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GST Enzyme Content of Wheat Landraces and Comparison with Modern Varieties

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ABSTRACT

The development of high-yielding modern wheat varieties to feed the growing population has had a negative impact on the production of ancestral and landrace crops. The use of modern varieties, which are very deficient in vitamins, minerals, antioxidants, and flavonoids, has caused people to turn to old varieties due to health problems that arise over time. In this study, which aimed to determine the glutathione S-transferase (GST) enzyme activity of registered varieties and landraces, the differences between the protein values and GST enzyme activity values of wheat were found to be statistically significant. When protein values were analysed among wheat varieties, einkorn wheat had the highest value with 15.53 mg/ml, and KUNDURU-1149 had the second highest value with 13.52 mg/ml. The lowest protein values were found in wheat landraces. Lr-4 had the highest GST enzyme activity with 299.7 mmol/min/mg protein and Lr-10 with 265.3 mmol/min/mg protein. A negative and high correlation was found between wheat protein values and GST enzyme activity, and it was determined that landraces were prominent in terms of GST enzyme activity.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the first plants cultivated by human beings; they are among the most important indicators of the transition to settled life (Uhri, 2011) and an important crop of the world and are considered an essential food for one-third of the World's population (Jamil et al., 2018). Turkey is an important gene center and is also the homeland of wheat (Kan et al., 2017). Today, there are about 25,000 varieties of wheat in the World, including 23 wild and nearly 400 cultivated varieties in Anatolia (Özberk et al., 2016). For thousands of years, farmers have identified, selected, propagated, and preserved landraces and have passed them down to the present day. Landraces are heterogeneous in their populations as they contain a large number of genotypes, all planted and harvested at the same time, and are specially adapted to the environmental conditions of the area where they are grown (e.g., tolerant to the biotic and abiotic stresses of the region) (FAO, 2019; Altunel et al., 2021). Landraces were not able to compete with modern varieties in terms of yield and profitability, and their cultivation areas declined, but nowadays they are regaining interest among farmers, consumers, and producers due to their higher genetic diversity and adaptability, as well as their desirable nutritional profile and palatability (Özberk et al., 2016; Kaplan Evlice, 2021; Živančev et al., 2023). Although natural antioxidant sources are animal- and plant-based, plant-based antioxidants are the most important natural antioxidants. Antioxidant activity is increased by protein hydrolysates in the content of many foodstuffs (Akıllıoğlu & Yalçın, 2010). In addition to being a cheap energy source and nutritious, wheat is also remarkable for its antioxidant properties (Doğan & Meral, 2006). When the antioxidant effects and free radical scavenging levels of gluten and germ, which are by-products of the wheat milling process, are examined, it is found that gluten is equivalent to vitamin E, butyl hydroxytoluene (BHT), and ascorbic acid, while germ is as antioxidant as α-tocopherol (Akıllıoğlu & Yalçın, 2010).

Glutathione (GSH), one of the important antioxidants that defends the body against free radicals, is a protein component and is composed of glycine, cysteine and glutamate amino acids (Aksoy, 2002). A low level of GSH in the cellular level or an increase in the number of free radicals leads to oxidative stress, which is the first stage of carcinogenic cell formation. Oxidative stress then causes damage to biological molecules such as fat, protein, carbohydrate and DNA. This damage plays an important role in the formation of cancer cells. In addition to this

function, GSH has many physiological functions such as neutralization of xenobiotics (drugs and toxic substances), transport of amino acids, keeping sulfhydryl groups in proteins in reduced state and acting as coenzyme in some enzymatic reactions (Aktaş et al., 2005). In order for GSH to accomplish its functions, it must bind to a molecule or an electron. The enzyme that makes this connection is the GST enzyme (Boyland & Chasseaud, 1969; Kumar & Trivedi, 2018).

The stationary life of plants has led to the evolution of a complex gridded antioxidant defence system constituting numerous enzymatic components, playing a crucial role in overcoming various stress conditions (Rajput et al., 2021). GSTs, which are ubiquitous (Shahrtash, 2013; Frova, 2023) and mainly cytosolic (Mohabatkar et al., 2009; Mohsenzadeh et al., 2011), are a large complex family of enzymes (EC 2.5.1.18) that play vital roles in flavonoid metabolism, response to abiotic stress (Board et al., 1990; Allocati et al., 2018; Gullner et al., 2018; Wang et al., 2019; Hasan et al., 2021; Jiang et al., 2022; Li et al., 2022), and plant growth and development (Gao et al., 2020). GSTs were discovered 50 years ago as enzymes capable of conjugating electrophilic organic substances with the thiol group of glutathione (Alan, 2013; Bengt, 2013). In addition, GST evolved from a gene duplication of an ancestral GSH-binding protein. They have been applied in various plant functions such as xenobiotic detoxification, growth, and development, and especially against biotic and abiotic stresses (Marrs, 1996; Laborde, 2010; Vaish et al., 2020; Zhuge et al., 2020; Hao et al., 2021). They are involved in a variety of intracellular events such as primary and secondary metabolisms, stress metabolism, herbicide detoxification (Gyamfi et al., 2004; Öztetik, 2010; Karpenko et al., 2019), and plant protection against ozone damage and heavy metals (Mohsenzadeh et al., 2011; Hacıoğlu, 2015; Kumar & Trivedi, 2018). Besides other functions, the results of GST transcript measurements in wheat leaves indicate that some GST isoenzymes have important roles in drought stress responses during both monocarpic senescence and grain filling (Galle et al., 2009). Furthermore, significant increases in GST levels in leaves in plant diseases is an important indicator that it has an effect on disease resistance (Mohammadı et al., 2000; Gullner et al., 2018; Galle et al., 2022). It is concluded that the activity of H_2O_2 scavenging enzymes and that of GST enzyme have a crucial role in detoxifying toxic compounds leading to more resistance against salt stress (Mohammadı et al., 2016).

GSTs also have different effects on human health. It mainly protects DNA (Stein et al., 2010) and proteins from damage by catalysing the binding of the sulfhydryl group of GSH to electrophilic substances. It increases cell resistance by inhibiting free radicals in the cell (Sun et al., 2023). Recently, GSTs have also been shown to act as modulators of signal transduction pathways that control cell proliferation and cell death (Laborde, 2010). Early on, GSTs were identified as prominent detoxication enzymes that protect cells against mutagens and carcinogens. It would appear that GSTs counter the effects of oxidative stress associated with numerous degenerative conditions such as Parkinson and Alzheimer disease, cataracts, atherosclerosis, diabetes, and cancer (Bengt, 2013). Also, oxidative stress plays an important role in the development of type 1 diabetes (T1D) and its complications. GST is one of the defense systems against the harmful effects of oxidative stress (Karkucak et al., 2012).

The increase in the number of free radicals can be tolerated by balancing the GSH level. In order for the level of GSH produced by the cells to not be insufficient, GSH must be taken by direct or indirect methods. Considering that wheat is the largest grain group consumed by humans, the GSH level in wheat is of great importance (Aksoy, 2002). The GST enzyme is an endogenous enzyme synthesized in the human body. It prevents the formation of free radicals in the body or protects the body from the harmful effects of these radicals by reducing the radicals formed (Ekici & Sağdıç, 2008). Considering the dietary habits and economic accessibility of today's societies, it can be said that wheat is the easiest and cheapest available source of GST. Compared to modern varieties, growing wheat landrace populations under organic conditions in their natural environment and making them available to consumers is important for healthy nutrition. It has the potential to fill an important gap in terms of healthy food supply, in particular infant nutrition, which is on the rise, as well as the problems of malnutrition, which is seen as the cause of many diseases, as well as foodstuffs produced using chemicals, where chronic diseases are increasing (Keçeli, 2019). In this study, it was aimed to determine and compare the protein values and GST activities of some 16 wheat landraces, 1 einkorn wheat, and some registered wheat varieties (Bayraktar-2000, Kunduru-1149, Ç-1252, Kıraç-66, Eminbey, Gün-91, Köse-220/39) obtained from local wheat farmers in different districts of Denizli province.

Materials and methods

Materials

Registered varieties and wheat landraces were used as material in the study. Samples of wheat landraces grown in Denizli province were collected from farmers (Table 1), and registered varieties were obtained from the Central Research Institute of Field Crops/ANKARA (GÜN-91 (Bread wheat/Red), KÖSE-220/39 (Bread wheat/Red), BAYRAKTAR-2000 (Bread wheat/White), Ç-1252 (Durum wheat), EMİNBEY (Durum wheat)) and Transitional Zone Agricultural Research Institute /ESKİŞEHİR (KIRAÇ-66 (Bread wheat/White), KUNDURU-1149 (Durum wheat)). Samples were kept at the Pamukkale University Faculty of Applied Sciences Seed and Genetic Stock Unit. Landraces and registered varieties are shown in Figure 1.

Methods

Sample Preparation for Measurement of GST Enzyme Activity

20–25 grains of landraces and registered wheat varieties collected for the study were placed in a mortar. Liquid nitrogen at -196ºC was added slowly and crushed with a porcelain mortar and pestle, and the addition of liquid nitrogen and crushing process were continued until the wheat was thoroughly crushed and turned into dry powder. Liquid nitrogen is used as a cooling agent to prevent the possible negative effects of the heat generated during grinding on the grain structure.

Figure 1. Registered wheat varieties and landraces used in the study

Table 1. Coordinates of the locations where the samples were collected

Landraces	Province / District / Village	Coordinates
*Lr-1	Denizli/Tavas/Balkıca	37.336972, 29.066686
Lr-2	Denizli/Tavas/Merkez	37.577063, 29.008511
Lr-3	Denizli/Tavas/Sarıabat	37.632638, 29.191802
Lr-4	Denizli/Pamukkale/Karataş	37.677967, 29.188153
Lr-5	Denizli/Tavas/Vakıf	37.613386, 28.983435
Lr-6	Denizli/Çameli/Gökçeyaka	37.044980, 29.286490
Lr-7	Denizli/Çivril/İğdir	38.376302, 29.783410
Lr-8	Denizli/Çivril/İğdir	38.331587, 29.758316
Lr-9	Denizli/Çameli/Gökçeyaka	37.066440,29.308330
Lr-10	Denizli/Tavas/Medet	37.515674, 29.010782
Lr-11	Denizli/Tavas/Balkıca	37.349183, 29.068396
Lr-12	Denizli/Tavas/Balkıca	37.334624, 29.063928
Lr-13	Denizli/Acıpayam/Kelekçi	37.211857, 29.290179
Lr-14	Denizli/Tavas/Balkıca	37.327446, 29.086800
Lr-15	Denizli/Tavas/Balkıca	37.345330, 29.066278
Lr-16	Denizli/Tavas/Balkıca	37.337100, 29.053566
Lr-17 (Einkorn)	Denizli/Güney/Eziler	38.192154, 28.945641

*Lr: Landrace

Each sample was labelled and placed in a tube. The powdered wheat sample tubes were kept at +2ºC until the next step. To start the extraction process, 0.5 g of each dry powdered wheat variety was weighed on a precision balance and placed in falcon tubes. To each sample, 3 ml of buffer solution [0.1 M phenylmethylsulfonyl fluoride (PMSF), 0.1 M aminocaproic acid (e-ACA), 0.4 M potassium phosphate (KPi), 10% Tritron X-100, and 0.1 M ethylenediaminetetraacetic acid (EDTA)] was added. In the homogenizer device, the tubes were stirred four times for one minute with a ten-second break, with the tubes constantly on ice. Then, centrifuged at 12000 rpm for 30 min at 4°C (Semiz et al. 2016), the upper liquid-clear parts were taken into Eppendorf tubes, passed through carbon dioxide gas, and sealed. Wheat extracts were stored in Eppendorf tubes at -86ºC until analysis.

Protein value determination

Conducting the analysis

Samples of wheat varieties and wheat landraces were ground and stored at -86ºC, then extracted and liquid extracts were taken, and protein values were determined according to the method of Lowry et al. (1951) using "Bovine Serum Albumin (BSA)" as a standard.

The protein standards obtained are mixed in balloon jugs. After dissolution, they are stored in plastic or glass bottles at 4°C. Wheat samples were diluted 1:200 (0.1 ml sample was completed to 20 ml with H_2O) in volumes ranging from 0.05 ml to 0.2 ml (0.05 ml, 0.1 ml, 0.2 ml), with a total volume of 0.2 ml.

After adding the alkaline copper reagent formed by mixing 0.1 N NaOH containing 2% sodium potassium tartrate, 2% sodium carbonate, and 2% copper sulphate into the tubes, it was mixed with a vortex. 8 or 10 minutes at room temperature with vortex mixing. Afterwards, 0.1 ml of folin reagent diluted 1:1 with distilled water was added to each tube and incubated at 50 °C for 30 min. After incubation, the intensity of the color in each tube was measured against the blind at 660 nm in a spectrophotometer. Protein values were calculated according to the following formula using the slope value obtained.

$$\text{Protein (mg/ml)} = \left[\frac{Abs(660nm)}{Slope}\right] * \left[\frac{1}{Sft}\right] * \left[\frac{1}{Sfa}\right]$$

Sft: In-tube dilution factor

Sfa: Original dilution factor

Spectrophotometric determination of GST activity with dichloro-4-nitrobenzene (DCNB) substrate

Conducting the analysis

Total GST activity was measured by placing the prepared wheat extracts in the spectrophotometer cuvette at 340 nm wavelength using 1,2-dichloro-4-nitrobenzene (DCNB) substrate in the order of components in the spectrophotometer cuvette, inverting and stirring several times, and recording the activity measurement between the 10th and 70th seconds. This procedure was repeated three times and calculated according to the following formula:

Activity (mmol/min/mg protein) = [(OD/min) / 0.0096 nmole]. 40 (1 mg/ml)

Solutions used in the study and preparations

To prepare 0.05 M GSH, 0.0153 g GSH was dissolved in 1 ml distilled water. It was prepared daily and stored on ice.

To prepare 0.02 M DCNB, 0.00384 g DCNB was dissolved in 1 ml EtOH. Prepared daily and stored on ice.

To prepare 0.4 M KPi, 13.601 g KH_2PO4 was weighed and dissolved in 250 ml distilled water. 17.418 g KH_2PO_4 was weighed and dissolved in 250 ml distilled water. K_2HPO_4 was titrated slowly with KH_2PO_4 to pH 7.5.

Statistical Analysis

All tests were run in three parallels. The statistical analysis software JMP 13.2.1 (2017) was used to do an analysis of variance (ANOVA). The LSD test resulted in a significant mean separation ($p < 0.01$).

Results and Discussion

Significant differences have been observed between wheat genotypes/varieties used in the study ($p<0.01$). Protein levels (in mg/ml) for the wheat cultivars examined in the study are provided. When the protein analysis findings were analyzed, Lr-17 einkorn wheat and Kunduru-1149 registered variety had higher protein levels than the other samples. Lr-17 (Einkorn) had the highest protein value of 15.53 mg/ml, while KUNDURU-1149 had the second highest at 13.52 mg/ml. Lr-10 had the lowest protein value of 7.04 mg/ml, with Lr-4 coming in second at 7.49 mg/ml. The average results of the other samples showed close values between 11.07 and 7.94 and were classified as Lr-15, Lr-1, EMIN BEY, Lr-11, Lr-5, Lr-16, KÖSE-220/39, Lr-14, GÜN-91, BAYRAKTAR-2000, KIRAÇ-66, Lr-8, Lr-9, Ç-1252, Lr-12, Lr-3, Lr-6, Lr-13, and Lr-2, from high to low. The average for registered wheat varieties was 10,27 mg/ml, but the average for wheat landraces was 9.65 mg/ml. While landrace protein averages were lower than the overall average, cultivar protein levels were higher. Landraces have lower protein content than cultivars, with the exception of einkorn (Figure 2).

Wheat genotypes have significantly different GST activity ($p < 0.01$). The GST activities of the wheat genotypes and varieties examined in the study were expressed in mmol/min/mg protein. When the GST-DCNB activity results were evaluated, the wheat varieties with the highest activity were Lr-4 (299.3 mmol/min/mg protein) and Lr-10 (265.7 mmol/min/mg protein). Lr-17 (Einkorn) wheat had the lowest GST activity value, measuring 118.1 mmol/min/mg protein (Table 2).

The values of other varieties and genotypes were between these two values. When GST-DCNB activity is ranked from higher to lower: Lr-13, Lr-2, Lr-3, Lr-6, Lr-9, Lr-8, Lr-14, Lr-12, GÜN-91, Ç-1252, Lr-7, BAYRAKTAR-2000, KIRAÇ-66, EMIN BEY, Lr-16, Lr-5, Lr-1, Lr-11, KÖSE-220/39, Lr-15, and KUNDURU-1149. While the average of registered wheat varieties was 178.9 mmol/min/mg protein, the average of wheat landrace varieties was 204.7 mmol/min/mg protein. While the average GST activity values of the registered varieties were below the general average, the GST activity values of the landrace varieties were above the average.

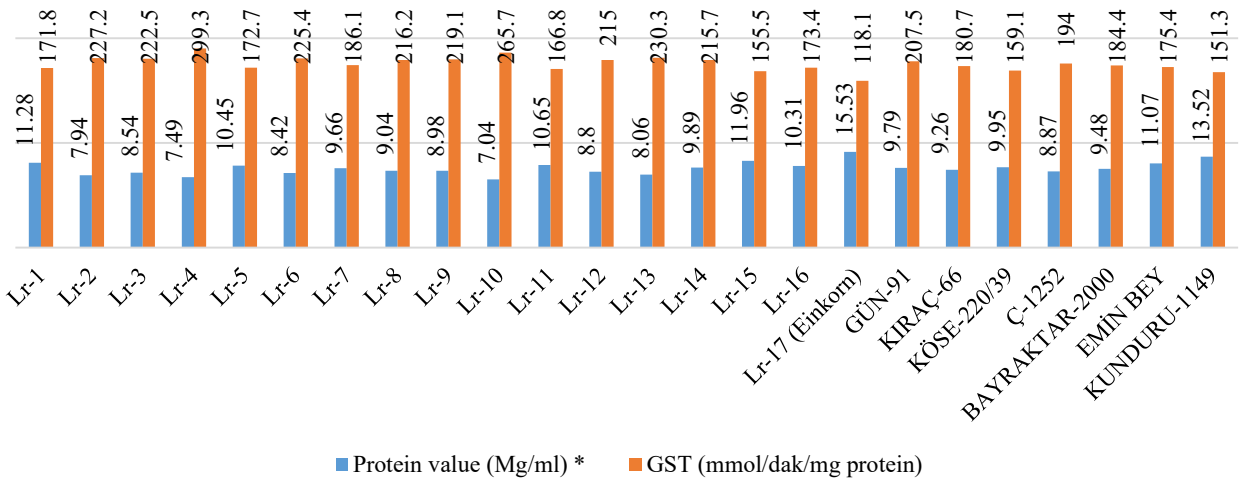

Figure 2. Graphic for protein content and GST enzyme results (Excel was used to create graphic)

Table 2 Protein and GST results

Variety / Genotype	Protein value (Mg/ml) *	Std. Dev.	GST (mmol/dak/mg protein)	Std. Dev.
Lr-1	11.28 ^{cd}	0.596494664	171.8^{gh}	9.951605
Lr-2	7.94 $^{m-o}$	2.27616374	227.2^{cd}	6.08376
Lr-3	8.54 $^{k-n}$	0.774767849	222.5^{cd}	30.01478
Lr-4	7.49 $^{n-o}$	0.325030874	299.3^{a}	20.06144
Lr-5	10.45 $^{d-f}$	0.323513963	172.7^{gh}	5.534154
Lr-6	8.42 $^{k-n}$	0.474054578	225.4^{cd}	18.01908
Lr-7	9.66 $^{e-j}$	1.03281816	186.1^{fg}	4.579707
Lr-8	9.04 $^{h-l}$	1.063061801	216.2^{cd}	15.64396
Lr-9	8.98 $^{h-l}$	1.654793806	219.1^{cd}	15.70883
Lr-10	7.04 ^{o}	0.28264429	265.7^{b}	21.34378
Lr-11	10.65 ^{de}	2.182561632	166.8$^{g-ı}$	8.417938
Lr-12	8.8 $^{j-m}$	0.480670457	215^{cd}	12.57165
Lr-13	8.06 $^{l-o}$	1.041301918	230.3^{c}	4.60356
Lr-14	9.89 $^{e-ı}$	0.690733851	215.7^{cd}	16.98057
Lr-15	11.96 ^{c}	0.046329683	155.5^{hı}	7.525971
Lr-16	10.31 $^{d-g}$	1.423860659	173.4^{gh}	8.347273
Lr-17 (Einkorn)	15.53 ^{a}	0.503835299	118.1^{j}	5.101781
GÜN-91	9.79 $^{e-j}$	1.637359962	207.5^{de}	20.682
KIRAÇ-66	9.26 $^{g-k}$	1.925215268	180.7^{fg}	3.813952
KÖSE-220/39	9.95 $^{e-h}$	0.707902921	159.1^{hı}	11.02349
Ç-1252	8.87 $^{ı-m}$	0.97531043	194^{ef}	12.28835
BAYRAKTAR-2000	9.48 $^{f-k}$	0.220065993	184.4^{fg}	1.635307
EMİN BEY	11.07 ^{cd}	0.040538472	175.4^{fh}	2.649001
KUNDURU-1149	13.52 ^{b}	1.321620939	151.3^{ı}	5.389533
Average	9.83		197.2	
Standart Deviation±	1.90		39.3	
CV	6.63		6.18	
LSD	1.07		20.04	

* Letters indicate different groups at 0.01 level, CV: Coefficient of variation.

The results show that landraces have a higher GST activity content than cultivars. The highest activity values were generally obtained from landraces. It is understood that 10 landrace cultivars gave higher values than the cultivars, and GÜN-91 cultivar showed the highest value among the cultivars (Figure 2).

There is a considerable negative association between protein values and GST activity in wheat varieties (Fig. 3). GST activity reduces with increasing protein value. Our findings support the fact that einkorn wheat has the highest protein value (15.53), 118.1 GST activity value, whereas Lr-10 has the lowest protein value (7.04), 265.7 GST activity value, and Lr-4 has the second lowest protein value (7.49), 299.3 GST activity value. A negative and high correlation (r=-0.8638) was found between protein amount and GST enzyme activity (Figure 3).

The results are consistent with Şanal (2017), who reported that the protein value of einkorn wheat was the highest with 15.53 mg/ml compared to wheat landraces and registered varieties. The overall average was also higher than the average of wheat landraces and registered varieties, and the selected landrace populations were higher than registered varieties in terms of protein content and quality based on sedimentation values.

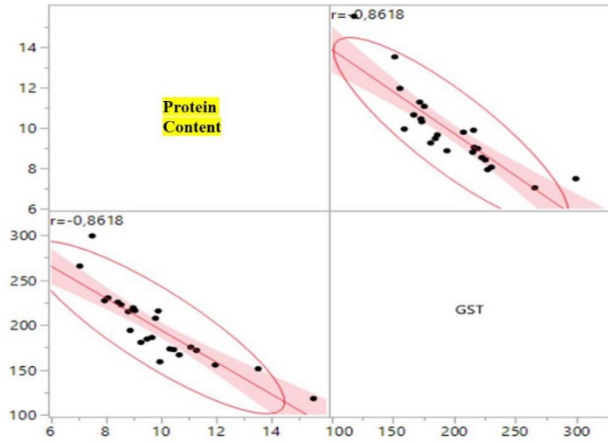

Figure 3. Correlation between protein content and GST

Our GST activity findings are consistent with those of Koyuncu (2009), who found that wheat landraces had higher lipoxygenase activity, polyphenol oxidase activity, and peroxidase oxidative activity than registered varieties in durum wheat landraces, as well as different protein activity, albeit not the same type of protein.

Previous research has examined the impact of plant diseases (*Fusarium graminearum*, *F. culmorum*) on GST in wheat (Mohammadi et al., 2000; Gallee et al., 2022; Guo et al., 2023), as well as the toxic effects of heavy metals (Cu, Pb, Cd, Se) on GST levels (Gökbulut, 2010; Hacıoğlu, 2015; Mohammadı et al., 2016; Jamil et al., 2018; Boukhalfa et al., 2019). Similarly, different researchers have reported that herbicides (atrazine, metachlor, promethrin, etc.) (Cataneo et al., 2002; Miteva et al., 2004; Jiang and Yang, 2009; Öztetik, 2010; Karpenko et al., 2019; Çanakcı Gülengül and Karabulut, 2021), herbicide safeners (Riechers et al., 2003; Theodoulou et al, 2003), polyethyleneglycol (PEG) (Gallée et al., 2005), and drought (Gallée et al., 2009; Sečenji et al., 2010) affect GST content in wheat plants. However, all of these investigations were done on plant roots, stems, and leaves.

GST enzyme activity in plants increases in cereals, particularly under biotic and abiotic stress conditions (Kömives et al., 1985; Pascal et al., 2000; Varga et al., 2012; Rezaei et al., 2013; Lukasik et al., 2015; Wang et al., 2019; Hao et al., 2021). Increasing the amount of GST in the plant as a defense mechanism against heat, cold, salinity, herbicide, and insect damage is a natural protective instinct. These findings can be explained by the fact that wheat landraces have more GST enzyme activity than modern varieties. To the best of our knowledge, no research has been conducted on the GST content of wheat grain and its close relatives. As a result, our research is unique in its field. Furthermore, because no research has been conducted on the subject, adequate discussion was not possible.

In conclusion, our findings demonstrate that while high protein value is a desirable property of cereals, the composition and levels of these components are also significant. It is plausible to argue that landraces are at the forefront of both breeding research and the provision of safe and nutritious food. Furthermore, studies conducted on einkorn, one of the ancient wheats with a rising value today, and intentionally enlightening society have led customers to this product. The same studies should be conducted for these landraces, which are scattered throughout our country, in order to improve their production in their local growing area and ensure that they take their position on the shelves for healthy individuals, allowing the producer to profit as well.

Declarations

The authors declare no conflict of interest.

Acknowledgments

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The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

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