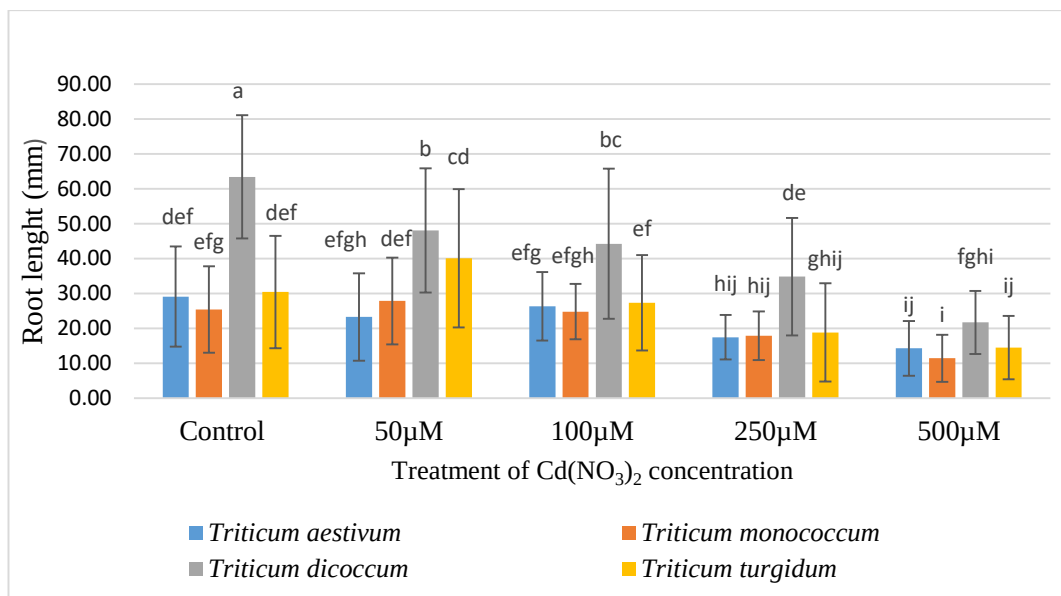


Figure 4.2. The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 μM, 50 μM, 100 μM, 250 μM and 500 μM) on root elongation (in mm) of a wheat landrace grown on Petri plates.

4.2 Effects of Cadmium Stress on Seed Germination Percentage and Seedling vigour in Wheat Landraces

Seed germination was observed to decrease with increasing doses of Cd(NO₃)₂, documenting the maximum germination inhibition 17% and 10% for *T. aestivum* and *T. turgidum*, respectively. At a (Cd(NO₃)₂) treatment of 500 μM, germination rate recorded at 70.0^d±0.0 for *T. aestivum* and 75.0^{bcd}±5.8 for *T. turgidum* compared with control rates of 87.5% (87.5^{abcd}±15.0) and 85% (85.0^{abcd}±10.0), respectively as shown in Figure 4.2 and Table 4.1. Conversely, at the same Cd(NO₃)₂ concentration of 500 μM, lower inhibition rates were noted for *T. monococcum* and *T. dicoccum* at 15% and 8% respectively, with germination rates of 80.0^{abcd}±8.2 and 92.5^{abc}±9.6 compared to control rates of 95% and 100%. Similarly, at concentrations of 100 and 250 μM, of Cd(NO₃)₂ treatment exhibited consistent effects on *T. dicoccum*, with observed germination rates of 90.00^{abcd}±8.2 and 90.0^{abcd}±8.2 for both concentrations. *T. aestivum* and *T. turgidum* also showed no significant difference between these concentrations, both recording germination rates of 72.50^{cd}±9.6 and 72.50^{cd}±9.6 respectively. However, *T. monococcum* displays a notable decrease in germination from 85.0^{abcd}±5.8 at 100 μM to 75.0^{bcd}±7.9 at 250 μM Cd(NO₃)₂. On the other hand, *T. dicoccum* demonstrated no

significant difference in response to varying treatments of $\text{Cd}(\text{NO}_3)_2$ concentration, as presented in Figure 4.3, Table 4.1. This suggests that *T. turgidum* may possess a higher tolerance to $\text{Cd}(\text{NO}_3)_2$ stress compared to the other species studied.

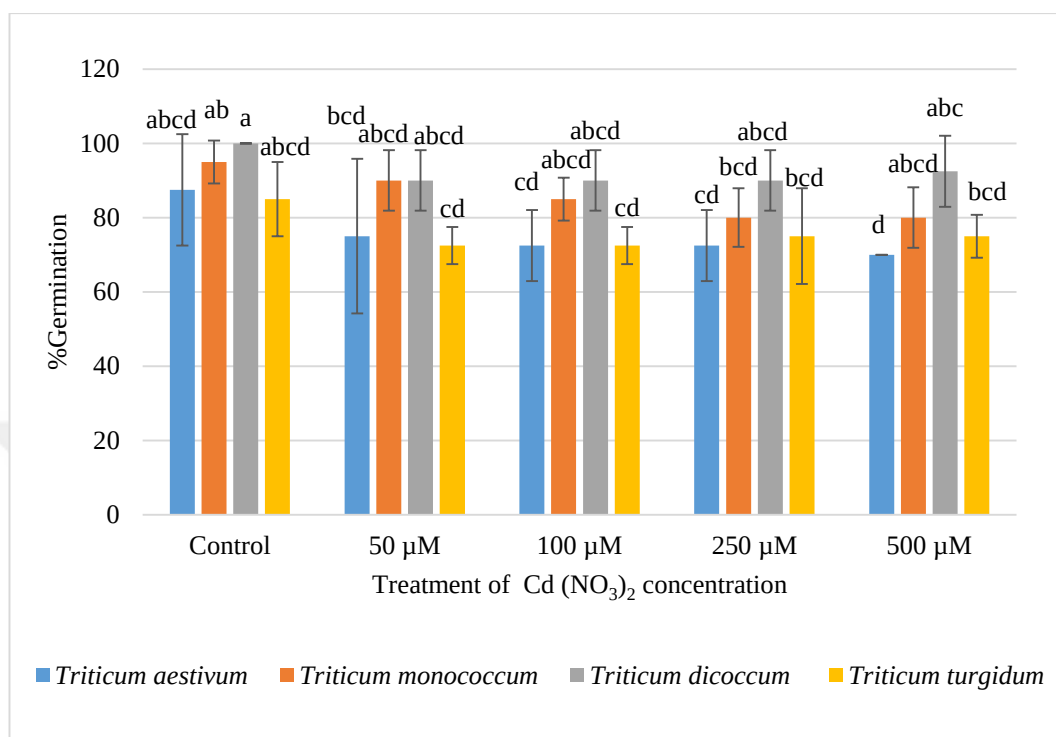


Figure 4.3. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on germination percentage of a wheat landrace grown in Petri plates.

The results of seed vigour of *Triticum aestivum* and *T. turgidum* showed more affected or decrease than *T. monococcum* and *T. dicoccum* by treatment with 500 μM of $\text{Cd}(\text{NO}_3)_2$ concentrations ($67.8^{\text{d}} \pm 2.2$, $65.9^{\text{d}} \pm 18.1$) and ($93.7^{\text{de}} \pm 23.8$ and $234.9^{\text{b}} \pm 54.0$) at 500 μM respectively as presented in the Figure 4.4 Table 4.1. On the other hand, there is no significant effect of *T. aestivum*, *T. turgidum* and *T. monococcum* at different concentrations of $\text{Cd}(\text{NO}_3)_2$ at 50, 100, 250 μM as presented in Figure 4.4, Table 4.1, but, in *T. turgidum* has the maximum decrease at 500 μM of $\text{Cd}(\text{NO}_3)_2$ ($65.9^{\text{d}} \pm 18.1$) with no effect compared with different concentration as presented on Figure 4.4, Table 4.1; on the other hand, *T. dicoccum* has higher tolerance between other species.

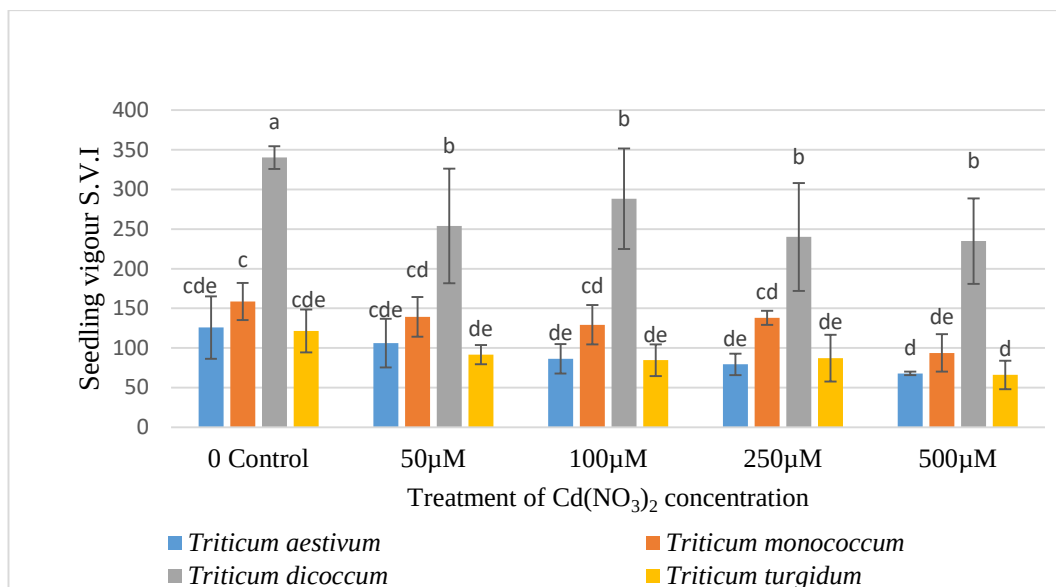


Figure 4.4. The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 µM, 50 µM, 100 µM, 250 µM and 500 µM) on seed vigour index (S.V.I) of a wheat landrace grown in Petri plates.

4.3 Effects of Cadmium Stress on the Fresh and Dry Root Weight of Wheat Landrace Cultivars on Petri Plates

The results revealed a significant reduction in the fresh root weight of *T. aestivum* under different concentrations of Cd(NO₃)₂ compared to the control. At 250 and 500 µM Cd(NO₃)₂, the reduction in fresh root weight was more pronounced, with values recorded as 53.5^{fg}±21.8 mg at 250 µM and 31.8^g±20.8 at 500 µM, respectively. Similarly, at 50 and 100 µM Cd(NO₃)₂, reductions of 115.3^{ef}±50.9 mg at 50 µM and 128^{def}±46.4 mg at 100 µM were observed, compared to the control (206^{bc}±52.4 mg) as shown in Figure 4.5 and Table 4.2. However, there was no significant effect on the dry root weight of *T. aestivum* under different concentrations, as depicted in Figure 4.6 and Table 4.2. For *T. monococcum*, the result shows that there is a significant reduction in fresh root weight at 250 and 500 µM Cd(NO₃)₂, with values of 113.3^{ef}±76.3 mg at 250 µM and 24.5^g±11.7 mg at 500 µM, respectively, compared to the control and other concentrations, as shown in Figure 4.5 and Table 4.2. Similar to *T. aestivum*, there is no significant effect on the dry root weight of *T. monococcum* under different concentrations, as presented in Figure 4.6 and Table 4.2. Regarding *T. dicoccum*, the highest decrease in fresh root weight was recorded (67.8^{efg}±16.8 mg) at 100 µM, with 250 µM Cd(NO₃)₂ showing

a reduction of $116.8^{ef} \pm 26.9$ mg, compared to other concentrations of $Cd(NO_3)_2$ and the control, as presented in Figure 4.5 and Table 4.2. Similarly, the highest decrease in root dry weight was observed at $100\mu M$ $Cd(NO_3)_2$, with no significant effect observed under different concentrations, as shown in Figure 4.6 and Table 4.2. However, both fresh root and dry root weight of *T. turgidum* showed no significant reduction under different stress conditions, as illustrated in Figure 4.5 and 4.6 and Table 4.2

Table 4.2. The effects of heavy metal cadmium stress ($Cd(NO_3)_2$) under different concentrations (0 μM , 50 μM , 100 μM , 250 μM , and 500 μM) on the fresh and dry weight of wheat root landraces in Petri plates are examined. Values are shown as the mean $\pm$ SD of four replicates.

Species of wheat landraces	$Cd(NO_3)_2$ treatment	Weight fresh root (mg)	Weight dry root (mg)
<i>Triticum aestivum</i> L. (Ekiz)	0 μM	$206^{bc} \pm 52.4$	$31.3^{cde} \pm 5.7$
	50 μM	$115.3^{ef} \pm 50.9$	$27.3^{cde} \pm 9.9$
	100 μM	$128^{def} \pm 46.4$	$33.5^{cd} \pm 10.2$
	250 μM	$53.5^{fg} \pm 21.8$	$27^{cde} \pm 11.2$
	500 μM	$31.8^g \pm 20.8$	$19^{cde} \pm 9.8$
<i>Triticum monococcum</i> spp. <i>monococcum</i> (Einkorn)	0 μM	$225^b \pm 54.5$	$32^{cde} \pm 4.1$
	50 μM	$207.5^{bc} \pm 67$	$36^{bc} \pm 5.8$
	100 μM	$202.5^{bcd} \pm 22$	$31.3^{cde} \pm 4.9$
	250 μM	$113.3^{ef} \pm 76.3$	$16.8^{de} \pm 11.5$
	500 μM	$24.5^g \pm 11.7$	$16.3^{de} \pm 7.5$
<i>Triticum dicoccum</i> ssp. <i>dicoccum</i> Schrank (Emmer)	0 μM	$320.5^a \pm 81.2$	$65^a \pm 19.8$
	50 μM	$229^b \pm 32.9$	$50^{ab} \pm 18.4$
	100 μM	$67.8^{efg} \pm 16.8$	$26.5^{cde} \pm 5$
	250 μM	$116.8^{ef} \pm 26.9$	$51^{ab} \pm 13.3$
	500 μM	$135.8^{cde} \pm 36.1$	$35.8^{bc} \pm 6.9$
<i>Triticum turgidum</i> L. var. <i>durum</i> (Durum)	0 μM	$113.5^{ef} \pm 57.9$	$29.5^{cde} \pm 14$
	50 μM	$113.5^{ef} \pm 57.9$	$18.3^{cde} \pm 9.6$
	100 μM	$62^{efg} \pm 28.5$	$30.3^{cde} \pm 14$
	250 μM	$82.8^{efg} \pm 38.6$	$19^{cde} \pm 6.1$
	500 μM	$79.3^{efg} \pm 46.9$	$14.3^e \pm 4.8$

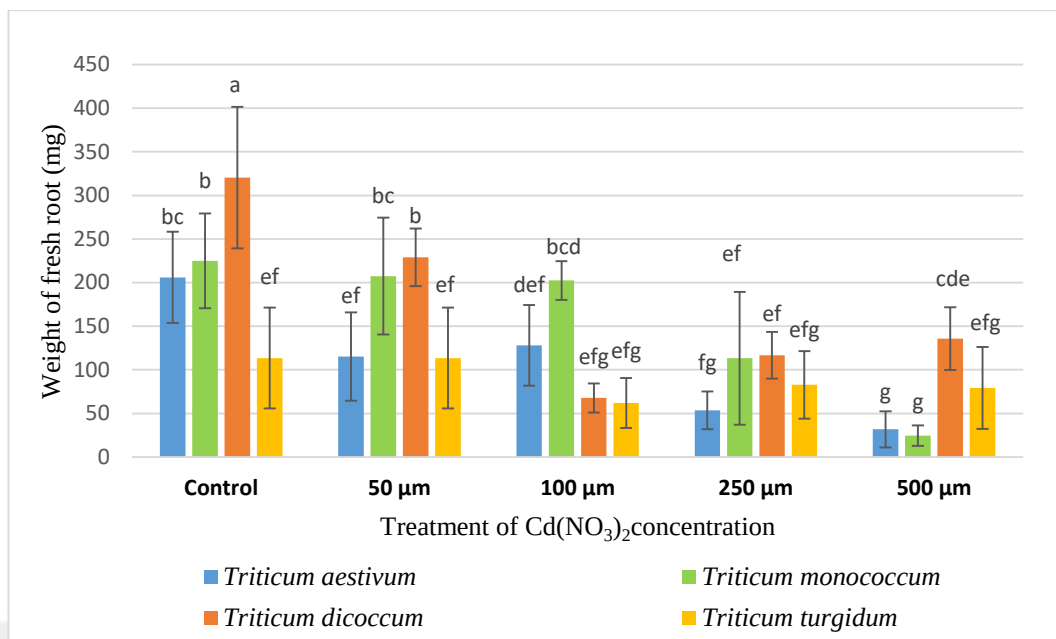


Figure 4.5. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on the fresh root weight of a wheat landrace on a Petri plate.

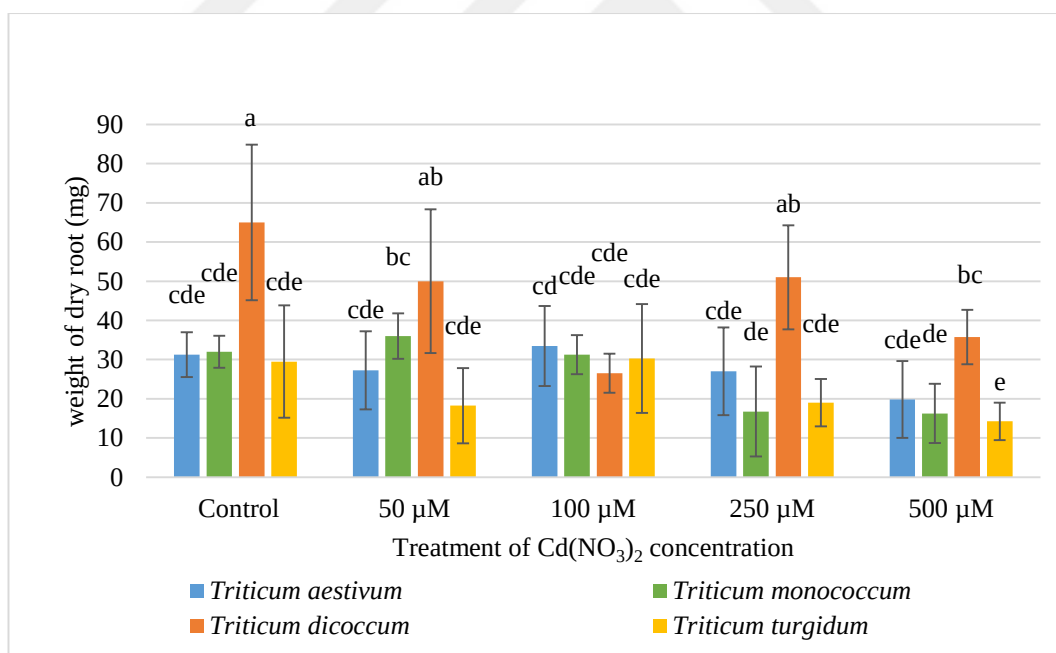


Figure 4.6. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on the dry root weight of a wheat landrace on a Petri plate.

4.4 Effects of Cadmium Toxicity on Growth Wheat Landraces Cultivars in Soil

4.4.1 Effects of Cadmium Stress ($\text{Cd}(\text{NO}_3)_2$) on Seed Germination Cultivars in Soil

The toxicity of cadmium had significant effects on both seedling germination and development. Cadmium phytotoxicity notably influenced seedling germination and growth across various wheat landrace cultivars (*T. aestivum*, *T. monococcum*, *T. dicoccum*, and *T. turgidum*). Furthermore, while the impact on germination percentage was noticeable, it was comparatively less drastic than on seedling growth. Moreover, the response to cadmium concentrations varied among all wheat landraces.

At a concentration of 500 μM $\text{Cd}(\text{NO}_3)_2$, an inhibitory effect on seed germination was evident, with *T. turgidum* exhibiting a rate of 78.3%, *T. dicoccum* at 70%, *T. monococcum* at 83% and *T. aestivum* at 90%. These rates corresponded to inhibitions of 22%, 30%, 14% and 8%, respectively, compared to the control, as presented in Figure 4.7 and Table 4.3. Similarly, at a concentration of 250 μM $\text{Cd}(\text{NO}_3)_2$, inhibitory effects on seed germination were observed with rates of 82% for *T. turgidum*, 72% for *T. dicoccum*, 88.3% for *T. monococcum*, and 87% for *T. aestivum*. These inhibitions compared to the control, were noted at 18%, 28%, 9%, and 11.3%, respectively, as presented in Figure 4.7. Furthermore, these research results underline the inhibitory impact of $\text{Cd}(\text{NO}_3)_2$ on seed germination across various wheat cultivars at higher concentrations, showing reduced germination rates compared to control groups, with variations between wheat landraces. Additionally, the effect of seed germination at low dose of $\text{Cd}(\text{NO}_3)_2$ (50 and 100 μM) was recorded 95% and 95% for *T. aestivum*, 86.7% and 75% for *T. monococcum*, 93.3% and 83.3% for *T. dicoccum* and 93.3% and 87% for *T. turgidum*, respectively. Thus, an increase in cadmium dose affected seed germination in some wheat species.

Table 4.3. The effects of heavy metal cadmium ($\text{Cd}(\text{NO}_3)_2$) stress under different concentrations (0 μM , 50 μM , 100 μM , 250 μM , and 500 μM) on the seed germination percentage and seedling growth development of wheat landrace cultivars in soil were observed after 28 days. Values are shown as the mean $\pm$ SD of three replicates.

Species of wheat landraces	$\text{Cd}(\text{NO}_3)_2$ treatment	Seed germination (%)	Shoot elongation (mm)	Root elongation (mm)
<i>Triticum aestivum</i> L. (Ekiz)	0 μM	98.3 ^{ab} $\pm$ 2.9	344 ^{ab} $\pm$ 39	99 ^{bc} $\pm$ 14
	50 μM	95 ^{abcd} $\pm$ 5.0	352 ^a $\pm$ 49	95 ^{cd} $\pm$ 12
	100 μM	95 ^{abcd} $\pm$ 5.0	346 ^a $\pm$ 43	96 ^c $\pm$ 13
	250 μM	86.7 ^{def} $\pm$ 5.8	296 ^{de} $\pm$ 44	105 ^{ab} $\pm$ 14
	500 μM	90.0 ^{bcde} $\pm$ 0.0	274 ^{fg} $\pm$ 36	94 ^{cd} $\pm$ 9
<i>Triticum monococcum</i> spp. <i>monococcum</i> (Einkorn)	0 μM	96.7 ^{abc} $\pm$ 5.8	336 ^{ab} $\pm$ 51	71 ^j $\pm$ 13
	50 μM	86.7 ^{def} $\pm$ 5.8	349 ^a $\pm$ 43	74 ^{ij} $\pm$ 22
	100 μM	75.0 ^{gh} $\pm$ 5.0	341 ^{ab} $\pm$ 45	72 ^j $\pm$ 17
	250 μM	88.3 ^{cde} $\pm$ 10.4	301 ^{de} $\pm$ 46	56 ^k $\pm$ 10
	500 μM	83.3 ^{efh} $\pm$ 2.9	298 ^{de} $\pm$ 61	56 ^k $\pm$ 12
<i>Triticum dicoccum</i> spp. <i>dicoccum</i> Schrank (Emmer)	0 μM	100 ^a $\pm$ 0.0	326 ^{bc} $\pm$ 39	89 ^{de} $\pm$ 17
	50 μM	93.3 ^{abcd} $\pm$ 2.9	313 ^{cd} $\pm$ 63	82 ^{fgh} $\pm$ 17
	100 μM	83.3 ^{efg} $\pm$ 7.6	309 ^{cd} $\pm$ 47	80 ^{ghi} $\pm$ 24
	250 μM	71.7 ^h $\pm$ 2.9	347 ^a $\pm$ 47	85 ^{efg} $\pm$ 24
	500 μM	70 ^h $\pm$ 0.0	325 ^{bc} $\pm$ 37	82 ^{efgh} $\pm$ 13
<i>Triticum turgidum</i> L. var. <i>durum</i> (Durum)	0 μM	100 ^a $\pm$ 0.0	305 ^d $\pm$ 41	109 ^a $\pm$ 23
	50 μM	93.3 ^{abcd} $\pm$ 2.9	313 ^{cd} $\pm$ 40	87 ^{ef} $\pm$ 20
	100 μM	86.7 ^{def} $\pm$ 5.8	295 ^{de} $\pm$ 45	87 ^{efg} $\pm$ 10
	250 μM	81.7 ^{efg} $\pm$ 5.8	284 ^{ef} $\pm$ 41	83 ^{efgh} $\pm$ 15
	500 μM	78.3 ^{fgh} $\pm$ 2.9	258 ^g $\pm$ 61	77 ^{hij} $\pm$ 21

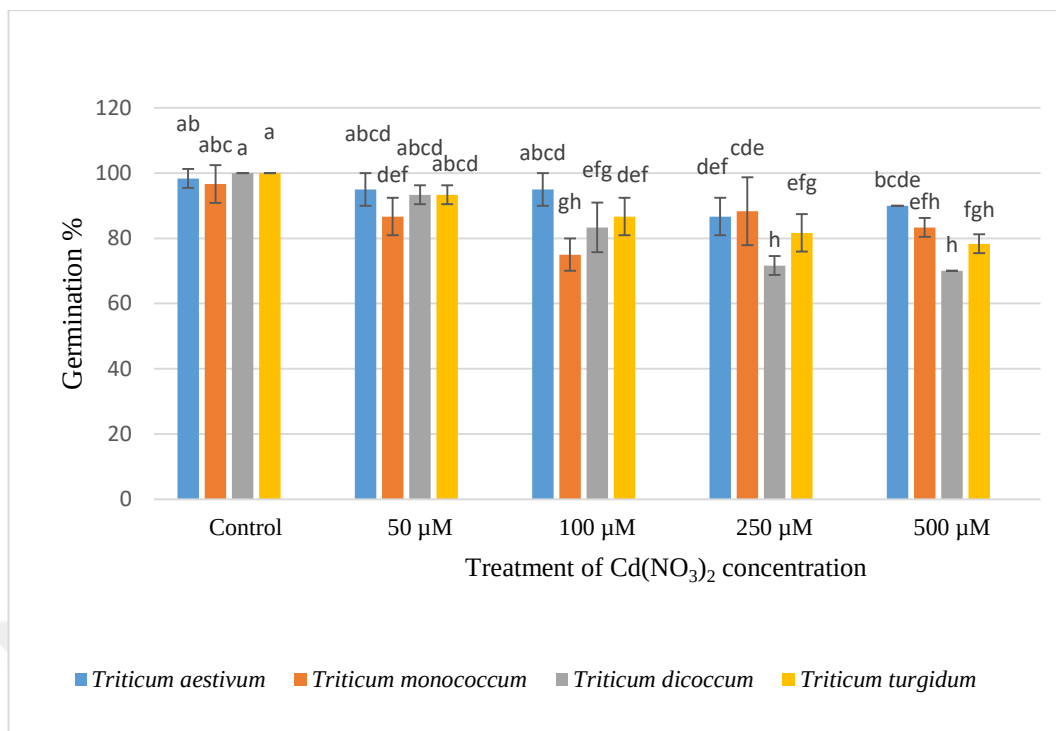


Figure 4.7. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on the germination percentage of wheat landrace cultivars in soil.

4.4.2 Effects of Cadmium Stress on Shoot and Root Elongation of Wheat Landraces Cultivars in Soil

This research study found that the effect of different $\text{Cd}(\text{NO}_3)_2$ treatment on shoot elongation was notably extended in *T. aestivum* and *T. monococcum*, recording $352^{\text{a}} \pm 49$ mm and $349^{\text{a}} \pm 43$ mm at a $\text{Cd}(\text{NO}_3)_2$ exposure rate of 50 μM , and $346^{\text{a}} \pm 43$ mm and $341^{\text{ab}} \pm 45$ mm at 100 μM , respectively. Additionally, *T. dicoccum* showed significant shoot elongation when treated with 250 μM $\text{Cd}(\text{NO}_3)_2$, showing an elongation of $347^{\text{a}} \pm 47$ mm compared to the control, as presented at Figure 4.8 and Table 4.3. On the other hand, no significant effect on shoot elongation was observed of in *T. dicoccum* at 500 μM compared with the control ($325^{\text{bc}} \pm 39$, $325^{\text{bc}} \pm 37$ mm) as presented in Figure 4.8 and Table 4.3. Moreover, *T. turgidum* exhibited a decrease in shoot elongation with an increase in $\text{Cd}(\text{NO}_3)_2$ dose to 500 μM ($258^{\text{g}} \pm 61$ mm), indicating a reduction in shoot elongation, as shown in Figure 4.8. These results suggest that there is no significant reduction in shoot elongation under different $\text{Cd}(\text{NO}_3)_2$ stress levels among wheat cultivars.

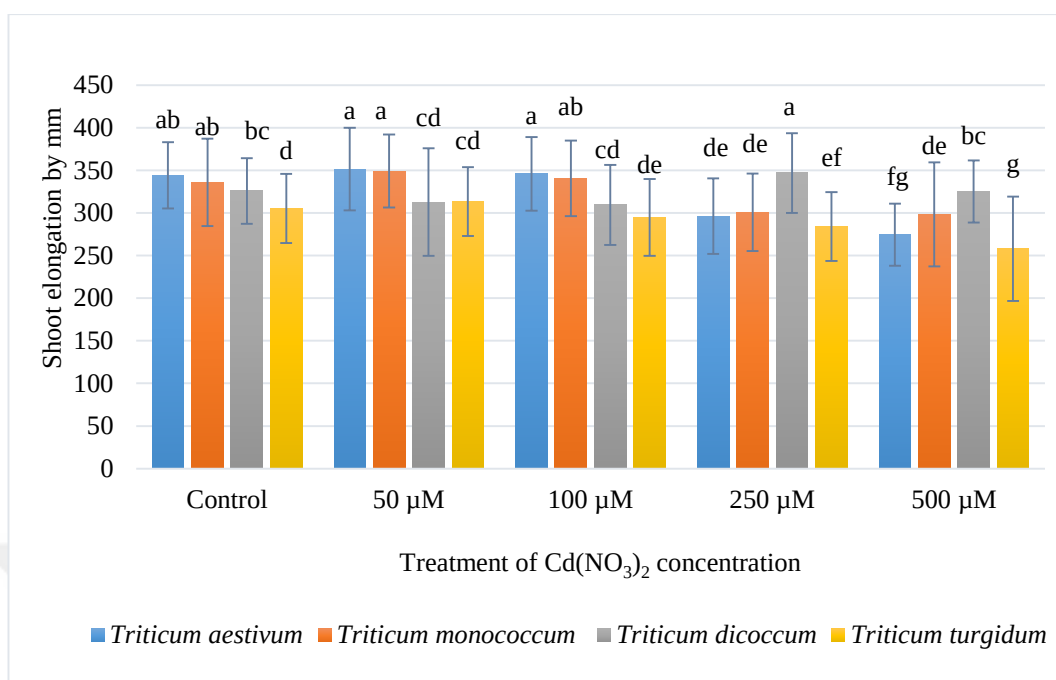


Figure 4.8. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on shoot elongation (measured in mm) of wheat landrace cultivars in soil.

Root elongation exhibited a similar pattern to shoot elongation under $\text{Cd}(\text{NO}_3)_2$ stress. For *T. monococcum*, root elongation was reduced at concentrations of 250 and 500 μM of $\text{Cd}(\text{NO}_3)_2$, recording lengths of $56^k \pm 10$ mm and $56^k \pm 12$ mm, respectively, compared with the control at $71^j \pm 13$ mm. Despite these reductions, the results indicate no significant effect on the root elongation of *T. monococcum* under cadmium stress, as presented in Figure 4.9 and Table 4.3. Across all cultivars, root elongation showed slight inhibition at varying concentrations (50 μM , 100 μM , 250 μM , and 500 μM) but displayed no significant effects when exposed to different $\text{Cd}(\text{NO}_3)_2$ concentrations compared to the control and amongst other cultivars, as detailed in Figure 4.9 and Table 4.3. Conversely, *T. aestivum* demonstrated an increase in root elongation at a 250 μM concentration, reaching $105^{ab} \pm 14$ mm, as shown in Figure 4.9 and Table 4.3.

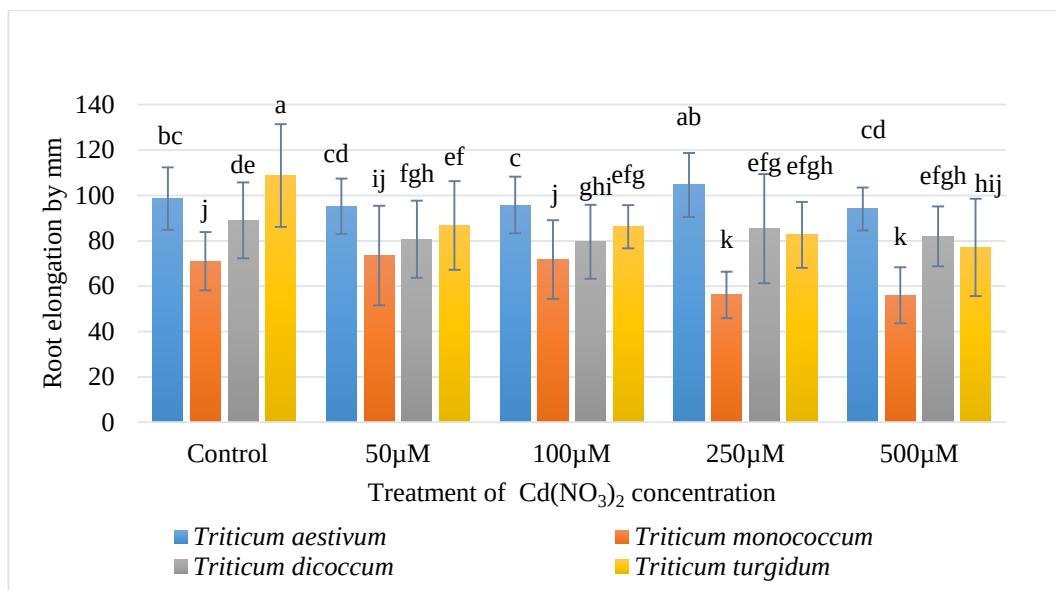


Figure 4.9. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on root elongation (measured in mm) of wheat landrace cultivars in soil.

4.4.3 Effects of Cadmium Stress on the Fresh and Dry Shoot of Wheat Landraces Cultivars in Soil

The effect of increased $\text{Cd}(\text{NO}_3)_2$ concentrations in soil on shoot fresh and dry weight toxicity varied among different varieties of wheat (*Triticum* species). *Triticum dicoccum* exhibited pronounced, decreases in shoot fresh and dry weight at different $\text{Cd}(\text{NO}_3)_2$ concentrations. Specifically, the shoot fresh weights were ($3060^{ijk} \pm 651$ mg, $2566.7^k \pm 334$ mg, $2753^{jk} \pm 339$ mg, $3253^{hijk} \pm 810$ mg and the shoot dry weight were ($356.7^{ghi} \pm 61.1$ mg, $280^{hi} \pm 10$ mg, $170^i \pm 11.6$ mg and $473.3^{efgh} \pm 5.77$ mg) when exposed to Cd concentrations of 50, 100, 250 as well as 500 μM , respectively compared with control and other varieties, as presented in Figure 4.10, 4.11 and Table 4.4. Similarly, *T. turgidum* showed a decrease in shoot fresh and dry weights, indicating sensitivity to elevated Cd concentrations. The shoot fresh weights were $5193^{def} \pm 645.3$ mg, $5530^{de} \pm 242.5$ mg, $4147^{ghij} \pm 332.5$ mg, and $3730^{ghijk} \pm 515.6$ mg, and shoot dry weights were $773.3^{abc} \pm 45.8$ mg, $650^{abcde} \pm 15.3$ mg, $573.3^{cdefg} \pm 11.5$ mg, and $500^{defgh} \pm 45.8$ mg when exposed to different Cd concentrations of 50, 100, 250 and 500 μM , respectively, compared with other varieties as shown in Figure 4.10, 4.11 and Table 4.4. In contrast, *Triticum*

monococcum demonstrated little decrease in shoot fresh and dry weights at different Cd concentrations (3850 ± 207^{ghij} mg, and $3753.3^{ghijk} \pm 1002$ mg, for shoot fresh weights; $699.7^{bcdef} \pm 120.5$ mg, and $700^{abcde} \pm 202.2$ mg, for shoot dry weights) when exposed to 250 μ M and 500 μ M Cd(NO₃)₂ concentrations, respectively, compared with the control and other varieties, as presented in Figure 4.10, 4.11 and Table 4.4 *Triticum monococcum* exhibited higher tolerance or resistance to Cd at higher doses in shoot and dry weights. On the other hand, *Triticum aestivum* showed no significant effect on shoot fresh and dry weights at 50 μ M and 100 μ M Cd concentrations, recording ($6973.3^a \pm 915$ mg, $70100^a \pm 345.9$ mg) for shoot fresh weights and ($823.3^{ab} \pm 70.9$ mg, $926.7^a \pm 141.5$ mg) for shoot dry weights. However, at 250 μ M and 500 μ M Cd(NO₃)₂ concentrations, there was a lower decrease in shoot fresh and dry weights, recording ($6083.3^{ab} \pm 830.7$ mg, $5783.3^{bc} \pm 586.2$ mg) for shoot fresh weights and ($690^{bcde} \pm 190$ mg, $580^{cdefg} \pm 70$ mg) for shoot dry weights, as presented in Figure 4.10, 4.11 and Table 4.4. These results indicate a lower decrease in *Triticum aestivum* shoot fresh weight with no significant effect on shoot dry weight, suggesting a potential adaptive response to Cd toxicity at different Cd(NO₃)₂ concentrations. These research results are based on different wheat varieties to Cd toxicity, demonstrated that the need for a nuanced explanation of the interactions amongst plant species as well as heavy metal stressors. The results showed *T. dicoccum* is more adversely affected by higher Cd(NO₃)₂ concentrations compared to other varieties, leading toward no significant reduction in shoot fresh and dry weight.

Table 4.4. The effects of heave metal cadmium stress ($\text{Cd}(\text{NO}_3)_2$) under different concentration (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on the fresh and dry weight (shoot and root) of wheat landraces cultivars in soil after 28days. Values are shows as the mean $\pm$ SD of three replicates

Species of wheat landraces	$\text{Cd}(\text{NO}_3)_2$ treatment	Weight fresh shoot (mg)	Weight dry shoot (mg)	Weight fresh root (mg)	Weight dry root(mg)
<i>Triticum aestivum</i> L. (Ekiz)	0 μM	9897 ^a $\pm$ 283	767 ^{abc} $\pm$ 67	1013 ^{cde} $\pm$ 242	130 ^{ab} $\pm$ 40
	50 μM	6973 ^a $\pm$ 915	823 ^{ab} $\pm$ 71	647 ^{fg} $\pm$ 75	143.3 ^a $\pm$ 25
	100 μM	7100 ^a $\pm$ 346	927 ^a $\pm$ 142	1123 ^{bc} $\pm$ 240	140 ^a $\pm$ 10
	250 μM	6083 ^{ab} $\pm$ 831	580 ^{bcde} $\pm$ 190	877 ^{cdef} $\pm$ 191	123 ^{ab} $\pm$ 5.8
	500 μM	5783 ^{bc} $\pm$ 586	19 ^{cdefg} $\pm$ 70	1113 ^{bcd} $\pm$ 326	117 ^{abc} $\pm$ 23
<i>Triticum monococcum</i> spp. (<i>monococcum</i> (Einkorn))	0 μM	4903 ^{def} $\pm$ 542	580 ^{cdef} $\pm$ 70	870 ^{cdef} $\pm$ 135	83 ^{def} $\pm$ 5.8
	50 μM	4780 ^{efg} $\pm$ 252	727 ^{abcd} $\pm$ 115	583 ^{fgh} $\pm$ 15	80 ^{def} $\pm$ 17
	100 μM	4310 ^{fgh} $\pm$ 898	440 ^{fgh} $\pm$ 17	943 ^{cdef} $\pm$ 98	107 ^{bcd} $\pm$ 5.8
	250 μM	3850 ^{ghij} $\pm$ 207	700 ^{bcdef} $\pm$ 120	757 ^{defg} $\pm$ 224	100 ^{bcde} $\pm$ 0.0
	500 μM	3753 ^{ghij} $\pm$ 1002	700 ^{abcde} $\pm$ 202	1127 ^{bc} $\pm$ 273	132 ^{ab} $\pm$ 16
<i>Triticum dicoccum</i> spp. (<i>dicoccum</i> Schrank (Emmer))	0 μM	4636 ^{efg} $\pm$ 508	540 ^{cdef} $\pm$ 27	1407 ^b $\pm$ 129	82 ^{def} $\pm$ 1
	50 μM	3060 ^{ijk} $\pm$ 651	357 ^{ghi} $\pm$ 61	647 ^{fg} $\pm$ 191	88 ^{cde} $\pm$ 16
	100 μM	2567 ^k $\pm$ 334	280 ^{hi} $\pm$ 10	257 ^h $\pm$ 91	51 ^{fg} $\pm$ 2.1
	250 μM	2753 ^{jk} $\pm$ 339	170 ⁱ $\pm$ 12	500 ^{gh} $\pm$ 105	55 ^{fg} $\pm$ 8.9
	500 μM	3253 ^{hijk} $\pm$ 810	473 ^{efgh} $\pm$ 6	767 ^{cdefg} $\pm$ 362	100 ^{bcde} $\pm$ 0.58
<i>Triticum turgidum</i> L. var. <i>durum</i> (Durum)	0 μM	7125 ^a $\pm$ 559	545 ^{cdef} $\pm$ 35	3055 ^a $\pm$ 177	90 ^{cde} $\pm$ 13
	50 μM	5193 ^{def} $\pm$ 645	773 ^{abc} $\pm$ 46	447 ^{gh} $\pm$ 72	83 ^g $\pm$ 32
	100 μM	5530 ^{de} $\pm$ 242	650 ^{abcde} $\pm$ 15	733 ^{efg} $\pm$ 153	84 ^{def} $\pm$ 10.3
	250 μM	4147 ^{ghij} $\pm$ 332	573 ^{cdefg} $\pm$ 12	440 ^{gh} $\pm$ 121	70 ^{efg} $\pm$ 10
	500 μM	3730 ^{ghijk} $\pm$ 516	500 ^{defgh} $\pm$ 46	437 ^{gh} $\pm$ 106	80 ^{def} $\pm$ 10

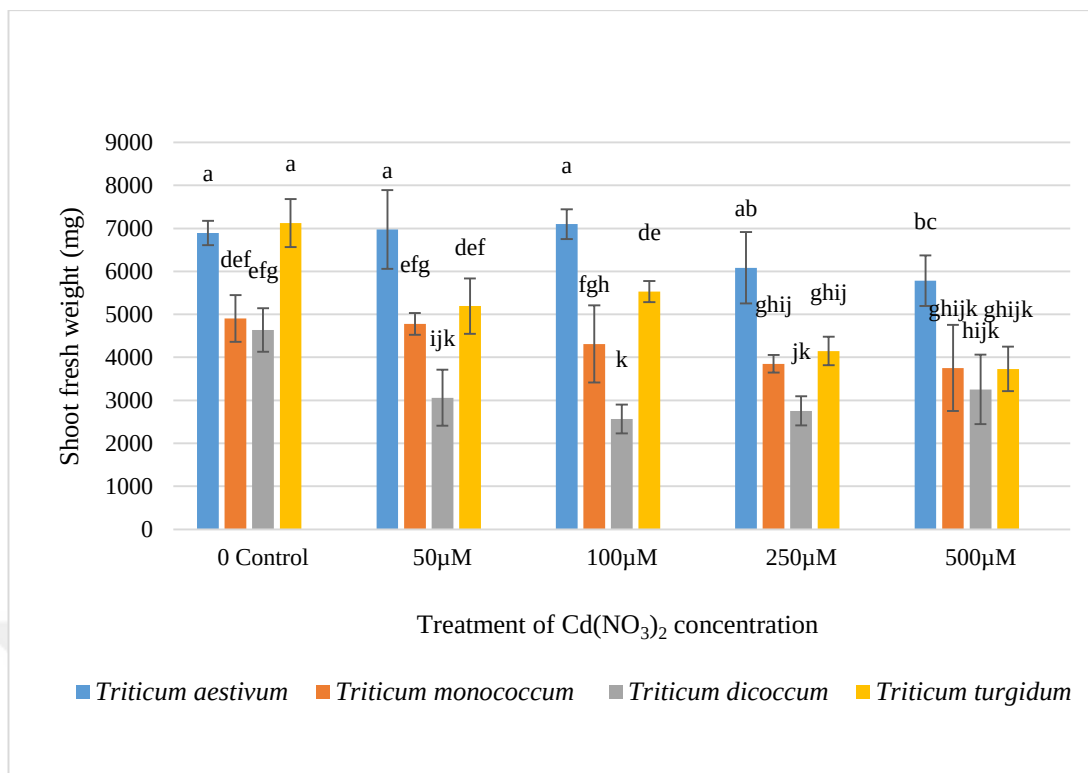


Figure 4.10. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on shoot fresh weight (mg) of wheat landrace cultivars in soil.

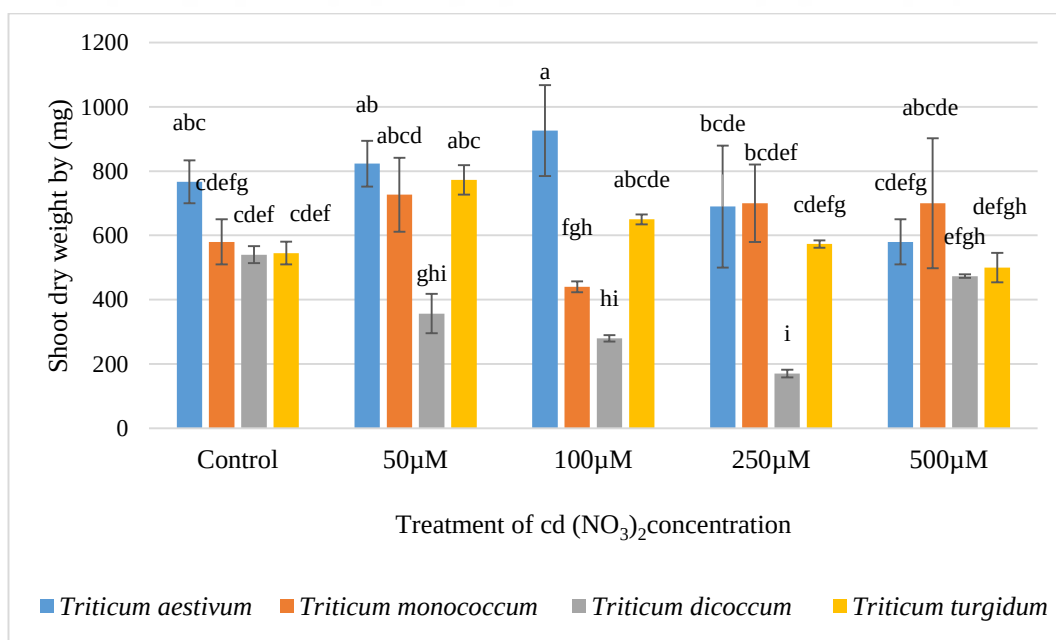


Figure 4.11. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on shoot dry weight (mg) of wheat landrace cultivars in soil.

4.4.4 Effects of Cadmium Stress on Fresh and Dry Root Weights of Wheat Landraces Cultivars in Soil

The effects of increased $\text{Cd}(\text{NO}_3)_2$ concentrations in soil on root fresh and dry weights varied among different varieties of *Triticum* species. In *T. aestivum*, an increase in fresh root weight was observed when exposed to higher $\text{Cd}(\text{NO}_3)_2$ concentrations (100 μM , 250 μM , and 500 μM), with recorded values of (1123.3^{bc}±239.7 mg, 876.6^{cdef}±191.4 mg, and 1113.3^{bcd}±325.9 mg) respectively, compared to the control, as shown in Figure 4.12 and Table 4.4. However, dry root weight showed no significant reduction at 50 μM and 100 μM $\text{Cd}(\text{NO}_3)_2$ concentrations (142.3^a±25.16 mg, 140^a±10.0 mg), and (123.3^{ab}±5.8 mg, 116.6^{abc}±23.1 mg) respectively, compared to the control, as presented in Figure 4.12 and Table 4.4.

Similarly, *T. monococcum* exhibited an increase in fresh root weight at a concentration 500 μM of $\text{Cd}(\text{NO}_3)_2$ (1126.7^{bc}±273.2 mg), compared to other concentrations and the control, as indicated in Figure 4.12 and Table 4.4. Conversely, at 100 μM $\text{Cd}(\text{NO}_3)_2$ concentration, no significant effect on fresh root weight was observed compared to the control (943.3^{cdef}±98.2 mg, 870^{cdef}±135.3 mg), as presented in Figure 4.12 and Table 4. 4.

Dry root weight also showed no significant reduction at low $\text{Cd}(\text{NO}_3)_2$ concentrations (100 μM and 250 μM) and the control (106.7^{bcd}±5.8 mg, 100^{bcd}±0.0 mg), (80^{def}±17.3 mg, 83.3^{def}±5.8 mg) respectively, but a significant increase was noted at 500 μM (131.7^{ab}±16.1 mg), compared to other concentrations and the control, as depicted in Figure 4.13 .1and Table 4.4.

For *T. turgidum*, a reduction in fresh root weight was observed at different concentrations of $\text{Cd}(\text{NO}_3)_2$ (50 μM , 100 μM , 250 μM , and 500 μM) compared to the control, as illustrated in Figure 4.12 and Table 4.4. However, there was no significant reduction in fresh root weight between concentrations of 50 μM , 250 μM , and 500 μM of $\text{Cd}(\text{NO}_3)_2$ (446.7^{gh}±72.3 mg, 440^{gh}±121.7 mg, 436.7^{gh}±105.9 mg) respectively. Interestingly, a slight increase in fresh root weight was observed at 100 μM $\text{Cd}(\text{NO}_3)_2$ concentration (733.3^{efg}±152.8 mg) compared to other concentrations, as depicted in Figure 4.12 and Table 4.4.

Dry root weight showed no significant difference at 100 μM , 250 μM , and 500 μM of $\text{Cd}(\text{NO}_3)_2$ ($84^{\text{def}} \pm 10.3$ mg, $80^{\text{def}} \pm 10$ mg, $70^{\text{efg}} \pm 10$ mg) respectively, compared to 50 μM of $\text{Cd}(\text{NO}_3)_2$ ($82.5^{\text{g}} \pm 31.8$ mg) and the control ($90^{\text{cde}} \pm 12.7$ mg), as presented in Figure 4.13 and Table 4.4.

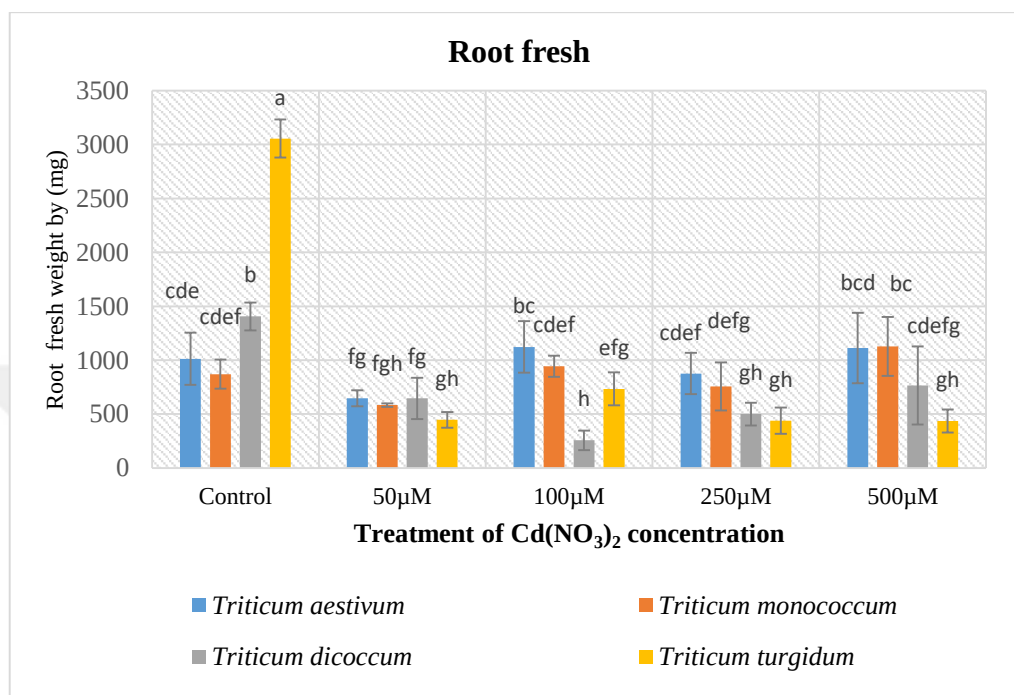


Figure 4.12. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on root fresh weight (mg) of wheat landrace cultivars in soil.

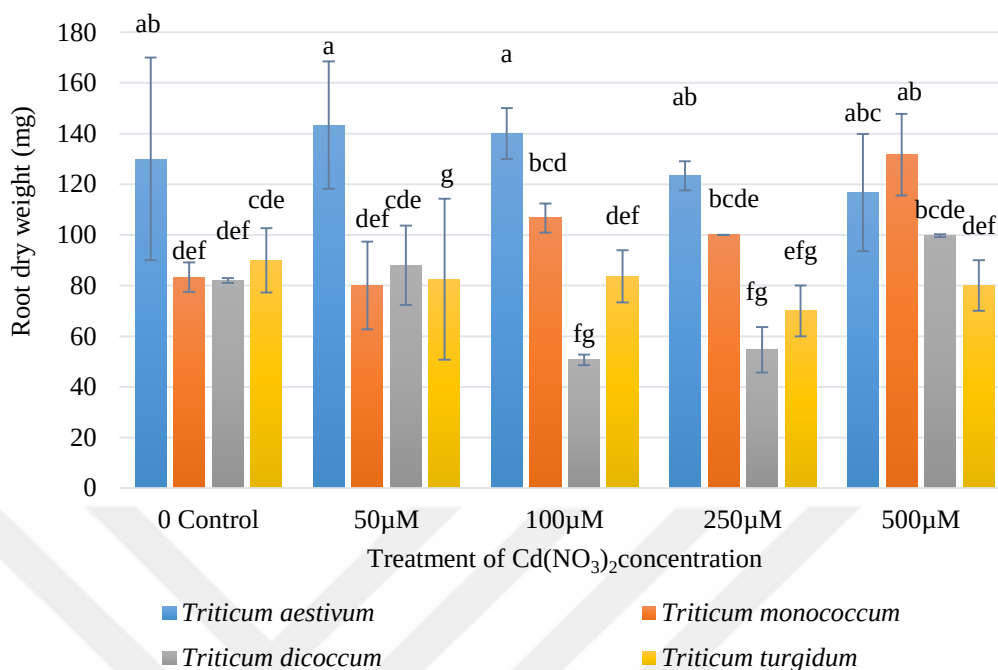


Figure 4.13. The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 μM, 50 μM, 100 μM, 250 μM and 500 μM) on root dry weight (mg) of wheat landrace cultivars in soil.

4.5 Effects of Cadmium Stress on Shoot Toxicity and Root Toxicity Percentage and Tolerance Index of Wheat Landrace Cultivars in Soil.

Shoot and root growth was adversely affected by the increased Cd(NO₃)₂ treatment concentrations in the soil. The extent of shoot toxicity depended on the wheat varieties and the dose of Cd(NO₃)₂ treatment exposure. As the Cd dose increased, the shoot toxicity also increased.

The research results indicated that shoot toxicity was most pronounced at 500 μM Cd(NO₃)₂ treatment concentration, with *T. dicoccum*, *T. turgidum*, and *T. aestivum* showing 34%, 33%, and 27% toxicity respectively, as presented in Figure 4.14 and Table 4.5. Similarly, at 250 μM Cd(NO₃)₂ treatment concentration, these species exhibited similar effects with 24.4%, 24%, and 24% toxicity respectively,

suggesting no significant difference between them at this concentration. However, at 250 μM and 500 μM $\text{Cd}(\text{NO}_3)_2$ treatments, *T. monococcum* showed lower shoot toxicity (18% and 22% respectively) and demonstrated greater tolerance compared to the other species, as shown in Figure 4.14 and Table 4.5. At 100 μM $\text{Cd}(\text{NO}_3)_2$ concentration, *T. dicoccum* and *T. monococcum* exhibited similar levels of shoot toxicity (21% and 21.2% respectively), while *T. aestivum* showed lower toxicity (3%) and greater tolerance. *T. turgidum* exhibited 17% toxicity at 100 μM $\text{Cd}(\text{NO}_3)_2$. In contrast, at 50 μM $\text{Cd}(\text{NO}_3)_2$ concentration, *T. turgidum* showed 5% toxicity, *T. dicoccum* 9%, *T. aestivum* 1.3%, and *T. monococcum* 7%, indicating lower toxicity levels at this lower $\text{Cd}(\text{NO}_3)_2$ concentration.



Table 4.5. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) at different concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on the fresh and dry weight (shoot and root) of wheat landraces cultivars in soil after 28days. Values are shows as the mean $\pm$ SD of three replicates.

Species of wheat landraces	$\text{Cd}(\text{NO}_3)_2$ treatment	Shoot toxicity (%)	Root toxicity (%)	Tolerance index (%)
<i>Triticum aestivum</i> L. (Ekiz)	50 μM	1.3 ^h $\pm$ 0.27	6.6 ^g $\pm$ 0.9	92 ^a $\pm$ 1.4
	100 μM	2.9 ^{gh} $\pm$ 0.35	6.1 ^g $\pm$ 0.26	91 ^a $\pm$ 1.9
	250 μM	24.4 ^{bc} $\pm$ 0.75	6.4 ^g $\pm$ 1.5	90 ^{ab} $\pm$ 3.2
	500 μM	27 ^b $\pm$ 1.1	13 ^f $\pm$ 0.5	86 ^b $\pm$ 1.9
<i>Triticum monococcum</i> spp. <i>monococcum</i> (Einkorn)	50 μM	6.8 ^{fg} $\pm$ 0.8	7.3 ^g $\pm$ 1.0	95 ^a $\pm$ 3.3
	100 μM	21.3 ^{cd} $\pm$ 3.8	22 ^e $\pm$ 2.2	78 ^c $\pm$ 2.1
	250 μM	18.2 ^{de} $\pm$ 2.6	28 ^{cd} $\pm$ 1.1	72 ^{de} $\pm$ 1.1
	500 μM	21.9 ^{cd} $\pm$ 1.5	30 ^c $\pm$ 1.3	69 ^e $\pm$ 1.3
<i>Triticum dicoccum</i> spp. <i>dicoccum</i> Schrank (Emmer)	50 μM	8.8 ^f $\pm$ 1.2	14 ^f $\pm$ 0.6	86 ^b $\pm$ 0.5
	100 μM	21 ^{cd} $\pm$ 2.3	25 ^{de} $\pm$ 1.8	75 ^{cd} $\pm$ 1.8
	250 μM	24 ^{bc} $\pm$ 0.8	31 ^c $\pm$ 1.7	69 ^e $\pm$ 1.7
	500 μM	34 ^a $\pm$ 4.4	39 ^b $\pm$ 6.1	62 ^e $\pm$ 5.2
<i>Triticum turgidum</i> L. var. durum (Durum)	50 μM	4.7 ^{gh} $\pm$ 1.6	26 ^{de} $\pm$ 0.5	74 ^{cd} $\pm$ 0.5
	100 μM	17 ^e $\pm$ 1.01	32 ^c $\pm$ 5.5	69 ^e $\pm$ 5.5
	250 μM	24.4 ^{bc} $\pm$ 4.5	38.1 ^b $\pm$ 1.8	62 ^f $\pm$ 1.6
	500 μM	32.7 ^a $\pm$ 2.2	43 ^a $\pm$ 2	57 ^f $\pm$ 5.2

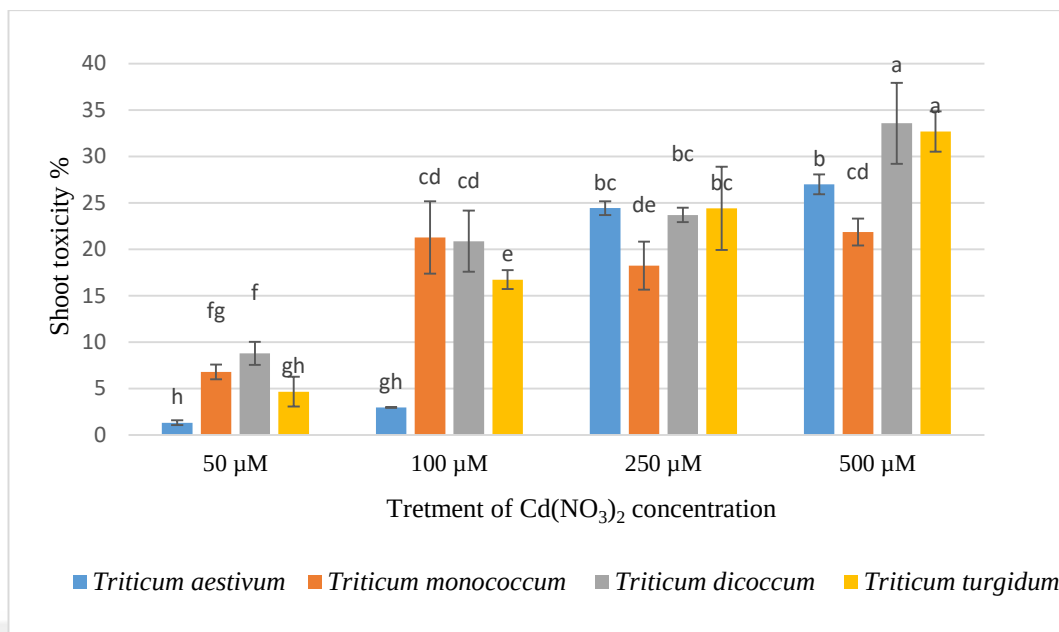


Figure 4.14. The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 µM, 50 µM, 100 µM, 250 µM and 500 µM) to evaluate the shoot toxicity percentage of wheat landrace cultivars in soil.

Root toxicity was more pronounced in all wheat landrace cultivars at higher Cd(NO₃)₂ concentrations. Specifically, at 500 µM Cd(NO₃)₂, root toxicity percentages were 43.4% for *T. turgidum*, 39% for *T. dicoccum*, 30.4% for *T. monococcum*, and 13% for *T. aestivum*. Similarly, at 250 µM Cd(NO₃)₂ concentration, root toxicity percentages were recorded as 38% for *T. turgidum*, 31% for *T. dicoccum*, and 28% for *T. monococcum*. These findings indicate an increase in root toxicity with higher concentrations, as illustrated in Figure 4.15 and Table 4.5. Furthermore, the results revealed that *T. aestivum* showed no significant effect at 50 µM, 100 µM, and 250 µM Cd(NO₃)₂ concentrations, demonstrating greater resistance to Cd(NO₃)₂ compared to other wheat cultivars, as presented in Figure 4.15 and Table 4.5. Conversely, *T. turgidum* exhibited greater sensitivity to root toxicity at all concentrations (50 µM, 100 µM, 250 µM, and 500 µM of Cd(NO₃)₂) compared to other wheat species.

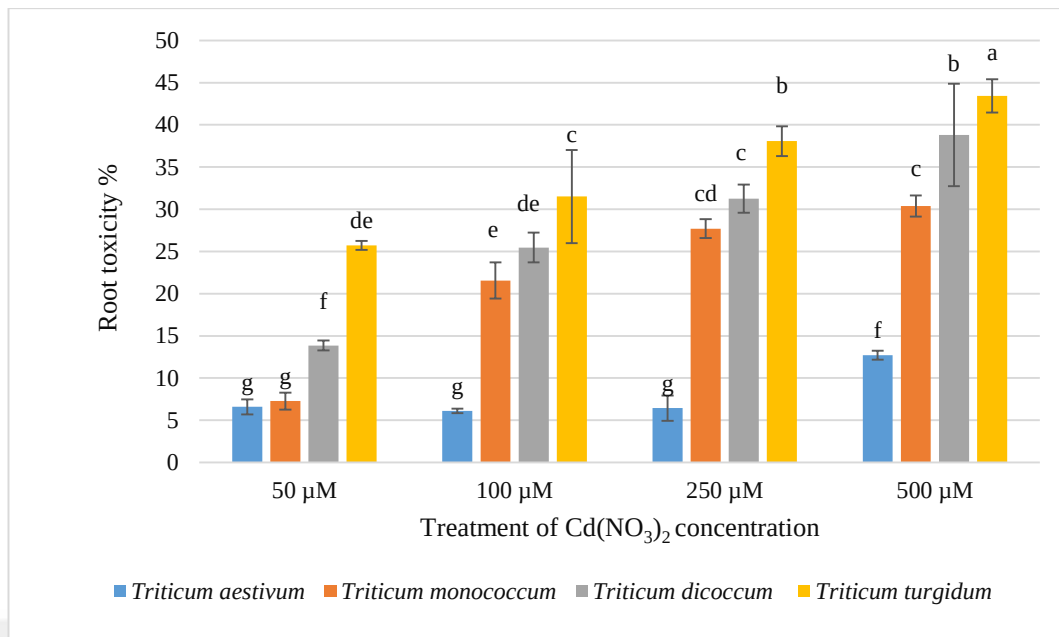


Figure 4.15. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 µM, 50 µM, 100 µM, 250 µM and 500 µM) to the evaluate of root toxicity percentage in wheat landrace cultivars

The increase in $\text{Cd}(\text{NO}_3)_2$ concentration led to a reduction in the tolerance index, as depicted in Figure 4.16. *T. aestivum* and *T. monococcum* exhibited greater tolerance to high $\text{Cd}(\text{NO}_3)_2$ concentrations at 250 µM and 500 µM, with tolerance indices of 90% and 72% and 86% and 69%, respectively. The maximum tolerance index was observed in *T. aestivum* at 50 µM (92%) and 100 µM (91%). Conversely, the lowest tolerance index was observed in *T. turgidum* at 50 µM (74%), 100 µM (68%), 250 µM (62%), and 500 µM (57%), as shown in Figure 4.16. Maximum tolerance was observed in *T. aestivum*, followed by *T. monococcum*, *T. dicoccum*, and *T. turgidum*, respectively, across various $\text{Cd}(\text{NO}_3)_2$ concentrations, as presented in Figure 4.16 and Table 4.5.

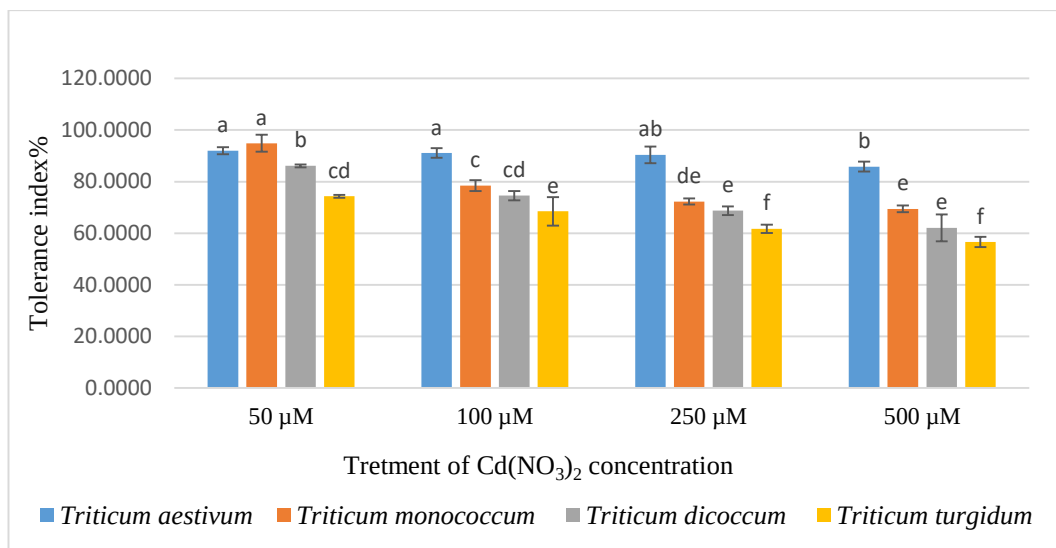


Figure 4.16. The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 µM, 50 µM, 100 µM, 250 µM and 500 µM) to evaluate the tolerance index percentage of wheat landrace cultivars in soil.

4.6 Photosynthetic Pigment Content: Chlorophyll a, b, and Carotenoids

Chlorophyll a (Chl a) content was highest in the leaves of *T. turgidum* in both control and other wheat landraces treated with different concentrations of Cd(NO₃)₂ (50 µM, 100 µM, 250 µM, and 500 µM). These findings suggest that exposure to Cd(NO₃)₂ toxicity had no significant effect on chlorophyll a levels across different wheat types. However, at Cd(NO₃)₂ concentration of 250 µM and 500 µM, the total chlorophyll a content was reduced in *T. aestivum* (17.4^{bc} ± 0.1, 17^c ± 0.1 mg/L) 250 µM and *T. monococcum* (17.1^c ± 1.7 mg/L) at 250 µM and *T. aestivum* (17.1^c ± 0.01 mg/L) at 500 µM, *T. monococcum* (18.3^{bc} ± 0.5 mg/L) at 500 µM as shown in Figure 4.17 and Table 4.6.

Table 4.6. The Effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) at various concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on photosynthetic pigments content (chlorophyll a, b and carotenoids) of wheat landrace cultivars in soil after 28days.

Values are shown as the mean $\pm$ SD of three replicates.

Species of wheat landraces	$\text{Cd}(\text{NO}_3)_2$ treatment	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Carotenoids (mg/L)
<i>Triticum aestivum</i> L. (Ekiz)	0 μM	18.4 ^{bc} $\pm$ 0.02	31 ^{abcd} $\pm$ 0.3	2187 ^{abcd} $\pm$ 27.8
	50 μM	18 ^{bc} $\pm$ 0.01	30.2 ^{abcde} $\pm$ 0.2	2118 ^{cdefg} $\pm$ 73.3
	100 μM	18 ^{bc} $\pm$ 0.01	29.4 ^{bcde} $\pm$ 0.2	2170 ^{abcde} $\pm$ 32.4
	250 μM	17.4 ^{bc} $\pm$ 0.01	29 ^{de} $\pm$ 0.2	2204 ^a $\pm$ 5.9
	500 μM	17.1 ^c $\pm$ 0.01	28 ^e $\pm$ 0.2	2155 ^{abcdeg} $\pm$ 53
<i>Triticum monococcum</i> spp. <i>monococcum</i> (Einkorn)	0 μM	18.4 ^{bc} $\pm$ 1.3	30.5 ^{abcd} $\pm$ 2.7	2200 ^{ab} $\pm$ 35.2
	50 μM	19 ^{bc} $\pm$ 0.4	32.2 ^a $\pm$ 0.6	2185 ^{abcd} $\pm$ 32.4
	100 μM	19 ^{bc} $\pm$ 0.5	32 ^{ab} $\pm$ 0.9	2163 ^{abcdeg} $\pm$ 8.1
	250 μM	17 ^c $\pm$ 1.7	29 ^{de} $\pm$ 2.7	2097 ^{efg} $\pm$ 3.8
	500 μM	18.3 ^{bc} $\pm$ 0.5	31 ^{abc} $\pm$ 0.9	2179 ^{abcd} $\pm$ 37.9
<i>Triticum dicoccum</i> spp. <i>dicoccum</i> Schrank (Emmer)	0 μM	18 ^{bc} $\pm$ 1	30 ^{bcde} $\pm$ 1.7	2091 ^{fg} $\pm$ 53.6
	50 μM	18.5 ^{bc} $\pm$ 0.3	31 ^{abcd} $\pm$ 0.5	2160 ^{abcdeg} $\pm$ 16.3
	100 μM	19 ^{bc} $\pm$ 0.3	30 ^{abcde} $\pm$ 0.5	2131 ^{abcdeg} $\pm$ 15
	250 μM	19 ^{bc} $\pm$ 0.7	31 ^{abc} $\pm$ 1	2112 ^{defg} $\pm$ 81.2
	500 μM	19 ^{bc} $\pm$ 0.1	32 ^{ab} $\pm$ 0.3	2124 ^{bcdeg} $\pm$ 41
<i>Triticum turgidum</i> L. var. <i>durum</i> (Durum)	0 μM	21.3 ^a $\pm$ 2.3	31 ^{abcd} $\pm$ 3.2	2117 ^{cdeg} $\pm$ 55.6
	50 μM	19.4 ^b $\pm$ 0.3	29 ^{cde} $\pm$ 0.2	2129 ^{abcdeg} $\pm$ 33
	100 μM	19 ^{bc} $\pm$ 0.2	32 ^{ab} $\pm$ 0.3	2193 ^{abc} $\pm$ 21
	250 μM	19 ^{bc} $\pm$ 0.2	30.4 ^{abc} $\pm$ 0.2	2081 ^g $\pm$ 13.1
	500 μM	18 ^{bc} $\pm$ 0.7	31 ^{abcd} $\pm$ 0.5	2197 ^{ab} $\pm$ 13.5

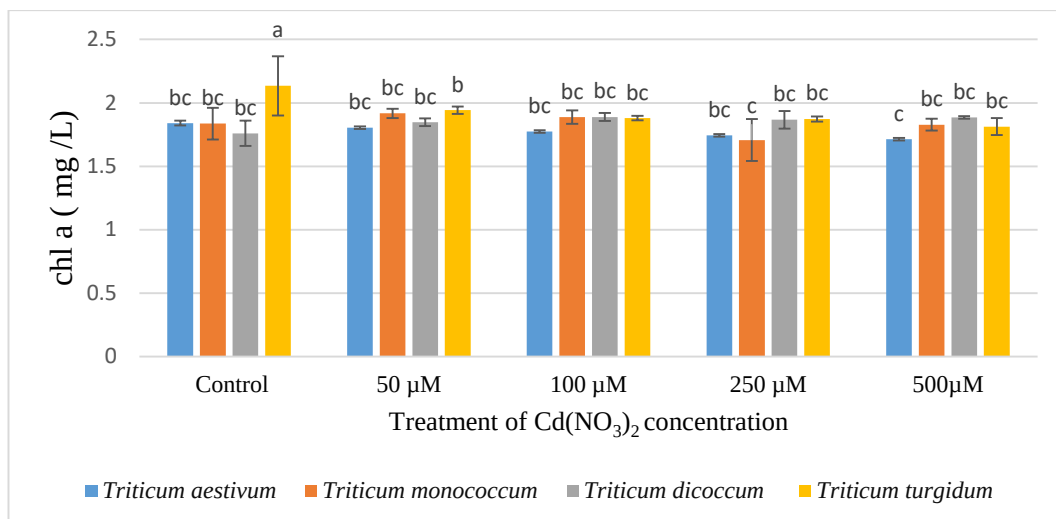


Figure 4.17. The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 μM, 50 μM, 100 μM, 250 μM and 500 μM) on the content of chlorophyll a (mg /L) in wheat landrace cultivars grown in soil.

On the other hand, chlorophyll b increased with the rise in the dose of Cd(NO₃)₂ toxicity in certain type of wheat landraces. In Figure 4.18, it can be observed that *T. monococcum* exhibited an increase to $32.2^{a} \pm 0.55$ mg/L, $31.7^{ab} \pm 0.9$ mg/L and $31.3^{abc} \pm 0.9$ mg/L at 50 μM, 100 μM and 500 μM of Cd(NO₃)₂ respectively, compared to the control. Moreover, *T. dicoccum* and *T. turgidum* showed an increase in chlorophyll b content at different concentrations of Cd(NO₃)₂ (50, 100, 250 and 500 μM). Specially, at 100 μM of Cd(NO₃)₂, *T. turgidum* was recorded, $31.6^{ab} \pm 0.27$ mg/L, and the highest content of chlorophyll b in *T. dicoccum* was $31.7^{ab} \pm 0.29$ mg/L at 500 μM. However, the results indicate that chlorophyll b was reduced at 250 μM and 500 μM of Cd(NO₃)₂ in *T. aestivum* ($28.7^{de} \pm 0.24$ mg/L, $27.9^{e} \pm 0.24$ mg/L) and *T. turgidum* ($29^{cde} \pm 0.23$ mg/L), with a reduction observed even at 50 μM of Cd(NO₃)₂ toxicity as presented in Figure 4.18. Thus, chlorophyll b exhibited a reduction with increased Cd toxicity.

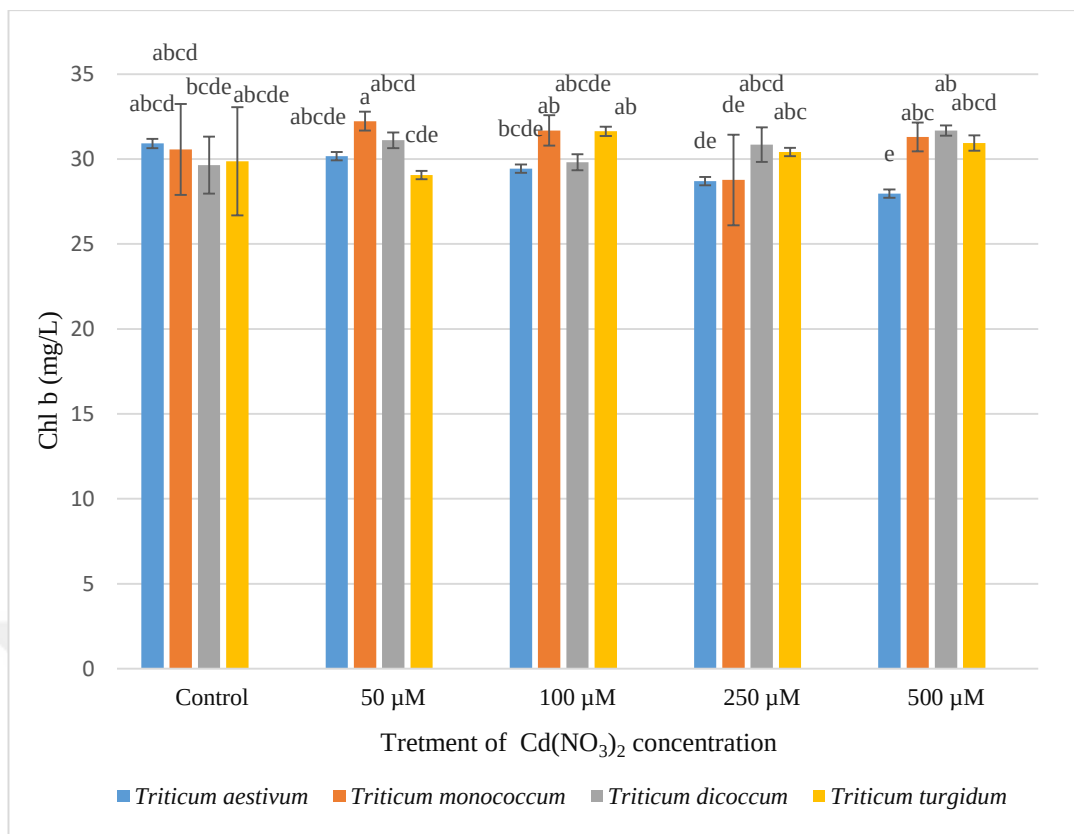


Figure 4.18. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on the content of chlorophyll b (mg /L) in wheat landrace cultivars grown in soil.

The highest carotenoid content was observed in the control plants. Additionally, an increase in carotenoid content was recorded, with levels reaching $2203^{\text{a}} \pm 5.94$ mg/L at 250 μM of $\text{Cd}(\text{NO}_3)_2$ in *T. aestivum*, and *T. turgidum*, and $2193^{\text{abc}} \pm 20.9$ mg/L and $2197^{\text{ab}} \pm 13.5$ mg/L at 100 and 250 μM , respectively. Furthermore, *T. dicoccum* exhibited an increase in carotenoid content when exposed to $\text{Cd}(\text{NO}_3)_2$ at different concentrations.

On the contrary, a decrease in carotenoid content with an increase of $\text{Cd}(\text{NO}_3)_2$ concentration was observed in *T. monococcum* partially at 250 μM of $\text{Cd}(\text{NO}_3)_2$ ($2097^{\text{efg}} \pm 3.8$ mg/L). In addition, the results indicate a show that significant effect of $\text{Cd}(\text{NO}_3)_2$ concentration on carotenoid content among different wheat types as presented in Figure 4.19 and Table 4.6.

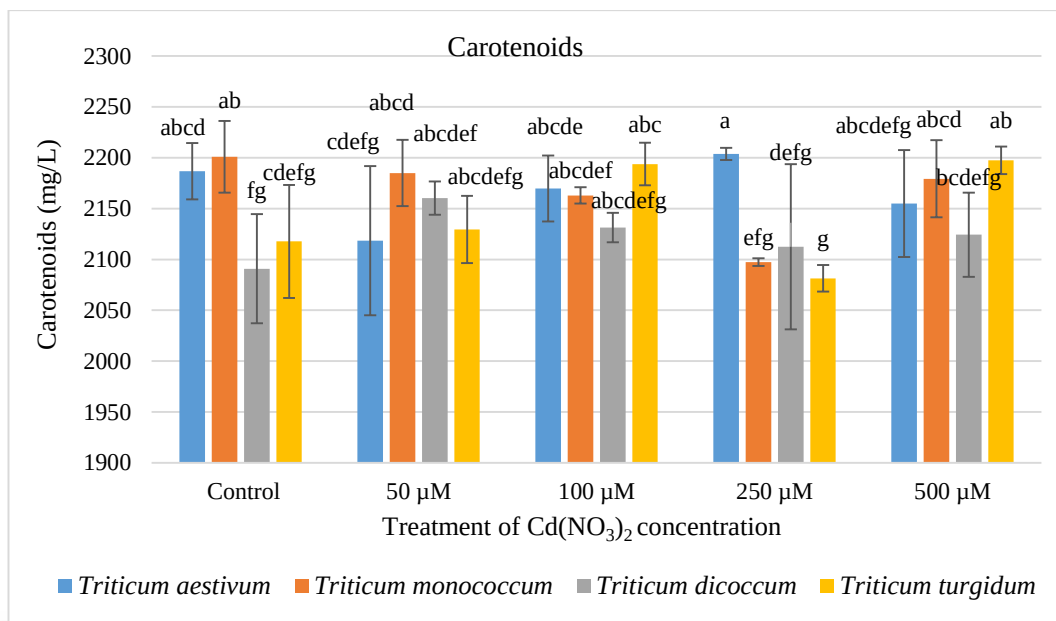


Figure 4.19. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on the content of carotenoids (mg/L) on wheat landrace cultivars grown in soil.

4.7 Accumulation of Total Phenolic Content on Leaves of Wheat Landraces

This research shows an increase in phenolic content in wheat landraces when exposed to Cd toxicity at different concentrations. This response is interpreted as a plant's defense mechanism to alleviate the toxic effects of $\text{Cd}(\text{NO}_3)_2$. *T. aestivum* exhibited an increase in phenolic content at high $\text{Cd}(\text{NO}_3)_2$ concentrations, with values of $1.25^{\text{efg}} \pm 0.022$ mg/g GA, $1.36^{\text{defg}} \pm 0$ mg/g GA, and $1.35^{\text{defg}} \pm 0.03$ mg/g GA, at 100, 250, and 500 μM of $\text{Cd}(\text{NO}_3)_2$, respectively. Similarly, *T. monococcum* showed an increase in phenolic content ($1.67^{\text{de}} \pm 0.02$ mg/g GA, $2.13^{\text{c}} \pm 0.087$ mg/g GA and $1.8^{\text{cd}} \pm 0.041$ mg/g GA) at 50, 100, and 500 μM of $\text{Cd}(\text{NO}_3)_2$ compared to the control, with the highest level observed at 100 μM . *T. dicoccum* exhibited increased phenolic content ($1.47^{\text{def}} \pm 0.008$ mg/g GA, and $1.63^{\text{de}} \pm 0.014$ mg/g GA) at 100, and 250 μM of $\text{Cd}(\text{NO}_3)_2$ compared to the control. However, there was a decrease in phenolic content at 50 and 500 μM of $\text{Cd}(\text{NO}_3)_2$. In contrast, *T. turgidum* showed a decrease in phenolic content ($1.37^{\text{defg}} \pm 0.054$ mg/g GA, $1.62^{\text{de}} \pm 0.47$ mg/g GA, and $1.62^{\text{de}} \pm 0.17$ mg/g GA) at different concentrations of $\text{Cd}(\text{NO}_3)_2$ (50, 250 and 500 μM , respectively) compared to the control, with a slight

decrease at 100 μM ($2.5^b \pm 0.09$). Overall, the results indicate a significant decrease in phenolic content under different concentrations of $\text{Cd}(\text{NO}_3)_2$, with no significant effect observed at 250 μM of $\text{Cd}(\text{NO}_3)_2$ among all wheat varieties.

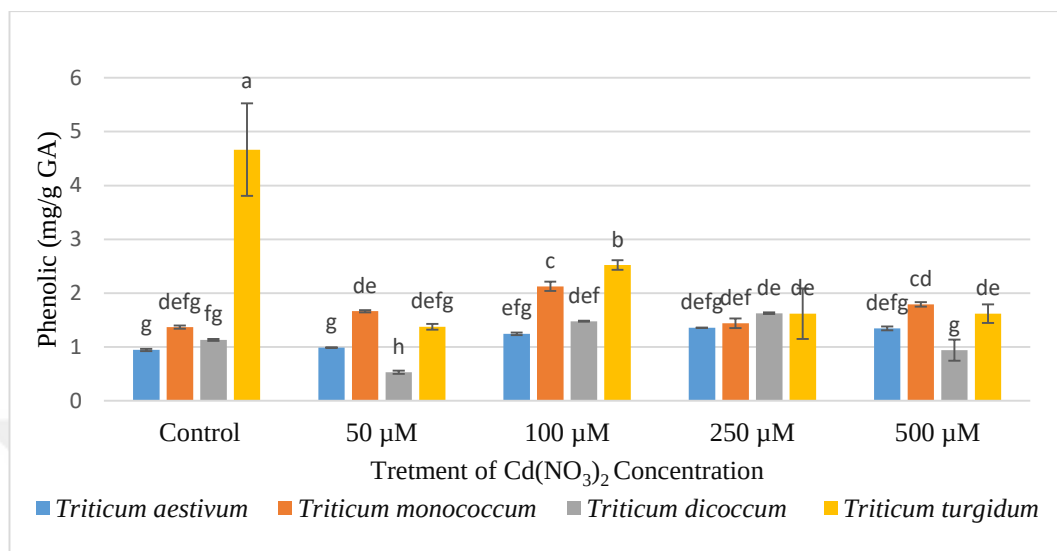


Figure 4.20. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on phenolic content (mg /g GA) in wheat landrace cultivars grown in soil.

4.8 Accumulation of Total Proline Content on Leaves of Wheat Landraces

Proline content was significantly increased with $\text{Cd}(\text{NO}_3)_2$ -stress treatment in wheat plant growth, as shown in Table 4.7 and Figure 4.21. The increase occurred in a dose-dependent manner, with the highest elevation observed in *T. turgidum* ($7.86^a \pm 0.02$ μmol proline /g fw, $7.35^{bc} \pm 0.07$ μmol proline /g fw) at 500, and 250 μM of Cd, respectively. Similarly, *T. dicoccum* exhibited increased proline levels ($6.90^c \pm 0.01$ μmol proline /g fw, and $7.16^{bc} \pm 0.22$ μmol proline /g fw) at 100 μM , and 500 μM , respectively. In contrast, *T. aestivum* showed an increase in proline content ($2.35^k \pm 0.29$ μmol proline /g fw, $2.68^j \pm 0.06$ μmol proline /g fw, and $3.64^h \pm 0.19$ μmol proline /g fw) at 50, 100, and 250 μM $\text{Cd}(\text{NO}_3)_2$ -treatments compared to the control. However, a slight decrease was observed in *Triticum aestivum* ($2.18^k \pm 0.04$ μmol proline /g fw) at 500 μM of $\text{Cd}(\text{NO}_3)_2$. Moreover, *T. monococcum* and *T. dicoccum* exhibited the highest proline levels ($7.15^{bc} \pm 0.04$, $6.90^c \pm 0.01$ μmol proline /g fw) at 100 μM and ($5.67^{bc} \pm 0.02$ μmol proline /g fw,

7.16^{bc}±0.22 µmol proline /g fw) at 500µM of Cd(NO₃)₂ compared to other concentrations. Furthermore, wheat *T. dicoccum*, exhibited increases in proline levels when exposed to higher concentrations of Cd(NO₃)₂ stress. However, in contrast, a decrease was found in proline levels at 250 µM the compared to 500 µM of Cd(NO₃)₂.

Table 4.7. The effects of cadmium stress (Cd(NO₃)₂) at various concentrations (0 µM, 50µM, 100µM, 250µM and 500µM) on phenolic and proline content wheat landrace cultivars grown in soil after 28 days. Values are shown as the mean ±SD of three replicates.

Species of wheat landraces	Cd(NO ₃) ₂ treatment	Phenolic content (mg/g) GA	Proline (µmol/g ⁻¹)
<i>Triticum aestivum</i> L. (Ekiz)	0 µM	0.95 ^g ±0.022	1.87±0.34
	50 µM	0.99 ^g ±0.008	2.35±0.297
	100 µM	1.25 ^{efg} ±0.022	2.68±0.062
	250 µM	1.36 ^{defg} ±0	3.64±0.19
	500 µM	1.35 ^{defg} ±0.03	2.18±0.037
<i>Triticum monococcum</i> spp. <i>monococcum</i> (Einkorn)	0 µM	1.37 ^{defg} ±0.03	4.64±0.037
	50 µM	1.67 ^{de} ±0.02	5.89±0.057
	100 µM	2.13 ^c ±0.087	7.15±0.04
	250 µM	1.44 ^{def} ±0.087	5.32±0.08
	500µM	1.8 ^{cd} ±0.041	5.66±0.02
<i>Triticum dicoccum</i> spp. <i>dicoccum</i> Schrank (Emmer)	0 µM	1.13 ^{fg} ±0.016	3.58±0.03
	50 µM	0.53 ^h ±0.029	4.5±0.08
	100 µM	1.47 ^{def} ±0.008	6.91±0.01
	250 µM	1.63 ^{de} ±0.014	5.11±0.05
	500 µM	0.94 ^g ±0.19	7.16±0.22
<i>Triticum turgidum</i> L. var. <i>durum</i> (Durum)	0 µM	4.67 ^a ±0.86	3.30i±0.43
	50 µM	1.37 ^{defg} ±0.054	2.67j±0.08
	100 µM	2.5 ^b ±0.09	4.08g±0.03
	250 µM	1.62 ^{de} ±0.47	7.36bc±0.06
	500 µM	1.62 ^{de} ±0.17	7.86a±0.02

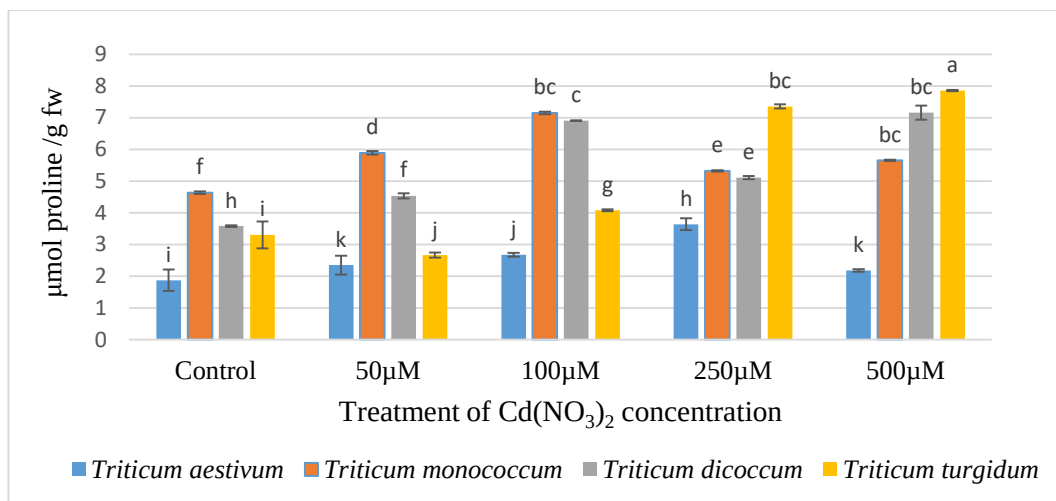


Figure 4.21. The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 μM, 50 μM, 100 μM, 250 μM and 500 μM) on total proline content (mg /g GA) in wheat landrace cultivars grown in soil.

4.9 Total Protein Content of Wheat Landraces

The total protein content of wheat landraces *T. aestivum*, *T. monococcum*, *T. dicoccum* and *T. turgidum* was measured to analyze the antioxidant enzymes CAT and SOD. It was observed that the total protein content significantly increased in leaves after exposure of wheat seedlings to different concentration of Cd(NO₃)₂ treatments (50, 100, 250 and 500 μM Cd(NO₃)₂). The results showed that the total of protein content increased with increasing Cd(NO₃)₂ concentration, depending on the wheat species. Specifically, *T. dicoccum* exhibited a higher total of protein content ($77.7^a \pm 2.80$ mg/ml,) at 250 μM. *T. turgidum* showed increased total protein content at 100 and 250 μM of Cd(NO₃)₂ ($57.7^b \pm 2.23$ mg/ml, and $44.8^c \pm 1.17$ mg/ml, respectively), while *T. aestivum* showed increased total protein content at 50 and 500 μM Cd(NO₃)₂ ($46.8^c \pm 2.73$ mg/ml, and $39.7^d \pm 2.20$ mg/ml, respectively), as presented in Figure 4.22 Table 4.8. On the other hand, the total protein content was significantly elevated in leaves under different concentrations of Cd(NO₃)₂. In contrast, *T. monococcum* showed a decrease in protein content in wheat leaf at 100 and 500 μM of Cd(NO₃)₂ compared to the control ($11.2^i \pm 0.76$ mg/ml, and $19.2^k \pm 0.42.9$ mg/ml), and ($28.4^{ghi} \pm 0.81$ mg/ml), respectively. Interestingly, there was a no change in protein content at 250 μM of Cd(NO₃)₂ compared to the control.

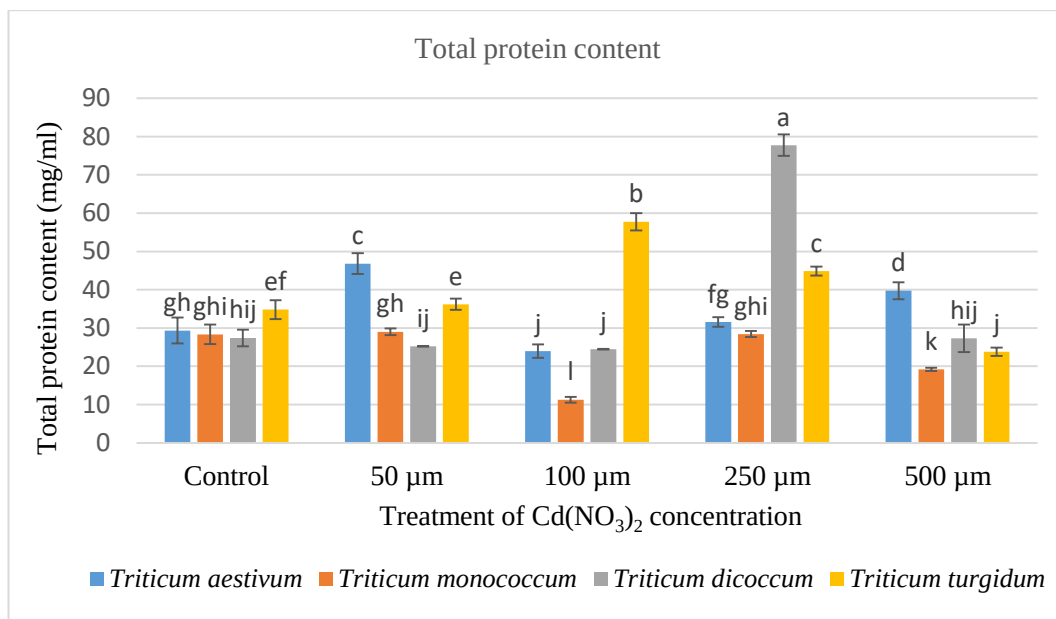


Figure 4.22 . The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 μM, 50 μM, 100 μM, 250 μM and 500 μM) on total protein content (mg /ml) in wheat landrace cultivars grown in soil.

4.10 Catalase (CAT) Activity

This investigation of antioxidant enzyme catalase activity in wheat species landraces under different Cd(NO₃)₂ concentrations (0, 50, 100, 250 and 500 μM) was revealed initially lower and nearly consistent levels after 28 days in the control groups for *T. aestivum*, *T. monococcum*, *T. dicoccum*, and *T. turgidum* with values of $2.9^{jk} \pm 0.4$ μmol H₂O₂/min/mg protein, $3.6^{ghi} \pm 0.52$ μmol H₂O₂/min/mg protein, $3.9^{ef} \pm 0.26$ μmol H₂O₂/min/mg protein, and $2.8^k \pm 0.25$ μmol H₂O₂/min/mg protein, respectively. However, a gradual increase in catalase activity was observed in *T. monococcum* ($10.29^a \pm 0.66$ μmol H₂O₂/min/mg protein, and $5.48^c \pm 0.08$ μmol H₂O₂/min/mg protein), at 100 and 500 μM Cd(NO₃)₂ stress, and in *T. aestivum* ($7.14^b \pm 0.29$ μmol H₂O₂/min/mg protein), at 250 μM Cd(NO₃)₂ stress compared to the control. Similarly, under both levels of Cd stress (100 μM and 250 μM) catalase activity in *T. turgidum* remained lower and nearly the same ($1.61^m \pm 0.09$ μmol H₂O₂/min/mg protein, and $2.22^i \pm 0.2$ μmol H₂O₂/min/mg protein), as presented in Table 4.8 and Figure 4.23. Moreover, the results show that CAT activity was similar at 50 and 500μM within different concentrations in *T. turgidum*. However, this research noted that CAT activity was increased at lower concentrations of Cd(NO₃)₂ (50 and 100μM) when exposed to *T. dicoccum* ($4.22^{de} \pm 0.12$ μmol H₂O₂/min/mg

protein, and $4.5^{de} \pm 0.09$ $\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$), and remained nearly the same at $3.9^{ef} \pm 0.26$ $\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$, and $4^{fg} \pm 0.35$ $\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$, in control and 500 μM of Cd. Additionally, the results demonstrated that the increase or decrease in CAT activity under different concentrations of Cd depends on the wheat plant species.

Table 4.8. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) at various concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on protein content, SOD and CAT activity of wheat landrace cultivars in soil after 28 days. Values are shown as the mean $\pm$ SD of three replicates.

Species of wheat landraces	$\text{Cd}(\text{NO}_3)_2$ treatment	Protein content (mg/g)	CAT Activity (U/mg protein)	SOD Activity (U/mg/protein)
<i>Triticum aestivum</i> L. (Ekiz)	0 μM	$29.3^{gh} \pm 3.4$	$2.9^{jk} \pm 0.4$	$8.18^{gh} \pm 0.4$
	50 μM	$47^c \pm 2.7$	$3.4^{fghi} \pm 0.2$	$9.5^g \pm 1.3$
	100 μM	$24^j \pm 1.7$	$3.32^{ghijk} \pm 0.3$	$24.2^e \pm 4.5$
	250 μM	$32^{fg} \pm 1.24$	$7.14^b \pm 0.3$	$4.5^h \pm 4.5$
	500 μM	$40^d \pm 2.2$	$3.12^{ijk} \pm 0.15$	$2.5^i \pm 0.2$
<i>Triticum monococcum</i> spp. <i>monococcum</i> (Einkorn)	0 μM	$28.3^{ghi} \pm 2.6$	$3.6^{fghi} \pm 0.5$	$6.5^{gh} \pm 1.08$
	50 μM	$29^{gh} \pm 0.9$	$3.2^{hijk} \pm 0.06$	$4.8^{hi} \pm 1.13$
	100 μM	$11.3^i \pm 0.8$	$10.3^a \pm 0.7$	$62^a \pm 4.4$
	250 μM	$28.4^{ghi} \pm 0.8$	$4^{fgh} \pm 0.06$	$41.4^c \pm 3.09$
	500 μM	$19^k \pm 0.4$	$5.5^c \pm 0.08$	$37.4^d \pm 1.6$
<i>Triticum dicoccum</i> spp. <i>dicoccum</i> Schrank (Emmer)	0 μM	$27.4^{hij} \pm 2.2$	$3.7^{ef} \pm 0.26$	$21.4^e \pm 1.5$
	50 μM	$25.2^{ij} \pm 0.1$	$4.2^{de} \pm 0.12$	$24^e \pm 2.02$
	100 μM	$25^l \pm 0.1$	$4.5^d \pm 0.08$	$62.5^a \pm 0.6$
	250 μM	$78^a \pm 2.8$	$1.5^m \pm 0.03$	$5.3^{hi} \pm 2.03$
	500 μM	$27.3^{hij} \pm 3.6$	$3.7^{fg} \pm 0.35$	$6.1^{ghi} \pm 0.5$
<i>Triticum turgidum</i> L. var. <i>durum</i> (Durum)	0 μM	$35^{ef} \pm 2.5$	$2.8^k \pm 0.25$	$9.2^g \pm 1.3$
	50 μM	$36.2^e \pm 1.5$	$3.7^{fg} \pm 0.1$	$16.1^f \pm 1.7$
	100 μM	$58^b \pm 2.2$	$1.6^m \pm 0.09$	$17.1^f \pm 1.7$
	250 μM	$45^c \pm 1.2$	$2.22^l \pm 0.1$	$22.42^e \pm 2.05$
	500 μM	$24^i \pm 1.1$	$3.7^{fg} \pm 0.2$	$47.3^b \pm 1.8$

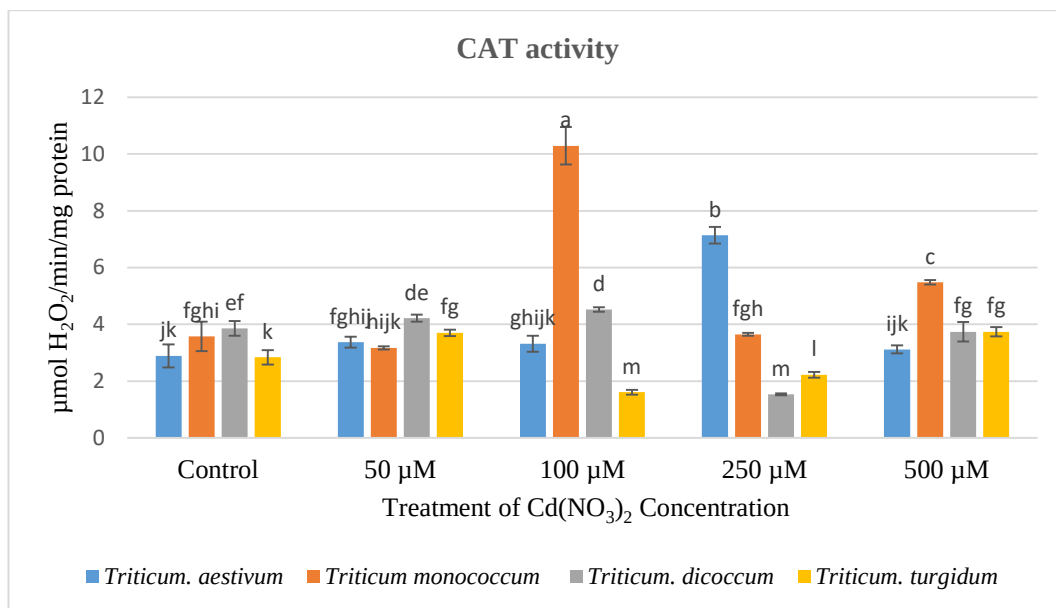


Figure 4.23. The effects of cadmium stress (Cd(NO₃)₂) concentration (0 μM, 50 μM, 100 μM, 250 μM and 500 μM) on CAT activity in wheat landrace cultivars grown in soil.

4.11 Superoxide Dismutase (SOD) Activity

In the present study, antioxidant molecules were determined in the leaves of wheat landrace cultivars to investigate the antioxidant enzyme SOD after exposure to Cd stress at different concentrations for 28 days. *T. monococcum* and *T. dicoccum* exhibited a significant increase in SOD activity under Cd stress at 100 μM, with values of $62^a \pm 4.35$ and $62.5^a \pm 0.6$ mg/ml protein, respectively. Furthermore, *T. monococcum* showed a slight increase in SOD activity when exposed to 250 μM Cd(NO₃)₂ ($41.4^c \pm 3.09$ mg/ml protein), and a decrease in activity at 500 μM of Cd(NO₃)₂ ($37.4^d \pm 1.6$ mg/ml protein), compared to the control. On the other hand, *T. dicoccum* exhibited a decrease in SOD activity when Cd(NO₃)₂ concentration increased ($5.34^{hi} \pm 2.03$ mg/ml protein, $6.1^{ghi} \pm 0.48$ mg/ml protein), at 250 μM and 500 μM, respectively, compared with the control, as presented in Table 4.8 and Figure 4.24. Similarly, SOD activity showed no significant effect at a Cd(NO₃)₂ concentration of 50 μM ($23.7^e \pm 2.03$ mg/ml protein), and compared to the control ($21.4^e \pm 1.5$ mg/ml protein), in *T. dicoccum*, indicating that Cd(NO₃)₂ had no impact at low concentrations (50 μM). However, at 250 and 500 μM, SOD activity remained similar ($22.4^e \pm 2.06$ mg/ml protein, and $47.3^b \pm 1.8$ mg/ml protein, respectively) compared to control. On the other hand, at 50 and 100 μM SOD

activity recorded the same content ($16.09^f \pm 1.7$ mg/ml protein, $17.1^f \pm 1.7$ mg/ml protein, respectively) compared to control, with no significant effect at low $\text{Cd}(\text{NO}_3)_2$ concentrations. *T. aestivum* also showed an increase in SOD activity, particularly at 100 μM ($24.15^e \pm 4.5$ mg/ml protein), compared to the control and other concentrations. However, as $\text{Cd}(\text{NO}_3)_2$ stress increased, SOD activity decreased compared to the control, as presented in Table 4.8. and Figure 4.24. Thus, the results of *T. aestivum* indicate a significant effect of content of SOD activity under different concentrations.

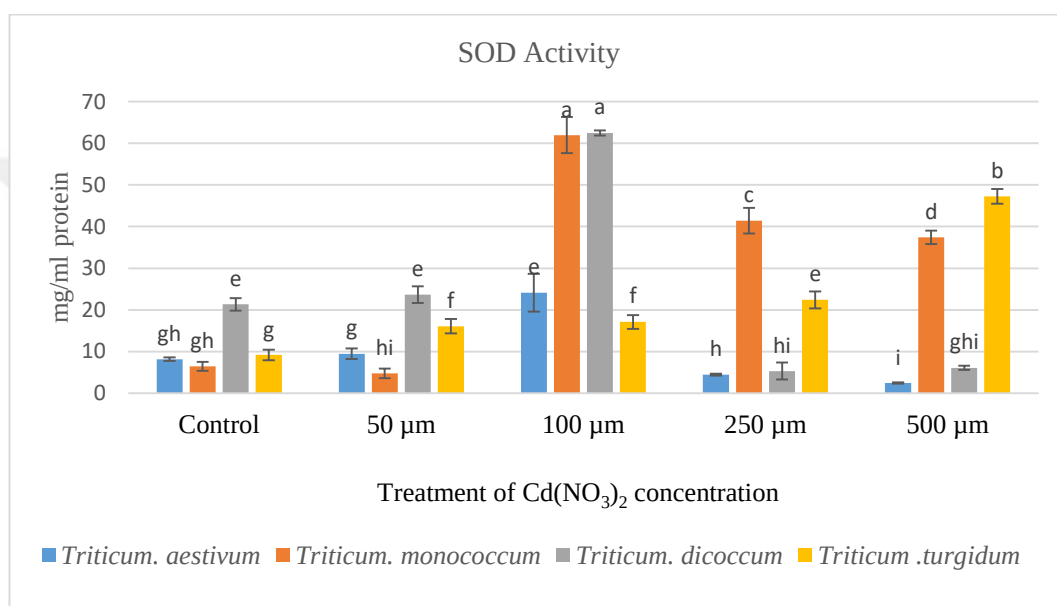


Figure 4.24. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) concentration (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on SOD activity in wheat landrace cultivars grown in soil.

4.12 Analysis of Total H_2O_2 Accumulation in Leaves of Wheat Landraces

This research study revealed variations in H_2O_2 accumulation detected in leaves subjected to control conditions versus cadmium treatment at different concentrations and across different wheat landrace cultivars, over a period of 28 days. These results indicated a significant increase in H_2O_2 content in the leaves of two wheat cultivars, *T. aestivum* and *T. monococcum*, at concentrations of 250 and 500 μM $\text{Cd}(\text{NO}_3)_2$, with values of $68.9^{bc} \pm 5.30$ $\mu\text{mol}/\text{H}_2\text{O}_2/\text{g}^{-1}$ fdw, $55^f \pm 0.42$ $\mu\text{mol}/\text{H}_2\text{O}_2/\text{g}^{-1}$ fdw, and $63.5^{de} \pm 0.42$ $\mu\text{mol}/\text{H}_2\text{O}_2/\text{g}^{-1}$ fdw, $52.2^f \pm 1.69$ $\mu\text{mol}/\text{H}_2\text{O}_2/\text{g}^{-1}$ fdw, respectively as presented in Table 4.9. In contrast, *T. turgidum* and *T.*

dicoccum exhibited an increase in H₂O₂ level at a lower concentration of Cd(NO₃)₂ (50 µM) with values of 72^{bc}±0.4 µmol/H₂O₂/g⁻¹ fdw and 65.2^d±1.5 µmol/H₂O₂/g⁻¹ fdw, respectively. However, at concentrations of 250 and 500µM of Cd(NO₃)₂, there was a decrease in H₂O₂ levels in *T. dicoccum* and *T. turgidum*, with values of 37^{hi}±5.0 µmol/H₂O₂/g⁻¹ fdw, 19.4^k±0.85 µmol/H₂O₂/g⁻¹ fdw, and 46^g±1.12 µmol/H₂O₂/g⁻¹ fdw, 30.2^j±2.4 µmol/H₂O₂/g⁻¹ fdw, respectively, as shown in Table 4.9 and Figure 4.25 Furthermore, *T. aestivum* exhibited no significant increase in H₂O₂ levels at 50 µM and 100 µM of Cd concentration, with values of 37.6^{hi}±0.52 µmol/H₂O₂/g⁻¹ fdw, and 37.5^{hi}±0.53 µmol/H₂O₂/g⁻¹ fdw, respectively.



Table 4.9. The effects of cadmium stress ($\text{Cd}(\text{NO}_3)_2$) at various concentrations (0 μM , 50 μM , 100 μM , 250 μM and 500 μM) on H_2O_2 content of wheat landrace cultivars grown in soil after 28 days. Values are shown as the mean $\pm$ SD of three replicates.

Species of wheat landraces	$\text{Cd}(\text{NO}_3)_2$ treatment	H_2O_2 Content ($\mu\text{mol} / \text{g}^{-1}$ Leaf dW)
<i>Triticum aestivum</i> L (Ekiz)	0 μM	41.1 ⁱ $\pm$ 2.6
	50 μM	38 ^{hi} $\pm$ 0.5
	100 μM	38 ^{hi} $\pm$ 0.4
	250 μM	69 ^{bc} $\pm$ 5.3
	500 μM	63.5 ^{de} $\pm$ 0.4
<i>Triticum monococcum</i> spp. <i>monococcum</i> (Einkorn)	0 μM	60.5 ^e $\pm$ 0.4
	50 μM	41 ^h $\pm$ 0.7
	100 μM	36 ⁱ $\pm$ 0.4
	250 μM	55 ^f $\pm$ 0.4
	500 μM	52.2 ^f $\pm$ 1.7
<i>Triticum dicoccum</i> spp. <i>dicoccum</i> Schrank (Emmer)	0 μM	75 ^b $\pm$ 1.12
	50 μM	65.2 ^d $\pm$ 1.5
	100 μM	46 ^g $\pm$ 5.5
	250 μM	37 ^{hi} $\pm$ 5.0
	500 μM	19.4 ^k $\pm$ 0.85
<i>Triticum turgidum</i> L. var. <i>durum</i> (Durum)	0 μM	79.4 ^a $\pm$ 2.1
	50 μM	72 ^{bc} $\pm$ 0.4
	100 μM	53.2 ^f $\pm$ 2.4
	250 μM	46 ^g $\pm$ 1.1
	500 μM	30.2 ^j $\pm$ 2.4

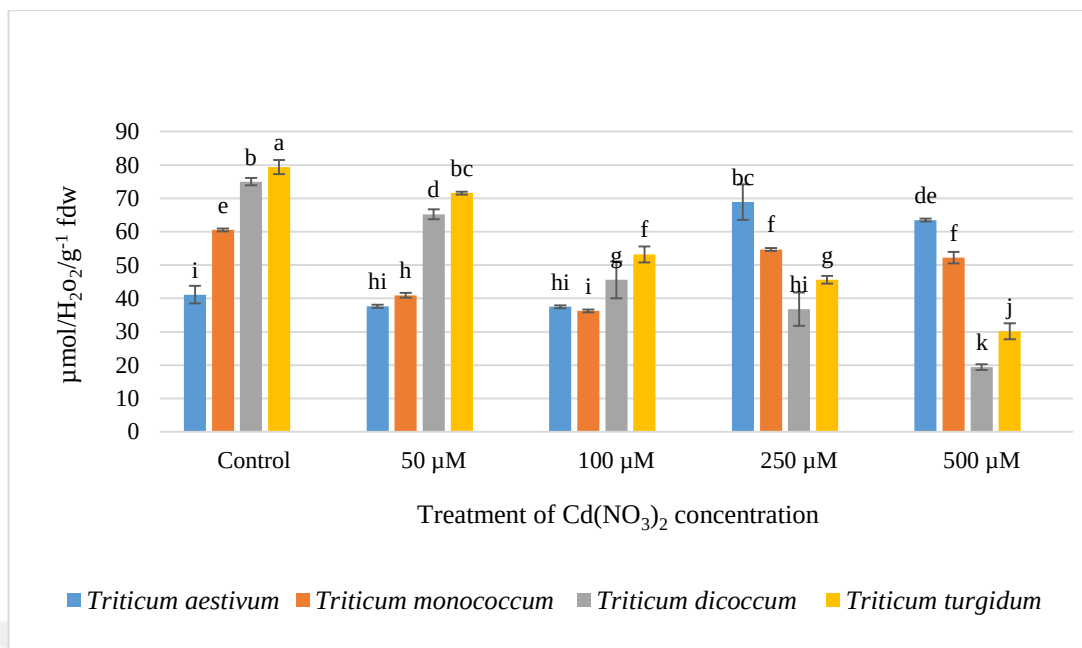


Figure 4.25. The effects of cadmium stress (Cd(NO₃)₂) concentrations (0 μM, 50 μM, 100 μM, 250 μM and 500 μM) on hydrogen peroxide (H₂O₂) accumulation in leaves of wheat landrace cultivars in soil.

4.13 Elemental Analysis of Wheat Landraces under Cd(NO₃)₂ stress

This study investigated the elemental composition of wheat landraces focusing on calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), and potassium (K), under cadmium (Cd(NO₃)₂) stress conditions. Table 4.10 presents the concentration of these elements in wheat samples.

The highest concentration of element was recorded in *T. aestivum* particularly in potassium (K), with a concentration of 59466.5 mg/kg under treatment with 500 μM Cd(NO₃)₂ compared to the control. Additionally, phosphorus (P) concentration reached 12544 mg/kg under treatment with 500 μM Cd(NO₃)₂. Calcium (Ca) concentrations ranged from 7758.3 mg/kg to 6081.1 mg/kg under 500 μM to 100 μM Cd(NO₃)₂ treatments, respectively. Treatment with 500 μM Cd(NO₃)₂ resulted in increased element concentrations in *T. aestivum*. However, moderate magnesium (Mg) concentrations were observed, ranging between 2121 mg/kg and 1823.2 mg/kg under 500 μM to 100 μM Cd(NO₃)₂ treatments, respectively, with a decrease in (Ca) concentration

with increasing Cd exposure. Iron (Fe) concentrations increased at 500 μM compared to 100 μM $\text{Cd}(\text{NO}_3)_2$.

In *Triticum monococcum*, the highest (K) concentration was recorded at 38844 mg/kg under 100 μM $\text{Cd}(\text{NO}_3)_2$ treatment, followed by (P) concentration at 8533.23 mg/kg under the same treatment. (Mg) concentration increased at 100 μM $\text{Cd}(\text{NO}_3)_2$ treatment. Additionally, (Ca) concentration increased at 500 μM compared to 100 μM $\text{Cd}(\text{NO}_3)_2$ and control.

For *Triticum dicoccum*, (K) concentration decreased to 20245.6 mg/kg under 500 μM $\text{Cd}(\text{NO}_3)_2$ treatment, while (Fe) concentration decreased to 132 mg/kg under the same treatment compared to control and 100 μM $\text{Cd}(\text{NO}_3)_2$. Conversely, concentrations of P, Mg, and (Ca) decreased at 100 μM $\text{Cd}(\text{NO}_3)_2$ compared to control, more so than at 500 μM $\text{Cd}(\text{NO}_3)_2$.

The results of *T. turgidum* show that the concentrations of P, Mg, Ca, and Fe were 5645.75, 1169.2134, 8267 and 57.5 mg/kg, respectively, decreased at 100 μM of Cd-treated compared to the higher concentration of 500 μM of $\text{Cd}(\text{NO}_3)_2$. However, the K element concentration decreased at 500 μM of $\text{Cd}(\text{NO}_3)_2$ as presented in Table 4.10.

These findings demonstrate the varied responses of wheat landraces to $\text{Cd}(\text{NO}_3)_2$ stress, with differential effects on elemental concentrations depending on the specific wheat species and $\text{Cd}(\text{NO}_3)_2$ concentration.

Additionally, the concentrations of Cd elements increased at 500 μM for all wheat species, including *T. turgidum*, *T. monococcum*, *T. aestivum*, and *T. dicoccum*.

Table 4.10. The concentration of elements in wheat landraces.

Wheat species	Cd(NO ₃) ₂ treatment	Cd (mg/kg)	Ca (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	P (mg/kg)	K (mg/kg)
<i>T. aestivum</i>	Control	0.076	8497.696	114.048	1926.745	10247.448	52536.303
	500 μ M	0.257	7758.332	74.406	2121.356	12544.453	59466.531
	100 μ M	0.238	6081.068	59.946	1823.156	11970.877	38144.910
<i>T. monococcum</i>	Control	0.056	5656.050	72.023	1875.993	5689.997	31815.925
	500 μ M	0.307	9818.287	155.045	2067.790	4996.067	19415.050
	100 μ M	0.201	9457.993	112.388	2131.758	8533.229	38844.859
<i>T. dicoccum</i>	Control	0.078	8245.875	132.406	2007.631	10027.268	27095.398
	500 μ M	0.231	14396.329	231.980	2831.863	7803.417	20245.573
	100 μ M	0.203	10320.39398	147.86104	2154.1793	6560.5085	24226.295
<i>T. turgidum</i>	Control	0.053	8863.692164	181.45539	1836.4298	9688.5025	33121.013
	500 μ M	0.507	8266.996474	57.50159	1169.2134	5645.7471	36417.874
	100 μ M	0.223	12402.76792	85.931312	1882.1049	6015.9825	27359.189

4.14 Phylogenetic Tree of Wheat Landraces in Türkiye

Characteristics of its region seventeen accessions of wheat landraces representing *Triticum* spp., collected from Türkiye as presented in Table 3.2 were sequenced for *ITS2*, *rbcL* and *matK* regions.

The aligned data sequences resulted from the length of the entire Internal Transcribed Spacer 2 (*ITS2*) region of *Triticum* species varied was 216 base pairs (bp) long when used for all (*T. monococcum*, *T. dicoccum*, and *T. aestivum*) as presented at Table 4.11. The length of product *rbcL* region was 602 pb when aligned sequences of the region of *Triticum* species (*T. monococcum*, *T. dicoccum*, and *T. aestivum*) as presented in Table 4.12. However, the alignment of sequences of the *matK* region was 837 pb were variable of *Triticum* species (*T. monococcum*, and *T. dicoccum*) as presented at Table 4.13. Regarding to this, two samples from genus *Hordeum* spp. within the Triticeae were selected as a potential out-group for *ITS2* from NCBI database accession number AJ607946.1 from *Hordeum jubatum*, and AJ607824.1 *H. bogdanii* and out-group for *rbcL* : NCBI database accession number KP210989.1 from *H. bogdanii* and JX848500.1 from *H. jubatum* addition, the out-group for *matK*: NCBI database number AB078106.1 from *H. jubatum* and AB078089.1 from *H. bogdanii* to construct the Maximum parsimony to analyzed phylogenetic trees used as an outgroup in all phylogenetic analyses.

Table 4.11. The length of product internal transcribed spacer 2 (ITS2) region of wheat landraces and GenBank accession numbers.

ITS2 region			
Sample number	Species of wheat landraces	Length	GenBank Accession number
Turkey 16	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816973
Turkey 30	<i>Triticum dicoccum</i>	216 bp	PP824371
Turkey 31	<i>Triticum dicoccum</i>	216 bp	PP824372
Turkey 32	<i>Triticum dicoccum</i>	216 bp	PP824373
Turkey 33	<i>Triticum dicoccum</i>	216 bp	PP824374
Turkey 34	<i>Triticum dicoccum</i>	216 bp	PP824375
Turkey 35	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816974
Turkey 36	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816975
Turkey 37	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816976
Turkey 39	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816977
Turkey 43	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816978
Turkey 44	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816979
Turkey 45	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816980
Turkey 47	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816981
Turkey 48	<i>Triticum monococcum</i> spp. <i>monococcum</i>	216 bp	PP816982
Turkey	<i>Triticum aestivum</i> Gallbasan	216 bp	PP814623
Turkey	<i>Triticum aestivum</i> Sariserez	216 bp	PP814624

Table 4.12. The length of product (rbcL) region of wheat landraces.

Sample number	Species of wheat landrace	Length
Turkey 16	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey30	<i>Triticum dicoccum</i>	607 bp
Turkey32	<i>Triticum dicoccum</i>	607 bp
Turkey31	<i>Triticum dicoccum</i>	607 bp
Turkey33	<i>Triticum dicoccum</i>	607 bp
Turkey34	<i>Triticum dicoccum</i>	607 bp
Turkey35	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey36	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey37	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey39	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey43	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey44	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey45	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey47	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey48	<i>Triticum monococcum</i> spp. <i>monococcum</i>	607 bp
Turkey	<i>Triticum aestivum</i> Gallbasan	607 bp
Turkey	<i>Triticum aestivum</i> Sariserez	607 bp

Table 4.13. The length of product (matk) region of wheat landraces.

Sample number	Species of wheat landrace	Length
Turkey16	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp
Turkey30	<i>Triticum dicoccum</i>	837 bp
Turkey31	<i>Triticum dicoccum</i>	837 bp
Turkey32	<i>Triticum dicoccum</i>	837 bp
Turkey33	<i>Triticum dicoccum</i>	837 bp
Turkey34	<i>Triticum dicoccum</i>	837 bp
Turkey35	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp
Turkey36	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp
Turkey37	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp
Turkey39	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp
Turkey43	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp
Turkey44	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp
Turkey45	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp
Turkey48	<i>Triticum monococcum</i> spp. <i>monococcum</i>	837 bp

4.14.1 Sequence Data Evaluation of the Nuclear Marker: Internal Transcribed Spacer 2 (ITS2) Region of Wheat Landraces

In this study investigation that, the evolutionary relationships of several *Triticum* species were established by sequencing their *ITS2* region. Wheat landraces reveals distinct clades corresponding to the in group species: *Triticum monococcum* (T.mon), *Triticum dicoccum* (T.dic), and *Triticum aestivum* (T.aes) were grouped into distinct clades, indicating their evolutionary relationships with *T. monococcum* at the base of the in-group cluster, therefore it might be the most ancestral species among the three wheats used. Within this species, the *T. monococcum* samples form

several clades, each supported by strong bootstrap values (100), indicating significant differentiation within the species. *Triticum aestivum* samples, including (T.aes_coli) and (T.aes_sac2), are grouped together with a bootstrap support of 100, indicating strong confidence in their close relationship. *Triticum dicoccum* samples (30_T.dic, 31_T.dic, 32_T.dic, 33_T.dic, and 34_T.dic) form a well-supported clade with high bootstrap values. Despite the presence of a polytomic structure among the *T. dicoccum* sequences, they are clearly separated from the monophyletic clade of *T. aestivum* sequences. The phylogenetic tree constructed using the *ITS2* DNA sequences successfully differentiates between *Triticum monococcum*, *Triticum dicoccum*, and *Triticum aestivum*. Most branches exhibit strong bootstrap support. Finally, the inclusion of *Hordeum* species as outgroups effectively roots the tree and clarifies the evolutionary relationships among the ingroup species.

The closer branching of *T. aestivum* and *T. dicoccum* compared to *T. monococcum* indicates a closer genetic relationship between the former two. This study concludes that *ITS2* provides a good resolution for revealing relationships between wheat landraces. Thus, through conducting detailed sampling of these species it is possible to reveal the exact relations of the wheat species in Türkiye. The *ITS2* region provides good resolution.

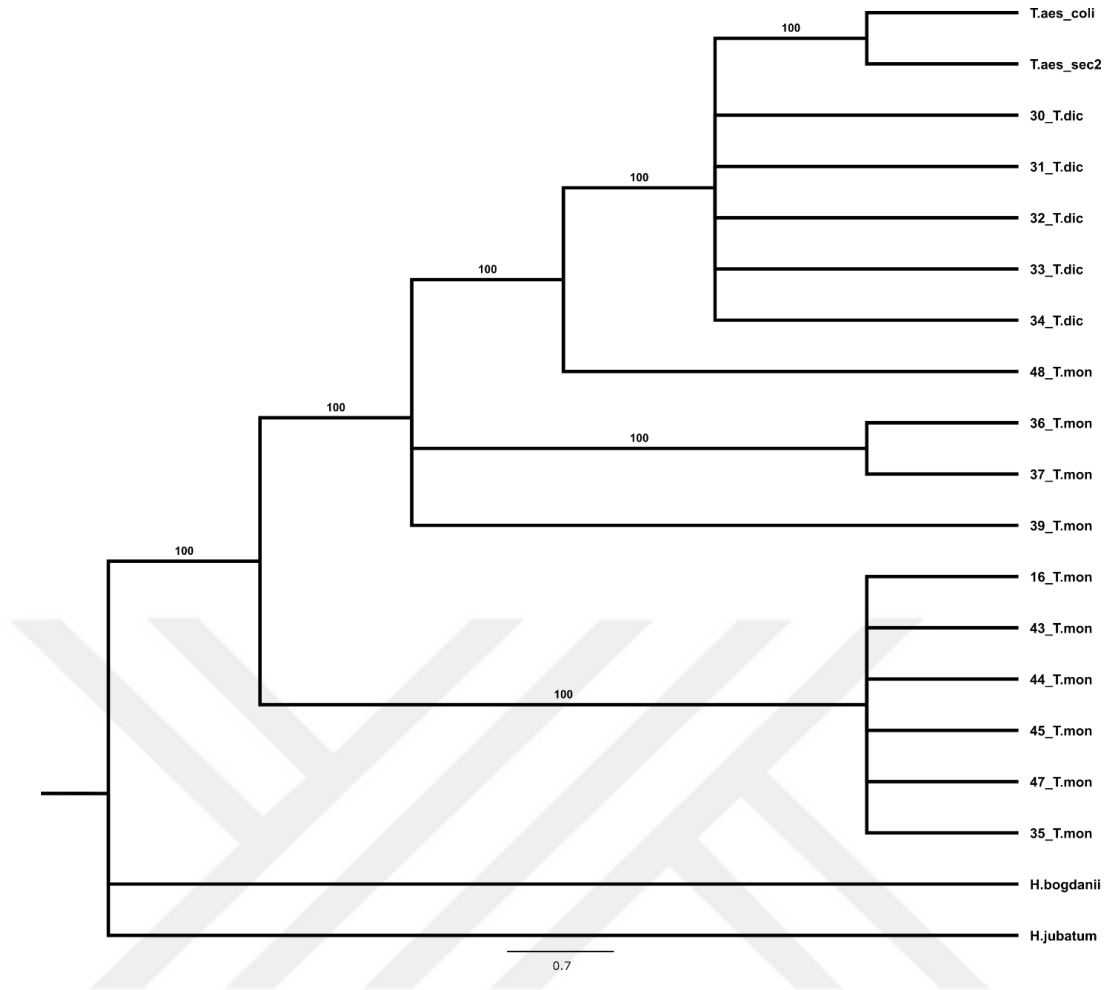


Figure 4.26. The Maximum parsimony 50% majority rule consensus tree of the ITS2 sequences of wheat landraces in Türkiye. The bootstrap values that are 50% support are shown over relevant branches.

4.14.2 Sequence Data Evaluation of the Chloroplast Marker *rbcL* Region of Wheat Landraces

The *rbcL* for phylogenetic tree, shows good and concordant results the *Triticum monococcum* ssp *monococcum*, with *Triticum dicoccum* and *T. aetvium*. *rbcL* gene shows good and with *ITS2* region, the ingroup species, *Triticum monococcum* (T.mon), *Triticum dicoccum* (T.dic), and *Triticum aestivum* (T.aes), are well-separated into distinct clades. In the same way, *Triticum aestivum* (T.aes) samples (T.aes_coli and T.aes_sec2) form a strongly supported clade with bootstrap values of 100. Both trees of *ITS2* and *rbcL* shows that *Triticum monococcum* is the most ancestral of the three wheat species used, with *T. dicoccum* and *T. aestivum* diverging later. Therefore, certain research studies should be conducted to study the

same or different results by other approaches such as Maximum Likelihood (ML) and Bayesian Inference (BI), to provide additional support for this conclusion.

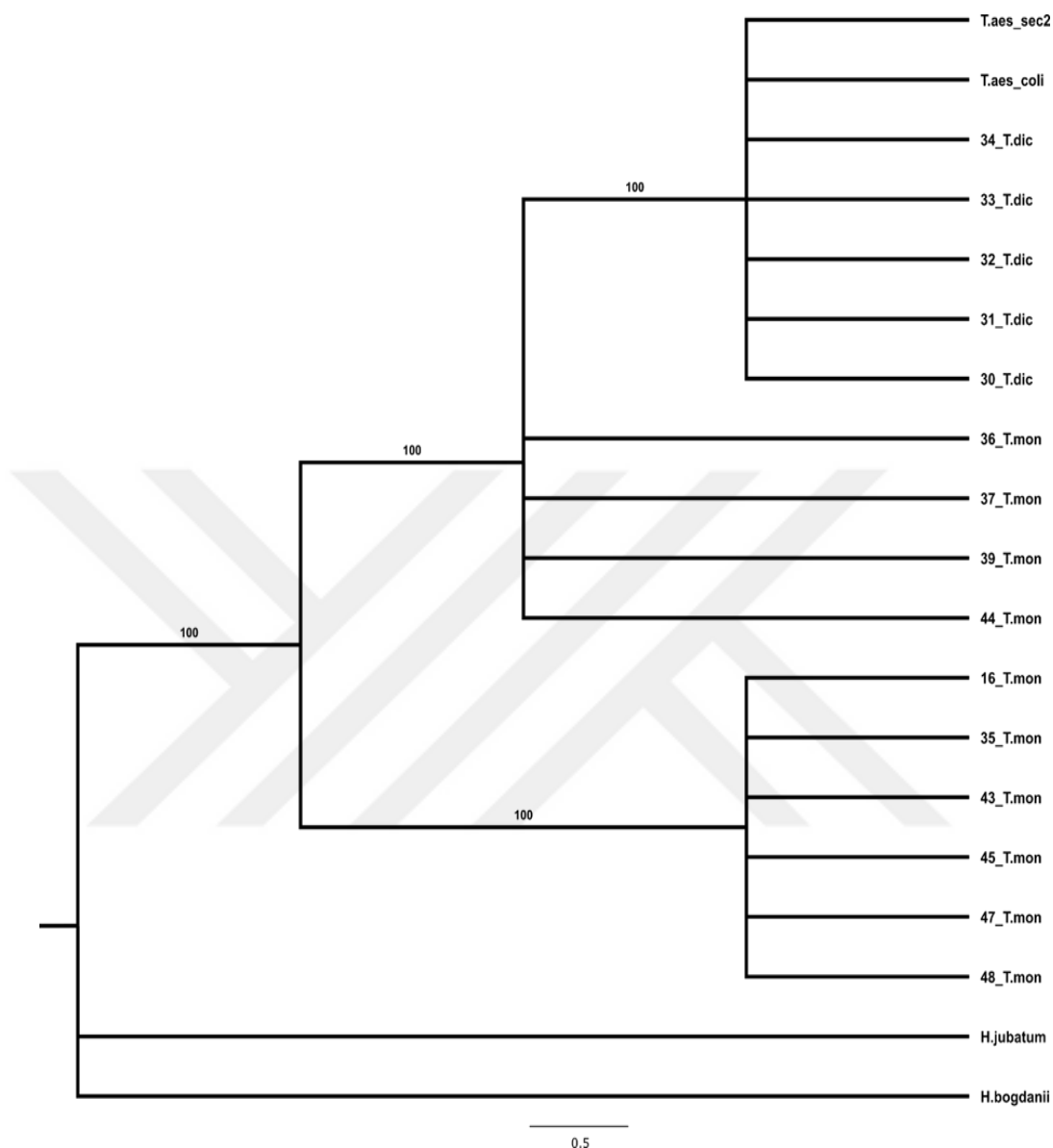


Figure 4.27. The Maximum parsimony 50% majority rule consensus tree of the rbcL sequences of wheat landraces in Türkiye. The bootstrap values that are 50% support are shown over relevant branches.

4.14.3 The Sequence Data Evaluation of the Chloroplast Marker *matk* Region of Wheat Landraces

The phylogenetic tree, shows one that cluster the *Triticum monococcum* ssp *monococcum*, with *Triticum dicoccum*.

Since *T. aestivum* samples for matK could not be successfully amplified and sequenced, the matK tree still provides valuable insights into the relationships between *T. monococcum* and *T. dicoccum*. The ingroup species, *Triticum monococcum* (T.mon) and *Triticum dicoccum* (T.dic), are clearly separated into distinct clades indicating their separate evolutionary lineages. These results contribute valuable insights into the phylogenetic relationships within the *Triticum* genus. However, the analysis is limited by the absence of *Triticum aestivum* (T.aes) sequences, future research should focus on obtaining and analyzing *T. aestivum* matK sequences to provide a more comprehensive phylogenetic framework for these important wheat species.

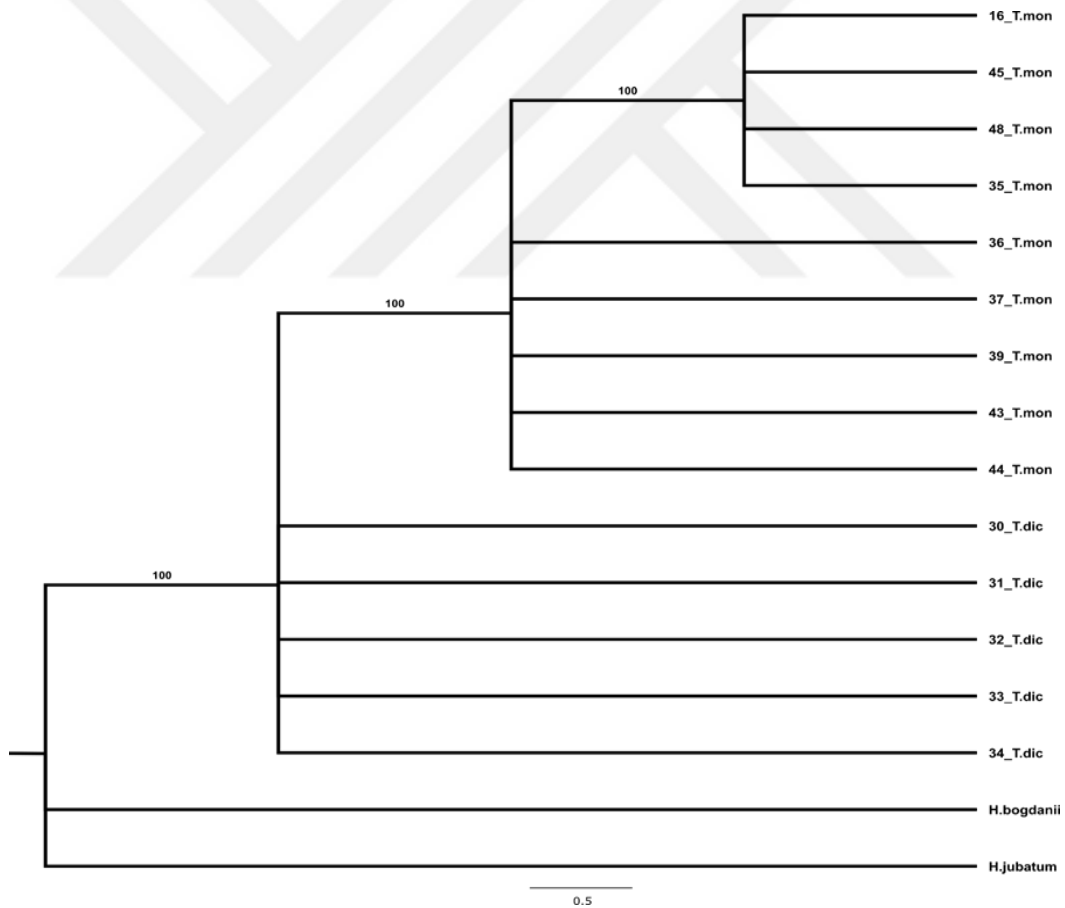


Figure 4.28. Maximum parsimony 50% majority rule consensus tree of the matK sequences of wheat landraces in Türkiye. The bootstrap values that are 50% support are shown over relevant branches.

5. DISCUSSION

5.1 Seed Germination and Seedling Vigour Percentage

The impact of Cd stress in seedling plant growth and development including seed germination percentage in wheat species, has been studied extensively. Researchers have explored the effects of varying Cd(NO₃)₂ concentrations (ranging from 50 µM to 500 µM) on different wheat species and observed both decreases and increases in germination percentage depending on the concentration and wheat genotype.

This research study has reported that exposure to Cd(NO₃)₂ generally reduces the germination percentage of various wheat plants, with no significant effect observed, which aligns with some research studies conducted by Hasanuzzaman et al. (2021) (77). These research results have found that *Triticum turgidum* exhibits higher tolerance than other types, which might indicate inherent differences in Cd(NO₃)₂ tolerance depending on wheat genotypes. The observed differences in seed germination, whether by increase or inhibition, might be attributed to the genetic variability among all cultivars, as declared by Amirjani (2012) (49) and Idrees et al. (2015) (65). This comparison helps isolate the specific effects of Cd and ensures the observed reduction in germination is directly attributable to Cd stress as declared by the research results.

Similarly, as declared by An, (2004) (79) the adverse effects of Cd(NO₃)₂ on demonstrate its phytotoxicity to wheat seedlings. Furthermore, seed germination under Cd(NO₃)₂ concentration stress can be decreased, accelerating the breakdown of on reserved food material in seed embryos. These findings align with research conducted by Ahmad et al. (2012) (39) which reported that Cd stress decreased seed germination, germination percentage, and vigour percentage in different crops. Wheat cultivars showed a non-significant effect of Cd(NO₃)₂ on germination consistent with the findings of this research study. Cd(NO₃)₂ exposure can induce the generation of harmful free radicals, damaging cellular components and inhibiting germination. Comparing the germination percentage of Cd-treated seeds to a control group germinated without Cd exposure is crucial. This comparison

helps isolate the specific effects of Cd and ensures that the observed reduction in germination is directly attributable to Cd stress. Germination percentage is only one aspect of Cd's impact on seed germination. Examining other parameters such as germination rate, seedling vigour, and root elongation provides a more comprehensive picture of Cd toxicity.

This result highlights the different tolerance levels between species, documenting that *T. turgidum* has a higher tolerance than other species of Cd(NO₃)₂ effect, as found by Shaikh et al. (2013)(80). The decrease in seed vigour and germination attributed to the increased breakdown of stored food in embryo, which is similar to the findings of this research and consistent with the results of Ahmad et al. (2012)(38). The initial health and vigour of the seeds can influence their tolerance to Cd(NO₃)₂ stress. Factors like temperature, light, and moisture can also influence germination success under Cd stress. Therefore, selecting or breeding wheat varieties with improved (Cd) tolerance could be beneficial for utilization in contaminated areas. Cd can interfere with essential metabolic processes crucial for seed germination and seedling development. Some genotypes might show a steeper decline in germination at lower Cd concentrations compared to others, indicating inherent differences in Cd tolerance among wheat varieties.

5.2 The Effect of Cadmium Stress on Root and Shoot Elongation

The research study results presented that Cd(NO₃)₂ had a more drastic effect on seedling growth with root, shoot and seedling elongation being the most sensitive parameters and considered good indicators of metal toxicity(61). According to this study, even a lower concentration of Cd (5 mg L⁻¹) significantly reduced these sensitive parameters. *T. dicoccum* showed greater tolerance and inhibition compared to the control and other concentrations, while *T. aestivum* showed a low effect with a slight increase in coleoptile, consistent with findings by Idrees (2015)(65). Ebrahimi and Madrid Díaz (2014) reported that copper negatively affected the growth and tolerance indices of *Festuca ovina*, with higher copper concentrations hampering seed germination, dry weight, and root and shoot length. Seedling growth was affected because of interactions of Cd contamination with enzymes and biochemical reactions inside the plant (81). When various wheat cultivars were exposed to Cd stress, their response varied across different

concentrations, possibly due to inherent differences in their ability to withstand Cd stress. The low tolerance indices observed might have resulted from changes in the physiological mechanisms of wheat plants during growth stages (82).

Roots are more affected by Cd impact. Liu et al. (2005) (83) reported that root are more sensitive than shoots to Cd contamination. This research results presented that roots are more sensitive, likely because roots are the first plant parts to absorb Cd, as reported by Rascio and Navari-Izzo (2011) (83). The research study has shown that an increase in Cd concentrations reduced shoot and root elongation of plants treated with different Cd concentrations, as presented in Table 4.4 and 4.5.

5.3 The Effect of Cadmium Stress on Dry and Fresh Wheat

The results showed that high Cd concentrations decreased seedling fresh and dry weights, as compared to the control Table 4.5. Similar findings were reported by Ashraf et al. (2011) (81) and Ahmad et al. (2012) (38). The decrease in the fresh and dry weight was due to the decreased metabolism caused by Cd interaction with enzymes in root elongation, as compared to the control. According to the Rascio et al. (2008)(84), root elongation was reduced under Cd stress because it alters the morphogenesis of the root. Similar results were reported by Ahmad et al. (2012) (38) in wheat. Root, shoot, and seedling elongation are the most sensitive morphologies of plants and are directly correlated to the effect of heavy metal. Cadmium treatments reduced the weights shoots and roots compared with the control. Thus, the results presented a reduction in shoot and root weight; the effect was significant for the highest Cd(NO₃)₂ treatments at 250 and 500 µM. With different Cd concentration measurements, there was a consistent reduction in both shoot and root elongation compared to the control. This reduction was particularly significant in the highest Cd treatment, indicating a dose-dependent effect of Cd on plant growth. Furthermore, Cd(NO₃)₂ treatments resulted in decreased root weights compared to the control, with the most significant effects observed in the highest Cd treatment. Differences were also evident in the fresh and dry weights of roots across varying treatments (50, 100, 250, and 500 µM). The highest weight was recorded in *T. dicoccum* at all concentrations while Cd(NO₃)₂ treatments significantly reduced root fresh and dry weights, with the most substantial reduction observed at 100 µM. These findings are consistent with previous studies by Ashraf

et al. (2011) (80) and Ahmad et al. (2012) (38), which demonstrated the adverse impact of increased $\text{Cd}(\text{NO}_3)_2$ concentrations on root growth and weights in wheat plants. In *T. aestivum*, $\text{Cd}(\text{NO}_3)_2$ treatment toxicity exhibited a low significance effect, indicating minimal impact on this variety. However, contrasting results were observed in *T. monococcum*, where root weight showed an increase at a concentration of 500 μM . The results also show that an increase in root dry weight at 50 and 500 μM in *T. dicoccum* while, there was a decrease in root dry weight at concentrations of 100 and 250 μM $\text{Cd}(\text{NO}_3)_2$ compared to the control. In *T. turgidum*, there was a consistent decrease in root dry weight across all concentrations compared to the control, indicating a negative impact of Cd treatment on this variety.

5.4 The Toxicity and Tolerance of Wheat

The specific chemical form of Cd can affect its bioavailability and toxicity to plants. This research study results figured out that the shoot toxicity of *T. aestivum* and *T. monococcum* show more resistance to Cd than *T. turgidum* and *T. dicoccum*. These results are similar to those obtained by Shaikh *et al.* (2013) (80). The selection or breeding of wheat varieties with improved Cd tolerance for utilization in contaminated areas. $\text{Cd}(\text{NO}_3)_2$ can interfere with essential metabolic processes crucial for seed germination and seedling development.

5.5 Photosynthetic Pigment Content

Observations of leaves chlorosis in wheat plants due to Cd toxicity have not been reported. The Chlorophyll a and b contents show no significant effect by Cd treatment across varying concentrations, as presented in Table 4.7. This study's results differ from previous findings as documented by Wu et al. (2003) (85). Additionally, Chlorophyll accumulation was inhibited by increasing $\text{Cd}(\text{NO}_3)_2$ concentration particularly in Chlorophyll b, more so than Chlorophyll a content, under different concentrations of $\text{Cd}(\text{NO}_3)_2$, similar to Amirjani (2012) (49).

According to the results of the study, an increase in carotenoid content was observed with the rise in $\text{Cd}(\text{NO}_3)_2$ concentrations. There was a notable increase in the carotenoid pigments in *T. turgidum*, *T. dicoccum* and *T. aestivum* at concentrations

of 250 μM and 500 μM $\text{Cd}(\text{NO}_3)_2$. These findings are consistent with results reported by Amirjani (2012) (49). Moreover, these results align with the findings of Bhatti et al. (2013) (39) and Idrees et al. (2015) (65), which documented an increase in carotenoids as Cd concentration increased. Carotenoid pigment serves as a secondary metabolite and a light-harvesting pigment, protecting cellular structures from damage and membranes from destabilization due to ROS damage. Thus, as Cd levels increase, plants produce more carotenoids to cope with the stress induced by Cd concentration, as stated by Singh et al. (2006) (86).

5.6 Phenolic

Phenolics are a diverse group of secondary metabolites naturally present in plants, playing a crucial role in plant defense mechanisms against various environmental stresses, including heavy metal toxicity. They can act as chelators, binding to heavy metal ions like Cd, and reducing their bioavailability within the plant. Essentially, they lock up these ions, preventing them from causing harm (86). They serve as antioxidants, aiding in the removal of free radicals generated by Cd exposure. Phenolics function as a plant defense against different environmental stresses, such as heavy metal toxicity like Cd. They are productive in scavenging free radicals that can damage plant cells by Cd exposure. In experimental settings, there is an observed increase in specific phenolic acids, such as ferulic acid and p-coumaric acid, in wheat plants exposed to Cd stress, serving as part of the plant's defense strategies (87). Some cultivars display a more pronounced increase in phenolic content compared to others. This variation may be attributed to inherent genetic differences in stress tolerance mechanisms. In this research study, it was found that *T. aestivum* showed an increase at high $\text{Cd}(\text{NO}_3)_2$ concentrations of 250 and 500 μM , while *T. monoccocum* also displayed an increase in phenolic content at concentrations of 50, 100, and 500 μM . Additionally, *T. monoccocum* recorded an increase in phenolic content at 100 and 250 μM . On the other hand, *T. turgidum* exhibited a decrease in phenolic content at concentrations of 50, 250, and 500 μM $\text{Cd}(\text{NO}_3)_2$ compared to the control. Therefore, this increase or decrease might be related to the genetic diversity inherent in stress tolerance mechanisms. Similarly, some research recommended that different wheat cultivars exhibit varying degrees of phenolic response to Cd exposure stress. Furthermore, the increased phenolic content in Cd-exposed wheat may offer potential benefits; for instance, enhanced

phenolic content can contribute to improved stress tolerance to better cope with Cd-exposure toxicity, as declared by Sharma et al. (2019) (87)

5.7 Proline

In this research, we investigated the influence of varying proline concentrations on the leaves of wheat landraces in the presence of $\text{Cd}(\text{NO}_3)_2$ different dosages (0, 50, 100, 250, and 500 μM) observed over 28 days. The findings indicated that increases in Cd levels were associated with rising levels of proline, which is consistent with findings from other studies, such as Wang et al. (2022)(88). Among the tested concentrations of $\text{Cd}(\text{NO}_3)_2$, proline at 100, 250, and 500 μM was found to be most effective in reducing Cd toxicity in wheat types like *T. turgidum* at 250, 500 μM of $\text{Cd}(\text{NO}_3)_2$, *T. dicoccum* at 100, 500 μM , and *T. aestivum* at 50, 100, and 250 μM , which is similar to the results that have been recorded by Wang et al. (2022) (88). The increase in proline levels might be related to an enhancement in tolerance to Cd by decreasing Cd accumulation and reestablishment in wheat types. Proline, an amino acid, plays a crucial role in various physiological processes in plants, including stress tolerance. In wheat, proline exhibits a complex and multifaceted relationship, influencing various aspects of its growth, development, and response to environmental challenges. Furthermore, proline is crucial in supporting cellular structures and membranes by lessening oxidative damage, particularly under the osmotic stress triggered by Cd exposure, as conducted by Kaya et al. (2019) (89) and Ashraf et al. (2018) (90). Moreover, proline signaling pathways of gene expression are associated with antioxidant defense mechanism systems, stress responses, and metal (Cd) transporters, thereby increasing overall Cd tolerance, as announced by Sharma et al. (2019) (87).

However, the exogenous application of proline resulted in a significant reduction in the endogenous levels of H_2O_2 . Proline treatment led to increased phenolic accumulation. Proline's ability to increase phenolic levels and activate the cellular antioxidant system might potentially contribute to its enhancement of induced metal tolerance, as reported by Ashraf et al. (2011) (81). Proline's chemical structure allows it to directly scavenge ROS like superoxide radicals and singlet oxygen, neutralizing their harmful effects (91). Proline helps stabilize cellular

membranes under Cd stress, reducing their vulnerability to oxidative damage caused by ROS (87).

5.8 The Protein Content

This comprehensive analysis conducted the impact of cadmium (Cd) on various concentrations and different wheat species, particularly concerning the total protein content and its distribution in different plant tissues. The study revealed that protein content in wheat species varied significantly under different Cd concentrations, ranging between 11 and 78mg/ml. Our investigation noted the highest concentration of Cd in the shoots, followed by leaves with the highest total protein found at 250 μ M of Cd on *T. dicoccum*, and *T. turgidum* at 100, 250 and 500 μ M of Cd(NO₃)₂ concentrations. This pattern of variation closely aligns with findings presented by Ghosh et al. (2022) (92), who documented a similar range in maize shoots under high Cd conditions. This distribution pattern can be attributed to root-to-shoot translocation and subsequent allocation of Cd within aboveground tissues. Cadmium stress, as observed, can cause extensive cellular damage and block protein synthesis, leading to a reduction in the overall protein content of wheat as found in these results, in *T. monoccocum* at 100 and 500 μ M of Cd(NO₃)₂-treatment. This finding is supported by the work of Rasheed (2014)(93), who also reported diminished protein levels in wheat under high Cd exposure. Similar to our findings, Jawad Hassan et al. (2020) (94) reported a decrease in total protein content in wheat subjected to elevated Cd levels, likely due to stress-induced protein degradation and disruptions in protein synthesis pathways. In general, Cd's impact is manifested at multiple levels within the plant system, from subcellular functions to ecosystem dynamics. Cd exposure increases the production of reactive oxygen species (ROS), leading to electrolyte leakage, overproduction of hydrogen peroxide, and increased levels of malondialdehyde. These biochemical changes are indicate significant stress within the plant system. High Cd concentrations impair enzyme performance and disrupt protein structures, resulting in potential redox-enzymatic regulatory interferences and misfiring of essential biochemical pathways.

5.9 CAT Activity

The effects of different cadmium (Cd) concentrations on seedling growth and the activity of antioxidant enzymes CAT in various wheat landraces cultivars were examined in this research study. This study observed an increase in CAT activity in *T. monococcum* at 100 and 500 μM and in *T. aestivum* at 250 μM of $\text{Cd}(\text{NO}_3)_2$ treatment. Initially CAT activity decreased but then exhibited a significant increase, especially noticeable in the *T. monoccocum* cultivar. This evidence might be related to the response to oxidative stress generated by induced robust enzyme activity.

However, catalase activity reduced to low-detectable levels during certain periods (28 days) of Cd treatment at 100 and 250 μM in *T. turgidum*, while *T. dicoccum* exhibited increased CAT activity at low concentration of $\text{Cd}(\text{NO}_3)_2$ (50 μM). This research finding similar to Jawad Hassan et al. (2020)(94). This dynamic change in catalase activity points to the complex nature of enzymatic responses under stress conditions. Additionally, peroxidase activity also exhibited a notable increase, especially under conditions of oxidative stress induced by H_2O_2 . This increment in peroxidase activity aligns with the role of peroxidase in mitigating oxidative damage in plant cells. This study contributes significantly to the existing knowledge by providing insights into the differential responses of antioxidant enzymes in wheat cultivars under Cd-induced stress. It sheds light on the intricates interplay between various enzymatic pathways and their crucial roles in plant stress tolerance. These findings not only enhance our understanding of plant physiology under heavy metal stress but also have implications for developing strategies to cultivate crops with improved resistance to environmental stressors.

5.10 SOD Activity

Another crucial aspect of this study involved exploring the effects of Superoxide Dismutase (SOD) under $\text{Cd}(\text{NO}_3)_2$ stress at different concentrations (50, 100, 250, and 500 μM) on wheat plants. It was observed that SOD activity increased in *T. monoccocum* and *T. dicoccum* at 100 μM , while *T. turgidum* showed an increase in SOD activity when the Cd concentration increased at 250 and 500 μM . Similiarly, SOD activity showed no significant effect on the content of SOD at the 50 μM of $\text{Cd}(\text{NO}_3)_2$ in *T. dicoccum*, indicating that Cd had no impact at low concentration 50 μM . This results aligns with findings from Hasanuzzaman et al.

(2020) (95). The results revealed a differential response, pointing to intrinsic variations in Cd tolerance across these wheat genotypes, potentially due to genetic diversity in their SOD isozymes and the regulatory pathways governing their expression. The SOD response among the wheat genotypes underscores the significance of genetic factors in determining plant resilience to heavy metal stress and enhanced tolerance to Cd, which are pivotal for breeding programs aimed at cultivating Cd-resistant wheat varieties. While SOD activity serves as a critical indicator of plant defence against oxidative stress, a comprehensive evaluation of Cd tolerance in wheat should also encompass other physiological parameters and aspects impacting grain quality. The study aligns with findings by Hasanuzzaman et al. (2020) (95) which reported an increase in SOD activity in wheat plants under Cd stress, viewed as a defence strategy against the damaging ROS induced by Cd.

5.11 H₂O₂ Hydrogen Peroxide

In this study, we investigated the impact of cadmium (Cd(NO₃)₂) on wheat leaves and the accumulation of certain antioxidant molecules, exploring how hydrogen peroxide H₂O₂ influences oxidative stress tolerance in plants. Our findings observed that oxidative stress induced by H₂O₂ showed that Cd application significantly elevated or increased the H₂O₂ content in the leaves of two wheat cultivars which are *T. aestivum*, and *T. monococcum*, at concentrations of 250 and 500 µM of Cd. In contrast, the wheat *T. turgidum* and *T. dicoccum* recorded an increase in H₂O₂ levels at a low concentration of Cd(NO₃)₂ (50 µM), which is similar to the results conducted by Nawaz et al. (2024) (96). Interestingly, the study also noted that under conditions of elevated H₂O₂ (20 µM), the peroxidase enzyme played a crucial role in mitigating the resultant oxidative stress, indicating the activation of alternative antioxidant mechanisms which observed a similar increase in peroxidase activity under H₂O₂ induced stress in rice leaves. Moreover, this research study observed a decrease in protein content of leaf under oxidative stress. The findings offer valuable perspectives on plant tolerance mechanisms under such stress conditions for future investigations aimed at enhancing plant resilience to environmental stresses (97).

5.12 The Effect of Cd treated on Elements Concentration of Wheat Landraces

The total concentration of elements such as K, P and Ca was found to be higher than that of Mg and Fe under different 100 and 500µM Cd(NO₃)₂ treated of all wheat species which is similar result obtained by Özkutlu and Kara (2019) found inhibition of the absorption of Mg, Ca and K under Cd stress, Cd ions have the potential to influence plant nutrient absorption and transport (98). Gonçalves et al. (2009) (99) discovered that Cd-stressed potatoes had limited absorption of Fe, Mn, Cu, and Zn, and that environmental factors and species tolerance affected the buildup of wheat.

5.13 Phylogenetic Tree on Wheat Landraces

In this study, the phylogenetic relationships among *Triticum* species, specifically *T. monococcum*, *T. dicoccum*, and *T. aestivum*, were explored using sequences from the *ITS2*, *rbcL*, and *matK* regions. Furthermore, *matK* regions have concentrated on the polygenetic relationship among *T. monococcum*, *T. dicoccum*.

The *ITS2* region, known for its variability and effectiveness in distinguishing closely related species, showed that *T. monococcum* is the most ancestral of the three species. This is consistent with the evolutionary history where *T. monococcum* (einkorn wheat) and *T. dicoccum* (emmer wheat) are considered progenitors of the more genetically complex *T. aestivum* (bread wheat). The high bootstrap values at the nodes among the species support these evolutionary relationships which similar to research results has been conducted by Golovnina et al. (2007) and Dizkirici et al. (2013) which are has similar identified *T. monococcum* as an ancestral of the three species (15) (100).

The *rbcL* gene sequences supported the findings from the *ITS2* region, showing a similar phylogenetic structure. The ingroup species formed distinct clades with high bootstrap values, reinforcing the genetic separation and ancestral positioning of *T. monococcum*. The congruence between the *rbcL* and *ITS2* trees enhances the reliability of these phylogenetic inferences.

The analysis of the *matK* region, although limited by the absence of *T. aestivum* sequences, provided valuable insights into the relationship between *T. monococcum* and *T. dicoccum*. These two species formed distinct clades, their

separate evolutionary lineages. Future research should focus to include *T. aestivum* sequences to provide a more comprehensive understanding of the phylogenetic framework Hsiao et al. (1995); Wang et al. (2000) (101) (102).

The distribution of all similar *Triticum* genes are grouped as *T. dicoccum* species (30_T.dic, 31_T.dic, 32_T.dic, 33_T.dic, and 34_T.dic). Also, *T. monococcum* spp. *monococcum* (36_T.mon, 37_T.mon, and 39_T.mon) are grouped together in another group.

This study delves into the intricate phylogenetic relationships within the genus *Triticum*, particularly focusing on the origins of wheat species. The research employs the Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA as a molecular marker to unravel the contributions of various diploid ancestors.

6. CONCLUSIONS AND RECOMMENDATIONS

In physiological research, the impact of heavy metal cadmium toxicity $\text{Cd}(\text{NO}_3)_2$ under different concentrations on the inhibition of seed germination and seedling growth of wheat landrace cultivars *T. aestivum*, *T. turgidum*, *T. dicoccum*, and *T. monococcum* was evaluated. The experiments were conducted under controlled conditions in growth chambers using Petri plates and soil. The study observed a gradual decrease in root and shoot elongation, as well as fresh and dry root and shoot weights, root and shoot toxicity, and tolerance index of seedling growth under conditions controlled by incubation in the growth chamber.

Cadmium adversely affects the morphological and physiological characteristics of wheat on Petri plates, a significant inhibition of coleoptile length was observed in *T. dicoccum*, indicating its high tolerance. Moreover, root elongation was decreased in all wheat species, and seed germination decreased with an increased dose of $\text{Cd}(\text{NO}_3)_2$. However, there was no significant reduction in shoot and root elongation.

The cultivars grown in soil under Cd stress had a significant effect on both seedling germination and development. The impact of $\text{Cd}(\text{NO}_3)_2$ showed no significant reduction in shoot and root elongation, as well as fresh and dry root and shoot weights among wheat cultivars. In addition, shoot and root toxicity were more pronounced in all wheat landraces at higher concentrations with *T. turgidum* exhibiting greater sensitivity to root toxicity at all concentrations. The increase in Cd dose led to a reduction in the tolerance index with maximum tolerance found in *T. aestivum*, followed by *T. monococcum*, *T. dicoccum*, and *T. turgidum*, respectively, across various $\text{Cd}(\text{NO}_3)_2$ concentrations.

Furthermore, the study examined the Cd stress and induced antioxidant enzyme activity (CAT and SOD), and H_2O_2 accumulation in wheat landraces. The reaction conditions of the different antioxidant assays may have a great influence on the effectiveness of antioxidants in the various plant extracts from leaves. Based on these analyses, methanol extracts were found to be potent radical scavengers and primary chain-breaking antioxidants.

Moreover, the total content of chlorophyll a was reduced, while chlorophyll b and carotenoids either increased or decreased with increasing doses of $\text{Cd}(\text{NO}_3)_2$, depending on wheat species. Results also indicated varying changes in oxidative enzyme activity, which could be related to protein damage or protective mechanisms, depending on the wheat plant types.

Cadmium, as a toxic heavy metal, detrimentally interferes with plant physiological processes, primarily through the generation of ROS. In this investigation on the role of phenolic and proline in wheat species under Cadmium (Cd) stress, the total content of phenolic and proline was significantly increased by Cd stress applications. It becomes increasingly clear that phenolic and proline accumulation is an adaptive strategy for plants facing environmental challenges. The critical function of proline in directly scavenging reactive oxygen species (ROS), boosting the activity of key antioxidant enzymes, and fortifying cell membranes collectively contributes to alleviating oxidative stress. Furthermore, proline application has been observed to stimulate antioxidant enzymes, enhancing the plant's internal defense mechanisms. However, the results indicate that cadmium exposure leads to a significant elevation in phenolic content within wheat plants. This increase is likely a defensive response to counteract the oxidative stress caused by Cd, as phenolics are known to possess strong antioxidative properties. The enhanced synthesis of phenolic compounds suggests an adaptive strategy to mitigate oxidative damage and stabilize cellular functions under metal stress (Cd). In contrast to the increase in phenolic compounds, the protein content in wheat plants exposed to Cd showed a notable decline. This reduction can be attributed to the inhibition of protein synthesis and the degradation of existing proteins caused by Cd-induced oxidative stress. The decrease in protein content may also reflect disruptions in nitrogen assimilation and amino acid metabolism, which are critical processes affected by Cd toxicity. This research study establishes that Cd exposure significantly alters the antioxidant defense system in wheat plants, with marked effects on phenolic content, protein synthesis, and antioxidant enzyme activities. The results enhanced the complexity of the responses involved in Cd detoxification and stress adaptation. This section is compulsory for the theses.

Future studies should prioritize unraveling the molecular genetic basis of these responses and exploring the potential for genetic engineering or breeding

programs to enhance Cd tolerance in wheat. This knowledge is crucial for ensuring crop productivity and sustainability in environments contaminated with heavy metals. By understanding the underlying genetic mechanisms involved in Cd tolerance, researchers can develop targeted strategies to breed wheat varieties with improved resilience to Cd stress. This can ultimately contribute to the maintenance of agricultural productivity and food security in areas affected by heavy metal contamination.

In the genetic study, a comprehensive investigation was conducted focusing on the genetic diversity of wheat landraces sampled from Türkiye regions as presented in Table 3.3. This was achieved through sequencing and amplification of regions of (*ITS2*, *mat K* and *rbc L*) by using PCR.

The study's findings provide significant insights into the phylogenetic relationships and genetic diversity among *T. monococcum*, *T. dicoccum*, and *T. aestivum*. Furthermore, the use of *ITS2*, *rbcL*, and *matK* markers has effectively illustrated the genetic distinctions between these species. These results contribute to the taxonomy and conservation strategies for wheat species and the importance of using multiple genetic markers in phylogenetic studies.

The use of *ITS2* and chloroplast markers like *rbcL* and *matK* has been shown to be effective in resolving phylogenetic relationships within the Triticeae. Furthermore, various Sequence data evaluations were tested for resolving the phylogenetic relationships among *Triticum* species. The present study was conducted with a small sampling size which includes 17 known species and subspecies in the genus *Triticum*. Even though this research has low sample size *ITS2* and *rbcL* regions provide good resolution.

The *ITS2* region, known for its conserved nature among closely related species, alongside the more variable *rbcL* gene, provided a complementary approach that enhanced our understanding of the phylogenetic relationships and evolutionary history of these taxa. Our results suggest that there is a considerable degree of genetic diversity within each species, which may reflect their adaptive responses to different environmental pressures and historical biogeographical events. The distinct genetic patterns observed between *T. monococcum*, *T. dicoccum*, and *T. aestivum*, support the notion of these being separate species, each

with unique evolutionary paths. This study not only contributes to the taxonomy and conservation strategies for wheat species but also focuses on to study relationships between species and the importance of using multiple genetic markers to obtain a comprehensive view of species diversity.

From an agricultural perspective, these findings can have significant implications for the conservation and utilization of wheat genetic resources. Understanding the genetic relationships and diversity among these species is crucial for breeding programs aimed at developing new varieties that can withstand environmental stressors, and disease, and yield high-quality grain.



7. REFERENCES

Vancouver citation system was used in this thesis.

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